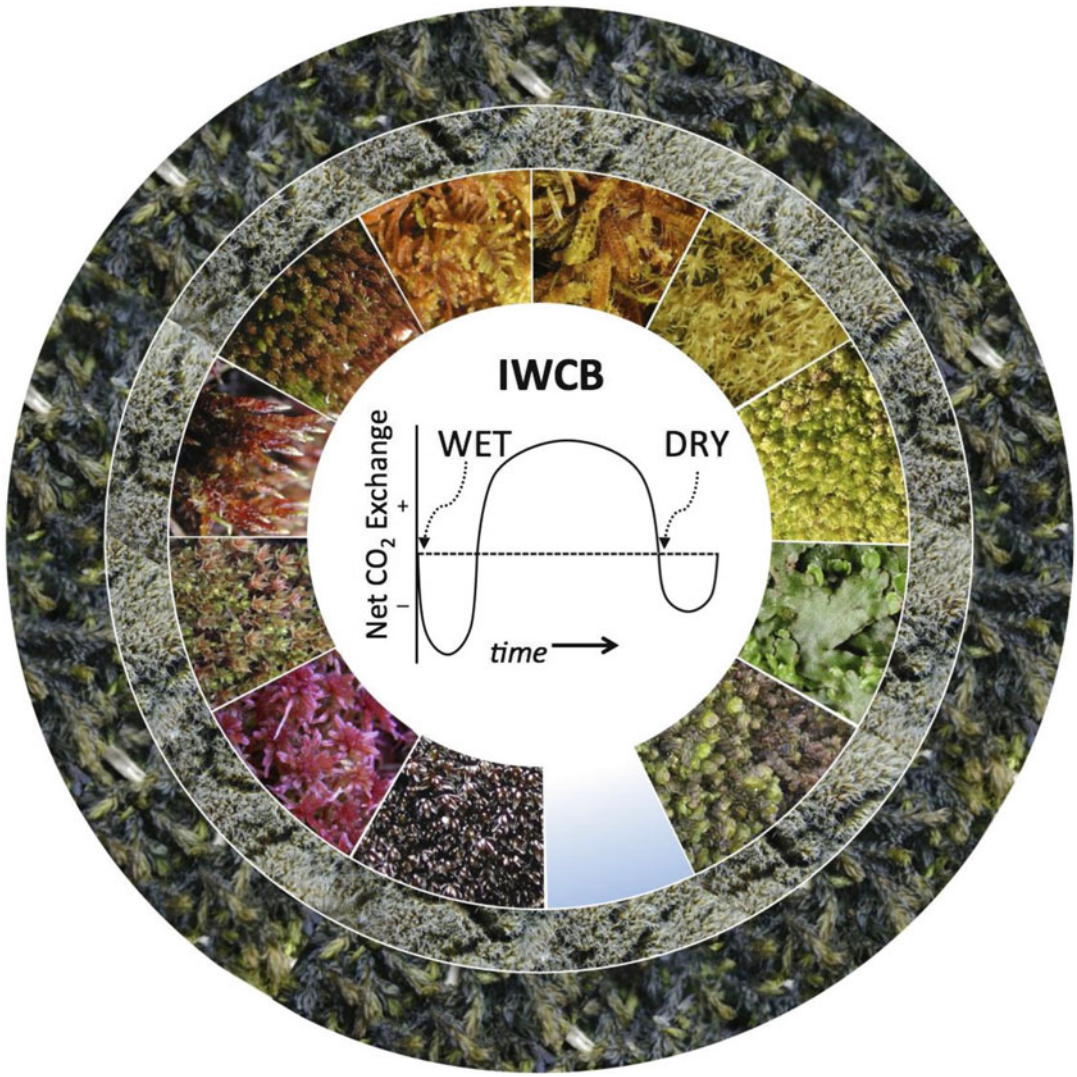
Advances in Photosynthesis and Respiration 37
Including Bioenergy and Related Processes

David T. Hanson
Steven K. Rice *Editors*

Photosynthesis in Bryophytes and Early Land Plants

Springer

Photosynthesis in Bryophytes and Early Land Plants



Bryophyte Color Wheel Bryophytes present diverse photosynthetic physiology and morphology, as evidenced by differences in the organization of the shoot system and by their varied pigmentation. Yet the vast majority are desiccation tolerant, represented by the Integrated Water-Carbon Budget (IWCB) model showing the photosynthetic response following hydration of a dry bryophyte (see Coe et al., Chap. 16, this volume). Bryophytes are arranged on a color wheel starting from the red on the left above the horizontal and progressing clockwise through orange, yellow, green, blue and violet with secondary colors also shown. No known bryophyte expresses blue pigmentation. Clockwise from red, species shown include *Calliergon sarmentosum*, *Bryum muehlenbeckii*, *Cratoneuron commutatum*, *Barbilophozia floerkei*, *Barbula enderesii*, *Bryum capillare*, *Conocephalum conicum*, *Frullania dilatata*, *Andreaea alpestris*, *Sphagnum warnstorfii* and *Bryum arcticum*, *with Grimmia funalis* in the white ring and *Cinclidotus riparius* in the black ring. Photographs by Michael Lüth and composition by Steven Rice.

Advances in Photosynthesis and Respiration
Including Bioenergy and Related Processes

VOLUME 37

The book series Advances in Photosynthesis and Respiration *Including Bioenergy and Related Processes* provides a comprehensive and state-of-the-art account of research in photosynthesis, respiration and related processes. Virtually all life on our planet Earth ultimately depends on photosynthetic energy capture and conversion to energy-rich organic molecules. These are used for food, fuel, and fiber. Photosynthesis is the source of almost all bioenergy on Earth. The fuel and energy uses of photosynthesized products and processes have become an important area of study, and competition between food and fuel has led to resurgence in photosynthesis research. This series of books spans topics from physics to agronomy and medicine; from femtosecond processes through season-long production to evolutionary changes over the course of the history of the Earth; from the photophysics of light absorption, excitation energy transfer in the antenna to the reaction centers, where the highly-efficient primary conversion of light energy to charge separation occurs, through the electrochemistry of intermediate electron transfer, to the physiology of whole organisms and ecosystems; and from X-ray crystallography of proteins to the morphology of organelles and intact organisms. In addition to photosynthesis in natural systems, genetic engineering of photosynthesis and artificial photosynthesis is included in this series. The goal of the series is to offer beginning researchers, advanced undergraduate students, graduate students, and even research specialists, a comprehensive, up-to-date picture of the remarkable advances across the full scope of research on photosynthesis and related energy processes. The purpose of this series is to improve understanding of photosynthesis and plant respiration at many levels both to improve basic understanding of these important processes and to enhance our ability to use photosynthesis for the improvement of the human condition.

For further volumes:
www.springer.com/series/5599

Photosynthesis in Bryophytes and Early Land Plants

Edited by

David T. Hanson
University of New Mexico
New Mexico
USA

and

Steven K. Rice
Union College
New York
USA

Springer

Editors
David T. Hanson
Department of Biology
University of New Mexico
Albuquerque
NM 87131, USA
dthanson@unm.edu

Steven K. Rice
Department of Biological Sciences
Union College
Schenectady
NY 12308, USA
rices@union.edu

ISSN 1572-0233
ISBN 978-94-007-6987-8 ISBN 978-94-007-6988-5 (eBook)
DOI 10.1007/978-94-007-6988-5
Springer Dordrecht Heidelberg New York London

Library of Congress Control Number: 2013949004

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

From the Series Editors

Advances in Photosynthesis and Respiration Including Bioenergy and Related Processes

Volume 37: Photosynthesis in Bryophytes and Early Land Plants

We are delighted to announce the publication of Volume 37 in this series. This is the third volume with the new cover and enhanced web presence. The series publisher, Springer, now makes the table of contents of all of the volumes freely available online. Links to each volume are given below. Readers may also see that this volume and the past few volumes have had significantly more color and the color figures are now better integrated into the chapters, instead of being collected in one section of the book. This improvement was possible because of changes in how the books are produced. Another change is that references to chapters in books are now tracked by bibliographic services. This will help authors provide evidence of the importance of their work. We hope that these updates will maintain the importance of these edited volumes in the dissemination of the science of photosynthesis and bioenergy.

This Book

This volume, *Photosynthesis of Bryophytes and Early Land Plants*, was conceived and edited by David T. (Dave) Hanson (University of New Mexico, Albuquerque, New Mexico, USA) and Steven K. (Steve) Rice (Union College, Schenectady, New York, USA). We are grateful to them for their timely submission of the book and to all the 33 authors, who contributed to this book that describes photosynthesis in bryophytes, plants that were, perhaps, the first to colonize the land, and that today are found in some of the harshest environments on land. Often, photosynthesis research focuses on land plants such as spinach and now *Arabidopsis*. Further, most often, photosynthesis is studied in aquatic organisms such as bacteria and algae (e.g., *Chlamydomonas*). This book focuses on the evolutionary transition between aquatic photosynthesis and terrestrial photosynthesis. As plants colonized the land, water availability, intense sunlight, and diffusion of CO_2 became important issues that determined which organisms would be successful. This book describes the latest information about how land plants adapted to the aerial environment. Fascination by one of us (TDS) with this topic began when Dave Hanson worked jointly in his laboratory and that of Linda Graham (see Chap. 2, this book). He is very grateful that Dave and Steve were willing to draw together so many experts to create this valuable look at the transition of photosynthesis from aquatic environments to aerial environments.

Authors

The book contains 18 chapters written by 33 authors from 8 countries [Australia (5); Canada (1); Estonia (2); Germany (4);

UK (6); The Netherlands (1); and USA (13)]. We thank all the authors for their valuable contribution to this book; their names (arranged alphabetically) are:

Maaike Y. **Bader** (Germany; Chap. 15); Jayne Belnap (USA; Chap. 16); Jessica Bramley-Alves (Australia; Chap. 17); Kirsten K. **Coe** (USA; Chap. 16); Martha Cook (USA; Chap. 2); J. Hans C. Cornelissen (The Netherlands; Chap. 5); David J. Cove (UK; Chap. 11); Andrew C. Cuming (UK; Chap. 11); Dianne **Edwards** (UK; Chap. 3); Lawrence B. **Flanagan** (Canada; Chap. 14); Linda **Graham** (USA; Chap. 2); Janice M. Glime (USA; Chap. 12); Tomáš **Hájek** (Czech Republic; Chap. 13); David T. Hanson (USA; Chaps. 1, 6, 10, 18); Diana H. **King** (Australia; Chap. 17); Martina Königer (USA; Chap. 8); Louise A. **Lewis** (USA; Chap. 2); Rebecca E. **Miller** (Australia; Chap. 17); Ülo **Niinemets** (Estonia; Chap. 9); Zach **Portman** (USA; Chap. 10); Michael C.F. Proctor (UK; Chap. 4); John A. **Raven** (UK; Chap. 3); Karen Renzaglia (USA; Chap. 6); Steven K. Rice (USA; Chaps. 1, 5, 10, 18); Sharon A. Robinson (Australia; Chap. 7, 17); Jed P. **Sparks** (USA; Chap. 16); Wilson **Taylor** (USA; Chap. 2); Mari Tobias (Estonia; Chap. 9); Juan Carlos **Villarreal** (Germany; Chap. 6); Sebastian **Wagner** (Germany; Chap. 15); Melinda J. Waterman (Australia; Chap. 7); Charles Wellman (UK; Chap. 2); Gerhard **Zotz** (Germany; Chap. 15).

Our Books: Now 37 Volumes

We list below information on the 36 volumes that have been published thus far (see http://www.springer.com/series/5599 for the series web site). We are pleased to note that Springer, our publisher, is now producing complete *Tables of Contents* of these books. Electronic access to individual chapters depends on subscription (ask your librarian) but Springer provides free downloadable front matter as well as indexes for nearly all volumes. The available web sites of the books in the Series are listed below.

- **Volume 36 (2013) Plastid Development in Leaves during Growth and Senescence**, edited by Basanti Biswal, Karin Krupinska and Udaya Biswal, from India and Germany. Twenty-eight chapters, 837 pp, Hardcover, ISBN: 978-94-007-5723-3 (HB) ISBN 978-94-007-5724-0 (e-book) [http://www.springerlink.com/content/978-94-007-5723-3/]
- **Volume 35 (2012) Genomics of Chloroplasts and Mitochondria**, edited by Ralph Bock and Volker Knoop, from Germany. Nineteen chapters, 475 pp, Hardcover, ISBN: 978-94-007-2919-3 (HB) ISBN 978-94-007-2920-9 (e-book) [http://www.springerlink.com/content/978-94-007-2919-3/]
- **Volume 34 (2012) Photosynthesis - Plastid Biology, Energy Conversion and Carbon Assimilation**, edited by Julian Eaton-Rye, Baishnab C. Tripathy, and Thomas D. Sharkey, from New Zealand, India, and USA. Thirty-three chapters, 854 pp, Hardcover, ISBN: 978-94-007-1578-3(HB)ISBN978-94-007-1579-0 (e-book) [http://www.springerlink.com/content/978-94-007-1578-3/]
- **Volume 33 (2012): Functional Genomics and Evolution of Photosynthetic Systems**, edited by Robert L. Burnap and Willem F.J. Vermaas, from USA. Fifteen chapters, 428 pp, ISBN: 978-94-007-1532-5 [http://www.springerlink.com/content/978-90-481-1532-5/]
- **Volume 32 (2011): C4 Photosynthesis and Related CO2 Concentrating Mechanisms**, edited by Agepati S. Raghavendra and Rowan Sage, from India and Canada. Nineteen chapters,425pp,Hardcover,ISBN:978-90-481-9406-3 [http://www.springerlink.com/content/978-90-481-9406-3/]
- **Volume 31 (2010): The Chloroplast**: **Basics and Applications**, edited by Constantin Rebeiz (USA), Christoph Benning (USA), Hans J. Bohnert (USA), Henry Daniell (USA), J. Kenneth Hoober (USA), Hartmut K. Lichtenthaler (Germany), Archie R. Portis (USA), and Baishnab C. Tripathy (India). Twenty-five chapters, 451 pp, Hardcover, ISBN: 978-90-481-8530-6 [http://www.springerlink.com/content/978-90-481-8530-6/]

- **Volume 30 (2009): Lipids in Photosynthesis: Essential and Regulatory Functions**, edited by Hajime Wada and Norio Murata, both from Japan. Twenty chapters, 506 pp, Hardcover, ISBN: 978-90-481-2862-4; e-book, ISBN: 978-90-481-2863-1 [http://www.springerlink.com/content/978-90-481-2862-4/]
- **Volume 29 (2009): Photosynthesis in Silico: Understanding Complexity from Molecules**, edited by Agu Laisk, Ladislav Nedbal, and Govindjee, from Estonia, The Czech Republic, and USA. Twenty chapters, 525 pp, Hardcover, ISBN: 978-1-4020-9236-7 [http://www.springerlink.com/content/978-1-4020-9236-7/]
- **Volume 28 (2009): The Purple Phototrophic Bacteria**, edited by C. Neil Hunter, Fevzi Daldal, Marion C. Thurnauer and J. Thomas Beatty, from UK, USA and Canada. Forty-eight chapters, 1053 pp, Hardcover, ISBN: 978-1-4020-8814-8 [http://www.springerlink.com/content/978-1-4020-8814-8/]
- **Volume 27 (2008): Sulfur Metabolism in Phototrophic** Organisms, edited by Christiane Dahl, Rüdiger Hell, David Knaff and Thomas Leustek, from Germany and USA. Twenty-four chapters, 551 pp, Hardcover, ISBN: 978-4020-6862-1 [http://www.springerlink.com/content/978-1-4020-6862-1/]
- **Volume 26 (2008): Biophysical Techniques Photosynthesis, Volume II**, edited by Thijs Aartsma and Jörg Matysik, both from The Netherlands. Twenty-four chapters, 548 pp, Hardcover, ISBN: 978-1-4020-8249-8 [http://www.springerlink.com/content/978-1-4020-8249-8/]
- **Volume 25 (2006): Chlorophylls and Bacteriochlorophylls: Biochemistry, Biophysics, Functions and Applications**, edited by Bernhard Grimm, Robert J. Porra, Wolfhart Rüdiger, and Hugo Scheer, from Germany and Australia. Thirty-seven chapters, 603 pp, Hardcover, ISBN: 978-1-40204515-8 [http://www.springerlink.com/content/978-1-4020-4515-8/]
- **Volume 24 (2006): Photosystem I: The Light-Driven Plastocyanin: Ferredoxin Oxidoreductase**, edited by John H. Golbeck, from USA. Forty chapters, 716 pp, Hardcover, ISBN: 978-1-40204255-3 [http://www.springerlink.com/content/978-1-4020-4255-3/]
- **Volume 23 (2006): The Structure and Function of Plastids**, edited by Robert R. Wise and J. Kenneth Hoober, from USA. Twenty-seven chapters, 575 pp, Softcover, ISBN: 978-1-4020-6570-6; Hardcover, ISBN: 978-1-4020-4060-3 [http://www.springerlink.com/content/978-1-4020-4060-3/]
- **Volume 22 (2005): Photosystem II: The Light-Driven Water: Plastoquinone Oxidoreductase**, edited by Thomas J. Wydrzynski and Kimiyuki Satoh, from Australia and Japan. Thirty-four chapters, 786 pp, Hardcover, ISBN: 978-1-4020-4249-2 [http://nwww.springerlink.com/content/978-1-4020-4249-2/]
- **Volume 21 (2005): Photoprotection, Photoinhibition, Gene Regulation, and Environment**, edited by Barbara Demmig-Adams, William W. Adams III and Autar K. Mattoo, from USA. Twenty-one chapters, 380 pp, Hardcover, ISBN: 978-14020-3564-7 [http://www.springerlink.com/content/978-1-4020-3564-7/]
- **Volume 20 (2006): Discoveries in Photosynthesis**, edited by Govindjee, J. Thomas Beatty, Howard Gest and John F. Allen, from USA, Canada and UK. One hundred and eleven chapters, 1304 pp, Hardcover, ISBN: 978-1-4020-3323-0 [http://www.springerlink.com/content/978-1-4020-3323-0/]
- **Volume 19 (2004): Chlorophyll *a* Fluorescence: A Signature of Photosynthesis**, edited by George C. Papageorgiou and Govindjee, from Greece and USA. Thirty-one chapters, 820 pp, Hardcover, ISBN: 978-1-4020-3217-2 [http://www.springerlink.com/content/978-1-4020-3217-2/]
- **Volume 18 (2005): Plant Respiration: From Cell to Ecosystem**, edited by Hans Lambers and Miquel Ribas-Carbo, from Australia and Spain. Thirteen chapters, 250 pp, Hardcover, ISBN: 978-14020-3588-3 [http://www.springerlink.com/content/978-1-4020-3588-3/]
- **Volume 17 (2004): Plant Mitochondria: From Genome to Function**, edited by David Day, A. Harvey Millar and James Whelan, from

Australia. Fourteen chapters, 325 pp, Hardcover, ISBN: 978-1-4020-2399-6 [http://www.springerlink.com/content/978-1-7923-2399-6/]

- **Volume 16 (2004): Respiration in Archaea and Bacteria: Diversity of Prokaryotic Respiratory Systems**, edited by Davide Zannoni, from Italy. Thirteen chapters, 310 pp, Hardcover, ISBN: 978-14020-2002-5 [http://www.springerlink.com/content/978-1-4020-2002-5]
- **Volume 15 (2004): Respiration in Archaea and Bacteria: Diversity of Prokaryotic Electron Transport Carriers**, edited by Davide Zannoni, from Italy. Thirteen chapters, 350 pp, Hardcover, ISBN: 978-1-4020-2001-8 [http://www.springerlink.com/content/978-0-7923-2001-8/]
- **Volume 14 (2004): Photosynthesis in Algae**, edited by Anthony W. Larkum, Susan Douglas and John A. Raven, from Australia, Canada and UK. Nineteen chapters, 500 pp, Hardcover, ISBN: 978-0-7923-6333-0 [http://www.springerlink.com/content/978-0-7923-6333-0/]
- **Volume 13 (2003): Light-Harvesting Antennas in Photosynthesis**, edited by Beverley R. Green and William W. Parson, from Canada and USA. Seventeen chapters, 544 pp, Hardcover, ISBN:978-07923-6335-4 [http://www.springerlink.com/content/978-0-7923-6335-4/]
- **Volume 12 (2003): Photosynthetic Nitrogen Assimilation and Associated Carbon and Respiratory Metabolism**, edited by Christine H. Foyer and Graham Noctor, from UK and France. Sixteen chapters, 304 pp, Hardcover, ISBN: 978-07923-6336-1 [http://www.springerlink.com/content/978-0-7923-6336-1/]
- **Volume 11 (2001): Regulation of Photosynthesis**, edited by Eva-Mari Aro and Bertil Andersson, from Finland and Sweden. Thirty-two chapters, 640 pp, Hardcover, ISBN: 978-0-7923-6332-3 [http://www.springerlink.com/content/978-0-7923-6332-3/]
- **Volume 10 (2001): Photosynthesis: Photobiochemistry and Photobiophysics**, edited by Bacon Ke, from USA. Thirty-six chapters, 792 pp, Softcover, ISBN: 978-0-7923-6791-8; Hardcover: ISBN: 978-0-7923-6334-7 [http://www.springerlink.com/content/978-0-7923-6334-7/]
- **Volume 9 (2000): Photosynthesis: Physiology and Metabolism**, edited by Richard C. Leegood, Thomas D. Sharkey and Susanne von Caemmerer, from UK, USA and Australia. Twenty-four chapters, 644 pp, Hardcover, ISBN: 978-07923-6143-5 [http://www.springerlink.com/content/978-0-7923-6143-5/]
- **Volume 8 (1999): The Photochemistry of Carotenoids**, edited by Harry A. Frank, Andrew J. Young, George Britton and Richard J. Cogdell, from USA and UK. Twenty chapters, 420 pp, Hardcover, ISBN: 978-0-7923-5942-5 [http://www.springerlink.com/content/978-0-7923-5942-5/]
- **Volume 7 (1998): The Molecular Biology of Chloroplasts and Mitochondria in Chlamydomonas**, edited by Jean David Rochaix, Michel Goldschmidt-Clermont and Sabeeha Merchant, from Switzerland and USA. Thirty-six chapters, 760 pp, Hardcover, ISBN: 978-0-7923-5174-0 [http://www.springerlink.com/content/978-0-7923-5174-0/]
- **Volume 6 (1998): Lipids in Photosynthesis: Structure, Function and Genetics**, edited by Paul-André Siegenthaler and Norio Murata, from Switzerland and Japan. Fifteen chapters, 332 pp, Hardcover, ISBN: 978-0-7923-5173-3 [http://www.springerlink.com/content/978-0-7923-5173-3/]
- **Volume 5 (1997): Photosynthesis and the Environment**, edited by Neil R. Baker, from UK. Twenty chapters, 508 pp, Hardcover, ISBN: 978-07923-4316-5 [http://www.springerlink.com/content/978-0-7923-4316-5/]
- **Volume 4 (1996): Oxygenic Photosynthesis: The Light Reactions**, edited by Donald R. Ort and Charles F. Yocum, from USA. Thirty-four chapters, 696 pp, Softcover: ISBN: 978-0-7923-3684-6; Hardcover, ISBN: 978-0-7923-3683-9 [http://www.springerlink.com/content/978-0-7923-3683-9/]
- **Volume 3 (1996): Biophysical Techniques in Photosynthesis**, edited by Jan Amesz and Arnold J. Hoff, from The Netherlands. Twenty-four chapters, 426 pp, Hardcover, ISBN: 978-0-7923-3642-6 [http://www.springerlink.com/content/978-0-7923-3642-6/]

- **Volume 2 (1995): Anoxygenic Photosynthetic Bacteria**, edited by Robert E. Blankenship, Michael T. Madigan and Carl E. Bauer, from USA. Sixty-two chapters, 1331 pp, Hardcover, ISBN: 978-0-7923-3682-8 [http://www.springerlink.com/content/978-0-7923-3681-5/]
- **Volume 1 (1994): The Molecular Biology of Cyanobacteria**, edited by Donald R. Bryant, from USA. Twenty-eight chapters, 916 pp, Hardcover, ISBN: 978-0-7923-3222-0 [http://www.springerlink.com/content/978-0-7923-3222-0/]

Further information on these books and ordering instructions can be found at http://www.springer.com/series/5599. Contents of volumes 1–31 can also be found at http://www.life.uiuc.edu/govindjee/photosynSeries/ttocs.html. (For volumes 33–35, pdf files of the entire Front Matter are available.)

Special 25 % discounts are available to members of the International Society of Photosynthesis Research, ISPR http://www.photosynthesisresearch.org/. See http://www.springer.com/ispr.

Future Advances in Photosynthesis and Respiration and Other Related Books

The readers of the current series are encouraged to watch for the publication of the forthcoming books (not necessarily arranged in the order of future appearance):

- Microbial BioEnergy: Hydrogen Production (Editors: Davide Zannoni and Roberto de Phillipis)
- The Structural Basis of Biological Energy Generation (Editor: Martin Hohmann-Marriott)
- Canopy Photosynthesis: From Basics to Applications (Editors: Kouki Hikosaka, Ülo Niinemets and Niels P.R. Anten)
- Saga of Non-Photochemical Quenching (NPQ) and Thermal Energy Dissipation In Plants, Algae and Cyanobacteria (Editors: Barbara Demmig-Adams, Győző Garab and Govindjee)
- ATP Synthase and Proton Translocation (Editor: Wayne Frasch)
- Cytochromes (Editors: William A. Cramer and Tovio Kallas)
- Photosynthesis for Bioenergy (Editors: Elizabeth A. Ainsworth and Stephen P. Long)

In addition to the above contracted books, the following topics are under consideration:

- Algae, Cyanobacteria: Biofuel and Bioenergy
- Artificial Photosynthesis
- Bacterial Respiration II
- Biohydrogen Production
- Carotenoids II
- Cyanobacteria II
- Ecophysiology
- Evolution of Photosynthesis
- Global Aspects of Photosynthesis
- Green Bacteria and Heliobacteria
- Interactions between Photosynthesis and Other Metabolic Processes
- Limits of Photosynthesis: Where Do We Go from Here
- Photosynthesis, Biomass and Bioenergy
- Photosynthesis under Abiotic and Biotic Stress
- Plant Respiration II

If you have any interest in editing/co-editing any of the above listed books, or being an author, please send an E-mail to Tom Sharkey at tsharkey@msu.edu. and/ or to Govindjee at gov@illinois.edu. Suggestions for additional topics are also welcome.

In view of the interdisciplinary character of research in photosynthesis and respiration, it is our earnest hope that this series of books will be used in educating students and researchers in Plant Sciences, Molecular and Cell Biology, Integrative Biology, Biotechnology, Agricultural Sciences, Microbiology, Biochemistry, Chemical Biology, Biological Physics, and Biophysics, but also in Bioengineering, Chemistry, and Physics.

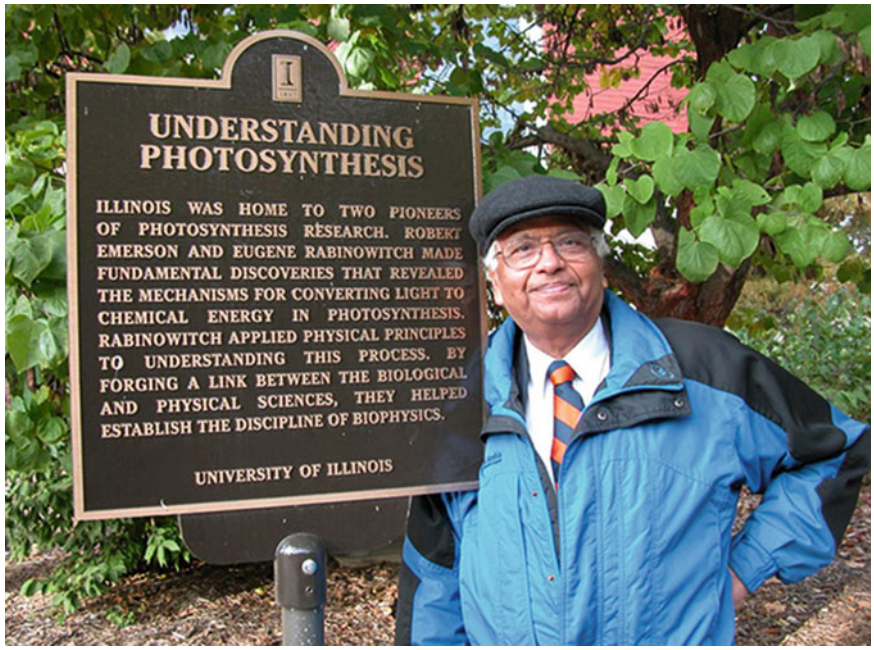
We take this opportunity to thank and congratulate Dave Hanson and Steve Rice for their outstanding editorial work; they have done a fantastic job, not only in editing, but also in organizing this book for all of us, and for their highly professional dealing with the reviewing process. We thank all the 33 authors of this book (see the list above): without their authoritative chapters, there would be no such volume. We give special thanks to I. Mohamed Asif, SPi Global, India, his directing the typesetting of this book; his expertise has been crucial in bringing this book to completion. We owe Jacco Flipsen, Andre Tournois, and Ineke Ravesloot (of Springer) thanks for their friendly working relation with us that led to the production of this book.

April 5, 2013 **Thomas D. Sharkey**

Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI, 48824, USA
tsharkey@msu.edu

Govindjee
Department of Plant Biology, Department of Biochemistry and Center of Biophysics and Computational Biology, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA
gov@illinois.edu

Series Editors

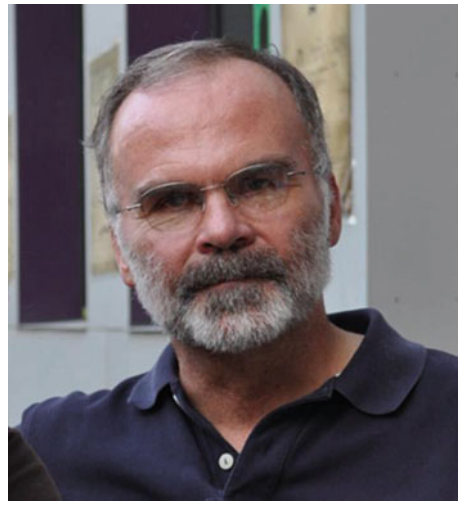


Govindjee with the plaque honoring his professors Robert Emerson and Eugene Rabinowitch.

Govindjee, who uses one name only, was born on October 24, 1932, in Allahabad, India. Since 1999, he has been Professor Emeritus of Biochemistry, Biophysics and Plant Biology at the University of Illinois at Urbana-Champaign (UIUC), Urbana, IL, USA. He obtained his B.Sc. (Chemistry and Biology) and M.Sc. (Botany; Plant Physiology) in 1952 and 1954, from the University of Allahabad. He studied 'Photosynthesis' at the UIUC, under two pioneers of photosynthesis Robert Emerson, and Eugene Rabinowitch, obtaining his Ph.D. in 1960, in Biophysics. He is best known for his research on excitation energy transfer, light emission (prompt and delayed fluorescence, and thermoluminescence), primary photochemistry and electron transfer in "Photosystem II" (PS II, water-plastoquinone oxido-reductase). His research, with many collaborators, has included the discovery of a short-wavelength form of chlorophyll (Chl) *a* functioning in what is now called PS II; of the two-light effect in Chl *a* fluorescence; and, with his wife Rajni Govindjee, of the two-light effect (Emerson Enhancement) in NADP reduction in chloroplasts. His major achievements, together with several other researchers, include an understanding of the basic relationship between Chl *a* fluorescence and photosynthetic reactions; an unique role of bicarbonate/carbonate on the electron acceptor side of PS II, particularly in the protonation events involving the Q_B binding region; the theory of thermoluminescence in plants; the first picosecond measurements

on the primary photochemistry of PS II; and the first use of Fluorescence Lifetime of Chl *a* fluorescence in understanding *photoprotection*, by plants, against excess light. His current focus is on the 'History of Photosynthesis Research', in 'Photosynthesis Education', as well as in the 'Possible Existence of Extraterrestrial Life'. He has served on the faculty of the UIUC for ~40 years. Govindjee's honors include: Fellow of the American Association of Advancement of Science (AAAS); Distinguished Lecturer of the School of Life Sciences, UIUC; Fellow and Lifetime member of the National Academy of Sciences (India); President of the American Society for Photobiology (1980–1981); Fulbright Scholar (1956), Fulbright Senior Lecturer (1997), and Fulbright Specialist (2012); Honorary President of the 2004 International Photosynthesis Congress (Montréal, Canada); the first recipient of the Lifetime Achievement Award of the Rebeiz Foundation for Basic Biology, 2006; Recipient of the Communication Award of the International Society of Photosynthesis Research, 2007; and the Liberal Arts and Sciences Lifetime Achievement Award of the UIUC, 2008. Further, Govindjee was honored (**1**) in 2007, through 2 special volumes of Photosynthesis Research, celebrating his 75th birthday and for his 50-year dedicated research in 'Photosynthesis' (Guest Editor: Julian Eaton-Rye); (**2**) in 2008, through a special International Symposium on 'Photosynthesis in a Global Perspective', held in November, 2008, at the University of Indore, India; and (**3**) Volume 34 of this Series "*Photosynthesis – Plastid Biology*, *Energy Conversion and Carbon Assimilation*", edited by Julian Eaton-Rye, Baishnab C. Tripathy, and one of us (TDS), was dedicated to Govindjee, celebrating his academic career. Currently, a special issue of *Photosynthesis Research* is being edited by Suleyman Allakhverdiev, Gerald Edwards and Jian-Ren Shen, celebrating his 80th birthday. Govindjee is coauthor of *Photosynthesis* (John Wiley, 1969); and editor of many books, published by several publishers including Academic Press and Kluwer Academic Publishers (now Springer).

Since 2007, each year a Govindjee and Rajni Govindjee Award (http://www.life.illinois.edu/plantbio/PlBiogiving.html; http://sib.illinois.edu/grants_Govindjee.htm) is being given to graduate students, by the UIUC, to recognize Excellence in Biological Sciences. For further information on Govindjee, see his website at http://www.life.illinois.edu/govindjee.



Thomas D. (Tom) **Sharkey** obtained his Bachelor's degree in Biology in 1974 from Lyman Briggs College, a residential science college at Michigan State University, East Lansing, Michigan. After 2 years as a research technician, Tom entered a Ph.D. program in the Department of Energy Plant Research Laboratory at Michigan State University under the mentorship of Klaus Raschke and finished in 1979. Post-doctoral research was carried out with Graham Farquhar at the Australian National University, in Canberra, where he coauthored a landmark review on photosynthesis and stomatal conductance. For 5 years he worked at the Desert Research Institute, Reno, Nevada. After Reno, Tom spent 20 years as Professor of Botany at the University of Wisconsin in Madison. In 2008, Tom became Professor and Chair of the Department of Biochemistry and Molecular Biology at Michigan State University. Tom's research interests center on the exchange of gases between plants and the atmosphere. The biochemistry and biophysics underlying carbon dioxide uptake and isoprene emission from plants form the two major research topics in his laboratory. Among his contributions are measurement of the carbon dioxide concentration inside leaves, an exhaustive study of short-term feedback effects in carbon metabolism, and a significant contribution to elucidation of the pathway by which leaf starch breaks down at night. In the isoprene research field, Tom is recognized as the leading advocate for thermotolerance of photosynthesis as the explanation for why plants emit isoprene. In addition, his laboratory has cloned many of the genes that underlie isoprene synthesis and published many papers on the biochemical regulation of isoprene synthesis. Tom has co-edited three books, the first on trace gas emissions from plants in 1991 (with Elizabeth Holland and Hal Mooney) and then volume 9 of this series (with Richard Leegood and Susanne von Caemmerer) on the physiology of carbon metabolism of photosynthesis in 2000 and volume 34 (with Julian Eaton-Rye and Baishnab C. Tripathy) entitled *Photosynthesis*: *Plastid Biology*, *Energy Conversion and Carbon Assimilation*. Tom has been co-series editor of this series since volume 31.

Contents

Preface

Why would anyone let you study moss? This question was put to one of us (David Hanson) by a well-known and well-respected plant biologist (who will not be named) in the middle of the 1990s when he was graduate student. This was not a question originating from malice, but rather benign ignorance and a genuine concern for the career of an aspiring biologist. It is also likely that many authors in this volume have had similar questions put to them since bryophyte photosynthesis, and even bryophyte biology in general, has suffered from a perceived lack of relevance or importance until recent decades. Much of the bryophyte dismissal came from out-dated views that bryophytes were just reduced vascular plants, an evolutionary dead-end with remnants that were essentially inconsequential ecologically except for the genus *Sphagnum*. Fortunately, these views have been turned on their head.

Bryophytes are now widely recognized as the earliest divergent land plants from phylogenetic evidence and ever-growing fossil data. This has cemented their important position in understanding the evolution of land plants. Ecologically, *Sphagnum* is still king and is probably the most important single genus of all land plants, playing a major, if not controlling, role in ecosystem function over 2–3 % of the continental surface. However, critical roles of other bryophytes as members of vast biological crust communities and as major components of high latitude and altitude ecosystems is undeniable, and their roles in many other ecosystems are more likely to be poorly understood rather than not being important. The value of bryophytes for understanding cellular and developmental biology of plants has also become much clearer and has helped immensely in the effort to garner respect for these misunderstood organisms. After centuries of relative neglect, it appears that the era of bryophyte biology is well underway.

This volume of *Advances in Photosynthesis and Respiration Including Bioenergy and Related Processes* brings together experts on bryophyte photosynthesis whose research spans the genome and cell, through whole plant and ecosystem function, and combines that with historical perspectives on the role of algal, bryophyte and vascular plant ancestors during the terrestrialization of the Earth. Many of the authors in this volume are responsible for ushering in a new era for bryophyte biology, while others are emerging as leaders for the future. There are also others in the field from both of these categories that we were not able to include in this volume, and we see that as evidence of a strong and growing field. Here we have tried to take a wide view on existing areas of research involving photosynthesis in bryophytes and early land plants (actual interpretations from fossil data, not just examination of modern representatives).

We begin this volume with an introductory chapter, followed by three multi-chapter sections, and we end the book with a final prospective chapter. The introduction (Chap. 1) provides an overview of why research in bryophyte photosynthesis is important; it is also a general guide to where each topic is addressed in the book. We hope that this will help newcomers to the field navigate to the material that is of greatest interest to them. In the first section of this volume, after the introduction, authors consider fossil, biogeochemical, systematic and comparative physiological evidence to understand three phases of terrestrialization: the transition to the land from aquatic algal ancestors (Chap. 2); the physiological adaptation of early land plants (Chap. 3); and the diversification of plants and environments (Chap. 4). The second section starts out with a discussion of the challenges involved in measuring photosynthesis of bryophytes and presents our view of what the best practices should entail (Chap. 5).

The section then introduces new perspectives and reviews photosynthetic physiology across spatial and temporal scales in six additional chapters that focus on the unique strategies of bryophytes in relation to carbon acquisition (Chap. 6), photoprotection (Chap. 7), chloroplast movement (Chap. 8), canopy structure (Chaps. 9 and 10) along with genetics and genomics of bryophytes (Chap. 11). This section also discusses novel approaches used in the investigation of bryophyte photosynthesis. The last section emphasizes the ecological setting, showing how the photosynthetic physiology of bryophytes plays out within aquatic (Chap. 12), peatland (Chaps. 13 and 14), tropical (Chap. 15), dryland (Chap. 16) and Antarctic (Chap. 17) settings with discussions of implications of global change. The volume ends with a forward-looking view (Chap. 18) of exciting opportunities for future work along with a list of some other books and websites that are valuable resources for researchers interested in bryophyte photosynthesis. Overall, the 18 well-illustrated chapters reveal unique physiological approaches to achieving carbon balance and dealing with environmental limitations and stresses that present an alternative, yet successful strategy for land plants.

We are grateful for the effort and patience of the authors and series editors in helping to bring this volume to fruition. The authors of this volume helped lay the foundations for this field of work and inspired both of us during our higher education and subsequent careers. We hesitate to single out specific authors in such a distinguished list, but we hope others will agree with us and take a moment to recognize and reflect upon the massive contributions of Michael Proctor and John Raven (to date!). We are especially grateful for their contributions to the field and to this volume. In addition, Govindjee and Tom Sharkey have long recognized the value of studying bryophyte photosynthesis and the value of their encouragement and advice is immeasurable. It has been a great pleasure to work with such an enthusiastic and knowledgeable group. We hope readers will be as invigorated as we are by this volume and will be inspired to participate in the advancement of research in bryophyte photosynthesis and respiration. Just remember, bryophytes rule! If you were unaware of this fact before reading this volume, we hope you are persuaded by the time you finish it.

David T. Hanson
Department of Biology
University of New Mexico
Albuquerque, NM 87131, USA

Steven K. Rice
Department of Biological Sciences
Union College, Schenectady,
NY 12308, USA

The Editors

David T. **Hanson** was born on April 25, 1972 in Baltimore, Maryland, USA. He is Associate Professor in the Department of Biology and Associate Curator of Bryophytes in the Museum of Southwestern Biology at the University of New Mexico in Albuquerque, New Mexico. He received his Bachelor of Science degree with Honors in Botany from the University of Iowa, Iowa City, Iowa. It was there that David's passion for bryophytes was ignited under the guidance of Prof. Diana G. Horton. His Honors thesis examined whether species of the genus *Atrichum* had sun and shade leaf forms based on the height of photosynthetic lamellae, and this initiated his life-long interest in both bryophytes and photosynthesis. During the summer of 1994, between undergraduate and graduate school, David was fortunate to take "Bryophytes" with one of the best known bryologists of his time, Prof. Howard Crum, along with "Boreal Flora" taught by the legendary Prof. Ed Voss at the University of Michigan Biological Station, cementing his love of the Northwoods, *Sphagnum* and bathtub Marys. These experiences colored his subsequent Ph.D., received in 1999, from the Botany Department at the University of Wisconsin-Madison, where he pursued research in photosynthesis, isoprene emission, and bryophyte biology under the guidance of Prof. Thomas D. Sharkey (co-editor of this series) and Prof. Linda E. Graham (contributor to this volume). His post-doctoral fellowship at the Australian National University from 2000 to 2002 was a joint appointment to work with Prof. T. John Andrews and Prof. Murray R. Badger on Rubisco kinetics and CO_2 concentrating mechanisms in hornworts and algae. David was appointed as Assistant Professor at the University of New Mexico started in 2002 and promoted to Associate Professor in 2008. In the area of bryophyte research, David is best known for his work on the evolution of isoprene emission from mosses and function of the hornwort pyrenoid. However, in 2006 he developed a new method for conducting high-frequency, online $^{13}CO_2$ gas exchange and since then his research has centered on using stable isotopes of CO_2 to study diffusion through photosynthetic tissues. This has led to several papers demonstrating an *in vivo* role for CO_2 transporting aquaporins. David is currently the Chair of the 2014 Gordon Conference on CO_2 Assimilation in Plants: Genome to Biome along with Prof. Christoph Peterhansel.

Steven K. Rice was born on April 12, 1961 in Ann Arbor, Michigan, USA, and is currently Professor in the Department of Biological Sciences and co-Program Director of the Bioengineering Program at Union College in Schenectady, New York. He received his Bachelor of Science degree in Biology from Yale University in 1983 and spent 5 years teaching science in museum and school settings. Following his deepening interests in plant biology, Steven returned to school at Duke University to earn his Master's of Science (1991) and Ph.D. (1994) degrees in Botany. At Duke, his interest in bryophyte structure-function relationships was stimulated by Brent Mishler, who co-advised his dissertation (with ecologist Norman Christensen) on "Form, Function and Phylogeny of Aquatic *Sphagnum*", and by Lewis Anderson, who led him on many collecting trips to the Coastal Plain, teaching Steven the idiosyncrasies of *Sphagnum* biology in that region. Following a post-doctoral position at University of North Carolina-Chapel Hill with the ecologist Robert Peet, and a teaching position at Wake Forest University, Steven came to Union College in 1998 as an Assistant Professor. He was promoted to Associate Professor in 2004 and to Professor in 2011. In his research he employs integrative and comparative approaches to understand the ecological and evolutionary significance of variation in plant form in bryophytes. His studies focus on understanding structure-function relationships with particular emphasis on how variation in structure influences water balance, carbon balance and plant productivity. With co-authors (N. Neal, J. Mango and K. Black), he received the 2012 Sullivant Award from the American Bryological and Lichenological Society for the best bryology publication in *The Bryologist*.

Contributors

Maaike Y. Bader
Department of Biology and Environmental Sciences, Functional Ecology of Plants, University of Oldenburg, Oldenburg, Germany

Jayne Belnap
U.S. Geological Survey, Southwest Biological Center, Canyonlands Research Station, Moab, UT, USA

Jessica Bramley-Alves
Institute for Conservation Biology and Environmental Management, University of Wollongong, Wollongong, NSW, Australia

Kirsten K. Coe
Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, NY, USA

Martha Cook
School of Biological Sciences, Illinois State University, Normal, IL, USA

J. Hans C. Cornelissen
Systems Ecology, Department of Ecological Science, VU University, Amsterdam, The Netherlands

David J. Cove
Centre for Plant Sciences, University of Leeds, Leeds, UK
Department of Biology, Washington University in St. Louis, St. Louis, MO, USA

Andrew C. Cuming
Centre for Plant Sciences, University of Leeds, Leeds, UK

Dianne Edwards
School of Earth and Ocean Sciences, Cardiff University, Cardiff, UK

Lawrence B. Flanagan
Department of Biological Sciences, Water and Environmental Science Building, University of Lethbridge, Lethbridge, Alberta, Canada

Janice M. Glime
Professor Emerita, Department of Biological Sciences, Michigan Technological University, Houghton, MI, USA

Linda Graham
Department of Botany, University of Wisconsin, Madison, WI, USA

Tomáš Hájek
Institute of Botany, Academy of Sciences of the Czech Republic, Třeboň, Czech Republic
Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic

David T. Hanson
Department of Biology, University of New Mexico, Albuquerque, NM, USA

Diana H. King
Institute for Conservation Biology and Environmental Management, University of Wollongong, Wollongong, NSW, Australia

Martina Königer
Department of Biological Sciences, Wellesley College, Wellesley, MA, USA

Louise A. Lewis
Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, CT, USA

Rebecca E. Miller
Institute for Conservation Biology and Environmental Management, University of Wollongong, Wollongong, NSW, Australia
School of Biological Sciences, Monash University, Clayton, VIC, Australia

Ülo Niinemets
Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Tartu, Estonia

Zach Portman
Department of Biology, Utah State University, Logan, UT, USA

Michael C.F. Proctor
Biosciences, College of Life and Environmental Sciences, University of Exeter, Exeter, UK

John A. Raven
Division of Plant Sciences, University of Dundee at the James Hutton Institute, The James Hutton Institute, Invergowrie, Dundee, UK

School of Plant Biology, University of Western Australia, Crawley, WA, Australia

Karen Renzaglia
Department of Plant Biology, Southern Illinois University, Carbondale, IL, USA

Steven K. Rice
Department of Biological Sciences, Union College, Schenectady, NY, USA

Sharon A. Robinson
Institute for Conservation Biology and Environmental Management, University of Wollongong, Wollongong, NSW, Australia

Jed P. Sparks
Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, NY, USA

Wilson Taylor
Department of Biology, University of Wisconsin-Eau Clair, Eau Claire, WI, USA

Mari Tobias
Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Tartu, Estonia

Juan Carlos Villarreal
Biology Department, Ludwig-Maximilians-Universität München, München, Germany

Sebastian Wagner
Department of Biology and Environmental Sciences, Functional Ecology of Plants, University of Oldenburg, Oldenburg, Germany

Melinda J. Waterman
Institute for Conservation Biology and Environmental Management, University of Wollongong, Wollongong, NSW, Australia

Charles Wellman
Department of Animal and Plant Sciences, Sheffield University, Sheffield, UK

Gerhard Zotz
Department of Biology and Environmental Sciences, Functional Ecology of Plants, University of Oldenburg, Oldenburg, Germany

Smithsonian Tropical Research Institute, Balboa, Panama

Author Index

Chapter 1

What Can We Learn From Bryophyte Photosynthesis?

David T. Hanson*
Department of Biology, University of New Mexico, Albuquerque, NM 87131, USA

Steven K. Rice
Department of Biological Sciences, Union College, Schenectady, NY 12308, USA

Summary

Bryophytes have been evolving in terrestrial and aquatic environments longer than any other group of land plants, surviving and thriving through an incredible range of climatic and environmental variation. Several of the bryophyte growth forms we find today closely resemble those found in ancient fossils whereas many of the other early land plant forms lack modern representatives. What is it about bryophyte growth form and physiology that has allowed them to persist through time and radiate into every terrestrial ecosystem, even dominating some of them? What can we learn from modern bryophytes to address this question and to predict how plants will respond to future environmental change? In this chapter, we briefly examine these questions as a preview to the volume as a whole.

*Author for correspondence, e-mail: dthanson@unm.edu

D.T. Hanson and S.K. Rice (eds.), Photosynthesis in Bryophytes and Early Land Plants, Advances in Photosynthesis and Respiration 37, pp. 1–8, DOI 10.1007/978-94-007-6988-5_1,

I. Introduction

Bryophytes have barely been tapped as a resource for understanding photosynthesis and respiration on land despite the fact that the bryophyte life form has achieved ecological success in varied environments that span every continent, occur across dramatic gradients of temperature and water availability and were present at the early stages of the transition from aquatic habitats to land, potentially as early as the Cambrian (see Chaps. 2 and 3). Critical aspects of life cycle evolution were involved in adapting bryophyte ancestors to life on land (e.g., protection of the embryo in protective maternal tissue, elaboration of two distinct multicellular stages specialized for different functions), but the colonization of land also required structural and physiological adaptations to succeed in habitats with high solar radiation, a drying atmosphere, high temperature fluctuations, and limited access to dissolved nutrients (Chaps. 4 and 7).

Although the primary architecture of the photosynthetic machinery was conserved from their algal ancestors, early land plants, as with contemporary bryophytes, likely facilitated carbon capture over short and long temporal scales in several ways. These include the reduction of external water films on leaf surfaces, which impede diffusion of carbon dioxide, the evolution of ventilated thalli or leaf structures, and the evolution of carbon concentrating mechanisms. In addition, they evolved desiccation tolerance, which allowed plants to equilibrate with a drying atmosphere and retain metabolic function upon rehydration along with adaptations to achieve positive carbon balance during wet—dry cycles. Bryophyte population and community structure also indirectly influences photosynthesis through alteration of canopy boundary layers and, thereby, retention of water and soil respired CO_2. The multiple scales over which bryophyte photosynthesis is measured (shoot, canopy, community) also raises the question of what is a functional photosynthetic unit in bryophytes, i.e. what can be treated as an analogue to the vascular plant leaf (Chaps. 5 and 9)? The amazing variation in form and function and the diverse range of micro-habitats that bryophytes occupy makes them an ideal, yet rarely utilized, system for studying the role of photosynthesis and respiration in the adaptive radiation of plants.

Abbreviation: Rubisco – ribulose-1,5-bisphosphate carboxylase/oxygenase

II. Terrestrialization

Terrestrialization is the adaptive radiation of aquatic organisms onto land. The organisms we consider here are grouped by function rather than by phylogeny. Interestingly, despite the existence of all major lineages of aquatic photosynthetic organisms, from cyanobacteria to green algae, being present when terrestrial photosynthetic organisms were becoming wide-spread, only one small corner of that aquatic diversity came to dominate land. For roughly the last 500 million years, descendants of Charophycean green algae, collectively called embryophytes, or land plants, have adaptively radiated onto land with greater success than any other lineage. Thus, the story of terrestrialization by photosynthetic organisms has been effectively limited to a single lineage. Bryophytes are at the base of this lineage, and in this volume we examine what features of bryophyte photosynthesis may have allowed them to be so successful.

A. Photosynthesis on Land

Despite their current dominance of terrestrial environments, land plants (embryophytes) are one of several groups of photosynthetic organisms that have terrestrial representatives. It is likely that cyanobacteria, many varieties of algae, and even lichens colonized land before or concurrently with land plants and each of these groups have extant terrestrial representatives

(Chaps. 2 and 3). Therefore, traits that allow persistence of photosynthetic organisms on land must be common to all these groups and be much broader than traits that allow photosynthetic organisms to thrive and compete successfully on land. Having low enough resistance to diffusion of CO_2 to provide adequate supply to the chloroplast interior and a mechanism for either maintaining water balance or dealing with the consequences of desiccation, seem to be the most basic criteria. Combining these traits with nutrient acquisition, photoprotection, thermotolerance and additions to C_3 biochemistry that improve CO_2 capture may be the adaptations that led to successful radiation into many habitats. These interactions among photosynthesis, phylogeny, climate, micro-habitat, and morphology clearly played important roles in generating the current situation where only the embryophyte lineage dominates land.

B. Tiny but Tenacious

So what is it about bryophytes that allowed them to succeed on land relative to other early photosynthetic organisms and that allows them to dominate the flora at high altitudes and latitudes today? The latter is much easier to answer as we can study extant organisms and their response to environmental conditions; however there are some intriguing patterns in fossil data that suggest the importance of scale to the organism's water and carbon economy. In Chap. 4, the author suggests that the successful photosynthetic organisms on land today are either small (<10 cm) and poikilohydric, or large (>10 cm) and homiohydric with few exceptions. Thus early bryophytes may have capitalized on their tiny size and the tenacious strategy afforded by poikilohydry, where harsh conditions are essentially avoided through desiccation-induced dormancy. This foothold could have then set the stage for evolution of the embryophyte lineage into the large, homiohydric strategy that works so well in less harsh environments.

C. Making Inferences from Extant Organisms

What made bryophytes more tenacious than other small early terrestrial photosynthetic organisms, leading to their ultimate success? Some of the other organisms, such as lichens, are also very tolerant of life on land but have been less successful. The answer to what features allowed the success of embryophytes over other lineages is probably unknowable as many of the early lineages have no extant relatives and there is a fairly poor fossil record. Perhaps the combination of multicellularity, protected sexual reproduction, and desiccation tolerance were the key for surviving through dramatic climate changes, or maybe UV and thermotolerance were essential. These speculations bring up three critical issues that affect our interpretation of historical events and we need to keep in mind that the farther back in time we go, the larger these issues become.

First, extant organisms are the product of evolution through time. We can observe the physiological properties of these organisms and can make predictions of the age of a lineage and the large-scale climate changes that occurred through that time range. However, evolution through natural selection occurs in response to local competition and local habitats acting on populations of individuals. When local conditions follow the larger-scale patterns, then our predictions are more meaningful, the problem is that we have no way of knowing when the large and small scale conditions align. Even for time periods and localities where we have a high degree of confidence about the environment and ecosystem, we still do not know the ancestral physiology.

Second, inferring ancestral physiology from phylogeny and comparative physiology of extant organisms is inherently problematic. Unlike gross morphology, there is very little information in the fossil record that informs us about photosynthetic physiology. Stable isotopes, chemistry, biophysics and in some cases even anatomy can put some reasonable bounds on physiology assuming that

the physiology of the ancient organism falls within the realm of known organism function. However, there is another fundamental issue, that of phylogeny itself. We are working from the tips of a tree to infer what organisms are at the base and how they branch. Even if we assume that we get the branching order correct, we do not have information about the evolutionary patterns along each branch so we do not know the physiological properties of a common ancestor between two groups. Also, fossils that could represent common ancestors or extinct lineages are highly variable, with older ones generally lacking DNA and often lacking much internal structure. As the number of characters decrease, our ability to even assess their relationships to extant plants becomes more difficult.

Third, historical data are inherently sparse as we only have material that was preserved, found, and analyzed. Molecular clock data help us predict what should have existed in the past in terms of ancestors of extant organisms, but that does not predict what other organisms might have existed and gone extinct. To quote Donald Rumsfeld (Feb. 12, 2002, Department of Defense news briefing), "As we know, there are known knowns. There are things we know we know. We also know there are known unknowns. That is to say we know there are some things we do not know. But there are also unknown unknowns, the ones we don't know we don't know."

Despite the uncertainties and problems with making past inferences, it is very important that we try because these inferences provide a platform for predicting plant responses to future climate change and environmental perturbations. In addition, understanding how plants have adapted to various environments gives us clues to the metabolic potential that exists in plants and how it could be tapped for agriculture, restoration of damaged ecosystems, and other uses. In the case of predicting future responses, we have the starting organisms to work with. Thus, we can test if these organisms can survive predicted past and future climates through plasticity. We can also initiate long-term selection experiments and track critical ecosystem responses using historical data to generate theory and inform experimental design. In the end, only tracking changes through time will truly show how plants evolve and adapt to life on land.

III. Biochemical and Cellular Biology

As extant representatives of the earliest land plants, modern bryophytes have an interesting mix of algal and seed plant features that are evident in their biochemistry and cellular biology. However, we have only scratched the surface of the potential diversity possessed by this group of organisms. The likelihood for discovering novel pathways and mechanisms for cellular function is high as bryophytes are not simply a mixture of algal and seed plant biology. The combination of pathways from algae and seed plants is itself unique and almost certainly requires novel mechanisms to maintain cell function. In addition, the long lineage of bryophytes has provided ample time and environmental variation to allow large divergences to have evolved from the common ancestors of bryophytes and other plants.

A. Are Bryophytes C_3?

To answer this, we have to first be clear what is meant by this deceptively simple question. Confusion often arises because the two well-known CO_2 concentrating mechanisms in plants, CAM and C_4, both initially form a stable four-carbon compound that is subsequently decarboxylated for fixation through the Calvin-Benson-Bassham cycle. Therefore, CAM and C_4, which initially fix CO_2 into a four-carbon intermediate, are seen as having biochemical add-ons to the C_3 pathway. However, in algae and cyanobacteria, it is common to have a CO_2 concentrating mechanism without the formation of a four-carbon intermediate. In these instances, the photosynthetic pathway is only C_3, while the function of CO_2 concentration is provided through the pumping of CO_2 and

bicarbonate into the cell along with locating carbonic anhydrase where it can facilitate CO_2 diffusion and availability for ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco).

To date, there is no clear evidence for C_4 metabolism in bryophytes despite a few attempts to find it (Chap. 12). In light of recent work identifying single-cell C_4 metabolism in seed plants and in some algae, a more concerted effort may be warranted. It is not clear if a common property of bryophyte biology, such as desiccation tolerance and the effects it has on cell structure, has constrained the evolution of single-cell C_4 metabolism. Currently, it appears that a few bryophytes have developed CO_2 concentrating mechanisms more like those in algae, with adaptations that control localization of carbonic anhydrase, utilize bicarbonate, and form pyrenoids (Chaps. 2, 6, and 12). It is also possible that the high CO_2 from respiration found in the soil boundary layer (Chap. 13) significantly reduces the benefits of investing in a CO_2 concentrating mechanism, and potentially even a role for mixotrophy assisting with positive carbon balance (Chap. 2).

B. The Terrestrial Pyrenoid: Unique Among Plants

The pyrenoid is a structure within the chloroplast, primarily composed of Rubisco and dissected by thylakoid membranes. The presence of a pyrenoid has been correlated with CO_2 concentrating mechanism function in algae and hornworts (Chap. 6), and is thought to function as a location where bicarbonate can be transported and subsequently converted into CO_2. This process elevates CO_2 around Rubisco, and disruption of the pyrenoid slows the rate of photosynthesis at ambient levels of CO_2. Interestingly, pyrenoids are common and wide-spread among algae but nearly absent in plants. Hornworts are the only group of land plants that contain pyrenoids, but the reason for this is not clear as pyrenoids have evolved and been lost multiple times in both algae and hornworts (Chap. 6). Recent discoveries regarding the role of the Rubisco small subunit in the formation of pyrenoids is spurring research into expressing them in seed plants to improve photosynthesis. However, there may be unique properties of hornworts, such as few, very large chloroplasts per cell, that may be necessary for the pyrenoid to properly function and it will be imperative to resolve such questions in the near future.

C. Drying Without Dying

A defining, though far from ubiquitous, property of bryophytes is their ability to tolerate desiccation (drying to equilibrium with air) and rapidly recover net positive photosynthesis in a matter of minutes (Chaps. 4 and 16). This is associated with expression of proteins important for managing both dehydration and re-hydration (dehydrins and re-hydrins, respectively). As one might expect, there is also a respiratory burst associated with repair that contributes to the overall carbon balance of the plant. This rapid recovery is essential for maximizing carbon uptake in the good times where water content is high enough for optimal cellular function, but not so high that diffusion of CO_2 is severely limited. Both plant form and community/canopy structure contribute significantly to the balance of water and carbon, making moss photosynthesis more structurally complex than higher plants over small scales, i.e. what one typically places in a measurement chamber (Chaps. 5, 10, and 13).

D. Tolerating Light

Managing high light is essential for the adaptive radiation of plants onto land and remains a major issue in many open habitats dominated by bryophytes today. The combination of cold and high light in polar and alpine regions and in peatlands (Chaps. 7, 13, 15, and 17) and the drying and re-hydrating in high light and often hot environments (Chap. 16) present some of the greatest challenges for a photosynthetic organism. Some tolerance of high light can be

accomplished through simple, rapid avoidance mechanism such as chloroplast movements (Chap. 8), production of UV absorbing compounds (Chap. 7), and longer-term dormancy through desiccation. However, bryophytes also have the land plant style xanthophyll cycle for dissipating excess light energy and algal-derived methods for dissipating heat (Chap. 7). In addition, most bryophytes lack multi-cell layer leaves so photoprotection mechanisms need to be active in all cells. Also, the combination of stresses experienced by bryophytes has likely led to the evolution of novel physiological mechanisms that we have yet to discover.

E. Bryophyte Genomics

The genomic era is upon us and bryophyte research is both leading and lagging. The genomic sequence of *Physcomitrella patens* was the first non-seed land plant to have its genome sequenced, and it can be transformed via homologous recombination as easily as yeast (see Chap. 11). Despite this remarkable toolbox for understanding fundamental properties of photosynthesis, this system has only been capitalized upon by developmental biologists. This presents an opportunity for readers of this volume as there is so much basic knowledge to be gained by applying the *P. patens* system to study photosynthesis and respiration.

IV. Organization of the Bryophyte Photosynthetic System

All bryophytes evaluated thus far utilize the Calvin-Benson-Bassham cycle, yet given their small stature that restricts their ability to maintain constant cellular water, their extra-cellular water pools that limit diffusion of carbon dioxide and their high rates of dark respiration relative to photosynthesis, achieving a positive carbon balance presents unique challenges. In order to do so, bryophytes have evolved physiological traits across multiple scales from biochemical through canopy-level that have allowed them to succeed ecologically in many habitats not suitable to other land plants.

Leafy bryophytes are organized hierarchically with leaves (i.e., phyllids), shoots or shoot systems (i.e., canopies) serving as the principal unit of gas exchange or light acquisition, depending on the species (Chaps. 5, 9 and 10). In shoots with large, well-spaced leaves as in the Polytrichaceae or Mniaceae and also in some aquatic species (e.g., *Fontinalis antipyretica*, *Sphagnum macrophyllum*), leaves may function individually enough to be considered appropriate photosynthetic units and are satisfactorily large enough to measure some physiological activity. At this scale, there has been much interest in understanding the physiological significance of leaf modifications like hair points (reduce rates of evaporation and affect carbon balance), papillae (enhance diffusion of carbon dioxide to chloroplasts contained within them), lamella (enhance photosynthetic leaf area and allow diffusion of carbon dioxide in gas phase) and leaf shape (long linear leaves enhance CO_2 uptake in aquatic species), and more recently comparative studies suggest that allocation to features like leaf costa or water storage cells present trade-offs in terms of carbon balance with rates of net photosynthesis (Chaps. 5 and 12).

However, even in species with large, well-spaced leaves, shoot system organization affects total leaf area, boundary layer properties that control water flux, microclimate, plant water status and light intensity and quality at the shoot surface, and thereby influences carbon dynamics of individual shoots as well as whole canopies. Consequently, in bryophytes, shoot-systems (i.e., the canopy) are normally considered the primary unit in studies of function (Chaps. 5, 9 and 10). Within the canopy, light gradients can be quite steep with light attenuating to levels below the light compensation point within the first few centimeters, although canopies with less dense shoot arrangements have thicker active canopies (Chap. 9). The branching structure of the canopy also influences canopy light

interception. In some pleurocarpous species like *Pleurozium schreberi*, new branches can form from buds within the canopy interior and take advantage of light if it is available, although it is not known if these are acclimated to low light, thereby optimizing whole-canopy photosynthetic rates (Chap. 9). Newly developed imaging techniques that measure physiological states based on thermal emission, spectral reflectance or fluorescence analyses can be used to evaluate variation in physiological activity across and within bryophytes canopies (Chaps. 9 and 10) and present exciting opportunities to understand how processes are integrated in two- and, even, three-dimensions within the canopy. At ecosystem scales, eddy covariance methods allow estimates of carbon and water fluxes, which allows linkage of small-scale physiological processes with large and long-term patterns of photosynthesis and respiration (Chap. 14).

V. Ecophysiology of Bryophyte Photosynthesis: Adapting to Environmental Stress

In habitats ranging from Antarctic pavements to boreal forests, arid temperate crusts and tropical ecosystems, the photosynthetic performance of bryophytes is dictated by achieving positive carbon balance over wet—dry cycles (Chaps. 13, 14, 15, 16 and 17) within the context of diverse stresses that vary among environments. Given the poikilohydric nature of their water relations, bryophyte carbon dynamics are affected by the respiratory demands of desiccation and rehydration as well as by the photosynthetic performance of active tissues when hydrated. The relative importance of environmental stresses varies from habitat to habitat mostly along gradients of radiation exposure, the intensity and duration of desiccation stress, and temperature, the latter of which has asymmetrical effects on respiration and photosynthesis (Chap. 15).

In habitats that cannot support a full cover of vascular plants, bryophytes can occupy openings and be subject to visible light intensities great enough to cause photodamage as well as exposure to high energy UV-B radiation (Chap. 7), which may alter DNA structure. Although many bryophytes possess mechanisms to dissipate excess energy once it is absorbed in photosystems, others also reduce radiation levels at the chloroplast by manufacturing cell wall pigments (Chaps. 7, 15 and 17) or by cellular mechanisms like chloroplast movement (Chap. 8) avoiding photodamage rather than tolerating its effects. In high latitude environments, plants are subject low solar angles minimizing the potential for photodamage from visible light, but are exposed to increased UV-B and bryophytes appear to possess mechanisms to avoid damage from the latter. When subject to elevated levels of UV-B light, bryophytes increase concentrations of photoprotective pigments and photosynthetic activity is often unaffected (Chap. 17). In boreal peatlands, light intensities are higher and species do not avoid light stress as they often maintain hydration during exposure to high light intensities. In these systems, *Sphagnum* species may dissipate excess absorbed light energy using photorespiration, which reduces net rates of photosynthesis and production and by energy-dependent quenching mechanisms, in addition to producing cell wall pigments that reduce light levels at the chloroplast (Chap. 13). Species of dryland environments escape potential damaging effects of high light by tolerating exposure in a desiccated state, which alters shoot structure and shades chlorophyll from high light. When hydrated, dryland species also possess photoprotective and energy dissipating mechanisms (Chap. 16) as found in more mesic species described above.

VI. Conclusion

In this volume, we have brought together experts on bryophyte photosynthesis that focus on scales that range from biochemistry to whole ecosystem with those interested in physiological issues associated with the early terrestrialization by plants. We hope that

these diverse perspectives provide the reader with a context to better understand the significance of the bryophyte functional syndrome in the past and in the present, how this understanding may direct biotechnological solutions to crop improvements, and to help predict responses of land plants to climate change scenarios.

Acknowledgements

We would like to thank all of the contributors to this volume for their inspirational and insightful work and comments. Their work and the encouragement of the series editors has been essential for formulating the framework we present here.

Chapter 2

Early Terrestrialization: Transition from Algal to Bryophyte Grade

Linda Graham*
Department of Botany, University of Wisconsin, Madison, WI 53706-1381, USA

Louise A. Lewis
Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, CT 06269-3943, USA

Wilson Taylor
Department of Biology, University of Wisconsin-Eau Claire, Eau Claire, WI 54702-4004, USA

Charles Wellman
Department of Animal and Plant Sciences, Sheffield University, Sheffield S10 2TN, UK

Martha Cook
School of Biological Sciences, Illinois State University, Normal, IL 61790-4120, USA

*Author for correspondence, e-mail: lkgraham@wisc.edu

D.T. Hanson and S.K. Rice (eds.), Photosynthesis in Bryophytes and Early Land Plants, Advances in Photosynthesis and Respiration 37, pp. 9–28, DOI 10.1007/978-94-007-6988-5_2,

Summary

Terrestrialization of planet Earth likely began more than a billion years ago with the colonization of land by bacteria, followed by eukaryotic algae much like those occupying modern soils and shallow freshwaters and the earliest embryophytes, close relatives of modern bryophytes. Colonization of land by algae and the first plants was prerequisite to the development of organic-rich soils that later supported more complex plant communities dominated by vascular plants, and the rise of land animals. Consequently, understanding terrestrialization sheds light on Earth's early biological carbon cycling processes, which aids our understanding of global biogeochemistry in particular, and planetary science in general.

Comprehending the process and pattern of ancient terrestrialization requires both neontological and paleontological approaches. Molecular phylogenetics provides the necessary scaffold upon which terrestrialization processes can be analyzed by comparing the structures, physiologies, microbiomes, and genomes of earliest-branching lineages of modern liverworts and mosses to those of plants' closest modern green algal relatives, the streptophyte algae (also known as charophyte algae or charophycean green algae). Such studies reveal that modern bryophytes inherited spore and body desiccation-resistance, degradation-resistant lignin-like phenolic cell wall polymers, and other physiological traits useful in terrestrial habitats from ancestral algae, indicating that such features were also traits of the earliest land plants.

Because modern algae and bryophytes possess degradation-resistant cells or tissues, artificially degrading them for comparison with enigmatic microscopic fossils has been a fruitful way to identify remains of early terrestrial photosynthesizers and thus illuminate terrestrialization patterns. Microfossils cited as evidence for terrestrial cyanobacteria

occur beginning more than 1,000 million years ago in the Precambrian, as do probable remains of freshwater and terrestrial eukaryotic algae. Some microfossils obtained from 499 to 511 million year old deposits closely resemble the modern complex streptophyte alga *Coleochaete* when it has been cultivated subaerially, suggesting that streptophytes were able to photosynthesize on land by the Middle Cambrian. Other microfossils observed in Cambrian and early Middle Ordovician deposits may also be remains of land plants. Remains of early liverwort-like land plants are confidently known from 470 million year old mid-Ordovician deposits, as are possible fossils of early-divergent mosses. Microfossils and macrofossils that have been compared to modern liverwort and moss taxa occur in Silurian to Devonian deposits laid down before and during the first major diversification of the vascular plants in the Late Silurian to Early Devonian, 407–418 million years ago. Such evidence, together with molecular phylogenies and clock analyses, demonstrates that bryophytes and streptophyte algal relatives were the dominant eukaryotic photosynthesizers on land from about 500–400 million years ago, prior to and during the earliest stages of vascular plant evolution.

Because bryophytes and streptophyte algae produce degradation-resistant carbon that can be sequestered, thereby reducing atmospheric carbon dioxide levels, models suggest that they had significant impacts on Earth's carbon cycle for at least 40 million years and perhaps more than 100 million years. We can thus predict that other Earth-like, habitable-zone planets may likewise experience long periods during which organisms equivalent to earthly terrestrial streptophyte algae and bryophytes impact planetary biogeochemistry.

I. Introduction

The sum of available evidence indicates that terrestrialization of planet Earth likely began with the colonization of land by photosynthetic prokaryotes such as cyanobacteria, followed by eukaryotic algae much like those occupying modern soils and shallow freshwaters and then the earliest embryophyte plants, which were probably quite closely related to modern bryophytes. Land colonization by algae and earliest plants was prerequisite to the development of soils that supported more complex plant communities and the later rise of land animals. Understanding terrestrialization is essential to comprehending Earth's early carbon cycling processes, which aids our understanding of modern biogeochemistry and planetary science. Comprehending the pattern of terrestrialization and the process by which photosynthesizers colonized land requires the analysis of both modern organisms and fossils, that is, both neontological and paleontological approaches (Graham 1993; Graham and Gray 2001).

This chapter begins with a brief overview of phylogenetic relationships that guide studies of terrestrialization (section "Molecular systematics provides a reasonably well-resolved framework for investigations of terrestrialization process and pattern"), continues with examples of trait evolution related to photosynthesis in early land plants and modern bryophytes and impacts on biogeochemistry that illuminate the terrestrialization process (section "Early-evolved physiological traits likely fostered the process by which streptophytes made the transition to land"), and concludes with a survey of what we currently know about the pattern of terrestrialization on Earth and the value of this information to the planetary sciences (section "Comparison of early-diverging modern photosynthesizers to Precambrian-Devonian fossils illuminates the pattern of terrestrialization").

II. Molecular Systematics Provides a Reasonably Well-Resolved Framework for Investigations of Terrestrialization Process and Pattern

Comparative physiological and molecular analyses that inform our understanding of terrestrialization process and pattern are possible because molecular phylogenetic studies (Qiu et al. 1998, 2006, 2007; Dombrovska and Qiu 2004; Crandall-Stotler et al. 2005; Forrest et al. 2006) have identified the earliest-branching lineages of modern liverworts and mosses–which serve as models of earliest land plants–and plants' closest modern algal relatives, the streptophyte algae (term used by Becker and Marin 2009) (Fig. 2.1).

Also known as the charophyte algae (Lewis and McCourt 2004) or charophycean algae (e.g. Graham et al. 2009), the streptophyte algae are a paraphyletic assemblage of green algae for which phylogenetic branching patterns are still being determined. The relatively basal positions of the unicellular flagellate *Mesostigma*, colonial *Chlorokybus*, and unbranched, filamentous Klebsormidiales seem well established, though the identity of the more complex modern streptophyte algal lineage (Charales, Coleochaetales, Desmidiales, Zygnematales, or some combination) that is sister to embryophytes continues to be debated (Turmel et al. 2007; Finet et al. 2010; Wodniok et al. 2011; Timme et al. 2012). For this reason, and because over the past several hundreds of millions of years since the divergence of embryophytes, the sister group has diverged substantially and consequently possesses traits not present in early embryophytes, comparative analyses aimed at defining the traits of earliest land plants should include representatives of multiple lineages of streptophyte algae. Streptophyte algae bequeathed diverse structural, reproductive; physiological, biochemical, and genetic traits to embryophyte descendants: (Graham 1993; Graham et al. 2000, 2004a, b). In the next section we focus on examples of photosynthesis-related physiological traits of modern streptophyte algae that (a) were likely also present in

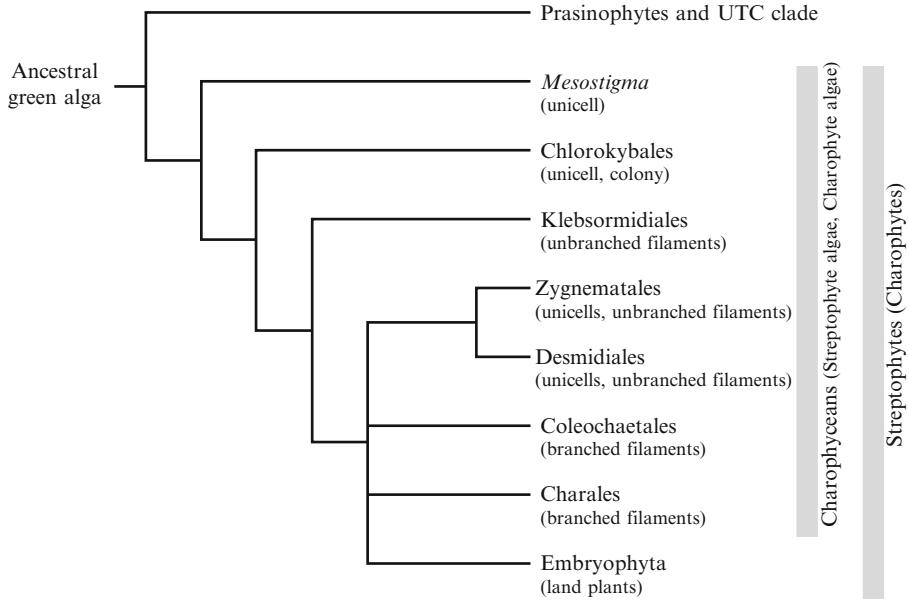


Fig. 2.1. Relationships of streptophyte algae to embryophytes. To date, molecular phylogenetic approaches have not allowed resolution of the modern streptophyte genus that is sister to embryophytes. UTC = classes Ulvophyceae, Trebouxiophyceae, Chlorophyceae, and the term Prasinophytes represents multiple classes.

ancient relatives, (b) were likely inherited by earliest embryophytes and modern bryophytes and (c) help to explain these organisms' past and present biogeochemical significance.

III. Early-Evolved Physiological Traits Likely Fostered the Process by Which Streptophytes Made the Transition to Land

Photosynthesis depends on the availability of (1) sufficient water as a source of reductant and as a medium from which dissolved mineral nutrients can be absorbed, (2) inorganic carbon, and (3) light. For earliest land plants, the transition from ancestral aquatic to more arid terrestrial habitats reduced accessibility to water, though CO_2 and light became more available, and coping with excess light became more challenging (see Chap. 3). In addition, earliest land plants likely interacted with microbial communities in new ways. Here, we focus on (A) acquisition of desiccation-resistance as a way of coping with water insufficiency, (B) modifications of light harvesting systems, (C) changes in traits related to carbon uptake and utilization, and (D) patterns of investment of photosynthate into protective body cell wall polymers.

A. Desiccation-Tolerance Is an Early-Evolved Streptophyte Trait

Photosynthesis on land is limited by the availability of water; consequently, the history of plant evolution has involved increasingly more effective adaptation to habitats and periods of limited hydration. Many plants, including many bryophytes, display desiccation-tolerance, a collection of traits that helps organisms to resist dying when in the dry state. The pattern of occurrence of desiccation tolerance in modern bryophytes (Wood 2007) suggests that early land plants were likely also tolerant of desiccation. Though some authors have proposed that streptophyte desiccation tolerance originated in early embryophytes, recent results described next indicate that desiccation tolerance likely was present in streptophyte algal ancestors and was inherited by early land plants–thereby fostering the process of ancient terrestrialization.

Though many modern streptophyte algal species occupy only or mainly freshwaters, representatives of several morphologically simple streptophyte orders (Chlorokybales, Klebsormidiales, Zygnematales, Desmidiales) commonly occur in terrestrial habitats (Graham et al. 2009). Here, they display desiccation-tolerance and so foster photosynthesis in subaerial ("under air") environments. The early-diverging species *Chlorokybus atmophyticus* has been isolated or identified from terrestrial habitats (Škaloud 2009), and Klebsormidiales are known for desiccation tolerance (Elster et al. 2008). *Klebsormidium crenulatum*, for example, shows full photosynthetic recovery after desiccation periods as long as 7 days (Karsten et al. 2010), using the osmolyte sucrose to cope with osmotic stress (Nagao et al. 2008). Certain unicellular desmidialeans have been reported to survive in dry soil for up to 3 months (Brook and Williamson 1998), and some zygnemataleans likewise are known to be desiccation tolerant (Holtzinger et al. 2010). Similar behavior is characteristic of some species of the chlorophyte clade, including members of Chlorophyceae and Trebouxiophyceae (Gray et al. 2007).

When grown in subaerial conditions such as on the surface of quartz sand, two species of the morphologically complex genus *Coleochaete* maintain intact green cells when air-dry for months, and (like *K. crenulatum* cited above) grow and asexually reproduce normally when moistened after having been air-dried for a week. These observations indicate that *Coleochaete* and earlier-diverging relatives possess the genetic potential for desiccation tolerance, indicating that ancient morphologically and reproductively complex streptophyte algae should have had the mechanisms to repair photosynthesis upon recovery and reproduce on land (Graham et al. 2012).

B. The Evolution of Distinctive Light-Harvesting Pigment-Protein Complexes May Have Accompanied the Streptophyte Transition to Land

Genome sequence projects have allowed comparisons of the photosynthetic light-harvesting complexes (LHCs) of higher plants with those of some green algae, revealing differences. Although prasinophyte and chlorophyte green algae and embryophytes (represented by *Arabidopsis*) display a number of homologous LHC genes, LHC1-like genes of these green algae show little homology to those of land plants, and homologs for higher plant Ll818 and some other genes have not been found in chlorophyte or prasinophyte genomes (Koziol et al. 2007). As noted earlier, several streptophyte algal species can be found in terrestrial locales, and many others inhabit quite shallow, nearshore freshwaters where they are exposed to high irradiance. To better understand the impacts of possible changes in light harvesting and photoprotection during the process of terrestrialization, it will be important to compare the LHCs of streptophyte algae to those of early-diverging lineages of bryophytes, aided by expressed sequence data being acquired for charophyceans and forthcoming genome sequences for the liverwort *Marchantia* and the early-diverging moss *Sphagnum*, as well as genomic information currently available for the more derived moss *Physcomitrella* (see Chap. 11). For example, the *Physcomitrella* genome includes sequence information for the antenna polypeptides Lhcb3 and Lhcb6. These proteins, as well as occurrence of an ortholog of PsbS and evidence for non-photochemical quenching, represent features hypothesized to be terrestrial adaptations (Alboresi et al. 2008).

C. Streptophyte Algae Bequeathed Carbon Acquisition Versatility to Embryophyte Descendants

The vast majority of modern streptophyte algae and bryophytes occupy freshwater or moist terrestrial habitats, suggesting that terrestrialization likely occurred by the transition from freshwater (rather than saline) habitats to land (Becker and Marin 2009). Carbon dioxide, the raw material for carbon fixation, can limit photosynthesis of algae and bryophytes that grow submerged in freshwaters when pH is low and the dominant photosynthesizers primarily use CO_2 as an inorganic carbon source. Such conditions prevail, for example, in modern humic lakes where desmidialean and zygematalean algae and peatmosses are diverse and abundant (see Chap. 13). Consequently, Graham (1993) and Graham and Gray (2001) proposed that CO_2-limitation was a major selective force driving streptophyte ecological transition from (1) inorganic C-limited deeper freshwater habitats to (2) shallower and more turbulent nearshore freshwaters richer in dissolved CO_2 to (3) the wave-splashed and unpredictably arid shores of freshwater ponds, lakes or streams. Atmospheric CO_2 has a much greater diffusivity in subaerial habitats (10^4 higher) than in water, and is thought to have been particularly abundant in pre-Carboniferous times (Berner 1997), including the Cambrian-Ordovician period commonly associated with the rise of pre-vascular land vegetation. Even so, the occurrence in modern streptophyte algae of additional carbon acquisition strategies suggests that earliest land plants and their direct ancestors may have also possessed flexibility in obtaining carbon, such as the ability to use bicarbonate in addition to CO_2 as an inorganic C source and capacity to utilize exogenous organic carbon.

1. Use of Bicarbonate as a Source of Dissolved Inorganic C

Streptophyte algae that occur in higher pH aquatic systems are known to produce intracellular and extracellular (periplasmic) carbonic anhydrases (CAs) that interconvert CO_2 and bicarbonate, thereby maintaining equilibrium levels of CO_2 for rubisco (Arancibia-Avila et al. 2000, 2001). These carbon-concentration systems endow plant relatives with considerable flexibility in

meeting challenges of inorganic carbon availability. For example, a strain of *Mougeotia* isolated from nuisance growths that form in acidic lakes but are also capable of growth in more alkaline waters appears to upregulate external CA activity when grown at pH 8, by comparison to growth at pH 5 (Arancibia-Avila et al. 2000). This change allows the alga to utilize bicarbonate, which is considerably more abundant at pH 8 than pH 5.

2. Origin of Beta-Type Carbonic Anhydrases

Immunolocalization analyses indicate that at least one species of *Chara* possesses a dispersed beta-type chloroplast stromal CA that is otherwise not known to occur in eukaryotes other than land plants. The apparent absence from a *Mougeotia* strain of beta-CA, whose encoding genes bear no sequence similarity to genes for more widely-distributed alpha-type periplasmic or thylakoidal CAs, suggests that the beta-type CA appeared in streptophyte algae after the divergence of Zygnematales and was inherited by earliest land plants (Arancibia-Avila et al. 2001).

During their evolutionary history, streptophytes have transitioned from a condition in which the carbon fixation enzyme rubisco is aggregated into intraplastidal spheres known as pyrenoids (the case for most streptophyte algae) to dispersal of rubisco throughout the chloroplast stroma (the case for most embryophytes). The acquisition of beta-type CA allows embryophytes to increase the degree of spatial association between CO_2-releasing CA and CO_2-binding rubisco. Such a change would have allowed streptophytes to transition from one or two larger, pyrenoid-bearing plastids to multiple, smaller, pyrenoidless plastids that can be rapidly repositioned for maximum light absorption or photoprotection (see Chap. 8). The presence of single, large pyrenoid-containing plastids in the cells of many streptophyte algae as well as early-diverging hornworts indicates that the transition to many, small, pyrenoidless plastids occurred more than once during early plant diversification and thus does not necessarily mark plants' closest algal relatives.

3. Mixotrophy

Several species of streptophyte algae and early-diverging bryophytes have been demonstrated capable of mixotrophy, the uptake and utilization of exogenous organic compounds (in addition to those produced endogenously by photosynthetic carbon fixation) (Graham et al. 1994, 2010a, b). These results suggest that mixotrophy is an early-evolved streptophyte trait that likely characterized earliest land plants, providing several possible advantages during the terrestrialization process. Absorbed sugars could be used as a substrate for cellulose biosynthesis, to cope with: (1) periods of reduced photosynthesis such as occur during drought, photoinhibition, or as the result of mineral nutrient deficiencies; and (2) to recover organic exudations. In addition, mixotrophy has been proposed to subsidize the energy costs of producing degradation-resistant cell wall polymers similar to lignin, which are discussed next.

D. Sporopollenin and Lignin-Like Vegetative Cell Wall Components Originated in Streptophyte Algae and Were Inherited by Earliest Land Plants, Influencing Their Carbon Cycle Impacts

Together, photosynthesis and uptake of organic carbon that might be available provide the organic resources needed by modern streptophyte algae and bryophytes to metabolize, grow, and reproduce, and likely also reflect the condition of earliest land plants. Sporopollenin and lignin-like phenolic wall polymers are examples of metabolic products known to enhance the survival of these organisms, but involve long biosynthetic pathways with high metabolic costs. Consequently, modern streptophyte algae and bryophytes, modeling earliest land plants, deploy sporopollenin and lignin-like wall polymers judiciously, in cells or tissues of greatest effectiveness.

1. Sporopollenin

The origin of sporopollenin-encased spores was a key embryophyte innovation allowing dispersal in dry air (Graham et al. 2004a). Some investigators have recently suggested that sporopollenin-walled spores might have arisen prior to the multicellular diploid (sporophyte) generation or its developmental precursor–the embryo (Taylor and Strother 2009; Brown and Lemmon 2011). Material similar in chemistry and ultrastructural appearance to sporopollen occurs as an inner zygote cell wall layer in *Coleochaete* (Delwiche et al. 1989) and other later-branching streptophyte algae, thereby indicating the origin of sporopollenin during streptophyte algal diversification, prior to the origin of embryophyte spores (Graham et al. 2004a). Streptophyte algal and bryophyte wall sporopollenin displays characteristic autofluorescence in violet or UV excitation, a property indicating presence of phenolic groups. Although the precise chemical composition of sporopollenin is uncertain, this material is regarded as the most degradation-resistant known biopolymer, helping to explain its protective role during the dispersal of streptophyte algal zygotes and embryophyte spores.

2. Lignin-Like Vegetative Cell Wall Polymers

In addition to sporopollenin, streptophyte algae appear to have bequeathed to earliest land plants and modern bryophytes a biochemically-distinct but likewise degradation-resistant, phenol-containing biopolymer that is deposited in the cell walls of vegetative body cells, rather than zygotes or spores. Gunnison and Alexander (1975a, b) were the first to discover that vegetative cell walls of some streptophyte algae possess the potential to resist microbial degradation in aquatic sediments, demonstrating the presence of "lignin-like" phenolics in decay-resistant cell walls of the desmidialean genus *Staurastrum*. Delwiche et al. (1989) subsequently revealed the occurrence of chemically similar, hydrolysis-resistant wall compounds in walls of *Coleochaete* vegetative cells positioned nearby zygotes, and also demonstrated that such wall compounds displayed specific fluorescence properties identical to those of vascular plant lignin, and were chemically, positionally, and ultrastructurally distinct from sporopollenin. Building on this work, Kroken et al. (1996) provided evidence that phenolic wall materials having lignin-like autofluorescence properties appear to confer hydrolysis-resistance upon positionally-equivalent tissues of bryophytes, namely specialized placental tissues located at the N/2 N interface. In the same project, a survey of the production of lignin-like phenolic wall polymers by representative streptophyte algae and bryophytes indicated that such materials likely evolved after the divergence of Klebsormidiales, and before the divergence of desmids such as *Staurastrum*, and were inherited by earliest land plants. The Kroken et al. (1996) survey also indicated that liverworts and mosses have commonly deployed phenolic materials in additional specialized tissues where degradation resistance is advantageous. Examples include the lower epidermis and rhizoid outgrowths (which lie in direct contact with substrate microbes) and sporangial epidermis, i.e. the capsule wall of the sporophyte generation, providing protection for developing spores. These evolutionary changes reflect differential regulatory control over the location of phenolic deposition in the bodies of different bryophyte species, thereby affecting the extent to which bodies and tissues are able to resist microbial and chemical degradation.

Recent application of thioacidolysis methodology to the liverwort *Marchantia* and the moss *Physcomitrella* has revealed the occurrence of lignin-specific monomers in these bryophyte model systems (as well as vascular plants and certain tested seaweeds) (Espiñeira et al. 2010). These findings help to explain the degradation resistance of liverwort and moss sporangial epidermis (Kroken et al. 1996), the *Polytrichum* calyptra (Kodner and Graham 2001), and the lower epidermis and rhizoids of *Marchantia*, as well as these

materials' close resemblance to particular cellular sheets and tube microfossils (Graham et al. 2004b) and Late Silurian to Late Devonian (420–370 million years ago) macrofossils of variable size known as *Prototaxites*. In the past, the latter have been interpreted in diverse ways, but were more recently suggested to represent rolled and slumped degradation-resistant lower body remains of *Marchantia*-like liverwort mats, which are known to occur in large dimensions both today and in the fossil record (Graham et al. 2010b, c).

Interpretation of at least some of the "dispersed cuticle" and tubular microfossils as liverwort or moss epidermal tissues explains why the cell sheet microfossils are invariably monostromatic (one cell layer thick), and why these particular tissues have survived degradation long enough to fossilize. The selective deposition of phenolic polymers into cell walls of some but not all bryophyte tissues explains why certain cells or tissues survive degradation and thus fossilize better than others. This suggests a testable explanation for the lack to date of intact body fossils of earliest bryophyte-like land plants, which are known only from spores and other microfossils. Results available to date (Graham et al. 2010a) predict that artificial degradation of a suite of modern bryophyte representatives chosen on the basis of key phylogenetic position and/or ecological criteria would reveal a pattern of adaptation to terrestrial stressors by increased production of protective lignin-like wall polymers.

Because there is cellular sheet ("dispersed cuticle") evidence for the existence of *Sphagnum*-like mosses as early as the mid-Ordovician (Kroken et al. 1996). Graham et al. 2004a generated quantitative estimates of degradation-resistant biomass for three modern, but relatively early-branching moss lineages (including *Sphagnum*) in order to model carbon sequestration for a conservatively-estimated 40 million year period prior to end-Ordovician glaciations and the subsequent rise of vascular plants. Such calculations indicate that bryophyte-like land plants played an important role in Earth's carbon cycle and played other key ecological roles for at least tens of millions of years prior to the rise of vascular plants, as bryophytes do today (Turetsky 2003).

IV. Comparison of Early-Diverging Modern Photosynthesizers to Precambrian-Devonian Fossils Illuminates the Pattern of Terrestrialization

Because modern algae (cyanobacteria and photosynthetic protists) and modern bryophytes possess degradation-resistant components, artificially degrading them for comparison with enigmatic microscopic fossils (microfossils) has proven to be a fruitful way to identify early terrestrial photosynthesizers and understand their structure. Artificial degradation allows investigators to identify the most hydrolysis-resistant and thus potentially preservable parts of modern algae and plants for comparison to ancient microfossils (Gensel 2008). This section describes how comparative studies of fossils and modern representatives of ancient lineages provide insight into photosynthetic and other characteristics of earliest terrestrial algae and land plants, beginning with the cyanobacteria.

A. Cyanobacteria Were Likely Earth's First Terrestrial Photosynthesizers

Earth's earliest terrestrial surfaces were almost certainly devoid of organic-rich soils typical of modern times, but rather consisted of rocky or sandy substrates, the latter generated by the weathering actions of wind and water. Geological features cited as evidence for terrestrial cyanobacteria have been described from the Precambrian. Horodyski and Knauth (1994) interpreted beads and cylinders of the iron oxide mineral hematite found in a 1.2 billion year old paleokarst as mineralized cyanobacteria. Prave (2002) cited 1.0 billion year old Scottish Torridonian sedimentary features similar to those associated

with modern microbial mats subject to periodic air exposure as indirect evidence for terrestrial cyanobacteria. Strother et al. (2011) report cyanobacterial sheaths and *Halothece*-like cells recovered from the same deposits by acid maceration. Though Cambrian and Ordovician records of terrestrial cyanobacteria have yet to appear, Early Silurian (Llandovery) microfossils have been interpreted as terrestrial cyanobacteria (Tomescu et al. 2009).

Such paleobiological observations are consistent with the widespread occurrence of cyanobacteria that are tolerant of high irradiance and drought in modern environments ranging from extremely cold dry valleys of Antarctica to deserts of varying aridity conditions (Hughes and Lawley 2003; see also review of terrestrial algae in Graham et al. (2009) and Chapter 16). In such locales, cyanobacteria can occur at the soil surface (e.g. Hu et al. 2003) or beneath or within translucent rocks such as quartzite, sandstone, or granite. Such rocks transmit sufficient light for photosynthesis and reduce the evaporation of moisture needed for active metabolism. For example, at a study site in the US Mojave Desert (California), all quartz pebbles thinner than 25 mm provided favorable light and moisture conditions for cyanobacteria growing beneath (Schlesinger et al. 2003). Likewise, the cyanobacterium *Chroococcidiopsis* was commonly found in hot and cold deserts of China associated with larger rocks in quartz stony pavements (Warren-Rhodes et al. 2007). Water amounts sufficient for at least some periods of active photosynthesis and growth are necessary for cyanobacterial survival. For example, in the hyperarid Atacama Desert of Chile, the lower precipitation limit allowing photosynthesis by cyanobacterial communities is 5 mm annually (Cockell et al. 2008).

Cyanobacteria of diverse types have adapted to modern terrestrial habitats (Fig. 2.2) and it is likely that many of their adaptive features are ancient, present in Precambrian representatives. Most cyanobacteria generate extracellular polysaccharide sheaths, which can aid in water absorption and retention and provide other functions useful in coping with terrestrial stresses (reviewed in Graham et al. 2009). For example, a number of cyanobacteria possess UV-protective sheath pigments (Sinha and Häder 2007) and mycosporine-like amino acids provide protection from UV radiation and diverse oxidative stresses (Oren and Gunde-Cimerman 2007; Sinha and Häder 2007). Intracellular carotenoids provide photoprotection in diverse cyanobacteria (Lakatos et al. 2001; Kirilovsky 2010). Other species display exceptional tolerance of their cellular components to severe water loss (Potts 1999). Cyanobacteria are also known to produce antibiotic compounds (e.g. Jaiswal et al. 2008), thereby slowing growth of degradative microorganisms.

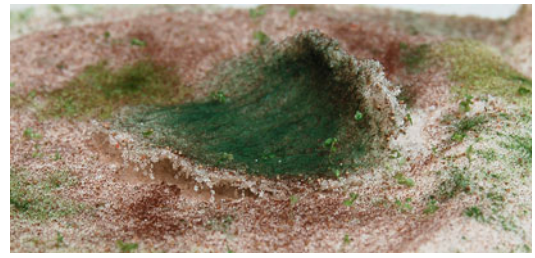


Fig. 2.2. Cyanobacterial mat growing on a sandy terrestrial surface.

B. Cyanobacterial and Other Microbial Associations Aid Bryophyte Photosynthesis

The geological evidence for early appearance of terrestrial cyanobacteria together with the physiological ability of many modern representatives to tolerate harsh environmental conditions suggests that cyanobacteria had colonized land well before the first land plants appeared. Consequently, well-established terrestrial cyanobacteria may have competed for resources with earliest plants, but likely also became associated with early plants in mutually beneficial partnerships. Cyanobacterial partners could contribute combined nitrogen generated by nitrogen-fixation metabolism (unique to certain prokaryotes), helping plants to cope with nutrient-poor substrates. Cyanobacterial

Fig. 2.3. The bacterial community within the gelatinous sheath of *Coleochaete pulvinata*.

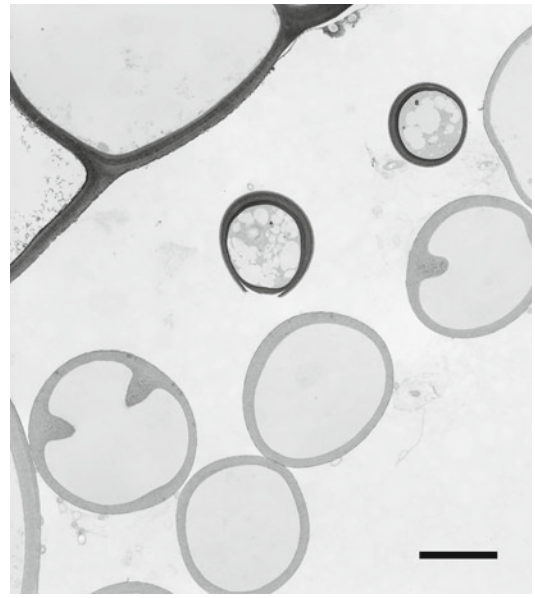


Fig. 2.4. TEM of the lower epidermis of a marchantialean liverwort and associated glomalean hyphae. Cells of the liverwort ventral surface (upper left corner) possess distinctively electron-dense cell walls, thought to reflect the presence of resistant phenolic polymers. Nearby sections of rhizoids reveal that compression resistance is a feature of these long, narrow cells bearing cell wall ingrowths. The smaller diameter, denser-walled sections are of the hyphae of glomalean fungi that are commonly associated with the lower epidermis of this liverwort. Scale bar = 6 μm.

partnerships also have the potential to provide protective benefits (water-holding mucilage, sunscreens, antibiotics). The potential value of cyanobacterial partners to early land plants is illustrated by symbiotic associations that have been documented for modern early-diverging liverworts and other bryophytes (Basilier 1980; West and Adams 1997; Mitchell et al. 2003; Gentili et al. 2005; Houle et al. 2006; Nilsson et al. 2006; Villarreal and Renzaglia 2006; Adams and Duggan 2008; Rikkinen and Vertanen 2008; Zackrisson et al. 2009).

Streptophyte algae commonly display communities with heterotrophic bacteria that live on algal cell walls or within extensive mucilaginous extracellular matrices generated by the algae (Fisher and Wilcox 1996; Fisher et al. 1998) (Fig. 2.3). Though little is currently known about their composition and function, such microbiomes are hypothesized to receive metabolizable exudates such as glycolate from the algae and contribute fixed nitrogen or vitamins to algal hosts. Heterotrophic prokaryotes also known to partner with bryophytes (Basile et al. 1969; Costa et al. 2001; Dedysh et al. 2002, 2006; Raghoebarsing et al. 2005; Opelt et al. 2007; Chen et al. 2008) can play important roles in carbon cycling (see Chap. 13).

Mycorrhizal-like fungal associations (Fig. 2.4), which occur widely in modern bryophytes, are suggested by several experts to have been critical to the success of early plants on land (Read et al. 2000; Bidartondo et al. 2002; Russell and Bulman 2004; Duckett et al. 2006; Wang and Qiu 2006; Ligrone et al. 2007; Pressel et al. 2008; Bidartondo and Duckett 2010; Bonfante and Selosse 2010). Specific genes known to be critical to the development of embryophyte-fungal symbioses are likely to have been features of early land plants (Wang et al. 2010).

As some experts have noted, at least some fungal-bryophyte associations may be of relatively recent origin, though certain fossil evidence supports the concept that bryophyte-fungal associations are ancient. The microfossil *Palaeoglomus grayi*–similar to modern glomaleans that today are not known to live independently from plant hosts and provide many modern plant species with mineral nutrients–occurs in mid-Ordovician deposits (Redeker et al. 2000, 2002). By fostering essential mineral acquisition, such beneficial

microbial associations likely fostered earliest plant photosynthesis.

C. Microfossils Indicate That Freshwater and/or Terrestrial Eukaryotic Algae Were Present in the Precambrian

Geochemical features have also provided indirect evidence for the occurrence of terrestrial eukaryotes in the Precambrian. For example, an increasing trend in smectitic clays seen in the rock record beginning around 850 Ma, presumably caused by retention of acidic water at the surfaces of weathering rocks, has been interpreted as the consequence of a primitive land biota including eukaryotes capable of secreting organic acids (Kennedy et al. 2006). Carbon isotopic data likewise indicate presence of terrestrial photosynthesizers in the late Precambrian (Knauth and Kennedy 2009). More recently, ancient lacustrine deposits from the 1.0 Ga Torridon Group (NW Scotland) were found to contain diverse assemblages of microfossils and structural organic fragments. In addition to previously-mentioned forms interpreted as prokaryotes, a diverse array of more complex structures mostly interpreted as eukaryotic were found (Strother et al. 2011). These shale deposits accumulated in lakes, but were frequently subaerially exposed as evidenced by numerous levels exhibiting desiccation cracks and possible raindrops. The dominant lifeforms are simple spherical cells and cell clusters (Zhang 1982; Brasier 2009), but more complex remains include forms with internal bodies, external processes and even flask-shaped and thalloid organisms (Strother et al. 2011). Some of these colonial or coenobial growth forms resemble those of modern eukaryotic algae. While many of the producers probably inhabited the water column, at least some were likely transported into the lake from surrounding streams, and some may even have derived from the land surface before being flushed into the river/lake. In addition, certain microfossil remains from Precambrian (Mesoproterozoic) to early Cambrian sites are said to resemble the modern unicellular freshwater green, zygnematealean algal genus *Spirotaenia* (Leiming et al. 2005; Leiming and Xunlai 2007).

These fossil observations are consistent with observations that diverse species of extant eukaryotic algae occupy a wide range of terrestrial and even arid habitats (Flechtner et al. 2008; Büdel et al. 2009) and display traits that aid in coping with high irradiance and desiccation (reviewed in Chapter 23 of Graham et al. 2009). Some green algae possess similar mechanisms to those found in cyanobacteria for dealing with high light stress, including the use of carotenoids and MAAs for photoprotection. The cellular mechanisms used by green algae for vegetative desiccation tolerance are not well studied, although the ability of vegetative cells to tolerate desiccation is phylogenetically widespread (e.g., Gray et al. 2007; Luttge and Büdel 2010).

D. Fossil Evidence Suggests That Streptophyte Algae Were Established on Land by the Middle Cambrian

As previously noted, like modern bryophytes, later-diverging streptophyte algae such as *Coleochaete* are known to be capable of producing degradation-resistant cell walls (Kroken et al. 1996). It has recently been observed that when terrestrially-grown *Coleochaete* has been subjected to artificial degradation, the remains closely resemble certain Middle Cambrian (499–511 million year old) microfossils (Graham et al. 2012), as well as some of the microfossil remains recovered from Early Middle Ordovician sediments (see figure 2h of Rubinstein et al. 2010). Such observations suggest that streptophytes had become terrestrial photosynthesizers by the Middle Cambrian and that terrestrial streptophyte algae continued to compete successfully with other terrestrial photosynthesizers well into the Ordovician.

E. Some Experts Think That Early Land Plants Had Evolved by the Middle Cambrian, Though the Concept Is Controversial

The most ancient remains that have been linked with earliest land plants are Cambrian-Early Devonian microscopic fossils that include: (A) spores and spore-like objects,

(B) small tubes of various types, and (C) sheets of cells often referred to as "dispersed cuticle" (Gensel et al. 1991). Many of the latter structures are clearly not homologous to the cuticles of vascular plant shoots (noncellular, superficial layers of cutin and wax), or to the chitin and protein cuticles (exoskeletons) of arthropods, and thus are better termed *cellular sheets*.

Identifying the sources of microfossils is important in comprehending early terrestrialization because larger pieces of earliest land plants, visible with the unaided eye and thus known as macrofossils, have so far not been identified from Cambrian through Ordovician deposits. This lack has been attributed to climatic conditions, occurrence of early plants in places where remains were not readily transported, or absence of degradation-resistant material other than sporopollenin-coated spores (reviewed by Gensel 2008). However, if earliest land plants resembled modern, early-diverging bryophytes, as suggested by molecular systematic data, they likely produced some degradation-resistant materials that could have survived as fragmentary remains (Kroken et al. 1996; Graham and Gray 2001; Kodner and Graham 2001; Graham et al. 2004a, b). Except under unusual environmental conditions, only degradation-resistant cells and tissues are likely to survive transport from the site of death to the site of deposition, burial in aquatic sediments, and fossilization as compressions, impressions, or petrifactions (Gensel 2008).

Tantalizing organic fragments – scraps of cuticle, wefts of tubes and sheets of cells/spores – are known from a number of Middle Cambrian – Early Ordovician deposits in North America. These may be early terrestrial multicellular autotrophs that disarticulated before becoming fossils. Given the half billion years between these organisms – whether algae or plants or something in between – and extant bryophytes, it is not surprising that the remains are enigmatic. One thing is certain however; something living in the Middle Cambrian produced spores with thick, multilayered walls, more robust than that produced by any extant alga, and nearly indistinguishable from some relatively advanced liverworts. Whether bryophytes had evolved even earlier than has been recognized, or thick, multilayered spore walls were among the first features to evolve among terrestrial algae is still an open question. Microfossils cited as remains of land plants also occur in early Middle Ordovician (473–471 million years ago) (Rubinstein et al. 2010).

The concept that the embryophyte clade originated in Cambrian or even Precambrian times is supported by some molecular clock estimates, which place the split between streptophyte algae and embryophytes at more than 700 million years ago (Hedges et al. 2006; Zimmer et al. 2007; Clarke et al. 2011). This timing is substantially earlier than the mid-Ordovician (about 460 million year) period commonly cited for the origin of land plants (e.g. Sanderson 2003). However, molecular clock methods are constantly improving, genome projects are contributing many new sequences, and additional fossils are being found and identified. Consequently, it may eventually be possible to reconcile embryophyte divergence dates that result from molecular and fossil approaches, as has recently been accomplished for the divergence of marsupial and placental mammals (Luo et al. 2011).

F. Microfossil and Macrofossil Evidence Indicates the Widespread Occurrence of Early Liverwort-Like and Moss-Like Land Plants by Mid-Ordovician Times, Extending into the Silurian and Devonian

Microfossils that have been confidently identified as remains of early liverwort-like land plants existed by the mid-Ordovician (Strother et al. 1996; Wellman 2010). Certain modern liverworts are known to disperse their spores in groups of four attached meiotic products, thereby resembling intriguing fossil spore tetrads known from the Middle Ordovician and later times (Gray 1985; Graham and Gray 2001). In pioneering the ultrastructural comparative analysis approach to the study of ancient and modern spore walls, Taylor (1995)

demonstrated that certain spores dispersed in pairs (dyads) were similar in cellular detail to those of modern liverworts. Spores lacking a Y-shaped trilete mark (characteristic of dispersed spores of vascular plants) were found within sporangia of Lower Devonian plants having certain bryophyte features (Edwards et al. 1999). Aggregates of fossil spores enclosed in resistant sporangial epidermal tissue (Wellman et al. 2003) suggested affinity with modern liverworts, which had previously been demonstrated to produce degradation-resistant sporangia (Kroken et al. 1996). Fourier transform infrared (FTIR) spectroscopy analysis shows that Silurian spores of types putatively identified as the reproductive dispersal units of early embryophytes are chemically similar to the trilete single spores characteristically dispersed by vascular land plants (Steemans et al. 2010).

Evidence that early mosses existed by the Ordovician is provided by 460 million year old cellular sheet microfossils (Gray et al. 1982) that: (1) closely resemble in cellular pattern and cell dimensions degradation-resistant sporangial epidermis produced by the early-divergent moss genus *Sphagnum* (Kroken et al. 1996), and (2) have not been otherwise classified. The observation that artificially degraded gametophytic cells that conspicuously cap sporophytes of modern *Polytrichum* mosses, known as calyptrae, closely resemble particular branched tube microfossils having unusually thick (8.5 μm) cell walls known from Silurian-Devonian sites (Kodner and Graham 2001 and articles referenced therein) provides evidence for the occurrence of somewhat more derived mosses by this time.

As earlier noted, lignin has been found in the complex thalloid liverwort *Marchantia*, explaining degradation resistance of the lower epidermis and rhizoids of *Marchantia*, and these materials' close resemblance to particular porose cellular sheets and tube microfossils (Graham et al. 2004b), as well as Late Silurian to Late Devonian (420–370 million years ago) macrofossils of variable size known as *Prototaxites* (Graham et al. 2010b, c). Macrofossils that have been compared to modern derived liverworts are known from the Middle Devonian (*Metzgeriothallus sharonae*) (Hernick et al. 2008) and lowermost Upper Devonian (*Hepaticites devonicus*) (Hueber 1961). The occurrence of confidently-identified macrofossils of derived liverwort lineages in these Devonian samples increases the likelihood that earlier-diverging liverworts existed considerably earlier.

Microfossil evidence indicates that early vascular land plants were likely present in the Ordovician, though were rare (Steemans et al. 2009). Vascular plant diversification, possibly delayed by Late Ordovician glaciations, did not proceed rapidly until the Late Silurian to Early Devonian, 407–418 million years ago (Gensel 2008). Consequently, Middle Cambrian (499–511 million years ago) fossil evidence for terrestrial *Coleochaete*-like streptophyte algae (Graham et al. 2012) and Ordovician evidence for liverwort-like early land plants (e.g., Wellman et al. 2003) indicate that non-vascular terrestrial streptophytes were successful for more than 100 million years prior to the rise of vascular plants and in addition left descendants through at least five major species extinction events.

A similar extremely long period of terrestrial domination by analogs of Earth's terrestrial streptophyte algae and bryophytes may generally characterize the development of planets similar to Earth that are located in habitable zones of other suns. Increasingly better-developed spectroscopic techniques are allowing astrobiologists to infer the properties of extrasolar planets (reviewed by Baraffe et al. 2010; Kaltenegger et al. 2010). Future planetary surveys may reveal spectroscopic evidence for terrestrialization, such as red reflectance suggesting chlorophyll and IR evidence for volatiles such as isoprene, the latter produced by Earth mosses (as well as some other plants) as an adaptation to terrestrial stressors (Hanson et al. 1999).

V. Perspective

- Understanding Earth terrestrialization is important in comprehending the origin of modern ecosystems, and requires analyses of modern bryophytes and their closest algal relatives as well as Precambrian-Devonian fossil remains attributed to earliest land plants, which are largely fragmentary. Artificial degradation of modern bryophytes and algae allows comparison of the remains with enigmatic microfossil fragments, aiding fossil classification.
- The sum of available molecular and microfossil evidence indicates that earliest land plants evolved from streptophyte algae that had accumulated physiological and other preadaptations relevant to land colonization, but the order of early embryophyte-specific trait acquisition has not as yet been well defined.
- Available molecular and microfossil evidence does not yet allow confident determination of the time when earliest land plants appeared. Some molecular clock and controversial microfossil data argue for a Precambrian (700 million years ago) to Middle Cambrian (500 million years ago) emergence time. Widely accepted microfossils indicate that liverwort-like early land plants existed by the Middle Ordovician (470 million years ago). Some microfossils indicate the presence of early-diverging mosses by the Mid-Ordovician and later-diverging mosses by the Silurian. Certain microfossil and macrofossils suggest the existence of *Marchantia*-like liverworts by the Late Silurian, and non-controversial macrofossils show that liverworts similar to those of other modern species existed by the Middle Devonian. Existing molecular systematic analyses and an increasing body of fossil evidence indicates that modern liverworts and mosses are *not* the descendants of degenerate vascular land plants.
- Models of carbon sequestration by early bryophyte-like land plants, constructed from data obtained from modern early-divergent bryophytes, indicate that prevascular land plants influenced Earth's carbon cycle for tens of millions of years before vascular plants became common, and help to explain how modern liverworts and mosses are biogeochemically significant. These results, together with microfossil evidence for earliest terrestrial streptophytes, suggest that Earth-like extrasolar planets that occupy habitable zones may likewise display an extended period of terrestrial carbon cycle dominance by streptophyte algae and bryophyte-like early land plants.

Acknowledgements

Sarah Friedrich provided assistance with illustrations. We thank Dr. James Graham and Christopher Cardona-Correa for helpful discussions. Grant support from the US National Science Foundation (NSF) and the United Kingdom Natural Environment Research Council (NERC) is much appreciated.

References

Adams DG, Duggan PS (2008) Cyanobacteria-bryophyte symbioses. J Exp Bot 59:1047–1058

Alboresi A, Caffarri S, Nogue F, Bassi R, Morosinotto T (2008) *In silico* and biochemical analysis of *Physcomitrella patens* photosynthetic antenna: identification of subunits which evolved upon land adaptation. PLoS One 3:e2033

Arancibia-Avila P, Coleman JR, Russin WA, Wilcox LW, Graham LE (2000) Effects of pH on cell morphology and carbonic anhydrase activity and localization in bloom-forming *Mougeotia* (Chlorophyta, Charophyceae). Can J Bot 78:1206–1214

Arancibia-Avila PA, Coleman JR, Russin WA, Graham JM, Graham LE (2001) Carbonic anhydrase localization in charophycean green algae: ecological and evolutionary significance. Int J Plant Sci 162:127–135

Baraffe I, Chabrier G, Barman T (2010) The physical properties of extra-solar planets. Rep Prog Phys 73:1–30

Basile DV, Slade LL, Corpe WA (1969) An association between a bacterium and a liverwort, *Scapania nemorosa*. J Torrey Bot Soc 96:711–714

Basilier K (1980) Fixation and uptake of nitrogen in *Sphagnum* blue-green algal associations. Oikos 34:239–242

Becker B, Marin B (2009) Streptophyte algae and the origin of embryophytes. Ann Bot 103:999–1004

Berner RA (1997) The rise of land plants and their effect on weathering and CO_2. Science 276:544–546

Bidartondo MI, Duckett JG (2010) Conservative ecological and evolutionary patterns in liverwort-fungal symbioses. Proc R Soc B Biol Sci 277:485–492

Bidartondo MI, Bruns TD, Weiss M, Sérgio C, Read DJ (2002) Specialized cheating of the ectomycorrhizal symbiosis by an epiparasitic liverwort. Proc R Soc Biol Sci Ser B 270:835–842

Bonfante P, Selosse M-A (2010) A glimpse into the past of land plants and of their mycorrhizal affairs: from fossils to evo-devo. New Phytol 186:267–270

Brasier MD (2009) Darwin's lost world: the hidden history of animal life. Oxford University Press, Oxford

Brook AJ, Williamson DB (1998) The survival of desmids on the drying mud of a small lake. In: Round FE (ed) Algae and the aquatic environment. BioPress, Bristol, pp 185–196

Brown RC, Lemmon BE (2011) Spores before sporophytes: hypothesizing the origin of sporogenesis at the algal-plant transition. New Phytol 190:875–881

Büdel B, Darienko T, Deutschewitz K, Dojani S, Friedl T, Mohr KI, Salisch M, Reisser R, Weber B (2009) Southern African biological soil crusts are ubiquitous and highly diverse in drylands, being restricted by rainfall frequency. Microb Ecol 57:229–247

Chen Y, Dumont MG, McNamara NP, Chamberlain PM, Bodrossy L, Stralis-Pavese N, Murrell JC (2008) Diversity of the active methanotrophic community in acidic peatlands as assessed by mRNA and SIP-PFLA analyses. Environ Microb 10:446–459

Clarke JT, Warnock RCM, Donoghue PCJ (2011) Establishing a time-scale for plant evolution. New Phytol 192:266–301

Cockell CS, McKay CP, Warren-Rhodes K, Horneck G (2008) Ultraviolet radiation-induced limitation to epilithic microbial growth in arid deserts – dosimetric experiments in the hyperarid core of the Atacama desert. J Photochem Photobiol B 90:79–87

Costa J-L, Paulsrud P, Rikkinen J, Lindblad P (2001) Genetic diversity of *Nostoc* symbionts endophytically associated with two bryophyte species. Appl Environ Microbiol 67:4393–4396

Crandall-Stotler BJ, Forrest L, Stotler RE (2005) Evolutionary trends in the simple thalloid liverworts (Marchantiophyta, Junnermanniopsida subclass Metzgeriidae). Taxon 54:299–316

Dedysh SN, Khmelenina VN, Suzina NE, Trotsenko YA, Semrau JD, Liesack W, Tiedje JM (2002) *Methylocapsa acidiphila* gen. nov., sp. nov., a novel methane-oxidizing and dinitrogen-fixing acidophilic bacterium from *Sphagnum* bog. Int J Syst Evol Microbiol 52:251–261

Dedysh SN, Pankratov TA, Belova SE, Kulichevskaya IS, Liesack W (2006) Phylogenetic analysis and in situ identification of bacteria community composition in an acidic *Sphagnum* peat bog. Appl Environ Microbiol 72:2110–2117

Delwiche CF, Graham LE, Thomson N (1989) Lignin-like compounds and sporopollenin in *Coleochaete*, an algal model for land plant ancestry. Science 245:399–401

Dombrovska O, Qiu Y-L (2004) Distribution of introns in the mitochondrial gene *nad1* in land plants: phylogenetic and molecular evolutionary implications. Mol Phylogenet Evol 32:246–263

Duckett JG, Carafa A, Ligrone R (2006) A highly-differentiated glomeromycotean association with the mucilage-secreting, primitive antipodean liverwort *Treubia* (Treubiaceae): clues to the origins of mycorrhizas. Am J Bot 93:797–813

Edwards D, Wellman CH, Axe L (1999) Tetrads in sporangia and spore masses from the Upper Silurian and Lower Devonian of the Welsh Borderland. Bot J Linn Soc 130:111–115

Elster JD, Peter D, L'ubomir K, Lucia V, Katarina Š, Antonio BP (2008) Freezing and desiccation injury resistance in the filamentous green alga *Klebsormidium* from the Antarctic, Arctic and Slovakia. Biologia 63:843–851

Espiñeira JM, Uzal EN, Gómez Ros LV, Carrión JS, Merino F, Ros Barceló A, Pomar F (2010) Distribution of lignin monomers and the evolution of lignification among lower plants. Plant Biol 13:59–68

Finet C, Timme RE, Delwiche CF, Marlétaz F (2010) Multigene phylogeny of the green lineage reveals the origin and diversification of land plants. Curr Biol 20:1–6

Fisher MM, Wilcox LW (1996) Desmid-bacterial associations in *Sphagnum*-dominated Wisconsin peatlands. J Phycol 32:543–549

Fisher MM, Wilcox LW, Graham LE (1998) Molecular characterization of epiphytic bacterial communities on charophycean green algae. Appl Environ Microbiol 64:4384–4389

Flechtner VR, Johansen JR, Belnap J (2008) The biological soil crusts of the San Nicolas Island: enigmatic algae from a geographically isolated ecosystem. West N Am Nat 68:405–436

Forrest LL, Davis EC, Long DG, Crandall-Stotler BJ, Clark A, Hollingsworth ML (2006) Unraveling the evolutionary history of the liverworts (Marchantiophyta): multiple taxa, genomes and analyses. Bryologist 109:304–334

Gensel PG (2008) The earliest land plants. Annu Rev Ecol Evol Syst 39:459–477

Gensel PG, Johnson NG, Strother PK (1991) Early land plant debris (Hooker's "waifs and strays"?). Palaios 5:520–547

Gentili F, Nilsson M-C, Zackrisson O, DeLuca TH, Sellstedt A (2005) Physiological and molecular diversity of feather moss associative N_2-fixing cyanobacteria. J Exp Bot 56:3121–3127

Graham LE (1993) The origin of land plants. Wiley, New York

Graham LE, Gray J (2001) The origin, morphology and ecophysiology of early embryophytes: neontological and palaeontological perspectives. In: Gensel P, Edwards D (eds) Plants invade the land. Columbia University Press, New York, pp 140–156

Graham LE, Graham JM, Russin WA, Chesnick JM (1994) Occurrence and phylogenetic significance of glucose utilization by charophycean algae: glucose enhancement of growth in *Coleochaete orbicularis*. Am J Bot 81:423–432

Graham LE, Cook ME, Busse JS (2000) The origin of plants: body plan changes contributing to a major evolutionary radiation. Proc Natl Acad Sci USA 97:4535–4540

Graham LE, Kodner RG, Fisher MM, Graham JM, Wilcox LW, Hackney JM, Obst J, Bilkey PC, Hanson DT, Cook ME (2004a) Early land plant adaptations to terrestrial stress: a focus on phenolics. In: Hemsley A, Poole I (eds) Evolution of plant physiology. Academic, New York, pp 155–170

Graham LE, Wilcox LW, Cook ME, Gensel PG (2004b) Resistant tissues of marchantioid liverworts resemble enigmatic Early Paleozoic microfossils. Proc Natl Acad Sci USA 101:11025–11029

Graham LE, Graham JM, Wilcox LW (2009) Algae. Benjamin Cummings/Pearson, San Francisco

Graham LE, Kim E, Arancibia-Avila P, Graham JM, Wilcox LW (2010a) Evolutionary and ecophysiological significance of sugar utilization by the peatmoss *Sphagnum compactum* (Sphagnaceae) and the common charophycean associates *Cylindrocystis brebissonii* and *Mougeotia* sp. (Zygnemataceae). Am J Bot 97:1485–1491

Graham LE, Cook ME, Hanson DT, Pigg KB, Graham JM (2010b) Structural, physiological, and stable carbon isotopic evidence that the enigmatic Paleozoic fossil *Prototaxites* formed from rolled liverwort mats. Am J Bot 97:1–8

Graham LE, Cook ME, Hanson DT, Pigg KB, Graham JM (2010c) Rolled liverwort mats explain major *Prototaxites* features: response to commentaries. Am J Bot 97:1079–1086

Graham LE, Arancibia-Avila P, Taylor WA, Strother PK, Cook ME (2012) Aeroterrestrial *Coleochaete* (Streptophyta, Coleochaetales) models early plant adaptation to land. Am J Bot 99:130–144

Gray J (1985) The microfossil record of early land plants: advances in understanding of early terrestrialization, 1970–1984. Philos Trans R Soc Lond B Biol Sci 309:167–195

Gray J, Massa D, Boucot AJ (1982) Caradocian microfossils from Libya. Geology 10:197–201

Gray DW, Lewis LA, Cardon ZG (2007) Photosynthetic recovery following desiccation of desert green algae (Chlorophyta) and their aquatic relatives. Plant Cell Environ 30:1240–1255

Gunnison D, Alexander M (1975a) Resistance and susceptibility to decomposition by natural microbial communities. Limnol Oceanogr 20:64–70

Gunnison D, Alexander M (1975b) Basis for the resistance of several algae to microbial decomposition. Appl Microbiol Biotechnol 29:729–738

Hanson DT, Swanson S, Graham LE, Sharkey TD (1999) Evolutionary significance of isoprene emission from mosses. Am J Bot 86:634–639

Hedges SB, Battistuzzi U, Blair JE (2006) Chapter 7. Molecular timescale of evolution in the Proterozoic. In: Xiao S, Kaufman AJ (eds) Neoproterozoic geology and paleobiology. Springer, New York

Hernick LVA, Landing E, Bartowski KE (2008) Earth's oldest liverworts – *Metzgeriothallus sharonae* sp. nov. from the Middle Devonian (Givetian) of eastern New York, USA. Rev Palaeobot Palynol 148:154–162

Holtzinger A, Tschaikner A, Remias D (2010) Cytoarchitecture of the desiccation-tolerant green alga *Zygogonium erecitorum*. Protoplasma 243:15–24

Horodyski RJ, Knauth LP (1994) Life on land in the Precambrian. Science 263:494–498

Houle D, Gauthier SB, Paquet S, Planas D, Warren A (2006) Identification of two genera of N_2-fixing cyanobacteria growing on three feather moss species in boreal forests of Quebec-Canada. Can J Bot 84:1025–1029

Hu CX, Zhang DL, Huang ZB, Liu YD (2003) The vertical microdistribution of cyanobacteria and green algae within desert crusts and the development of the algal crusts. Plant Soil 257:97–111

Hueber FM (1961) *Hepaticites devonicus* a new fossil liverwort from the Devonian of New York. Ann Mo Bot Gard 48:125–131

Hughes KA, Lawley B (2003) A novel Antarctic microbial endolithic community within gypsum crusts. Environ Microbiol 5:555–565

Jaiswal P, Singh PK, Prasanna R (2008) Cyanobacterial bioactive molecules – an overview of their toxic properties. Can J Microbiol 54:701–717

Kaltenegger L, Selsis F, Fridlund M, Lammer H, Beichman C, Danchi W, Eiroa C, Henning T, Herbst T, Léger A, Liseau R, Lunine J, Paresca F, Penny A, Quirrenbach A, Röttgering H, Schneider J, Stam D, Tinetti G, White GJ (2010) Deciphering spectral fingerprints of habitable exoplanets. Astrobiology 10:89–102

Karsten U, Lütz C, Holzinger A (2010) Ecophysiological performance of the aeroterrestrial green alga *Klebsormidium crenulatum* (Charophyceae, Streptophyta) isolated from an alpine soil crust with an emphasis on desiccation stress. J Phycol 46:1187–1197

Kennedy M, Droser M, Mayer LM, Pevear D, Mrofka D (2006) Late Precambrian oxygenation; inception of the clay mineral factory. Science 311:1446–1449

Kirilovsky D (2010) The photoactive orange carotenoid protein and photoprotection in cyanobacteria. In: Hallenbeck PC (ed) Recent advances in phototrophic prokaryotes. Springer, Philadelphia, pp 139–159

Knauth LP, Kennedy MJ (2009) The late Precambrian greening of the earth. Nature 460:728–732

Kodner RB, Graham LE (2001) High-temperature, acid-hydrolyzed remains of *Polytrichum* (Musci, Polytrichaceae) resemble enigmatic Silurian-Devonian tubular microfossils. Am J Bot 88:462–466

Koziol AG, Borza T, Ishida K-I, Keeling P, Lee RW, Durnford DG (2007) Tracing the evolution of the light-harvesting antennae in chlorophyll a/b-containing organisms. Plant Physiol 143:1802–1816

Kroken SB, Graham LE, Cook ME (1996) Occurrence and evolutionary significance of resistant cell walls in charophytes and bryophytes. Am J Bot 83:1241–1254

Lakatos M, Bilger W, Büdel B (2001) Carotenoid composition of terrestrial cyanobacteria: response to natural light conditions in open rock habitats in Venezuela. Eur J Phycol 36:367–375

Leiming Y, Xunlai Y (2007) Radiation of Meso-Neoproterozoic and early Cambrian protists inferred from the microfossil record of China. Palaeogeogr Palaeoclimatol Palaeoecol 254:350–361

Leiming Y, Xunlai Y, Fanwei M, Jie H (2005) Protists of the Upper Mesoproterozoic Ruyang group in Shanxi Province, China. Precambrian Res 141:49–66

Lewis LA, McCourt RM (2004) Green algae and the origin of land plants. Am J Bot 91:1535–1556

Ligrone R, Carafa A, Lumini E, Bianciotto V, Bonfante P, Duckett JG (2007) Glomeromycotean associations in liverworts: a molecular, cellular, and taxonomic analysis. Am J Bot 94:1756–1777

Luo Z-X, Yuan CX, Meng Q-J, Ji Q (2011) A Jurassic eutherian mammal and divergence of marsupials and placentals. Nature 476:442–445

Luttge U, Büdel B (2010) Resurrection kinetics of photosynthesis in desiccation-tolerant terrestrial green algae. Plant Biol 12:437–444

Mitchell EAD, Gilbert D, Buttler A, Amblard C, Grosvernier P, Gobat J-M (2003) Structure of microbial communities in *Sphagnum* peatlands and effect of atmospheric carbon dioxide enrichment. Microb Ecol 46:187–199

Nagao MK, Matsui K, Uemura M (2008) *Klebsormidium flaccidum*, a streptophyte algae green alga, exhibits cold acclimation that is closely associated with compatible solute accumulation and ultrastructural changes. Plant Cell Environ 31:872–875

Nilsson M, Rasmussen U, Bergman B (2006) Cyanobacterial chemotaxis to extracts of host and nonhost plants. FEMS Microbial Ecol 55:382–390

Opelt K, Chobot V, Hadacek F, Schönmann S, Eberl L, Berg G (2007) Investigations of the structure and function of bacterial communities associated with *Sphagnum* mosses. Environ Microbiol 9:2795–2809

Oren A, Gunde-Cimerman N (2007) Mycosporines and mycosporine-like amino acids: UV protectants or multipurpose secondary metabolites? FEMS Microbiol Lett 269:1–10

Potts M (1999) Desiccation tolerance in cyanobacteria. Eur J Phycol 34:319–328

Prave AR (2002) Life on land in the Proterozoic: evidence from the Torridonian rocks of northwest Scotland. Geology 30:811–814

Pressel S, Ligrone R, Duckett JG, Davis EC (2008) A novel ascomycetous endophytic association in the rhizoids of the leafy liverwort family Schistochilaceae (Jungermanniidae, Hepaticopsida). Am J Bot 95:531–541

Qiu Y-L, Cho Y, Cox JC, Palmer JD (1998) The gain of three mitochondrial introns identifies liverworts as the earliest land plants. Nature 394:671–674

Qiu Y-L, Li L, Bin W, Chen Z, Knoop V, Groth-Malonek M, Dombrovska O, Lee J, Kent L, Rest J, Estabrook GF, Hendry TA, Taylor DW, Testa CM, Ambros M, Crandall-Stotler B, Duff RJ, Stech M, Frey W, Quandt D, Davis CC (2006) The deepest divergences in land plants inferred from phylogenomic evidence. Proc Natl Acad Sci USA 103:15511–15516

Qiu Y-L, Li L, Wang B, Chen Z, Dombrovska O, Lee J, Kent L, Li R, Jobson RW, Hendry TA, Taylor DW, Testa CM, Ambros M (2007) A nonflowering land plant phylogeny inferred from nucleotide sequences of seven chloroplast, mitochondrial, and nuclear genes. Int J Plant Sci 168:691–708

Raghoebarsing AA, Smolders AJP, Schmid MC, Riipstra WIC, Wolters-Arts M, Derksen J, Jetten MSM, Schouten S, Damsté S, Lamers LPM, Roelofs JGM, Op den Camp HJM, Strous M (2005) Methanotrophic symbionts provide carbon for photosynthesis in peat bogs. Nature 436:1153–1156

Read DJ, Duckett JG, Francis R, Ligrone R, Russell A (2000) Symbiotic fungal associations in 'lower' land plants. Philos Trans R Soc Lond B Biol Sci 355:815–831

Redeker D, Kodner R, Graham LE (2000) Glomalean fungi from the Ordovician. Science 289:1920–1921

Redeker D, Kodner R, Graham LE (2002) *Palaeoglomus grayi* from the Ordovician. Mycotaxon 84:33–37

Rikkinen J, Vertanen V (2008) Genetic diversity in cyanobacterial symbionts of thalloid bryophytes. J Exp Bot 59:1013–1021

Rubinstein CV, Gerrienne P, de la Puente GS, Astini RA, Steemans P (2010) Early Middle Ordovician evidence for land plants in Argentina (Eastern Gondwana). New Phytol 188:365–369

Russell J, Bulman S (2004) The liverwort *Marchantia foliacea* forms a specialized symbiosis with arbuscular mycorrhizal fungi in the genus *Glomus*. New Phytol 165:567–569

Sanderson MJ (2003) Molecular data from 27 proteins do not support a Precambrian origin of land plants. Am J Bot 90:954–956

Schlesinger WH, Pippen JS, Wallenstein MD, Hofmockel KS, Klepeis DM, Mahall BE (2003) Community composition and photosynthesis by photoautotrophs under quartz pebbles, southern Mohave desert. Ecology 84:3222–3231

Sinha RP, Häder D-P (2007) UV-protectants in cyanobacteria. Plant Sci 174:278–289

Škaloud P (2009) Species composition and diversity of aero-terrestrial algae and cyanobacteria of the Boreč Hill ventaroles. Fottea 9:65–80

Steemans P, Le Hérissé A, Melvin J, Miller MA, Paris F, Verniers J, Wellman CH (2009) Origin and radiation of the earliest vascular land plants. Science 324:353

Steemans P, Lepot K, Marshall CP, Le Hérissé A, Javaux EJ (2010) FTIR characterization of the chemical composition of Silurian miospores (cryptospores and trilete spores) from Gotland, Sweden. Rev Palaeobot Palynol 162:577–590

Strother PK, Al-Hajri S, Traverse A (1996) New evidence for land plants from the lower Middle Ordovician of Saudi Arabia. Geology 24:55–58

Strother PK, Battison L, Brasier MD, Wellman CH (2011) Earth's earliest non-marine eukaryotes. Nature 473:505–509

Taylor WA (1995) Spores in earliest land plants. Nature 373:391–392

Taylor WA, Strother PK (2009) Ultrastructure, morphology, and topology of Cambrian palynomorphs from the Lone Rock Formation, Wisconsin, USA. Rev Palaeobot Palynol 153:296–309

Timme RE, Bachvaroff TR, Delwiche CF (2012) Broad phylogenomic sampling and the sister lineage of land plants. PLoS One 7(1):e29696

Tomescu AMF, Rothwell GW, Honeggar R (2009) A new genus and species of filamentous microfossil of cyanobacterial affinity from Early Silurian fluvial environments (lower Massanutten Sandstone, Virginia, USA). Bot J Linn Soc 160:284–289

Turetsky MR (2003) The role of bryophytes in carbon and nitrogen cycling. Bryologist 106:395–409

Turmel M, Pombert J-F, Charlebois P, Otis C, Lemieux C (2007) The green algal ancestry of land plants as revealed by the chloroplast genome. Int J Plant Sci 168:679–689

Villarreal JCA, Renzaglia KS (2006) Structure and development of *Nostoc* strands in *Leiosporoceros dussii* (Anthocerotophyta): a novel symbiosis in land plants. Am J Bot 93:693–705

Wang B, Qiu Y-L (2006) Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza 16:299–363

Wang B, Yeun LH, Xue J-Y, Liu Y, Ané J-M, Qiu Y-L (2010) Presence of three mycorrhizal genes in the common ancestor of land plants suggests a key role of mycorrhizas in the colonization of land by plants. New Phytol 186:514–525

Warren-Rhodes KA, Rhodes KL, Boyle LN, Pointing SB, Chen Y, Liu S, Zhuo P, McKay CP (2007) Cyanobacterial ecology across environmental gradients and spatial scales in China's hot and cold deserts. FEMS Microbiol Ecol 61:470–482

Wellman CH (2010) The invasion of the land by plants: when and where? New Phytol 188:306–309

Wellman CH, Osterloff PL, Mohiuddin U (2003) Fragments of the earliest land plants. Nature 425:282–285

West NJ, Adams DG (1997) Phenotypic and genotypic comparison of symbiotic and free-living cyanobacteria from a single field site. Appl Environ Microbiol 63:4479–4484

Wodniok S, Brinkmann H, Glöckner G, Heidel AJ, Phillippe H, Melkonian M, Becker B (2011) Origin of land plants: do conjugating green algae hold the key? BMC Evol Biol 11:104–111

Wood AJ (2007) The nature and distribution of vegetative desiccation-tolerance in hornworts, liverworts, and mosses. Bryologist 110:163–177

Zackrisson O, DeLuca TH, Gentili F, Sellstedt A, Jäderlund A (2009) Nitrogen fixation in mixed

Hylocomium splendens moss communities. Oecologia 160:309–319

Zhang Z (1982) Upper Proterozoic microfossils from the Summer Isles, NW Scotland. Palaeontology 25:443–460

Zimmer A, Lang D, Richardt S, Frank W, Reski R, Rensing SA (2007) Dating the early evolution of plants: detection and molecular clock analyses of orthologs. Mol Genet Genom 278:393–402

Chapter 3

Photosynthesis in Early Land Plants: Adapting to the Terrestrial Environment

John A. Raven*
Division of Plant Sciences, University of Dundee at the James Hutton Institute, The James Hutton Institute, Invergowrie, Dundee DD2 5DA, UK

School of Plant Biology, University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia

Dianne Edwards
School of Earth and Ocean Sciences, Cardiff University, Park Place, Cardiff CF10 3AT, UK

Summary

The embryophytic land plants evolved from charophycean green algae, one of the three clades of green algae which are important components of the microflora of present-day terrestrial habitats. The earliest embryophytes are recognised in the fossil record from their characteristic spores, with little evidence as to their vegetative structure. These earliest

*Author for correspondence, e-mail: j.a.raven@dundee.ac.uk

D.T. Hanson and S.K. Rice (eds.), Photosynthesis in Bryophytes and Early Land Plants, Advances in Photosynthesis and Respiration 37, pp. 29–58, DOI 10.1007/978-94-007-6988-5_3,

embryophytes presumably resemble the extant terrestrial green algae in being desiccation tolerant and poikilohydric. Only the embrophytes subsequently developed the homoiohydry which characterised the organism which today contribute most of the biomass and primary productivity on land, and allowed many of the organisms to become desiccation intolerant in the vegetative phase. Pre-Carboniferous land plant fossils have very few examples of bryophytes other than spores: exceptions are the Middle Devonian *Metzgeriothallus* and the Upper Devonian *Pallaviciniites*. Many of the other fossils are recognisable as polysporangiophytes, including vascular plants. Homoiohydry in some of these plants is shown by the occurrence of cuticle and stomata, although there is no fossil evidence bearing on desiccation tolerance/intolerance. In addition to the embryophytes there are many other fossils, e.g. *Pachytheca, Parka*, *Protosalvinia, Prototaxites* and *Spongiophyton*, which are probably photosynthetic organisms, but are not readily classified: algae, bryophytes and lichens have been suggested, in addition to the possibility that some represent terrestrial fungi. The high atmospheric CO_2 concentrations in the early Phanerozoic would have permitted higher rates of photosynthesis than occurs today on the basis of the surface area of the plant exposed to the gas phase because large concentration gradients from the atmosphere to the carboxylase driving diffusive entry of CO_2 are possible. Relatively complex morphologies (several layers of photosynthetic structures) and/or anatomy (ventilation within the organisms using gas spaces) are required if the light-harvesting capacity is to be matched by the CO_2 assimilation capacity.

I. Introduction

The extant land flora ranges in complexity, as judged by the number of kinds of cells in the organism, from unicellular and colonial cyanobacteria and (mainly) green eukaryotic algae (free living and lichenized) to bryophytes and vascular plants (Bell and Mooers 1997). The homoiohydric and (generally) desiccation intolerant (see Definitions) seed plants, with sexual reproduction independent of liquid water on the above-ground plant surface are the most successful components of the extant land flora as judged by the number of described species and their global productivity and biomass. However, the 'lower' land plants, i.e. cyanobacteria, eukaryotic algae and embryophytes of the bryophyte and pteridophyte grades, are significant components of extant terrestrial vegetation, and are more representative of the degree of complexity of the earliest photosynthetic organisms on land. In seeking present day analogues of the plants represented by the early fossils of photosynthetic organisms on land it is important to take into account differences between the present and past environments.

II. Extant Terrestrial Cyanobacteria, Algae and Embryophytes

Many higher taxa of algae, and cyanobacteria, have terrestrial representatives (Table 3.1; van den Hoek et al. 1995; Graham and Wilcox 2000; Lewis and Lewis 2005). "Terrestrial" here includes epi-, endo- and hypolithic algae as well as those living on finer-grained substrates, such as soil and desert sand (Lewis and Lewis 2005; Cardon et al. 2008; Cockell et al. 2009). The embryophytes are, apparently, ancestrally terrestrial: at least the earliest knowledge we have of them is of aerially dispersed meiospores

Abbreviations: CAM – Crassulacean Acid Metabolism; CCM – CO_2 concentrating mechanism; $\delta^{13}C$ – quantitative measure of the stable carbon isotope ratio relative to the standard carbon in the VPDB (Vienna Peee Dee Belemnite). $\delta^{13}C = \{[(^{13}C/^{12}C)_{sample}/(^{13}C/^{12}C)_{standard}] - 1\} \times 1{,}000$; Ga – 10^9 years; Ma – 10^6 years; Rubisco – Ribulose Bisphosphate Carboxylase-Oxygenase

Table 3.1. Characteristics of cyanobacterial and algae related to the evolution of complex life on land[a].

Taxon	Lifeform	Terrestrial	Desiccation tolerant	Oldest fossils	References
Cyanobacteria	Unicellular, colonial, multicellular	*Nostoc*, many lichenized	Yes	2.13 Ga (biomarkers), 2.45 Ga (O_2)	Tomitani et al. (2006) and Rasmussen et al. (2008)
Chlorophyta Prasinophyceae	Unicellular, colonial	No	No	1.3 Ga	Teyssèdre (2006) and Becker and Marin (2009)
Chlorophyta Chlorophyceae	Unicellular, colonial, multicellular	*Fritschiella*	Yes	(450 Ma)[b]	Lewis and Lewis (2005), Gray et al. (2007), Becker and Marin (2009), Caisova et al. (2011), and Lüttge and Büdel (2009)
Chlorophyta Trebouxiophyceae[b]	Unicellular, colonial, multicellular	*Prasiola*, *Trebouxia* Many lichenized	Yes	450 Ma	Derenne et al. (1992), Senousy et al. (2004), Lewis and Lewis (2005), Gray et al. (2007), Zhang et al. (2007), Bescker and Marin (2009), and Lüttge and Büdel (2009)
Chlorophtyta Ulvophyceae	Colonial, coenocytic, multicellular	*Trentopohlia*	Yes	540 Ma	Org et al. (1992), Gupta and Agrawal (2004), Lewis and Lewis (2005), Rindi et al. (2009a, b), and Lüttge and Büdel (2009)
Streptophyta Charophyceae	Unicellular colonial multicellular	*Klebsormidium*	Yes	450 Ma	Lewis and Lewis (2005), Becker and Marin (2009), and Karsten et al. (2010)
Streptophyta Embryophytes	Multicellular		Yes; bryophytes > non-seed vascular plants > seed plants	475 Ma	Oliver et al. (2005), Rubinstein et al. (2010), Wellman (2010), and Brown and Lemmon (2011)
Rhodophyta Bangiophyceae	Unicellular, multicellular	*Porphyridium*; *Porphyra* high Intertidal	Yes	1.2 Ga	Cole and Sheath (1990) and Butterfield (2000)
Rhodophyta Florideophyceae	Multicellular	*Bostrychia, Caloglossa, Catanella* high Intertidal	Yes	540 Ma (570 Ma?)	Cole and Sheath (1990) and Yuan et al. (2011)
Ochrista Bacillariophyceae	Unicellular, colonial (filamentous)	Some diatoms	Yes (resting cells)	120 Ma	Souffreau et al. (2010, 2013)

(continued)

Table 3.1. (continued)

Taxon	Lifeform	Terrestrial	Desiccation tolerant	Oldest fossils	References
Ochrista Fucophyceae	Multicellular	No; *Pelvetia* (high intertidal) drowns if continuously submerged (*Petrodrema* photobiont in high intertidal lichen *Verrucaria* spp.)	Yes	(570 Ma?)	Rugg and Norton (1987), Xu (2001), Sanders (2004), and Yuan et al. (2011)
Ochrista Tribophyceae	Unicellular, colonial muticellular, coenocytic (acellular)	*Botrydium, Vaucheria* (*Heterocapa* photobiont in high intertidal lichen *Verrucaria* spp.)	?	600 Ma	Trschermak (1941), Javanau et al. (2003), and Butterfield (2004, 2007)

[a]General references: Hoffman (1989), Van den Hoek et al. (1995), Kenrick and Crane (1997), Gensel (2008), Lewis (2007), Graham and Wilcox (2009), Taylor et al. (2009), and Paffrey et al. (2011)

[b]Based on the finding of Trebouxiophyceae in the Ordovician (Derenne et al. 1992), and branching order of the Chlorophyceae, Trebouxiophyceae and Ulvophyceae from molecular phylogenetics Lewis and McCourt (2004)

Table 3.2. The distribution of desiccation tolerance, desiccation intolerance, poikilohydry and homoiohydry among oxygenic photosynthetic organisms. The terms dessication tolerance, desiccation tolerance, homoiohydric and poikilohydric are defined in 'Definitions' at the end of this Table.

	Desiccation tolerant	Desiccation intolerant
Poikilohydric	Terrestrial Cyanobacteria, free-living and lichenized. Terrestrial (and some aquatic) Charophyceae, Chlorophyceae (free-living and lichenized), Trebouxiophyceae (free-living and lichenized),Ulvophyceae, intertidal Trebouxiophyceae, Ulvophyceae, Bangiophyceae, Florideophyceae, Fucophyceae Many dispersed spores, pollen and seeds of embryophytes; vegetative stages of many terrestrial (and some aquatic) bryophytes	Most aquatic cyanobacteria and algae. Some aquatic bryophytes. Aquatic vascular plants
Homoiohydric	Vegetative stage of many heterosporous lycopods (Isoetales, Selaginellales),some ferns, a few flowering plants	Vegetative stage of terrestrial members of the lycopods, many ferns, gymnosperms, almost all angiosperms

It is important to realise that there is a continuum of the extent of desiccation tolerance from intolerance to long-term tolerance of equilibration with an atmosphere of low relative humidity, and between poikilohydry and homoiohydry. From Raven (1977, 1994a, b, 1995, 1996, 1997a, b, 1999a, b, 2002a, b, 2003), Woodward (1998), Raven and Edwards (2001, 2007), Raven and Andrews (2010), Edwards et al. (1996, 1997, 2003), Proctor et al. (1992, 2007), Alpert (2005), Oliver et al. (2005), Watkins et al. (2007b), Wang et al. (2009), and Table 3.1

Definitions

Desiccation Intolerance The inability of an organism, or phase in the life cycle of an organism, to recover from dehydration to some specified level, e.g. equilibration with an atmosphere of 50 % relative humidity.

Desiccation Tolerance The ability of an organism, or phase in the life cycle of an organism, to recover from dehydration to some specified level, e.g. equilibration with an atmosphere of 50 % relative humidity.

Homoiohydry The ability of an organism to regulate water loss, and hence remain hydrated, despite a lack of water supply equal to the potential rate of water loss to an unsaturated atmosphere. For plants homiohydry involves a water uptake system (commonly a root system in the soil), an endohydric water transport system from the site of water uptake to the site of transpiratory water vapour loss, intercellular gas spaces, cuticle and stomata.

Poikilohydry The inability of an organism to regulate water loss, and hence remain hydrated, despite a lack of water supply equal to the potential rate of water loss to an unsaturated atmosphere. Poikilohydric plants may have one or more of the components of the homiohydric apparatus (water uptake system (commonly a root system in the soil), an endohydric water transport system from the site of water uptake to the site of transpiratory water vapour loss, intercellular gas spaces, cuticle and stomata) but lack the full suite of components functioning in an integrated manner.

References Raven (1977), Csintalan et al. (2000), Alpert (2005), Turnbull and Robinson (2009).

(Csotonyi et al. 2010; Rubinstein et al. 2010; Wellman 2010; Brown and Lemmon 2011).

Most of these algal taxa with terrestrial species have at least some desiccation tolerant terrestrial representatives, both free-living and lichenized (Tables 3.1 and 3.2). Many algae which live in the marine intertidal and in small bodies of inland water are also desiccation tolerant, though some of them (Fucophyceae, Floridiophyceae) do not have terrestrial representatives. Furthermore, some terrestrial algae are known not to be desiccation tolerant (Bacillariophyceae (diatoms) Soffreau et al. 2010), while others have apparently not been examined for desiccation tolerance (the unicellular bangiophycean *Porphyridium*). Terrestrial members of the cyanobacteria (free and lichenized), Chlorophyceae (free living and lichenized), Charophyceae, Trebouxiophyceae (free living and lichenized) and Ulvophyceae (free living and lichenized) have desiccation tolerant members (Tables 3.1 and 3.2).

The embryophytes seem to be ancestrally desiccation tolerant (Oliver et al. 2005) some in the vegetative state (sporophyte and

gametophyte) and more generally in dispersed spores (including pollen grains) and seeds (Brown and Lemmon 2011). Ancestral desiccation tolerance in embryophytes is of phylogenetic interest in relation to the desiccation tolerance (or otherwise) of extant representatives of the clades most likely to have given rise to the embryophytes (see Chap. 2). Among the embryophytes, most terrestrial bryophytes have desiccation tolerance in the vegetative phases as well as spores, and there is a greater fraction of organisms that are desiccation tolerant in the vegetative phase among plants at the pteridophyte grade of organization than among seed plants (Tables 3.1 and 3.2).

Most of the terrestrial algae and cyanobacteria are unicellular. Exceptions are the unbranched heterocystous filaments of the cyanobacterium *Nostoc*, the branched filamentous chlorophycean *Fritschiella*, the thalloid trebouxiophycean *Prasiola*, the branched filamentous ulvophycean *Trentepohlia* and the unbranched filamentous charophycean *Klebsormidium* (Table 3.1). The capacity for differentiation is clearly significant in the evolution and function of embryophytes, e.g. in the evolution of homoiohydry (see below). The red and brown algae, with no terrestrial representatives, have many genera with considerable structural complexity (Van den Hoek et al. 1995; Bell and Mooers 1997; Graham and Wilcox 2000).

All terrestrial algae and cyanobacteria, free-living and lichenized, are poikilohydric, as is the vegetative phase of bryophytes and of a few vascular plants, and many spores, pollen grains and seeds (Table 3.2). The photosynthetically competent sporophytes of some bryophytes have more homoiohydric features than do the gametophytes, but since the sporophyte depends on the poikilohydric gametophyte for water and soil-derived nutrients (matrotrophy) it is functionally poikilohydric. It is important to emphasize that there is a continuum of conditions between poikilohydry and homoiohydry (Proctor and Tuba 2002), just as there is a continuum for desiccation tolerance, ranging from intolerance of even very limited water loss for a short period to tolerance of equilibration with an atmosphere of very low relative humidity over a long period. Homoiohydry demands a certain minimum size and complexity (number of cell types), while poikilohydry does not (Raven 1999a, b; Boyce 2008).

III. The Time of Origin of Photosynthetic Taxa with Emphasis on Those Which Occur on Land

Table 3.1 lists the earliest recorded fossils for major taxa of photosynthetic organisms. The dates come from body fossils (e.g. dasycladalean green algae from the Ulvophyceae and coralline red algae from the Florideophyceae in the Cambrian), from taxon-specific biomarkers (e.g. cyanobacteria from the Palaeoproterozoic, and Trebouxiophyceae from the Ordovician), and the accumulation of O_2 earlier in the Palaeoproterozoic (evidence of oxygenic photosynthesis, hence cyanobacteria) (Table 3.1). Recent evidence (Sánchez-Baracaldo et al. 2005; Blank and Sánchez-Baracaldo 2010) suggests that the earliest cyanobacteria were from freshwater habitats, and that global oxygenation could only occur after cyanobacteria had colonized the ocean. In two cases the occurrence of green algal clades (Charophyceae, Chlorophyceae (Fig. 3.1a, b)) before the earliest known fossil for that clade is inferred from the occurrence of other clades of green algae (Trebouxiophyceae, Ulvophyceae) and the branching order of the green algae and embryophytes indicated by molecular phylogenetic studies (Lewis and McCourt 2004; Turmel et al. 2008). For the Chlorophyceae and Ulvophyceae there are body fossil indications of their presence 700–800 Ga ago (Butterfield et al. 1988). Table 3.1 shows that cyanobacteria occurred before the origin of embryophytes, as did all four of the green algal clades (Charophyceae, Chlorophyceae, Trebouxiophyceae, Ulvophyceae) with terrestrial representatives, as well as the

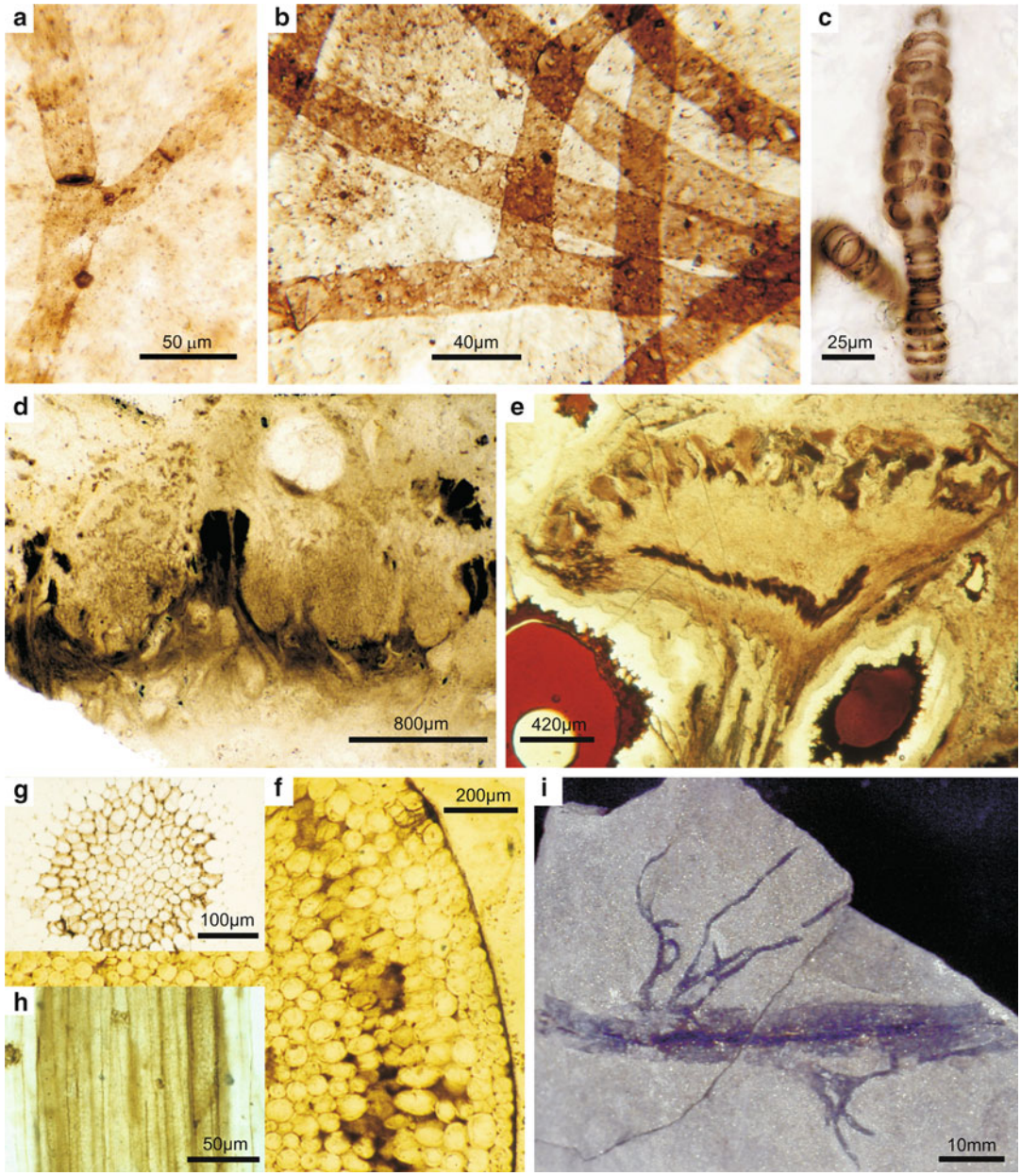


Fig. 3.1. (**a**) *Proterocladus*, a siphonocladalean green alga, Svanbergfjellet Formation, Spitsbergen, late Proterozoic (c. 750 myr). (**a-c** courtesy of Dr Nick Butterfield, Cambridge). (**b**) *Palaeovaucheria*, a vaucheriacean alga, Lakhanda Formation, Siberia, late Mesoproterozoic (c. 1000 myr). (**c**) *Bangiomorpha pubescens*, a red alga, Hunting Formation, Arctic Canada, Mesoproterozoic (c. 1200 myr). (**d**) *Winfrenatia reticulata*, an early lichen, showing a section of a thallus with hyphal pockets (arrows), with cyanobacteria inside. Rhynie Chert, Scotland, Pragian-Emsian (Lower Devonian, c. 404 myr). (Courtesy of Prof. Tom Taylor, Kansas). (**e**) *Kidstonophyton discoides*, longitudinal section through the distal regions of a male gametophyte with antheridia of ?Nothia aphylla, Rhynie Chert, Scotland, Pragian (Lower Devonian, c. 404 Myrs). (Courtesy of Prof. Hans Kerp, Münster). (**f–h**) *Aglaophyton major*, sections through upright stems of sporophyte; (**f**) transverse section of ground tissues with cuticle and zone of arbuscular mycorrhizae in cortex, (**g**) TS of central conducting cells, (**h**) LS of central conducting cells lacking conventional thickenings, Rhynie Chert, Scotland, Pragian – Emsian (Lower Devonian, c. 404 myr). (**i**) *Drepanophycus spinaeformis*, a lycophyte represented here by rhizome and lateral roots. Strathmore Group, Scotland, Emsian (Lower Devonian, c. 400 myr).

Prasinophyceae with no known terrestrial representatives. This provides a wide range of photosynthetic organisms that could have been on land before the embryophytes evolved. As is indicated in Table 3.1 and in the discussion above, all of these algae (including cyanobacteria) have desiccation tolerant members as well as representatives with some differentiation of their thalli. It would seem that there were other clades of chlorophyll b-containing organisms than the Charophyceae which had some of the attributes of the ancestor of the embryophytes, e.g. desiccation tolerance, terrestrial habit, presence of plasmodesmata, localized growing points (Raven 1977, 2005; Sarkar et al. 2009; Popper et al. 2011).

Other clades of algae are less likely to have been represented among terrestrial biota at or just before the time that the embryophytes evolved. For the Rhodophyta the two classes considered here (Bangiophyceae and Florideophyceae) have fossil records dating from pre-Ordovician times (Fig. 3.1c; Table 3.1); the acidophilic Cyanidiophyceae are not considered here. Extant unicellular bangiophycean red algae (e.g. *Porphyridium*) which can grow in terrestrial habitats are not known to be desiccation tolerant; the most complex member of the Bangiophyceae is less complex than the most complex member of the Florideophyceae, a class with no terrestrial members: some live in fresh waters but none on land.

Among the Ochrophyta (= Heterokontophyta) the oldest known fossils are of the Tribophyceae from the Neoproterozoic: the extant tribophytes that live on land are apparently not desiccation tolerant, and the lichenized (presumably) desiccation tolerant tribophyte is intertidal rather than terrestrial (Table 3.1). The diatoms are only known from much later in the fossil record, and fossil-calibrated phylogenies suggest that (unpreserved) ancestral diatoms probably arose less than 250 Ma ago (Table 3.1; see also Guillou 2011; Ichiniomiya et al. 2011). While fossils from as early as the Ediacaran have been assigned to the Fucophyceae these organisms could have been members of the Rhodophyta (Table 3.1).

As for fossils of lichenized algae, no terrestrial lichens are known in the fossil record until *Winfrenatia* (Fig. 3.1d) from the 410 Ma Rhynie Chert (Taylor et al. 1995), although what seems to be a marine lichen was found in 600 Ma marine sediments (Yuan et al. 2005). *Winfrenatia* has no parallels among extant lichens since, although the inhabitant was a cyanobacterium as in many modern lichens, the exhabitant was a glomeromycote rather than an ascomycote or a basidiomycote (Karatygin et al. 2009). Whether the ascomycotes and basidiomycotes had evolved by 470 Ma, with the earliest fossil evidence of embryophytes, is not clear; although Raven and Andrews (2010) suggest that the basidiomycotes had not evolved by the Silurian. However Hueber (2001) had earlier placed Lower Devonian *Prototaxites* in the basidiomycotes and this genus is also recorded in the mid Silurian. Work in progress in Cardiff suggests the presence of terrestrial lichens before 410 Ma in the Welsh Borderland.

It should be noted that molecular clock extrapolations to the time of origin of clades (see Heckman et al. 2001) can be subject to considerable error (Graur and Martin 2003), and such extrapolations have been avoided in the preceding four paragraphs. Clarke et al. (2011) present a time-scale for plant evolution using fossil calibration and a critical use of molecular calibrations.

The possible taxa of terrestrial oxygenic photosynthetic organisms at the time leading up to earliest known embryophytes is of unicellular and filamentous (including heterocystous) cyanobacteria, with green alga from the four clades Charophyceae, Chlorophyceae, Trebouxiophyceae and Ulvophyceae. Some of the cyanobacteria and algae could have been lichenized. It must be emphasised that these suggestions are based on fossils from marine or lacustrine sediments, and include range extensions based on the use of dated fossils of a sister clade to infer the date of

origin of a clade which lacks a fossil record at the relevant time.

IV. Evidence of Primary Productivity on Land Before and Contemporary with the First Evidence of Embryophytes

We can appeal to geochemical evidence, i.e. the occurrence of biologically stimulated weathering, suggesting the occurrence of terrestrial primary productivity prior to 470 Ma ago. It might be expected that minerotrophic photosynthetic organisms, i.e. those obtaining nutrient elements other than carbon from weathered rocks, would have more effect on weathering than ombrotrophic photosynthetic organisms, i.e. those obtaining nutrient elements by dry (gaseous, e.g. ammonia, nitrogen dioxide, acquired in a similar manner to carbon dioxide) or wet (aqueous solution) deposition from the atmosphere. Examples of predominantly ombrotrophic organisms are terrestrial algae, many terrestrial bryophytes (Ayres et al. 2006; Jones et al. 2007a, b) and a few (e.g. epiphytic) vascular plants, while some bryophytes and almost all vascular plants are predominantly minerotrophic. Baars et al. (2008) show that the bryophytes grown on a peat/sand substrate do not have a significant effect on chemical weathering via provision of CO_2 to ground water, in contrast to vascular plants with their greater vertical extent of below-ground structures. Lenton et al. (2012) have explored the extent of weathering produced by the moss *Physcomitrella patens*. This limitation may also apply to the endohydric sporophytes of *Aglaophyton* (with an atypical form of water conducting tissues, Fig. 3.1f–h) which has more or less horizontal rhizomes with little vertical penetration. The vascular plant model is structurally exemplified by the lycophyte *Drepanophycus* (Fig. 3.1i) with rooting structures which penetrated at least several cm into the sediment, but in a depositional environment composed of material which had been previously weathered. To have a major effect on weathering the plant must have underground structures penetrating at least several cm into the regolith or cracks in rock. Photosynthate produced in the above-ground structures using atmospheric CO_2 is conducted to underground structures where it is used in growth and maintenance, both of which generate CO_2. Herbivores, parasites and decomposers also produce CO_2 below ground. There is restricted diffusion of CO_2 back to the atmosphere, so CO_2 accumulates to a higher steady-state concentration than in the atmosphere, thus increasing the rate of chemical weathering. It would be expected that microalgae have even less effect on weathering than do bryophytes. Lichens, by contrast, can carry out significant weathering (Gadd and Raven 2010).

A number of lines of evidence have been bought forward which are consistent with biologically enhanced weathering on land before the first fossil evidence for embryophytes. Lenton and Watson (2004) have modelled phosphate weathering and have outcomes consistent with biotically enhanced weathering in the Neoproterozoic. Kennedy et al. (2006; see Derry 2006) have investigated the occurrence of pedogenic (soil-produced) clay minerals in mudstones from the Neoproterozoic and the Cambrian, and found a five-fold increase from 750 to 500 Ma, again consistent with biotically enhanced weathering on land. The more widely used technique of measuring the ^{87}Sr:^{86}Sr in marine carbonates (Lenton and Watson 2004; Derry 2006; Kennedy et al. 2006) as an indicator of terrestrial weathering has been refined to take into account seawater processes which alter the ^{87}Sr:^{86}Sr of carbonates (Shields 2007). While the data indicate increased terrestrial weathering in the Neoproterozoic and into the Cambrian, there is a long-term decrease from the late Cambrian onwards (Shields 2007). The decreasing weathering takes us into the Silurian and Devonian when there were terrestrial embryophytes in the form of vascular plants plus non-vascular polysporangiophytes (Raven and Edwards 2001) and uncharacterized organisms with substantial

underground structures, which are possible fungi (Hillier et al. 2008) An important point about biological effects on weathering is that the long-term (geological) variations in weathering are coupled to the volcanic production of CO_2 in the exogenic cycle (Shields 2007). The changes in atmospheric CO_2 content over geological time intervals reflect the difference between the inputs of CO_2 by vulcanism and removal by weathering, with subsequent $CaCO_3$ precipitation and long-term incorporation into sediments in the ocean. It should be remembered that abiotic weathering is temperature dependent, and that trace gases could have had a larger forcing effect on global temperature in greenhouse worlds such as occurred in parts of the Early and Mid Palaeozoic than occurs today, so abiotic weathering could have been greater than expected for atmospheric CO_2 levels (Beerling et al. 2011). Overall, the evidence for pre-Ordovician photosynthetic life on land, based largely on weathering rates, is indirect and can be subject to other interpretations.

Consideration of the possible functioning of the organisms represented by early terrestrial fossils requires that their structure is understood. Fossils of macroscopic eukaryotes from the Proterozoic include a bangiophycean red alga (Butterfield et al. 1990; Butterfield 2000), possible chlorophycean and ulvophycean green algae (Butterfield et al. 1988), a vaucheriacean tribophycean (Chromista) alga (Butterfield 2004) and a number of probable red and/or brown seaweeds (Yuan et al. 2011), as well as a probable lichen (Yuan et al. 2005). However, this evidence for Proterozoic structural complexity of free-living and symbiotic algae relates almost entirely to fossils from marine sediments.

There are few continental fossils of photosynthetic organisms, other than the meiospores of embryophytes (cf. Wellman et al. 2003), of undisputed terrestrial rather than aquatic origin, before the Mid to Late Silurian. Strother et al. (2011) report fossils of multicellular eukaryotic organisms which are apparently photosynthetic and which occurred in continental rocks just over a billion years old. These organisms probably lived in a lake, but could have been exposed to the atmosphere during seasonal or other drawdowns of the water level. Yang et al. (2004) report a bryophyte-like fossil, *Parafunaria sinensis*, from the Early-Middle Cambrian of China, although there is no evidence as to internal structure. Tomescu et al. (2006) report continental cyanobacterial macrophytes from the Early Silurian (Llandovery), although these seem as likely to be aquatic as terrestrial. Tomescu and Rothwell (2006; see also Strother 2010; Strother et al. 2011) discuss an Early Silurian wetland flora of thalloid organisms from less than 1 cm to greater than 10 cm long (see Bomfleur et al. 2010 for Triassic occurrence of thalloid organisms). These organisms were (presumably) photosynthetic and amphibious. Tomescu et al. (2009) used the closely similar values of the natural abundance of carbon isotopes in two of these thalloid organisms with that of the bulk carbon in the strata to suggest that these organisms were major primary producers in what, from the quantity of organic carbon, could have been a productive wetland (Tomescu et al. 2009). Tomescu et al. (2009) point out that carbon isotope ratios for the thalloid organisms are similar to those for ventilated (Meyer et al. 2008) liverworts such as *Lunularia* and *Marchantia* predicted by the BRYOCARB model (Fletcher et al. 2004). Of course, there could be other primary producers with closely similar carbon stable isotope ratios; since nothing is known of such producers, the conclusions of Tomescu et al. (2009) can be tentatively accepted. The non-marine status of the wetland flora was substantiated by Tomescu et al. (2009) on the basis of significant differences between the carbon isotope ratio of the thalloid organisms and the local bulk organic carbon on the one hand, and of organic carbon in marine sediments of the Early Silurian on the other. However, this averaged marine carbon isotope value presumably relates to phytoplankton, while the thalloid organisms would be more closely related, morphologically and

functionally if not necessarily phylogenetically, to benthic marine macroalgae. The present $\delta^{13}C$ for marine phytoplankton is about −23 ‰ for low and medium latitudes with lower (more negative) values at high latitudes. However, the range of $\delta^{13}C$ for extant marine macrophytes is from −3 to −35 ‰ (Raven et al. 2002a, b; Marconi et al. 2011), so it would be difficult today to distinguish these organisms from terrestrial or many freshwater organisms (see Fletcher et al. 2004). Wu et al. (2011) describe a Lower-Middle Cambrian fossil which they tentatively relate, in overall morphology, to the chlorophycean sub-aerial alga *Fritschiella*, although the fossil was from a marine deposit, and the heterotrichous morphology is also found in several other clades of algae (Fritsch 1945). As with the marine macroalgae of Yuan et al. (2011) it is not possible to attribute the thalloid organisms of Tomescu and Rothwell (2006) and Tomescu et al. (2009) to higher taxa.

V. Terrestrial Photosynthetic Organisms in the Upper Silurian and Devonian

A. Upper Silurian

Upper Silurian strata have fossils which are clearly the sporophyte phase of embryophytes, e.g. *Baragwanathia* (Fig. 3.2a), *Bathurstia* and *Cooksonia* (Edwards 1996; Kotyk et al. 2002; Taylor et al. 2009). These organisms are polysporangiophytes and, where data are available, they have tracheophyte attributes. As for phylogenetic attribution, there are examples of lycophytes (*Baragwanathia*), rhyniophytes (*Cooksonia,* Fig. 3.2c–e) and zosterophyllophytes (*Bathurstia*). Edwards (1996, 2000) describes fossils of vegetative structures and sporangia (and are thus sporophytes) from Upper Silurian and Lower Devonian strata which are characterized by small size: these too all seem to be polysporangiophytes. Functionally the Upper Silurian sporophytes are widely considered to be homoiohydric: however, Boyce (2008) points out that the smaller plants, including smaller specimens of *Cooksonia* (Fig. 3.2b, g), are unlikely to have been homoiohydric since there is a minimum size limit for homoiohydry (Raven 1999a, b). The Upper Silurian fossils give no obvious evidence as to the gametophyte phase of the plants (but see Gerrienne and Gonez 2011) nor of monosporangiophytic sporophytes, which characterize bryophytes.

The Upper Silurian tracheophytes do not help to resolve the order in which homoiohydric attributes arose, since this time interval had the first occurrence of xylem and of stomata (and, presumably, intercellular gas spaces). The molecular phylogenetic and other cladistic evidence generally favours 'stomata first' (Edwards et al. 1997; Kenrick and Crane 1997; Renzaglia et al. 2000; Shaw and Renzaglia 2004, 2011; Bowman 2011), and it is plausible that fitness increases can be attributed to the occurrence of stomata with functionality as in extant seed plant stomata on thalloid organisms without an endohydric conducting system (Raven 1984, 1993a, b, 1996, 2002a, b; see also Edwards et al. 1997; Woodward 1998; Berry et al. 2010; Beerling and Franks 2009; Hartung 2010; Khandelwal et al. 2010). The only extant analogues of this situation are in the gametophytes of marchantialean liverworts, where the anatomically complex pores show only a passive, reactive, response to thallus water status rather than the proactive, active response of extant seed plant stomata whose opening and closing responds to soil water availability, the desiccating properties of the atmosphere, and whether photosynthesis is possible at the plastid level. Using stomatal anatomy to suggest function in fossil stomata in non-seed plants is complicated by the present uncertainly on the extent to which moss, lycophyte and fern stomata show a proactive rather than a passive response to their environment (Doi and Shimazaki 2008; Doi et al. 2008; Brodribb et al. 2010; Bowman 2011; Brodribb and MacAdam 2011; Chater et al. 2011; McAllister and Bergman 2011; Ruszala et al. 2011), and the extent to which stomatal density and index

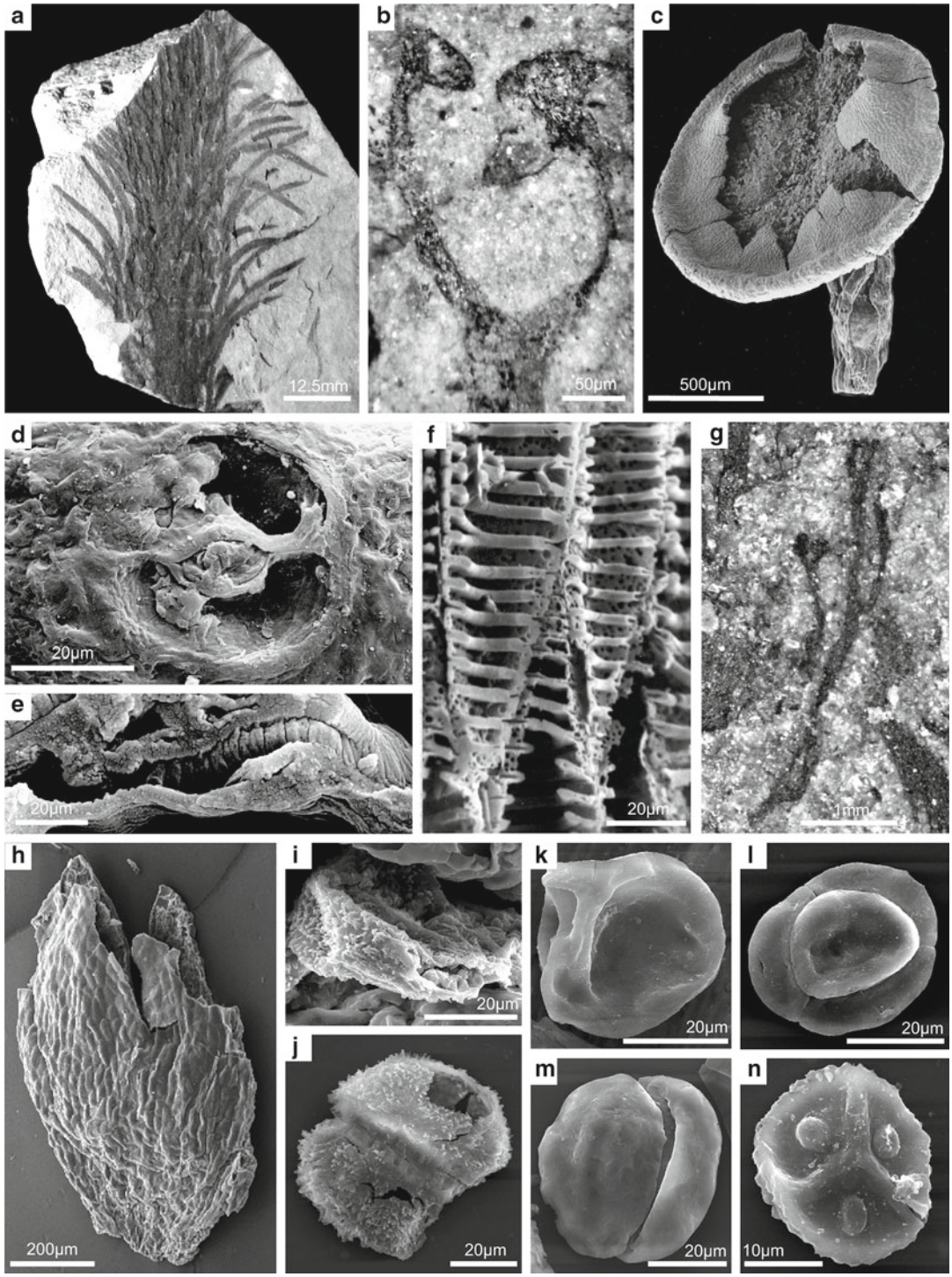


Fig. 3.2. **(a)** *Baragwanathia longifolia,* an herbaceous lycophyte, Wilson Creek Shale, Victoria, Australia, Pragian (Lower Devonian, c. 410 myr). (**b**) *Cooksonia* sp. its earliest record, Cloncannon Formation, Ireland, upper Wenlock (Silurian, c. 425 myr). (**c–e**) *Cooksonia pertoni*, SEM of charcoalified specimen, Ditton Group, Welsh Borderland, England, lower Lochkovian (Lower Devonian, c. 414 myr), (**d**) Surface view of

in non-seed plants is controlled by their environment (Baars and Edwards 2008; Ruszala et al. 2011).

Endohydry, but not true xylem, occurred in several now-extinct polysporangiophytes; xylem seems to have had a single origin in the ancestor of tracheophytes (Kenrick and Crane 1997; see Raven 2003). Much recent attention has been focussed on the evolution of xylem at the functional level (Fig. 3.2f; Edwards 2003; Sperry 2003; Brodribb et al. 2007; Pitterman 2010; Wilson and Fischer 2011) and the origin of lignin (Ligrone et al. 2008; Weng and Chapple 2010; Espiñaire et al. 2011; Popper et al. 2011), including the possible role of horizontal gene transfer (Emiliani et al. 2009) and the occurrence of lignin in red algae (Martone et al. 2009).

The Upper Silurian plants also had cuticles. While essential for homoiohydry, cuticles have been found in earlier deposits, although it is not clear what organisms they belonged to, whether cuticularization is polyphyletic, and what were the function(s) of early cuticles (Edwards et al. 1996; Budke et al. 2011). There is no evidence as to the occurrence of below-ground structures of the sporophytes. To summarize, the order of acquisition of structures related to homoiohydry suggest that the cuticle was the first, followed by stomata and intercellular gas spaces, and finally by xylem. Roots, on fossil evidence, first appeared in the Lower Devonian in zosterophyllophytes and lycophytes and the Middle Devonian in the euphyllophytes. The Upper Silurian lycophyte *Baragwanathia* has microphylls, but there is no evidence in the Upper Silurian of any of the polyphyletic planar lateral structures ('megaphylls') found in euphyllophytes from the Lower Devonian onwards.

While much attention has been focussed on the Upper Silurian embryophytes, it must be remembered that there were also plant-like thalloid organisms (Taylor et al. 2009; Strother 2010). An example is the estimate by Strother (2010) of the fraction of pixels in scans of the Downtonian (Upper Silurian) slabs used by Lang (1937) which showed thalloid rather than axial fossils; the thalloid organisms compose 0.941–0.999 of the area occupied by plant fossils (Strother 2010). *Prototaxites*, a bulky axial organism recently attributed to the basidiomycotes which appeared in the Wenlock (Silurian) and extended into the Upper Devonian, is dealt with below.

B. Lower Devonian

The Lower Devonian has a number of sites with excellent preservation, e.g. the Rhynie and Windyfield cherts, although it has been pointed out that the preservation depends on geological factors relating to hot springs which could in turn mean that the preserved plants have specialized physiologies and so might not be representative of most terrestrial vegetation (Channing and Edwards 2009). In addition to the three genera mentioned for the Late Silurian, a number of other genera of polysporangiophytes in these and other clades have been found. As well as tracheophytes, i.e., rhyniophytes, zosterophyllophytes, lycophytes and trimerophytes, there were also polysporangiophytes lacking tracheids but with some other form of endohydric conducting system, e.g. *Aglaophyton* (Fig. 3.1f–h), and a range of dispersed

Fig. 3.2. (continued) stoma and stem subtending sporangium in Fig. 3.2c, (**e**) fractured LS with tracheids from stem in Fig. 3.2c. (**f**) Longitudinally fractured tracheids of *Gosslingia breconensis*, a zosterophyll, Senni Beds, Brecon Beacons, Wales, Pragian (Lower Devonian, c. 410 myr). (**g**) Much branched coalified compression with terminal sporangia, St. Maughans Formation, Brecon Beacons, lower Lochkovian (Lower Devonian, c. 414 myr). (**h–i**) *Partitatheca splendida*, SEM of coalified sporangium with valvate dehiscence and stomata, Ditton Group , lower Lochkovian (Lower Devonian, c. 414 myr), (**i**) *Cymbohilates horridus* var. *splendida*, a permanent sculptured dyad from sporangium in Fig. 3.2h. (**j–n**) SEMs of dispersed spores, St. Maughans Formation, Brecon Beacons, lower Lochkovian (Lower Devonian, c. 414 myr). (**j**) *Cymbohilates horridus* var. A, a permanent sculptured dyad, (**k**) *Tetrahedraletes medinensis*, a permanent laevigate tetrad, (**l**) tetrad of laevigate trilete monads, ? *Ambitisporites*, (**m**) *Dyadospora murusdensa,* a separating laevigate dyad, (**n**) *Aneurospora* sp., a trilete monad.

embryophyte spores (Taylor et al. 2011). As well as sporophytes there are also the associated gametophytes for some of the organisms, identified by the possession of antheridia and archegonia (Fig. 3.1d). Although gametophytes defined in this way have only so far been found in the Rhynie Chert (Taylor et al. 2009), somewhat similar structures, a few with putative gametangia, have been found in other Lower Devonian deposits (Gerrienne and Gonez 2011). The sporophytes and the gametophytes both have homoiohydric characteristics. Other structures found in fossils from the Early Devonian are roots in zosterophyllophytes and lycophytes, and leaves (Raven and Edwards 2001; Taylor et al. 2009; Raven and Andrews 2010; Hao et al. 2010; see Grebe 2011; Ropello et al. 2011), as well as secondary xylem (Gerienne et al. 2011) in euphyllophytes. The very small size of the earliest known leaves in a Lower Devonian euphyllophyte has been related to problems with heat dissipation from larger leaves when there is little loss of energy as the latent heat of evaporation in the prevailing high atmospheric CO_2 concentrations (Beerling 2005). While all the Rhynie Chert mycorrhizal fungi are glomeromycotes (Fig. 3.1f), i.e. as are extant arbuscular mycorrhizas (Smith and Read 2008), molecular phylogenetic studies show that *Endogone*-like mucoromycotes form symbioses with several earliest-branching land plants (Bidartondo et al. 2011; see also Bidartondo and Duckett 2010). However, there is no fossil evidence for associations between embryophytes and mucoromycotes. There are some Lower Devonian fossils which have been attributed to embryophytes at the bryophyte grade of organization. *Sporogonites* resembles a thalloid liverwort or hornwort, with several sporophytes associated with a thalloid putative gametophyte (Taylor et al. 2009).

Finally for the Lower Devonian embryophytes are the small much branched plants (Fig. 3.2g) from South Wales investigated by Morris et al. (2011). These plants are probably not large enough to be capable of homoiohydry (Boyce 2008). Many of these plants produced the cryptospores (so named because the affinities of the producers were then unknown, when compared with the trilete spores produced by tracheophytes) (Fig. 3.2h–n) that provide evidence for land plants in the Ordovician and Silurian (Edwards et al. 2012).

While not dismissing the challenges that remain in understanding the functioning of the tracheophytes and, more generally, the other polysporangiophytes, it is also important to examine the functioning of Lower Devonian macroscopic organisms which are not necessarily embryophytes and which are believed to be photosynthetic. Examples are *Pachytheca, Parka* and *Spongiophyton* (Taylor et al. 2009), and tiny much branched plants of uncertain affinities (Morris et al. 2011).

Pachytheca (Fig. 3.3b) is found in Upper Silurian-Lower Devonian sediments as spheres 1–10 mm in diameter with a medulla of densely spaced intertwined tubes and a cortex of radial tubes with (possibly) a cuticle on the surface (Taylor et al. 2009). After early suggestions that *Pachytheca* was a green alga it has been variously suggested to be a stage in the life cycle of *Parka* or to be the propagules of *Prototaxites* (Taylor et al. 2009). Chemical analyses suggest affinities (aromatic hydrocarbons and alkylphenols) between *Pachytheca* and *Prototaxites* (Abbott et al. 1998). Such an association is consistent with the similar $\delta^{13}C$ values for *Pachytheca* (-27.5 ± 0.7 ‰) and for *Prototaxites* from the same locality (-28.0 ± 1.0 ‰) (Abbott et al. 1998). In view of the diversity of $\delta^{13}C$ values for *Prototaxites* (Boyce et al. 2007; Hobbie and Boyce 2010) it would be useful to have more paired $\delta^{13}C$ values of *Pachytheca* and *Prototaxites* for further testing the possible relationship. Paired analyses from potential underground root-like structures also would be helpful.

Parka decipiens (Fig. 3.3a) is common in the Old Red Sandstone (Upper Silurian-Lower Devonian) as disks some 70 mm in diameter, with pockets of spore-like structures (monads?) lacking any haptotypic feature but suggestive of a reproductive function (Hemsley 1989; Taylor et al. 2009).

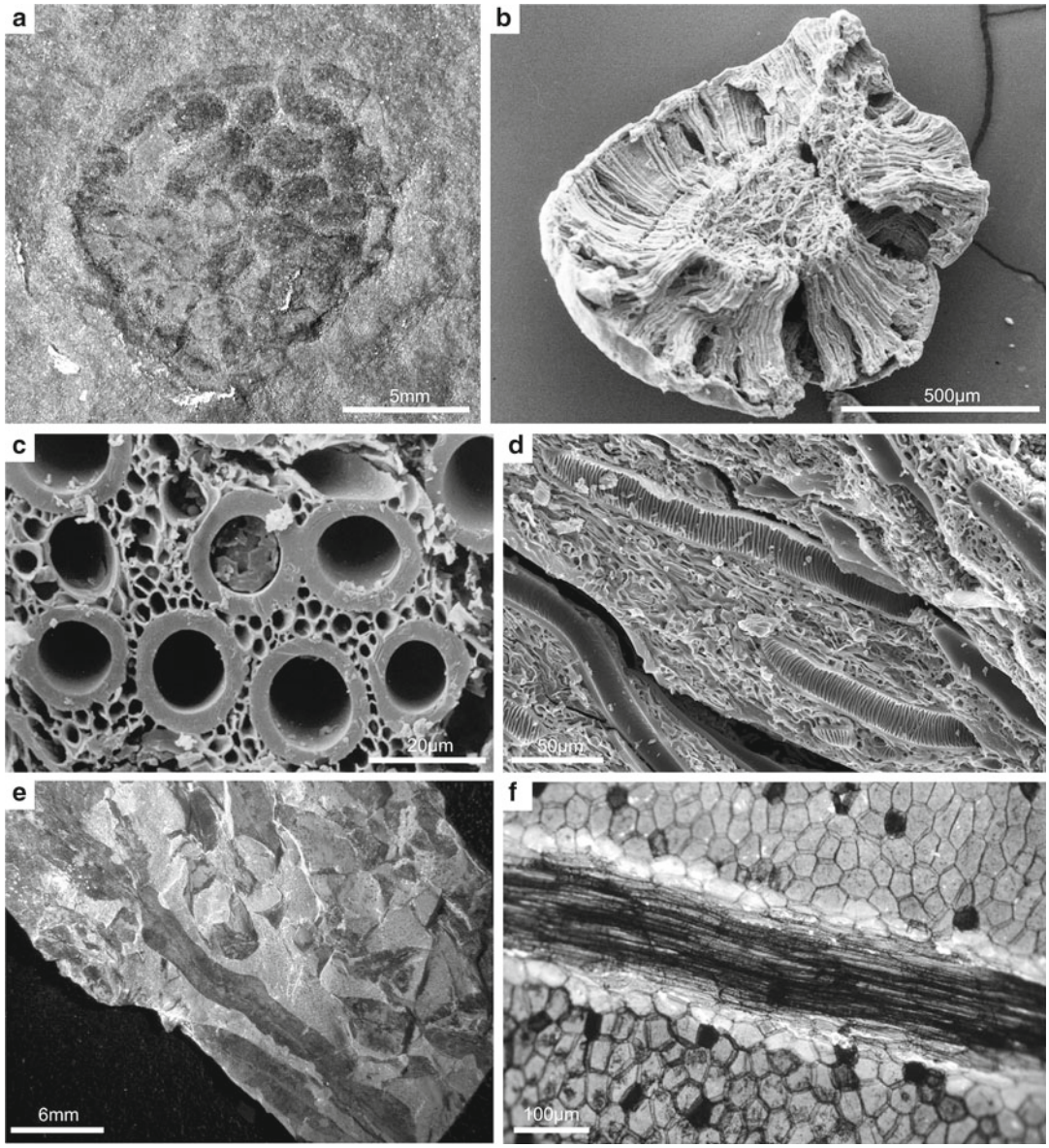


Fig. 3.3. (**a**) *Parka decipiens*, compression fossil of uncertain affinity. Dundee Formation, Myreton, Scotland, Lochkovian (Lower Devonian, c. 415 myr). (**b**) *Pachytheca* sp., SEM of fractured sphere showing cortex and medulla. Ditton Group, Welsh Borderland, England, lower Lochkovian (Lower Devonian, c. 414 myr). (**c**) *Prototaxites* sp., SEM of transverse fracture, Ditton Group, Welsh Borderland, England, lower Lochkovian (Lower Devonian, c. 414 myr). (**d**) *Nematasketum* sp., SEM of longitudinal fractured specimen, St. Maughans Formation, Brecon Beacons, lower Lochkovian (Lower Devonian, c. 414 myr). (**e**) *Metzgeriothallus sharonae,* coalified jungermanniopsid liverwort, Plattekill Formation, Cairo, N.Y. State, Givetian (upper Middle Devonian, c. 388 myr), (**f**) isolated thallus of liverwort in Fig. 3.3e, showing cells of central costa and thallus wings. (**e–f** courtsey of Linda Hernick, NY State).

The simplicity of their vegetative structure allows comparisons with the charophycean alga *Coleochaete* and the protonemal stage of the gametophyte of the moss *Sphagnum*, though without definitive assignment to these or any other higher taxa. The purportedly

reproductive structures of *Parka* have no obvious extant analogues. These organisms could have lived in wetlands, in which case they would presumably function like the organisms described by Tomescu and Rothwell (2006) and Tomescu et al. (2009). There seem to be no carbon isotope measurements on *Parka*. The organic macromolecules of *Parka* are distinct from those of *Pachytheca* and *Prototaxites* (Abbott et al. 1998).

The dichotomizing terrestrial *Spongiophyton* has been considered as an intermediate between algae and tracheophytes: it has tubular dichotomizing morphology, a thick cuticle and a scattered surface pores; specimens are some 25 by 25 mm (Gensel et al. 1991; Jarhren et al. 2003; Fletcher et al. 2004; Taylor et al. 2009). *Spongiophyton* has also been the subject of carbon isotope studies (Javaneau et al. 2003; Fletcher et al. 2004). Javaneau et al. (2003) claimed that the $\delta^{13}C$ values of *Spongiophyton* showed that their photosynthetic metabolism was similar to that of extant lichens. However, a more detailed analysis of the $\delta^{13}C$ values of *Spongiophyton* and comparison with extant hornworts, lichens, liverworts and mosses show that the *Spongiophyton* $\delta^{13}C$ values are statistically indistinguishable from the values from the four extant groups, although the range for *Spongiophyton* spp. is less than that for each of the four extant groups. It can be said that *Spongiophyton* does not exhibit the most positive $\delta^{13}C$ values seen for those lichens and hornworts with CCMs (Smith and Griffiths 1996a, b, 1998; Hanson et al. 2002), which might be expected granted the high atmospheric CO_2 levels in the Early Devonian. *Spongiophyton* spp. also do not show the most negative $\delta^{13}C$ values for extant lichens, liverworts and mosses. The most negative $\delta^{13}C$ values for extant organisms are a function of C_3 physiology with a high CO_2 conductance relative to biochemical conductance values related to the structure of the organism and also to the absence of an external water film decreasing CO_2 conductance. A further possible contributory factor is the enrichment of the local atmosphere in ^{13}C-depleted CO_2 from soil respiration of organic carbon produced by taller plants, which is unlikely to be a major factor in the Early Devonian. The high CO_2 level in the Early Devonian atmosphere would, for a given ratio of CO_2 diffusive conductance to biochemical conductance in organisms lacking CO_2 concentrating mechanisms (CCMs), give lower $\delta^{13}C$ values for organic carbon than with the present CO_2 level.

C. Middle Devonian

The Middle Devonian shows further elaboration of vascular plants (euphyllophytes with roots, more widespread secondary thickening with forests of cladoxylopsids (Stein et al. 2012), leaves, heterospory), and the loss of non-vascular polysporangiophytes such as *Aglaophyton* as well as several tracheophytes (Stein et al. 2007; Taylor et al. 2009; Raven and Andrews 2010; Gerrienne et al. 2011). The Middle Devonian also has the first unequivocal fossils of the vegetative structure of a bryophyte (Hernick et al. 2008; cf. Chang and Graham 2011). The organism, *Metzgeriothallus sharonae*, is a liverwort of the order Metzgeriales in the Jungermannopsida. As Hernick et al. (2008) point out, the discovery of *Metzgeriothallus sharonae* (Fig. 3.3e, f) puts the separation of the Metzgeriales and Jungermanniales, and hence of the Jungermannopsida and the Marchantiopsida, at no later than the Middle Devonian (cf. Chang and Graham 2011).

D. Upper Devonian

In the Upper Devonian there were gymnosperms among the euphyllophyte vascular plants (Taylor et al. 2009; Raven and Edwards 2004). Among non-tracheophytes there was the liverwort *Pallaviciniites* (= *Hepaticites*) *devonicus* (Hueber 1961). There is also the enigmatic *Protosalvinia* (= *Foerstia*) (Taylor et al. 2009), a thalloid, apparently terrestrial photosynthetic organism of unknown affinities (Romankiw et al. 2007). The thalli have an apical notch and conceptacles containing spores; it is not known if these are meiospores. *Protosalvinia* has been suggested to

be an alga, a bryophyte or a fern, but no firm conclusions have been drawn. There seem to have been no carbon stable isotope natural abundance studies of the possible photosynthetic pathway of *Protosalvinia*, although there have been ^{13}C-NMR studies of the chemistry of the organism which were interpreted as showing vascular plant affinities (Romankiw et al. 2007).

E. Prototaxites

Prototaxites (Fig. 3.3c) is found in Middle Silurian to Upper Devonian strata; it is made up of tubes which occur in cylindrical structures, apparently above ground, up to a metre in diameter and several metres long (Taylor et al. 2009). Some large Lower Devonian below-ground structures may be attributable to *Prototaxites*, but these are preserved as casts (Hillier et al. 2008). Suggestions as to the nature of *Prototaxites* include a fungus, an alga, a lichen or, most recently, rolled-up liverwort mats (Hueber 2001; Boyce and Hotton 2010; Graham et al. 2010a, b; Taylor et al. 2010; Edwards and Axe 2012). Carbon isotope values for *Prototaxites* have been interpreted in several ways (Boyce et al. 2007; Graham et al. 2010a, b; Hobbie and Boyce 2010; Taylor et al. 2010). There is a large diversity of δ^{13}C values among specimens (Boyce et al. 2007), and the interpretation of Hobbie and Boyce (2010) that this organism was a fungus obtaining organic carbon from a wide range of terrestrial and amphibious primary producers and, after floods, aquatic primary producers and that of Graham et al. (2010a, b) involving mixotrophic nutrition of the liverworts contributing to *Prototaxites* specimens are equally plausible. Edwards and Axe (2012) suggest fungal affinities for *Nematasketum*, an organism similar to *Prototaxites* (Fig. 3.3d). An alternative explanation for the variation in carbon isotope ratio among *Prototaxites* spp. can be found in the very significant developmentally or environmentally determined variations in δ^{13}C within a species showing facultative expression of Crassulacean Acid Metabolism (−14 to −28‰) (Winter and Smith 1996). However, there is no precedent for Crassulacean Acid Metabolism in the Palaeozoic or in lichens (Winter and Smith 1996).

VI. Photosynthetic Capacities

A. Extant Organisms

The discussion above shows that there is large range of morphologies and anatomies among Silurian and Devonian embryophytes and other terrestrial organisms thought to have been photosynthetic. Raven (1995; see also Raven 1992, 1993a, b) summarized photosynthetic rates on a ground area basis for extant terrestrial plants with a range of morphologies and mechanisms of CO_2 assimilation (Table 3.3). The values from Raven (1995) are supplemented in Table 3.3 with data on the rates of photosynthesis of a hornwort with a CCM (Smith and Griffiths 1996a, b) and emersed intertidal fucoid brown macroalgae (Surif 1989; Surif and Raven 1989, 1990) as indicators of the photosynthetic capacity of additional thalloid organisms at close to their optimal degree of hydration for photosynthesis, i.e. sufficiently hydrated to allow the maximum biochemical capacity for photosynthesis but without sufficient surface water to significantly restrict diffusive supply of CO_2 from the atmosphere to the surface of photosynthetic structures. There is also recognition of the amplification of surface area by the occurrence of vertical photosynthetic lamellae on the leaves of polytrichaceous mosses (Marschall and Proctor 2004; Proctor 2005; Waite and Sack 2010), the role of CCMs in hornworts (Smith and Griffiths 1996a, b; Hanson et al. 2002; Griffiths et al. 2006; Meyer et al. 2008), and additional information on the role of intercellular gas spaces in marchantiaceous liverworts (Griffiths et al. 2006; Proctor 2010). A final addition is the area-based photosynthetic rates of moss populations for comparison with the predictions based on the photosynthetic rate per unit leaf area and the leaf area index (Rice et al. 2008; Waite and Sack 2010).

Table 3.3. Photosynthetic rates on a ground area basis for extant oxygenic photosynthetic organisms exposed to the present atmosphere under optimal hydration, light and temperature conditions.

Morphological and Anatomical Organization	Organism, and phases in the life cycle when appropriate	Presence of carbon concentrating mechanism	Net photosynthetic rate μmol CO_2 m^{-2} s^{-1} substrate area
Unicellular, filamentous or multicellular. No organised intercellular gas spaces communicating with the atmosphere in multicellular organisms	Mat of filamentous cyanobacteria	Yes	0.97
	Cryptoendolithic crust of unicellular green algae	Yes or no	1.1
	Epiphytic green unicellular algae (>1 layer)	Yes or no	3.8
	Epilithic supratidal green alga with a flat unistratose thallus (*Prasiola*)	Yes	3.3
	Epilithic intertidal brown algae in turfs or as several layers of thalli; when present, gas bladders do not communicate with the atmosphere.	Yes	3.2–6.6
	Thalloid liverwort lacking intercellular gas spaces communicating with the atmosphere (gametophyte)	No	0.8
	Hornwort (gametophyte)	No	0.8–1.2
	Hornwort with CCM (gametophyte)	Yes	0.7–1.4
Multicellular. No intercellular gas spaces communicating with the atmosphere; multilayer structures (Leaf area index >1)	Measurement of unistratose leaf of a moss	No	1.4
	Predictions for moss shoot with ten layers of unistratose leaves based on measured projected area-based rate for leaves (gametophyte)	No	≤14
	Prediction for moss shoot with ten layers of leaves with vertical photosynthetic lamellae (*Dawsonia, Polytrichum*) (gametophyte)	No	≥20
	Measurements on single leaves of mosses with vertical photosynthetic lamellae on leaves (*Dawsonia, Polytrichum*) (gametophyte)	No	2.3–8.7
	Measurements for population of *Sphagnum* spp. (terrestrial/amphibious)	No	2.1–7.0
	Measurements for populations of terrestrial and epiphytic mosses other than Polytrichaceae	No	0.6–6.0
Multicellular. Intercellular gas spaces communicating with the atmosphere; no multilayer structure	Thalloid liverwort with intercellular gas spaces (gametophyte)	No	3.6
	Crustose or foliose lichens with intercellular gas spaces	Yes or no	4.7
Multicellular. Intercellular gas spaces communicating with the atmosphere via stomata; multilayer structures (Leaf area index >1)	Fruticose lichens with intercellular gas spaces	Yes or no	≤15.1
	C_3 leaf with intercellular gas spaces (sporophyte)	No	34
	C_4 leaf with intercellular gas spaces (sporophytes)	Yes	51

Based on Raven et al. (1998), Raven (1992, 1995), with additional material from Surif (1989) and Surif and Raven (1989, 1990) for intertidal brown algae, Smith and Griffiths (1996a, b, 2000), Hanson et al. (2002), and Griffiths et al. (2006) for hornworts and crustose lichens, Griffiths et al. (2006) and Proctor (2010) for liverworts, Marschall and Proctor (2004), Proctor (2005) and Waite and Sack (2010) for polytrichaceous mosses, and Nobel (1977), Rice and Giles (1996), Williams and Flanagan (1996, 1998), Rice (2000), Pannewitz et al. (2005), Rice et al. (2008), Waite and Slack (2010, 2011) for data on a ranges of non-polytrichaceous mosses

There is only one rate of net photosynthesis on a ground area basis for organisms or associations without multiple layers and photosynthetic tissue separated by atmospheric gases, and also lacking both CCMs and developmentally produced intercellular gas spaces which can distribute external CO_2 to photosynthetic cells (i.e. are unventilated) (Table 3.3); the measured rate is less than 1 μmol CO_2 m^{-2} s^{-1}. All the rates for unventilated organisms with CCMs are higher (Table 3.3), with the exception of the hornwort with a CCM. However, rates of photosynthetic electron transport through photosystem II estimated from chlorophyll fluorescence (Hanson et al. 2002; Griffiths et al. 2006) shows substantially greater photosynthetic capacity in a hornwort with a CCM but with an unventilated thallus than in an unventilated hornwort lacking a CCM, with an intermediate photosynthetic capacity for a ventilated thalloid liverwort without a CCM.

Turning to organisms lacking CCMs and with two or more layers of unventilated photosynthetic tissue separated by atmospheric gases, the measured rates for mosses (other than the Polytrichaceae) are up to 6 μmol CO_2 m^{-2} substrate area s^{-1} (Table 3.3). This value is lower than that predicted from the photosynthetic rate of individual leaves and the occurrence of ten layers of leaves (Table 3.3), perhaps as a result of shading of lower leaves by upper leaves and/or of photoinhibition of the upper leaves. The photosynthetic rate per unit leaf projected area is higher for the leaves of polytrichaeous mosses with vertical photosynthetic lamellae on the adaxial leaf surface than for other mosses lacking such lamellae, leading to the prediction of higher photosynthetic rates by a canopy of polytrichaceous mosses than of other mosses with the same leaf area index.

The final category involving bryophytes is that of organisms lacking CCMs with only a single layer of thalloid photosynthetic tissue, but with ventilation of the thallus involving non-stomatal pores linking the gas spaces and the atmosphere. Table 3.3 shows that the marchantiaceous liverworts have higher rates of photosynthesis than do the thalli of unventilated liverworts; this conclusion is borne out by measurements of electron transport rate discussed above (Griffiths et al. 2006) as well as from estimates of a light saturation parameter (Marschall and Proctor 2004; Proctor 2010). Comparison of the marchantiaceous liverworts with ventilated crustose or foliose lichens is complicated by the variable presence of CCMs in lichens, but the rate is similar to that of the ventilated thalloid liverworts. The photosynthetic sporophytes of hornworts and most mosses are ventilated and have stomata but, since they are permanently dependent on the poikilohydric gametophyte to supply water and inorganic nutrients, they are not homoiohydric, and the quantitative importance of sporophyte photosynthesis is uncertain.

Organisms with ventilated photosynthetic tissues and a leaf (or thallus) area index in excess of 1 are the sporophytes of vascular plants, some of which (C_4 and Crassulacean Acid Metabolism (CAM) plants) have CCMs. There are also fruticose lichens, again some with photobionts expressing CCMs. Table 3.3 shows that the poikilohydric fruticose lichens have net photosynthetic rates on a substrate area basis higher than those measured for bryophytes, but less than that for C_3 and, especially, C_4 plants.

Water content is a very significant determinant of the photosynthetic rate for poikilohydric organisms on land. There is water content (water per unit dry matter) at which the maximum rate of photosynthesis using atmospheric CO_2 is achieved. As the water content increases above this optimum value the rates of photosynthesis are increasing, limited by diffusion of CO_2 from the air-water interface to the chloroplasts through an increasing thickness of water, while decreasing water contents below the optimum value leads to increasing restriction on photosynthesis by limitations within the intracellular photosynthetic machinery (e.g. Williams and Flanagan 1998). The effect of the water film in restricting CO_2 diffusion at supraoptimal water contents is possibly less significant for endohydric bryophytes with a

less wettable (cuticle with wax) surface (Raven 1977, 2002a, b).

In addition to the variations in the rates of photosynthesis on a substrate area basis among the extant organisms which can photosynthesize using atmospheric CO_2, there are also measurements and predictions of the extent to which the various organisms use resources in assimilating CO_2. Well characterized effects among homoiohydric plants are the small transpiratory water loss per unit CO_2 assimilated in CAM plants relative to that in C_3 plants, with C_4 plants intermediate, and the potential for a greater photosynthetic rate per unit nitrogen in the photosynthetic apparatus in C_4 and CAM plants than for C_3 plants. These increases in the photosynthesis per unit water lost, and in photosynthetic rate per unit nitrogen, in plants with CCMs are not limited to homoiohydric plants; the arguments also apply to poikilohydric plants (Surif and Raven 1989; Griffiths et al. 2006). A greater water use efficiency of CO_2 assimilation in poikilohydric photosynthetic organisms with CCMs would mean that the organism could assimilate more CO_2 in a hydration – desiccation cycle than would an otherwise similar organisms lacking a CCM, assuming that the organism with a CCM has the same sensitivity to desiccation as organisms lacking CCMs. Maberly and Madsen (1990) have shown that a quarter of the photosynthesis by the high intertidal fucoid brown alga *Fucus spiralis* occurs when the alga is immersed rather than submersed; the extent to which this depends on the occurrence of a CCM in this alga is not clear. This also applies to the variety of microscopic green algae from four classes which occur in desert crusts: some have CCMs, at least in so far as these can be expected to occur in organisms with pyrenoids (Table 3.1). All cyanobacteria, including those in desert crust, have CCMs.

B. Relevance to the Colonization of Land by Photosynthetic Organisms

In examining the implications for colonization of the land we must consider both the structure of the fossils of (putatively) photosynthetic organisms, and the environmental conditions. Of the environmental conditions the most significant is probably the higher CO_2 partial pressure in the Ordovician, Silurian and at least the Early Devonian. Such higher CO_2 concentrations means that the Form IB Rubiscos of C_3 embryophytes would be operating at closer to saturation with CO_2, granted similar conductance to CO_2 to those in extant organism of similar structure. This provides the potential for more rapid photosynthesis per unit photosynthetic tissue and per unit nitrogen in the photosynthetic tissue. A proviso here is that many extant vascular plants downregulate expression of Rubisco when grown in high CO_2, although overall the response is usually an increase in photosynthetic rate in the higher CO_2 level. In addition, in extant vascular plants in high CO_2 there is a decreased diffusive conductance to CO_2; this decrease could permit a rate of photosynthesis which was still greater than is found in the present atmosphere but with a significantly smaller water vapour loss per unit of CO_2 assimilated, and a correspondingly higher temperature of the photosynthetic structures relative to that of the atmosphere. The temperature difference argument has been used in the context of the small size of the earliest euphyllophyte leaves in the relatively high temperatures of the Early Devonian: larger leaves would have a higher leaf temperature. Waite and Sack (2010) found that bryophytes have a lower photosynthetic nitrogen use efficiency than neighbouring C_3 vascular plants, although this could well be related to some extent to some other attributes of bryophytes. One of these is the greater general shade adaptation in the bryophytes than in vascular plants, although the data in Waite and Sack (2010) refer to bryophytes and neighbouring (in similar environments) habitats. Acclimation to low irradiance in *Tortula ruralis* involves, as in many other photosynthetic organisms, an increase in nitrogen in dry matter (Hamerlynk et al. 2002). A further possibility is greater nitrogen content (possibly storage, in evolutionary

terms the accumulation against some future deterministic or stochastic event) in desiccation-tolerant organisms, remembering that a greater fraction of bryophytes than vascular plants exhibit desiccation tolerance (Proctor et al. 2007; Tymms and Ganff 1979; Wilson et al. 2001; Oliver et al. 2011). A final consideration is a smaller possibilities for nitrogen retranslocaton within bryophytes than within vascular plants (Raven 2003).

Turning to the photosynthetic significance of the range of morphologies in the early photosynthetic organisms on land, much attention has been paid to organisms whose structures most closely resemble those of extant plants. These are the sporophytes of fossil vascular plants such as lycophytes (including zosterophyllophytes) and rhyniophytes, and rhyniophytoid plants, whose mode of preservation allows quantitative estimates to be made of stomatal frequency, maximum stomatal conductance, and the conductance of the endohydric (xylem or xylem-like) conducting system (Raven 1977, 1984, 2003; Konrad et al. 2000; Roth-Nebelsick et al. 2000; Roth-Nebelsick 2001, 2005; Roth-Nevelsick and Konrad 2005; Wilson and Fischer 2011; Boyce 2010; Edwards 2004). Matching of the conductance of the endohydric conducting system and of the stomatal conductance (Raven 1977, 1984; Konrad et al. 2000; Roth-Nebelsick et al. 2000) has been suggested as at least a partial explanation of the low stomatal density and stomatal conductance in *Asteroxylon*: the low stomatal conductance restricts the likelihood of failure by cavitation or embolism of the low conductance xylem by restricting the potential for transpiration (Wilson and Fischer 2011). While maximum stomatal conductance can be computed for the earliest stomata-bearing plants, the effectiveness of stomata in decreasing the possibility of xylem failure is increased pre-emptive closure, i.e. the capacity to sense a large evaporative demand by the atmosphere and a limited water supply from the soil and to decrease stomatal opening. The occurrence of these pre-emptive responses was discovered in, and well characterized from, seed plants (predominantly angiosperms); however, their occurrence in non-seed embryophytes is still controversial (Brodribb et al. 2010; Bowman 2011; Brobribb and MacAdam 2011; Chater et al. 2011; Ruszala et al. 2011). Until this question is resolved we must hesitate in attributing the full seed plant range of environmental responses of stomata to extant and fossil sporophytes of non-seed plants, with the possibility that their homoiohydric mechanisms show less precise responses to the environment. One way of addressing the question of the outcome of stomatal activity in regulating water loss per unit dry matter increase is to examine the natural abundance of carbon stable isotopes (Farquhar et al. 1989). Extant C_3 ferns and C_3 flowering plants from the same epiphytic habit have closely similar carbon isotope ratios (Hietz et al. 1999), with similar results from more spatially wide-ranging surveys (Smith and Epstein 1971; Watkins et al. 2007a).

As we have already seen, not all the Silurian and Lower Devonian plants can be readily assigned to an extant ecophysiological category. Staying initially with the sporophyte phase of embryophytes, some of the Devonian polysporangiophytes (e.g. cooksonioids) have axial diameters which are too small (Boyce 2008) to function in homoiohydric gas exchange (Raven 1999a, b). This led Boyce (2008) to suggest that these structures, with their pronounced peripheral stereome, were matrotrophic on the gametophytes phase. Boyce (2008) followed suggestions of Edwards (1996) and Edwards et al. (1996, 1998), that the rare stomata on these plants were involved in increasing solute flow to the sporangium in the transpiration stream, and then in drying out the sporangium prior to spore dispersal with continued support from the stereome (cf. Duckett et al. 2009, 2010).

The embryophyte gametophytes from the Rhynie Chert have somewhat different vegetative morphologies from those of the corresponding sporophytes (Taylor et al. 2009). However, the gametophytes resemble the sporophytes in having the suite of homoiohydric characteristics, i.e. endohydric conducting

system, cuticle, intercellular gas spaces and stomata. These anatomical findings are consistent with both the sporophyte and the gametophyte phase, a situation without parallel in other known extinct, or extant, plants. Among other things, the occurrence of homoiohydry in both phases means that the initially matrotrophic sporophyte is nourished by a homoiohydric gametophyte, rather than a poikilohydric gametophyte as in all extant free-sporing embryophytes.

The more thalloid terrestrial or amphibious photosynthetic organisms were presumably poikilohydric. The optimum water content per unit dry matter for photosynthesis using atmospheric CO_2 would be expected to be lower, or the range of water contents at which photosynthesis was maximal would be larger, because of the decreased diffusive restriction on CO_2 supply to chloroplast for a given water film thickness with the higher atmospheric CO_2 concentration.

VII. Conclusion

In addition to the dominant vascular plants on land today there are ecologically and biogeochemically significant contributions to primary productivity on land from bryophytes, lichens and free-living cyanobacteria and algae. The limited primary productivity on land before fossil evidence of embryophyte spores (470 Ma ago) presumably involved free living, and possibly lichenized, cyanobacteria and green algae. The first fossils of the vegetative phase of bryophytes do not occur until the Middle Devonian (380 Ma ago), yet tracheophytes are known from 420 Ma ago, thalloid terrestrial or amphibious primary producers are known from even earlier, and molecular phylogenetic studies show that bryophytes preceded vascular plants. The absence of early fossils makes it difficult to comment on the photosynthetic capacities of early bryophytes, and we rely on extrapolations of photosynthetic rates of extant bryophytes of various life forms to indicate their possible contribution.

Acknowledgements

The authors are grateful to the editors for helpful comments. The University of Dundee is a registered Scottish charity, No SC015096

References

Abbott GD, Ewbank G, Edwards D, Wang G-Y (1998) Molecular characterization of some enigmatic Lower Devonian fossils. Geochim Cosmochim Acta 62:1407–1418

Alpert P (2005) The limits and frontiers of desiccation-tolerant life. Integr Comp Biol 45:685–695

Ayres E, van der Wal R, Sommerkorn M, Bardgett RD (2006) Direct uptake of soil nitrogen by mosses. Biol Lett 2:286–288

Baars C, Edwards D (2008) Effects of elevated atmospheric CO_2 on spore capsules of the moss *Leptobryum pyriforme*. J Bryol 30:26–40

Baars C, Jones TH, Edwards D (2008) Microcosm studies on the role of land plants in elevating carbon dioxide and chemical weathering. Glob Biogeochem Cycl 22(3):Art No. GB3019

Becker B, Marin B (2009) Streptophyta algae and the origin of embryophytes. Ann Bot (Oxford, UK) 103:999–1004

Beerling DJ (2005) Leaf evolution: gases, genes and geochemistry. Ann Bot (Oxford, UK) 96:3345–3352

Beerling DJ, Franks PJ (2009) Evolution of stomatal function in 'lower' land plants. New Phytol 183:921–925

Beerling DJ, Fox A, Stevenson DS, Valdes PJ (2011) Enhanced chemistry-climate feedbacks in past greenhouse worlds. Proc Natl Acad Sci USA 108:9770–9775. www.pnas.org/cgi/101073/pnas.1102409108

Bell G, Mooers AO (1997) Size and complexity among multicellular organisms. Biol J Linn Soc 60:345–363

Berry JA, Beerling DJ, Franks PJ (2010) Stomata: key players in the earth system, past and present. Curr Opin Plant Biol 13:233–240

Bidartondo MI, Read DJ, Trappe JM, Merckx V, Ligrone R, Duckett JG (2011) The dawn of symbiosis between plants and fungi. Biol Lett 7:574–577

Bidartondo MI, Duckett JG (2010) Conservative ecological and evolutionary patterns in liverwort-fungal symbioses. Proc R Soc Lond Ser B 277:485–492

Blank CE, Sánchez-Baracaldo P (2010) Timing of morphological and ecological innovations in the

cyanobacteria – a key to understanding the rise of atmospheric oxygen. Geobiology 8:1–23
Bomfleur B, Krings M, Kerp H (2010) Thalloid organisms and the fossil record. New perspectives from the Transantarctic Mountains. Plant Signal Behav 5:293–295
Bowman JL (2011) Stomata: active portals for flourishing on land. Curr Biol 21:R540–R541
Boyce CK (2008) How green was *Cooksonia*? The importance of size in understanding the early evolution of physiology in the vascular plant lineage. Paleobiology 34:179–194
Boyce CK (2010) The evolution of plant development in a palaeontological context. Curr Opin Plant Biol 13:102–107
Boyce CK, Hotton CL (2010) *Prototaxites* was not a taphonomic artefact. Am J Bot 97:1073
Boyce CK, Hotton CL, Fogel ML, Cody GD, Hazen RM, Knoll AH, Hueber FM (2007) Devonian landscape heterogeneity recorded by a giant fungus. Geology 35:399–401
Brodribb TJ, McAdam SAM (2011) Passive origins of stomatal control in vascular plants. Science 331:582–585. doi:10.1126/science.1197985
Brodribb TJ, Field TS, Jordan GJ (2007) Leaf maximum photosynthetic rate and venation are linked by hydraulics. Plant Physiol 144:1890–1898
Brodribb TJ, McAdam SAM, Jordan GJ, Field TS (2010) Evolution of stomatal responsiveness of water-use efficiency among land plants. New Phytol 183:839–847
Brown RC, Lemmon BE (2011) Spores before sporophytes: hypothesizing the origin of sporogenesis at the algal-plant transition. New Phytol 192:266–301. doi:10.1111/j.1469-8137.03709.x
Budke JM, Goffinet B, Jones CS (2011) A hundred-year-old question: is the moss calyptra covered by a cuticle? A case study of *Funaria hygrometrica*. Ann Bot (Oxford, UK) 107:1279–1286
Butterfield NJ (2000) *Bangiomorpha* n. gen. n. sp.: implications for the evolution of sex, unicellularity, and the Mesoproterozoic/Neoproterozic radiation of eukaryotes. Paleobiology 26:386–404
Butterfield NJ (2004) A vaucheriacean alga from the middle Neoproterozoic of Spitsbergen: implication for the evolution of Proterozoic eukaryotes and the Cambrian explosion. Paleobiology 30:231–252
Butterfield NJ (2007) Macroevolution and macroecology through deep time. Palaeontology 50:41–55
Butterfield NJ, Knoll AH, Swett K (1988) Exceptional preservation of fossils in an upper Proterozoic shale. Nature 334:424–427
Butterfield NJ, Knoll AH, Swett K (1990) A bangiophyte red alga from the Proterozoic of Arctic Canada. Science 250:104–107
Caisova L, Marin B, Sausen N, Pröschold T, Melkonian M (2011) Polyphyly of *Chaetophora* and *Stigeoclonium* within the Chaetophorales (Chlorophyceae), revealed by sequence comparison on nuclear-encoded SSU rRNA genes. J Phycol 47:164–177. doi:10.1111/j.1529-8817.2010.00949.x
Cardon ZG, Gray DW, Lewis LA (2008) The green algal underground: evolutionary secrets of desert cells. BioScience 58:114–122
Chang Y, Graham SW (2011) Inferring the higher-order phylogeny of mosses (Bryophyta) and relatives using a large, multigene plastid data set. Am J Bot 98:839–849
Channing A, Edwards D (2009) Yellowstone hot spring environments and the palaeo-ecophysiology of Rhynie Chert plants: towards a synthesis. Plant Ecol Divers 2:111–143
Chater C, Kamisugi Y, Movahedi M, Fleming A, Cuming AC, Gray JE, Beerling DJ (2011) Regulatory mechanism controlling stomatal behaviour conserved across 400 million years of land plant evolution. Curr Biol 21:1025–1029
Clarke JT, Warnock RCM, Donoghue PCJ (2011) Establishing a time-scale for plant evolution. New Phytol 192:266–301. doi:10.1111/j.1469-8137.2011.03794.x
Cockell CS, Kaltenegger L, Raven JA (2009) Cryptic photosynthesis – extrasolar planetary oxygen without a surface biological signature. Astrobiology 9:623–636. doi:10.1089/ast.2008.0273
Cole KH, Sheath RG (1990) Biology of the red algae. Cambridge University Press, Cambridge/New York
Csintalan Z, Takáks Z, Proctor MCF, Nagy Z, Tiuba Z (2000) Early morning photosynthesis in the moss *Tortula ruralis* following summer dew fall in the Hungarian dry sandy grassland. Plant Ecol 151:51–54
Csotonyi JT, Swiderski J, Stackebrandt E, Yurkov V (2010) A new extreme environment for aerobic phototrophs: biological soil crusts. In: Hallenbrock PC (ed) Recent advances in phototrophic prokaryotes. Advances in experimental medicine and biology, vol 657. Springer, Berlin, pp 3–14
Derenne S, Metzger P, van Berber PF, Galletier JP, Sinninghe Damsté JS, De Leeuw JW, Berkaloff C (1992) Similar morphological and chemical variations of *Gloecapsomorpha prisca* in Ordovician sediments and cultures of *Botryococcus braunii* as a response to changes in salinity. Org Geochem 19:299–313
Derry LA (2006) Fungi, weathering, and the emergence of animals. Science 311:1386–1387
Doi M, Shimazaki K (2008) The stomata of the fern *Adiantum capillus-veneris* do not respond to CO_2 in the dark and open by photosynthesis in guard cells. Plant Physiol 147:922–930

Doi M, Wada M, Shimazaki K (2008) The stomata of the fern *Adiantum capillus-veneris* lack stomatal responses to blue light. Plant Cell Physiol 47:748–755

Duckett JG, Pressel S, P'ng KMY, Renzaglia KS (2009) Exploding a myth: the capsule dehiscence mechanism and the function of pseudostomata in *Sphagnum*. New Phytol 183:1053–1063

Duckett JG, Pressel S, P'ng KMY, Renzaglia KS (2010) The *Sphagnum* air-gun mechanism resurrected? Not with a closer look. New Phytol 185:89–891

Edwards D (1996) New insights into early land ecosystems: a glimpse of a lilliputian world. Rev Palaeobot Palynol 90:159–174

Edwards D (2000) The role of Mid-Palaeozoic mesofossils in the detection of early bryophytes. Philos Trans R Soc B 355:733–754

Edwards D (2003) Xylem in early tracheophytes. Plant Cell Environ 26:57–72

Edwards D (2004) Embryophytic sporophytes in the Rhynie and Windyfield cherts. Trans R Soc Edinb Earth Sci 94:397–410

Edwards D, Axe L (2012) Evidence for a fungal affinity for *Nematasketum* Burgess and Edwards, a close ally of *Prototaxites* Dawson. Bot J Linn Soc 168:1–18

Edwards D, Abbott GD, Raven JA (1996) Cuticles in early land plants: a palaeoecophysiological evaluation. In: Kierstens G (ed) Plant cuticles. Bios Scientific Publishers, Oxford, pp 1–31

Edwards D, Kerp H, Hass H (1997) Stomata in early land plants: an anatomical and ecophysiological approach. J Exp Bot 49:255–278

Edwards D, Axe L, Duckett JG (2003) Diversity of conducting cells in early land plants and comparisons with extant bryophytes. Bot J Linn Soc 141:297–347

Edwards D, Richardson JB, Axe L, Davies KL (2012) A new group of Early Devonian plants with valvate sporangia containing sculptured permanent dyads. Bot J Linn Soc 168:229–257

Emiliani G, Fondi M, Fani R, Gribaldo S (2009) A horizontal gene transfer at the origin of phenylpropanoid metabolism: a key adaptation of plants to land. Biol Direct 4:7. doi:10.1186/1745-6150-4-7

Espiñeira JM, Uzal EO, Ros LVG, Carrión JS, Merino F, Barceló AR (2011) Distribution of lignin monomers and the evolution of lignification among lower plants. Plant Biol 13:59–68

Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. Annu Rev Plant Physiol Plant Mol Biol 40:503–537

Fletcher BJ, Beerling DJ, Chaloner WG (2004) Stable carbon isotopes and the metabolism of the terrestrial Devonian organism *Spongiophyton*. Geobiology 2:107–119

Fritsch FE (1945) Studies in the comparative morphology of the algae. IV. Algae and archegoniate plants. Ann Bot (Oxford, UK) New Series 9:1–30

Gadd GM, Raven JA (2010) Geomicrobiology of eukaryotic microorganisms. Geomicrobiol J 27:491–519

Gensel PG (2008) The earliest land plants. Annu Rev Ecol Evol Syst 39:439–477

Gensel PG, Chaloner WG, Forbes WH (1991) *Spongiophyton* from the late Lower Devonian of New Bruswick and Quebec, Canada. Palaeontology 34:149–168

Gerrienne P, Gomez P (2011) Early evolution of life cycles in embryophytes: a focus on the fossil evidence of gametophyte/sporophyte size and morphological complexity. J Syst Evol 49:1–16

Gerrienne P, Gensel PG, Strullu-Derienne C, Lardeux H, Steemans P, Prestianni C (2011) A simple type of wood in two early Devonian plants. Science 333:837

Graham LE, Wilcox L (2000) Algae. Prentice Hall, Upper Saddle River

Graham LE, Cook ME, Hanson DT, Pigg KB, Graham JM (2010a) Structural, physiological, and stable isotope evidence that the enigmatic Palaeozoic fossil *Prototaxites* formed from rolled liverwort mats. Am J Bot 97:1079–1086

Graham LE, Cook ME, Hanson DT, Pigg KB, Graham JM (2010b) Rolled liverwort mat can explain major *Prototaxites* features: response to commentaries. Am J Bot 97:1079–1086

Graur D, Martin W (2003) Reading the entrails of chickens: molecular timescales of evolution and the illusion of precision. Trends Genet 20:80–86

Gray DW, Lewis LA, Cardon ZG (2007) Photosynthetic recovery following desiccation of desert green algae (Chlorophyta) and their aquatic relatives. Plant Cell Environ 30:1240–1245

Grebe M (2011) Unveiling the Casparian strip. Nature 473:294–295

Griffiths H, Maxwell K, Richardson D, Robe W (2006) Turning the land green: inferring photosynthetic physiology and diffusive limitations in early bryophytes. In: Hemsley A, Poole I (eds) The evolution of plant physiology. Elsevier Academic Press, Kew, pp 33–6

Guillou L (2011) Characterization of the Parmales: much more than resolution of a taxonomic enigma. J Phycol 47:2–4

Gupta S, Agrawal SC (2004) Vegetative survival and reproduction under submerged and air-exposed conditions and vegetative survival as affected by salts, pesticides and metals in aerial green alga *Trentepohlia aura*. Folia Microbiol 49:37–40

Hamerlynck EP, Csintalen Z, Nagy Z, Tuba Zm Goodin D, Henebry GM (2002) Ecophysiological consequences of contrasting microenvironments on the desiccation tolerant moss *Tortula ruralis*. Oecologia 131:498–505

Hanson D, Andrews TJ, Badger MR (2002) Variability of the pyrenoid-based CO_2-concentrating mechanism in hornworts (Anthocerophyta). Funct Plant Biol 28:407–416

Hao S, Xue J, Guo D, Wang D (2010) Earliest rooting system and root:shoot ratio from a new *Zosterophyllum* plant. New Phytol 185:217–225

Hartung W (2010) The evidence of abscisic acid (ABA) and ABA function in lower plants, fungi and lichen. Funct Plant Biol 37:806–812

Heckman DS, Geiser DM, Eidell BR, Stauffer RL, Kardos NL, Hedges SB (2001) Molecular evidence for the early colonization of land by fungi and plants. Science 293:1129–1133

Hemsley AR (1989) The ultrastructure of the Devonian plant *Parka decipiens*. Anns Bot (Oxford, UK 64:359–367

Hernick LV, Landing E, Bartowski KE (2008) Earth's oldest liverworts – *Metzgeriothallus sharonae* sp. nov. from the Middle Devonian (Givetian) of eastern New York, USA. Rev Palaeobot Palynol 148:154–162

Hietz P, Wanek W, Popp M (1999) Stable isotope composition of carbon and nitrogen and nitrogen content in vascular epiphytes along an altitudinal transect. Plant Cell Environ 22:1435–1443

Hillier RD, Edwards D, Morrisey LP (2008) Sedimentological evidence for rooting structures in the Early Devonian Anglo-Welsh Border (UK), with speculation on their producers. Palaeogeogr Palaeoclimatol Palaeoecol 270:366–380

Hobbie EA, Boyce CK (2010) Carbon sources for the Paleozoic giant fungus *Prototaxites* inferred from modern analogues. Proc R Soc Lond Ser B 277:2149–2156

Hoffmann L (1989) Algae of terrestrial habitats. Bot Rev 55:77–105

Hueber FM (1961) *Hepaticites devonicus*, a new fossil liverwort from the Devonian of New York. Ann Mo Bot Gard 48:125–132

Hueber FM (2001) Rotted wood-alga-fungus: the history and life of *Prototaxites* Dawson. Rev Palaeobot Palynol 116:125–158

Ichiniomiya M, Yochikowa S, Kamiya M, Ohki K, Takaichi S, Kuwater A (2011) Isolation and characterization of Parmales (Heterokonta/Heterokontophyta/Stramenopiles) from the Oyashi region, Western North Pacific. J Phycol 47:144–151

Javaneau EJ, Knoll AH, Walter MR (2003) Recognizing and interpreting the fossils of early eukaryotes. Orig Life Evol Biosph 33:75–94

Jones MR, Leith ID, Fowler D, Raven JA, Sutton MA, Nemiz E, Cape JN, Sheppard LJ, Smith RI, Theobald MR (2007a) Concentration-dependent NH_3 deposition processes for mixed moorland semi-natural vegetation. Atmos Environ 41: 2049–2060

Jones MR, Leith ID, Fowler D, Raven JA, Sutton MA, Nemiz E, Cape JN, Sheppard LJ, Smith RI (2007b) Concentration-dependent NH_3 deposition processes for stomatal and non-stomatal moorland species. Atmos Environ 41:8980–8994

Karatygin IV, Snigirevskaya NS, Vikulin SV (2009) The most ancient terrestrial lichen *Winfrenatia reticulata*: a new find and new interpretation. Palaeont J 43:107–114

Karsten U, Lütz C, Holzinger A (2010) Ecophysiological performance of the aeroterrestrial green alga *Klebsormidium crenulatum* (Charophyceae, Streptophyta) isolated from an alpine soil crust with an emphasis on desiccation stress. J Phycol 56:1187–1197

Kennedy M, Droser M, Mayer LM, Pevear D, Mrofka D (2006) Late Precambrian oxygenation; inception of the clay mineral factory. Science 311:1446–1449

Kenrick P, Crane PR (1997) The origin and early evolution of plants on land. Nature 389:33–39

Khandelwal A, Cho SH, Marella H, Sakata Y, Perroud P-F, Pan A, Quatrano RS (2010) Role of ABA and ABI3 in desiccation tolerance. Science 327:546

Konrad W, Roth-Nebelsick A, Kerp H, Hass H (2000) Transpiration and assimilation of Early Devonian land plants with axially symmetric telomes – simulations at the tissue level. J Theor Biol 206:91–107

Kotyk ME, Basinger JF, Gensel PG, Freitas TF (2002) Morphologically complex plant macrofossils from the late Silurian of Arctic Canada. Am J Bot 89:1004–1013

Lang WH (1937) On the plant-remains from the Downtonian of England and Wales. Philos Trans R Soc B 227:245–291

Lenton TM, Watson AJ (2004) Biotic enhancement of weathering, atmospheric oxygen and carbon dioxide in the Neoproterozoic. Geophys Res Lett 31:L05202. doi:10.1029/2003GL018802

Lenton TM, Crouch MJC, Johnson MT, Pires N, Nolan (2012) Enhanced weathering by the first land plants caused Late Ordovician global change. Nat Geosci 5:86–89

Lewis LA (2007) Chlorophyta on land. Independent lineages of green eukaryotes from arid lands. N: algae and cyanobacteria in extreme environ-

ments. Cellular origin, life in extreme habitats and astrobiology, vol 11, Part 6. Springer, Berlin, pp 569–582

Lewis LA, Lewis PO (2005) Unearthing the molecular phylodiversity of desert green algae (Chlorophyta). Syst Biol 54:936–947

Lewis LA, McCourt RM (2004) Green algae and the origin of land plants. Am J Bot 91:1535–1556

Ligrone R, Caraf A, Duckett JG, Renzaglia KS, Ruel K (2008) Immunocytochemical detection of lignin-related epitopes in cell walls in bryophytes and the charalean alga *Nitella*. Plant Syst Evol 270:257–272. doi:10.1007/s00606-007-0617-z

Lüttge U, Büdel B (2009) Resurrection kinetics of photosynthesis in desiccation-tolerant terrestrial green algae (Chlorophyta) on tree bark. Plant Biol 12:437–444

Maberly SC, Madsen TV (1990) The contribution of air and water to the carbon balance of *Fucus spiralis* L. Mar Ecol Progr Ser 62:175–183

Marconi M, Giordano M, Raven JA (2011) Impact of taxonomy, geography and depth on $\delta^{13}C$ and $\delta^{15}N$ variation in a large collection of macroalgae. J Phycol 47:1025–1035. doi:10.1111/j.1529-8817.2011.01045.x

Marischal M, Proctor MCF (2004) Are bryophytes shade plants? Photosynthetic light response and proportions of chlorophyll a and total carotenoids. Ann Bot (Oxford, UK) 94:593–603

Martone PT, Estevez JM, Lu F, Ruel K, Denny MW, Somerville C, Ralph J (2009) Discovery of lignin in seaweed reveals convergent evolution of cell-wall architecture. Curr Biol 19:169–175

McAllister CA, Bergman DC (2011) Sequence and formation of basic helix-loop-helix proteins required for stomatal development in Arabidopsis are deeply conserved in land plants. Evol Dev 13:182–189

Meyer M, Seibt U, Griffiths H (2008) To concentrate or ventilate? Carbon acquisition, isotope discrimination and physiological ecology of early land plant life forms. Philos Trans R Soc B 363:2767–2778

Morris JL, Richardson JB, Edwards D (2011) Lower Devonian plant and spore assemblages from the Lower Old Red Sandstone strata of Tredomen Quarry, South Wales. Rev Palaeobot Palynol 185:183–208

Nobel PS (1977) Internal leaf area and cellular CO_2 resistance. Photosynthetic implications of variations with growth conditions and plant species. Physiol Plant 40:137–144

Oliver MJ, Velter J, Mishler BD (2005) Desiccation tolerance in bryophytes: a reflection of the primitive strategy for plant survival in dehydrating habitats? Integr Comp Biol 45:788–799

Oliver MJ, Guo L, Alexander DC, Ryals JA, Wore BWM, Cushman JC (2011) A sister group contract using untargeted global metabolomic analysis delineates the biochemical regulation underlying desiccation tolerance in *Sporobolus stapfianus*. Plant Cell 23:1231–1248

Org B-L, Lim M, Wee Y-C (1992) Effects of desiccation and illumination on photosynthesis and pigmentation of an edaphic population of *Trentepohlia odorata* (Chlorophyta). J Phycol 28:768–772

Paffrey LW, Lahr DJG, Knoll AH, Katz LA (2011) Estimating the timing of early eukaryotic diversification with multigene molecular clocks. Proc Natl Acad Sci USA 108:13624–13629

Pannewitz S, Green TAG, Maysek K, Schensog M, Seppelt R, Sancho LG, Türk R, Schoetter B (2005) Photosynthetic responses of three common mosses from continental Antarctica. Antarct Sci 17:341–352

Pittermann J (2010) The evolution of water transport in plants: an integrated approach. Geobiology 8:112–139

Popper ZA, Michel G, Hervé C, Domozych DS, Willats WGT, Tuohy MG, Kloareg B, Stengel D (2011) Evolution of plant cell walls: from algae to flowering plants. Annu Rev Plant Biol 62:567–590

Proctor MCF (2005) Why do Polytrichaceae have lamellae? J Bryol 27:221–229

Proctor MCF (2010) Trait correlation in bryophytes: exploring an alternative world. New Phytol 185:1–3

Proctor MCF, Tuba Z (2002) Poikilohydry and homoiohydry: antithesis or spectrum of possibilities? New Phytol 156:327–349

Proctor MCF, Raven JA, Rice SK (1992) Stable carbon isotope discrimination measurements in *Sphagnum* and other bryophytes: physiological and ecological implications. J Bryol 17:193–202

Proctor MCF, Oliver MJ, Wood AJ, Alpert P, Stark LR, Cleavitt NL, Mishler BD (2007) Dessication-tolerance in bryophytes: a review. Bryologist 110:595–621

Rasmussen B, Fletcher IR, Brocks JJ, Kilburn MR (2008) Reassessing the first appearance of eukaryotes and cyanobacteria. Nature 455:1101–1104

Raven JA (1977) The evolution of vascular land plants in relation to supracellular transport processes. Advances in Botanical Research 5:153–219, Academic Press, London

Raven JA (1984) Physiological correlates of the morphology of early vascular plants. Bot J Linn Soc 88:105–126

Raven JA (1993a) Energy and nutrient acquisition by autotrophic symbioses and their asymbiotic ancestors. Symbiosis 14:33–60

Raven JA (1993b) The evolution of vascular land plants in relation to quantitative functioning of dead water-conducting cells and stomata. Biol Rev Camb Philos Soc 68:337–363

Raven JA (1994a) Physiological aspects of the functioning of vascular tissue in early vascular plants. Bot J Scotl 47:49–64

Raven JA (1994b) The significance of the distance from photosynthesising cells to vascular tissue in extant and early vascular plants. Bot J Scotl 47:65–82

Raven JA (1995) The early evolution of land plants: aquatic ancestors and atmospheric interactions. Botan J Scotl 47:151–175

Raven JA (1996) Into the voids: the distribution, function, development and maintenance of gas spaces in plants. Ann Bot (Oxford, UK) 78:137–142

Raven JA (1997a) The role of marine biota in the evolution of terrestrial biota: gases and genes. Biogeochemistry 39:139–164

Raven JA (1997b) Multiple origins of plasmodesmata. Eur J Phycol 32:95–101

Raven JA (1999a) The minimum size of seeds and spores in relation to the ontogeny of homoiohydry. Funct Ecol 13:5–14

Raven JA (1999b) The size of cells and organisms in relation to the evolution of embryophytes. Plant Biol 1:2–12

Raven JA (2002a) Putting the fight in bryophytes. New Phytol 156:321–326

Raven JA (2002b) Tansley review: selection pressures on stomatal evolution. New Phytol 153:371–386

Raven JA (2003) Long-distance transport in non-vascular plants. Plant Cell Environ 26:73–85

Raven JA (2005) The evolution of plasmodesmata. In: Oparka K (ed) Plasmodesmata. Blackwell Publishers, Oxford, pp 33–52

Raven JA, Andrews M (2010) Evolution of tree nutrition. Tree Physiol (Oxford, UK) 30:1050–71

Raven JA, Edwards D (2001) Roots: evolutionary origins and biogeochemical significance. J Exp Bot 52:381–401

Raven JA, Edwards D (2004) Physiological evolution of lower embryophytes: adaptations to the terrestrial environment. In: Hemsley AR, Poole I (eds) The evolution of plant physiology: from whole plants to ecosystems, Linnean Society symposium series 21. Elsevier Academic Press, London, pp 17–41

Raven JA, Griffiths H, Smith EC, Vaughn KC (1998) New perspectives in the biophysics and physiology of bryophytes. In: Bates JW, Ashton NW, Duckett JG (eds) Bryology for the twenty-first century. Proceedings of the centenary symposium of the British Bryological Society. Maney Publishing and The British Bryological Society, Leeds, pp 261–275

Raven JA, Johnston AM, Kübler JE, Korb RE, McInroy SG, Handley LL, Scrimgeour CM, Walker DI, Beardall J, Vanderklift M, Fredricksen J, Dunton KH (2002a) Mechanistic interpretation of carbon isotope discrimination by marine macroalgae and seagrasses. Funct Plant Biol 29:355–378

Raven JA, Johnston AM, Kübler JE, Korb RE, McInroy SG, Handley LL, Scrimgeour CM, Walker DI, Beardall J, Clayton MN, Vanderklift M, Fredriksen S, Dunton KH (2002b) Seaweeds in cold seas: evolution and carbon acquisition. Ann Bot (Oxford, UK) 90:525–536

Renzaglia KS, Duff RJ, Nickrent DI, Garbury DI (2000) Vegetative and reproductive innovations for a unified phylogeny. Philos Trans R Soc B 355:769–793

Rice SK (2000) Variation in carbon isotope discrimination within and among *Sphagnum* species in a temperate wetland. Oecologia 123:1–8

Rice SK, Giles L (1996) The influence of water content and leaf anatomy on carbon isotope discrimination and photosynthesis in *Sphagnum*. Plant Cell Environ 19:118–124

Rice SK, Aclander L, Hansen DT (2008) Do bryophyte shoot systems function like vascular plant leaves or canopies? Functional trait relationship in *Sphagnum* mosses (Sphagnaceae). Am J Bot 95:1366–1374

Rindi F, Lam DW, López-Bautista JM (2009a) Phylogenetic relationships and species circumscription in *Trentepohlia* and *Printzina* (Trentepohliales, Chlorophyta). Mol Phylogenet Evol 52:329–339

Rindi F, Allali HA, Lam DW, López-Bautista JM (2009b) An overview of the biodiversity and biogeography of terrestrial green algae. In: Rescigno V et al (eds) Biodiversity hotspots. Nova Science Publishers, New York, pp 1–25

Romankiw LA, Hatcher PG, Roen JB (2007) Evidence of land plant affinities for the Devonian fossil *Protosalvinia* (*Foerstia*). Lethaia 21:417–423

Ropello D, De Rybel B, Tendon VD, Püster A, Alassimone J, Vermeer JEM, Yamazaki M, Stierhof Y-D, Beeckman T, Geldner M (2011) A novel protein family mediates Casparian strip formation in the endodermis. Nature 473:380–383

Roth-Nebelsick A (2001) Heat transfer of rhyniophytic plant axes. Rev Palaeobot Palynol 116:112–139

Roth-Nebelsick A (2005) Reconstructing atmospheric carbon dioxide with stomata: possibilities and limitations of a botanical CO_2 sensor. Trees Struct Funct 19:251–265

Roth-Nebelsick A, Konrad W (2005) Assimilation and transpiration capabilities of rhyniophytic plants from the Lower Devonian and their implications for

palaeoatmospheric CO_2 concentrations. Palaeogeogr Palaeoclimatol Palaeoecol 202:153–178

Roth-Nebelsick A, Grimm G, Mosbrugger V, Hass H, Kerp H (2000) Morphometric analysis of *Rhynia* and *Asteroxylon*: testing functional aspects of early land plant evolution. Palaeobiology 26:406–418

Rubinstein CV, Gerrienne P, de la Puente GS, Astini RA, Steemans P (2010) Early middle Ordovician evidence for land plants in Argentina (Eastern Antarctica). New Phytol 188:365–369

Rugg DA, Norton TA (1987) *Pelvetia canaliculata*, a high-shore seaweed that shuns the sea. In: Crawford RMM (ed) Plant life in aquatic and amphibious habitats. Blackwell, Oxford, pp 347–358

Ruszala EM, Beerling DJ, Franks PJ, Chater C, Casson SA, Gray JE, Hetherington AM (2011) Land plants acquired active stomatal control early in their evolutionary history. Curr Biol 21:1030–1035

Sánchez-Baracaldo P, Hayes PK, Blank CE (2005) Morphological and habitat evolution in the Cyanobacteria using a compartmentalization approach. Geobiology 3:145–165

Sanders WS (2004) The intertidal marine lichen formed by the pyrenomycete fungus *Verrucaria tavaresiae* (Ascomytina) and the brown alga *Petroderma maculiforme* (Phaeophyceae) thallus organisation and symbiont interaction. Am J Bot 91:511–522

Sarkar P, Bosneaga E, Auer M (2009) Plant cell walls throughout evolution: towards a molecular understanding of their design principles. J Exp Bot 60:3615–3635

Senousy HH, Beakes GW, Hack E (2004) Phylogenetic placement of *Botryococcus braunii* (Trebouxiophyceae) and *Botryococcus sudatis* isolate UTEX 2629 (Chlorophyceae). J Phycol 40:412–423

Shaw AJ, Szövény P, Shaw B (2011) Bryophyte diversity and evolution: windows into the early evolution of land plants. Am J Bot 98:352–369

Shields GA (2007) A normalised seawater strontium isotope curve: possible implications for Neoproterozoic-Cambrian weathering rates and the further oxygenation of the Earth. eEarth 3:35–42

Smith BN, Epstein E (1971) Two categories of $^{13}C/^{12}C$ ratios for high plants. Plant Physiol 47:380–384

Smith EC, Griffiths H (1996a) The occurrence of the chloroplast pyrenoid is correlated with the activity of a CO_2-concentrating mechanism and carbon isotope discrimination in lichens and bryophytes. Planta 198:6–16

Smith EC, Griffiths H (1996b) A pyrenoid-based carbon concentrating mechanism is present in terrestrial bryophytes of the class Anthocerotae. Planta 200:203–212

Smith EC, Griffiths H (2000) The role of carbonic anhydrase in photosynthesis and the activity of the carbon concentrating mechanisms in bryophytes of the class Anthocerotae. New Phytol 145:29–37

Smith SE, Read DJ (2008) Mycorrhizal symbiosis, 3rd edn. Elsevier Academic Press, Amsterdam

Souffreau C, Vanormelingen P, Verleyen E, Sabbe K, Wyverman W (2010) Tolerance of benthic diatoms from temperate aquatic and terrestrial habitats to experimental desiccation and temperature stress. Phycologia 49:309–324

Souffreau C, Vanormelingen P, Sabbe K, Vyverman W (2013) Tolerance of resting cells of freshwater and terrestrial benthic diatoms to experimental -desiccation and freezing is habitat-dependent. Phycologia 52:246–255

Sperry JS (2003) Evolution of water transport and xylem structure. Int J Plant Sci 164:S115–S127

Stein WE, Mannolini F, VanAller Hernick L, Landing E, Berry CM (2007) Giant cladoxylopsid trees resolve the enigma of the earth's earliest tree stumps at Gilboa. Nature 446:904–907

Stein WE, Berry CM, VanAller Hernick L, Mannolini F (2012) Surprisingly complex community discovered in mid-Devonian fossils forest at Gilboa. Nature 483:78–81

Strother PK (2010) Thalloid carbonaceous incrustations and the asynchronous evolution of embryophyte characters during the Paleozoic. Int J Coal Geol 83:154–161

Strother PK, Battison L, Brasier MD, Wellman CH (2011) Earth's earliest non-marine eukaryotes. Nature 473:505–509. doi:10.1038/nature09943

Surif MB (1989) Physiology of photosynthesis in relation to the taxonomy of the Phaeophyta. PhD Thesis, University of Dundee

Surif MB, Raven JA (1989) Exogenous inorganic carbon sources for photosynthesis in seawater by members of the Fucales and the Laminariales (Phaeophyta): ecological and taxonomic aspects. Oecologia 78:97–105

Surif MB, Raven JA (1990) Photosynthetic gas exchange under emersed conditions in eulittoral and normally submersed members of the Fucales and Laminariales: interpretation in relation to C isotope ratio and N and water use efficiency. Oecologia 82:68–80

Taylor TN, Hass H, Remy W, Kerp H (1995) The oldest fossil lichen. Nature 378:244

Taylor TN, Taylor EL, Krings M (2009) Paleobotany: the biology and evolution of fossil plants, 2nd edn. Academic, Amsterdam

Taylor TN, Taylor EL, Decorbeix A-L, Schwenderer A, Serbet R, Escapa I, Krings M (2010) The enigmatic Devonian fossil *Prototaxites* is not a rolled-up liverwort mat. (comment on paper by Graham et al.). Am J Bot 97:268–275

Taylor WA, Gensel PG, Wellman CH (2011) Wall ultrastructure in three species of dispersed spore *Emphanisporites* from the Early Devonian. Rev Palaeobot Palynol 163:264–280

Teyssèdre B (2006) Are the green algae (Phylum Viridiplantae) two billion years old? Carnets de Geologie 2006(3):1–15

Tomescu AMF, Rothwell GW (2006) Wetlands before tracheophytes: thalloid terrestrial communities of the Early Silurian Passage Creek biota (Virginia). Geol Soc Am Spec Pap 399:41–56

Tomescu AMF, Rothwell GW, Honegger R (2006) Cyanobacterial macrophytes in an Early Silurian (Llandovery) continental biota: Passage Creek, lower Massanuten Sandstone, Virginia, USA. Lethaia 39:329–338

Tomescu AMF, Pratt LM, Rothwell GW, Strother PK, Nadon GC (2009) Carbon isotopes support the presence of extensive land floras pre-dating the origin of vascular plants. Palaeogeogr Palaeoclimatol Palaeoecol 283:45–59

Tomitani A, Knoll AH, Cavanaugh CM, Ohno T (2006) The evolutionary diversification of cyanobacteria: molecular phylogenetic and palaeontological perspectives. Proc Natl Acad Sci USA 103:5442–5447

Tschernak E (1941) Untersuchungen über die Beziehungen von Pilze und Alge in Flechtenthallus. Ost Bot Z 90:233–307

Turmel M, Brouard J-S, Gagron C, Otis C, Lemieux C (2008) Deep division in the Chlorophyceae (Chlorophyta) revealed by chloroplast phylogeny analyses. J Phycol 44:739–750

Turnbull JD, Robinson SA (2009) Desiccation protects two Antarctic mosses from ultraviolet-B induced DNA damage. Funct Plant Biol 36:214–221

Tymms MJ, Ganff DF (1979) Proline accumulation during water stress in resurrection plants. J Exp Bot 30:165–168

Van den Hoek C, Mann BG, Jahns HM (1995) Algae. An introduction to phylogeny. Cambridge University Press, Cambridge

Waite M, Sack L (2010) How does moss photosynthesis relate to leaf and canopy structure? Trait relationships for 10 Hawaiian species of contrasting light habitats. New Phytol 185:156–172

Waite M, Sack L (2011) Does global stoichiometric theory apply to bryophytes? Tests across an elevation x soil age ecosystem matrix on Mauna Loa, Hawaii. J Ecol 99:122–134

Wang XQ, Yang PF, Liu Z, Liu WZ, Hu Y, Chen H, Kuang TY, Reis ZM, Shen SH, He YK (2009) Exploring the mechanism of *Physcomitrella patens* desiccation tolerance through a proteomic strategy. Plant Physiol 149:1739–1750

Watkins JE, Rundell PW, Cardelús CL (2007a) The influence of life forms on carbon and nitrogen relationship in tropical rainforest ferns. Oecologia 153:225–232

Watkins JE, Mack MC, Sinclair TR, Mulkey SS (2007b) Ecological and evolutionary consequences of desiccation tolerance in tropical fern gametophytes. New Phytol 176:708–717

Wellman CH (2010) The invasion of the land by plants: when and where? New Phytol 188:306–309

Wellman CH, Osterloff PL, Mohluddin U (2003) Fragments of the earliest land plants. Nature 425:282–285

Weng J-K, Chapple C (2010) The origin and evolution of lignin biosynthesis. New Phytol 187: 273–295

Williams TG, Flanagan LB (1996) Effect of changes in water content on photosynthesis, transpiration and discrimination against (CO_2)-C-13 and (COO)-18O-16O in *Sphagnum* and *Pleurozium*. Oecologia 108: 38–46

Williams TG, Flanagan IB (1998) Measuring and modelling environmental influences on photosynthetic gas exchange in *Sphagnum* and *Pleurozium*. Plant Cell Environ 21:555–564

Wilson JP, Fischer WW (2011) Hydraulics of *Asteroxylon mackei*, an early Devonian vascular plant, and the early evolution of water transport in vascular plants. Geobiology 9:121–130

Wilson D, Stock WD, Hedderson F (2001) Historical nitrogen content of bryophyte tissue as an indicator of increased nitrogen deposition in Cape Metrapolitan Area, South Africa. Environ Pollut 157:938–945

Winter K, Smith JAC (eds) (1996) Crassulacean acid metabolism. Biochemistry, Ecophysiology and Evolution. Springer, Berlin, pp xxxii–449

Woodward FI (1998) Do plants really need stomata? J Exp Bot 49:471–480

Wu MY, Zhao YL, Tong JN, Yang RD (2011) New macroalgal fossils of the Kaili Biota in Guizhou Province, China. Sci China Earth Sci 54:93–100

Xu ZL (2001) New discoveries of phaeophycean fossils in the Early Cambrian, Haikou, Kunming, Yunnan, Southwest China. Acta Botanica Sinica 43: 1072–1076

Yang R-D, Mao J-R, Zhang W-H, Jiang L-J, Gao H (2004) Bryophyte-like fossil (*Parafunaria sinensis*) from Early-Middle Cambrian Kaili Formation of Guizhou Province, China. J Integr Plant Biol 46:180–185

Yuan X, Xiao S, Taylor TN (2005) Lichen-like symbiosis 600 million years ago. Science 308:1017–1020

Yuan X, Chen Z, Xiao S, Zhou C, Hua H (2011) An early Ediacaran assemblage of morphologically differentiated eukaryotes. Nature 470:390–393

Zhang Z, Metzger P, Sachs JP (2007) Biomarker evidence for the co-occurrence of three races (A, B and C) of Botryococcus braunii in El Junco Lake, Galápagos. Organic Geochem 38:1459–1478

Chapter 4

The Diversification of Bryophytes and Vascular Plants in Evolving Terrestrial Environments

Michael C.F. Proctor*
Biosciences, College of Life and Environmental Sciences, University of Exeter, Geoffrey Pope Building, Stocker Road, Exeter EX4 4QD, UK

Summary

This chapter covers the basic needs of plants and the constraints of the physical environment on a pioneer land flora, including acquisition of CO_2, nutrients, and coping with the intermittent availability of water. The radiation climate, heat and mass transfer, laminar and turbulent boundary layers, heat budgets, and the control of evaporation and temperature are briefly discussed. The importance of scale is emphasized; the vascular-plant strategy is optimal at

*Author for correspondence, e-mail: M.C.F.Proctor@exeter.ac.uk

D.T. Hanson and S.K. Rice (eds.), Photosynthesis in Bryophytes and Early Land Plants, Advances in Photosynthesis and Respiration 37, pp. 59–77, DOI 10.1007/978-94-007-6988-5_4,

large scales (> a few cm), but the poikilohydric strategy is optimal at smaller scales. A scenario is envisaged for evolution of the "vascular-plant package", allowing a transition from reliance on evaporative cooling close to the ground surface, to convective cooling of erect axes. The changing physical environment and vegetation through successive periods of geological time is briefly sketched in relation to the evolution of bryophyte diversity. Vascular plants have been an important part of the environment for bryophyte evolution since the early history of plant life on land.

I. Introduction

All photosynthetic organisms need water, light, CO_2, and other chemical elements that are essential for their structure and functioning – N, P, K, Mg, Ca, Fe and others ("micronutrients") in smaller quantities. These all have to be acquired from the environment. The main challenge to an aquatic photosynthetic organism is remaining within the photic zone; other requirements are met from the surrounding water. On land, water is only locally and intermittently available. Above ground there is generally light, and atmospheric CO_2, but other nutrients must come either from rain (or other airborne sources), or from the substratum. Resources from two different media have to be brought together. Furthermore, there are many problems associated with differences in interactions at the plant surface in water and in air.

That applies to all plants, however primitive or highly evolved, and we assume it has been true throughout geological time. Plants at every step in evolution change the habitat for organisms around them. Environments evolve hand-in-hand with the organisms that inhabit them; for any one species, all the other species in the same habitat are part of its environment. The complexity of modern forests with their multitude of ecological niches did not arise ready made; it evolved. "The past is a foreign country; they do things differently there" may apply to human behavior, but it is not a basis on which useful scientific work can be done. The basic principles of physics, and physiological and ecological knowledge from present-day plants and ecosystems, should inform our view of the geological past.

II. Beginnings: The Transition from Water to Land

We can only conjecture when, and in what habitats, the first land-plants evolved. There must always have been an interface between water and land, both in the oceans and in rivers and lakes, and plants occupying that interface. The fossil evidence is sparse, and tantalizing (Chaps. 2 and 3). Of the diverse groups of photosynthesizing organisms in water, Chlorophyta (in the broad sense) are overwhelmingly predominant on land, with Cyanobacteria as a widely-pervasive poor second. The heterokont groups that are dominant in the sea, and prominent in freshwater aquatic habitats, probably evolved too late, and the land habitat was preempted by highly-evolved, green, land vegetation (Palmer et al. 2004).

This points to fresh (or brackish) water habitats, most probably wet mud on river banks or pool margins, as the likeliest origin of land vegetation as we know it. One of the first selection pressures at the land–water interface must have been for desiccation tolerance – manifested widely in different bacterial and algal groups, at least in desiccation-tolerant spores or resting stages. We can envisage the earliest land-plants forming a crust over the land surface, after the manner of "biological soil crusts" in present-day desert, polar and high-mountain environments, and as pioneer communities in all climates (Belnap and Lange 2001). As with present-day bryophytes and lichens, physical considerations would have limited their size to a few centimeters at the most – the *Racomitrium* mats of polar regions, and

the dense lichen growth in the coastal mist zone of the Namibian desert come to mind – so it would inevitably have been a Liliputian world (Edwards 1996).

III. Exchanges of Matter and Energy at the Earth's Surface

A. The Climate Near the Ground: Gradients at the Interface

The climate close to the ground surface can be very different from that of the air a meter or two above it. If the ground is wet the air in contact with it will be saturated with water vapour, with a gradient away from the surface to the concentration of water vapour in the ambient air. There will be lesser gradients of oxygen (O_2) and carbon dioxide (CO_2) due to the photosynthesis and respiration of the plants and soil. Under most conditions there will be temperature gradients too. On sunny days incoming solar radiation predominates, and the ground surface is warmer than the air. On clear nights, thermal infra-red radiation from the ground predominates, and the ground surface is cooler than the air: if the ground-surface temperature falls below freezing a ground-frost results.

B. Transfers of Heat and Matter to and from the Atmosphere

These temperature and concentration differences drive transfers of heat and gases between the ground (or plant) and the atmosphere. Temperature is a measure of the concentration of kinetic energy in the molecules in a gas, so transfers of heat and gases are analogous molecular diffusion processes. Rate of heat flow (J m^{-2} s^{-1}) is directly proportional to temperature difference (K), and inversely proportional to the diffusion resistance to heat transfer in air (r_H, units sm^{-1}). The rate of diffusion of a gas (mol m^{-2} s^{-1}) is proportional to the concentration difference (mol m^{-3}), and inversely proportional to the diffusion resistance (r_C, units sm^{-1}). Light molecules diffuse faster than heavy molecules, so each gas has its own characteristic diffusion resistance. In some simple situations the diffusion resistances can be calculated from relevant dimensions (m) and the thermal conductivity of air, or the diffusivity of the particular gas in air (Campbell and Norman 1998; Gates 1980; Jones 1992; Monteith and Unsworth 1990). In many cases the only recourse is to measure, e.g. water loss, under a particular set of conditions and to estimate diffusion resistances from the measurements.

When air flows past a solid object, the air in contact with the object is stationary, and there is a gradient of velocity away from the surface. Close to the surface viscous forces in the fluid predominate and the streamlines are parallel with the surface – *laminar* flow, which creates a laminar boundary-layer. Farther from the surface, or at higher windspeeds, inertial forces become predominant, and the flow breaks up into eddies – leading to *turbulent* flow. The ratio of inertial to viscous forces is expressed by Reynold's Number $(Re) = Vl/\nu$, where V is the velocity of flow, l is a characteristic dimension (length or diameter for a flat plate, diameter for a rod or flow in a pipe), and ν is the kinematic viscosity of air. In practical situations, if Re is much over 10,000 the flow is likely to generate turbulence, if less the flow is likely to be laminar. For a flat plate in laminar flow, the ratio of the effective ("displacement") depth, δ, of the laminar boundary-layer to l is approximated by $\delta/l = 1.72/\sqrt{Re}$; thus the boundary layer depth is proportional to the square root of l, and inversely proportional to the square root of V (Monteith and Unsworth 1990). For a flat plate 5 cm wide in air flowing at 1 ms^{-1}, the effective thickness of the laminar boundary layer is around 1.5–2 mm. At 0.1 ms^{-1} (conventionally taken as "still air"), the thickness of the laminar boundary-layer will be c. 5–6 mm. Even if conditions are such that turbulence is being generated, there will always be a laminar sub-layer close to the surface.

The importance of these considerations in the present context is that exchange of heat and gases in the laminar boundary

layer is by molecular diffusion, which is slow. In turbulent air exchange processes are very much faster, in proportion to the size and vigour of the eddies – though of course the top of the laminar boundary-layer is not sharp but merges gradually with the turbulent air above. Bryophytes are often comparable in size to the laminar boundary layer of their substratum, and may be immersed within it, so molecular diffusion governs most exchange processes in their immediate environment. Vascular plants are generally much larger; exchange in the air spaces in the mesophyll and diffusion of water-vapour and gases through the stomata are governed by molecular diffusion, but exchange processes outside the leaves and stems mostly take place in turbulent air.

C. The Heat Budget and Penman's Equation

The intensity of solar radiation reaching the earth's upper atmosphere is about 1,370 W per square meter (Wm^{-2}); the peak energy of sunlight is at about 450 nm, in the middle of the visible spectrum. Some of this incoming radiation is absorbed by the atmosphere, and some is reflected back into space by clouds. On a clear day around 1,000 Wm^{-2} reaches the ground. When sunlight is absorbed by a solid surface it is transformed into heat, which can leave the surface in only three ways. It may be *conducted* into the ground (or plant) raising its temperature, it may heat the air close to the surface and be *convected* away by gaseous diffusion and air currents, or it may be re-*radiated* back to the environment as thermal infrared radiation (with peak energy far outside the visible spectrum at a wavelength of around 10,000 nm).

The evaporation of water is driven by the concentration difference of water vapor between the air in contact with the wet surface (saturated) and the ambient air. For water to evaporate, the latent heat of evaporation must be supplied. The latent heat comes from some combination of radiative energy exchange at the surface, conduction from the substrate, and convective transfer from the air (Campbell and Norman 1998; Gates 1980; Jones 1992; Monteith and Unsworth 1990). Penman (1948), making some simplifying assumptions, derived an equation to estimate the rate of evaporation from a wet surface:

$$\lambda E = [s(R_n - G) + \rho c_p(\chi_s - \chi)/r_H]/(\gamma^* + s)$$

where E is the rate of evaporation, λ is the latent heat of evaporation, R_n is the net radiation balance of the surface, G is storage of heat by the substratum, r_H is the diffusion resistance to heat transfer, s is the slope of the saturation vapor-density curve, ρ is the density of air, c_P is the specific heat of air, $(\chi_s - \chi)$ is the saturation deficit of the ambient air, and γ^* is the apparent psychrometer constant. The quantities, λ, ρ, c_P, s and γ^* are "constants" which vary somewhat with temperature and can be looked up in tables. R_n, G, $(\chi_s - \chi)$ and r_H are variables, which can be measured or estimated.

The left-hand term in the numerator is the supply of heat by radiation or conduction. The right-hand term is the heat drawn by convective transfer from the air. If the net radiation income is small and heat cannot be drawn from the substratum, the rate of evaporation will be determined mainly by the saturation deficit of the air and the boundary layer diffusion resistance of the bryophyte. The latent heat of evaporation will be drawn mostly from the air, and the bryophyte surface will be cooler than air temperature. In a humid sheltered situation in sun the position is reversed. The right-hand term is now small, and evaporation is determined mainly by the net radiation income; the bryophyte will be warmer than the air. Evaporation will be at a minimum when net radiation income and saturation deficit are low, and boundary-layer resistances are high (implying low windspeed), as in sheltered, shady, humid forests. Evaporation will be maximal in full sun, in exposed situations, with dry air. Dry surfaces in full sun can easily reach 50–60 °C, and temperature can only be kept within tolerable limits for most life if evaporative cooling is added to the heat budget.

IV. Selection Pressures on Early Land Plants

A. Water Loss and CO_2 Uptake

A plant cannot acquire CO_2 from the atmosphere without at the same time losing water. However, the pathways for CO_2 acquisition and water loss are significantly different. Water is lost from the wet cell surfaces to the bulk atmosphere, so the diffusion resistance to water-loss is entirely in the gas phase (Nobel 1977; Jones 1992). Carbon dioxide is taken up through the wet walls of the photosynthesizing cells, and must then diffuse in the liquid phase from the absorbing cell surface to the chloroplasts. The CO_2 diffusion resistance in water is higher than that in air by a factor of around 10^4; a diffusion barrier 1 μm of water is equivalent to about 10 mm of still air. This means that CO_2 acquisition is almost wholly diffusion-limited, and that a large part of the resistance to CO_2 uptake is in the liquid phase within the cell. The diffusion resistance of the air will still be an important factor affecting evaporation, but water loss is under more complex micrometeorological control. Selection pressure will tend to increase area for CO_2 acquisition relative to projected area intercepting radiation and governing water loss. Hence selection pressures for maximizing CO_2 acquisition and for minimizing water loss are not diametrically opposed. The evolution of ventilated photosynthetic tissues (mesophyll and analogous structures), and probably of much of the diversity of bryophyte life-form, is driven by this difference.

B. Desiccation Tolerance

Drying-out is an ever-present hazard on land, and desiccation-tolerance, the ability to lose most of the cell water without harm, suspend metabolism, and recover normal function on re-wetting (poikilohydry) is very common amongst small terrestrial plants including cyanobacteria, chlorophycean algae, bryophytes and lichens. Desiccation tolerance has a voluminous literature (Oliver et al. 2005; Alpert 2005, 2006; Proctor et al. 2007b), which will not be explored further here, beyond noting that in the dry state desiccation-tolerant organisms can tolerate far higher temperatures than when hydrated (Hearnshaw and Proctor 1982).

C. Disseminule Dispersal

Spores (or other propagules) shed into a laminar boundary layer would almost certainly be deposited close to the point of release. To stand a chance of wide dispersal they need to be shed into air with at least a modest level of turbulence. The effect of this is easy to visualize when a moss such as *Mnium hornum* is fruiting in spring. The carpet of gametophyte leaves is photosynthesizing in relatively still air within a few millimeters of the ground, while the ripe sporophytes are dancing in the slightest wind on their wiry 5 cm-long setae. It is tempting to see spore dispersal in other groups of bryophytes too, as adaptations to get spores out of the relatively stagnant air close to the ground – the upstanding slender apically-dehiscing sporophytes of hornworts (Anthocerophyta), the dehiscence of *Sphagnum* (by whatever mechanism; Ingold 1965; Duckett et al. 2009; Whittaker and Edwards 2010), or the "catapult" mechanism of the elaters of liverworts (Marchantiophyta).

V. The Evolution of Vascular Plants

It now seems to be the consensus that the liverworts (Marchantiophyta) are the sister group of all other archegoniate land plants (Edwards et al. 1995; Frey and Stech 2005; Qiu et al. 2006), and they probably originated in the mid to late Ordovician perhaps 450 million years ago (mya) (Chaps. 2 and 3). There is still doubt whether the Bryopsida or the Anthocerotopsida diverged next from the line leading to the vascular plants, but it seems certain that both groups were established by the end of the Ordovician. The fossil record over this period (c. 30–40 million years) is of dispersed (liverwort-like)

"cryptospores" and fragmentary plant remains. In the early Silurian, perhaps earlier in Gondwana, cryptospore abundance and diversity diminished as trilete spores appeared, became abundant, and underwent rapid diversification. This change coincides approximately with the appearance of vascular plant megafossils and probably represents the origin and adaptive radiation of vascular plants (Edwards et al. 1995, 1998b; Edwards 2000; Steemans et al. 2009).

A. The Evolution of Complexity of Form, and Conducting Systems

No doubt organisms competed from the start in the early land flora, and even at the relatively high levels of atmospheric CO_2 in the early Palaeozoic (Berner and Kothavala 2001; Bergman et al. 2004) complexity of form favoring CO_2 uptake relative to evaporation probably evolved early – branched filaments, multi-cellular plant bodies with air spaces (Raven 1996) or with filamentous, plate-like or leaf-like outgrowths. Modern terrestrial algae and bryophytes provide plenty of models, such as *Trentepohlia*, *Petalophyllum*, *Fossombronia*, the Marchantiales (Proctor 2010), *Crossidium*, *Aloina*, and the Polytrichales (Proctor 2005). Did an epidermis (with pores) arise first for mechanical protection of photosynthetic structures, which had to be thin walled to maximize CO_2 capture? Modern Marchantiales and Polytrichales provide two suggestive models in which protective layers (evolved in quite different ways) seem primarily to serve this function (Figs. 4.1 and 4.2).

Any organized multi-cellular plant body presupposes conduction of water, food materials and growth regulators (Raven 1977, 1984; Raven and Handley 1987). Extant bryophytes supplement diffusion through cell walls and general cell-to-cell transport of solutes (Proctor 1959; Pressel et al. 2010) by various specialized conducting systems. The water-conducting elements (hydroids) of mosses and similar water-conducting strands in Calobryales, Metzgeriales and *Takakia* probably all evolved independently, and none is homologous with the tracheids

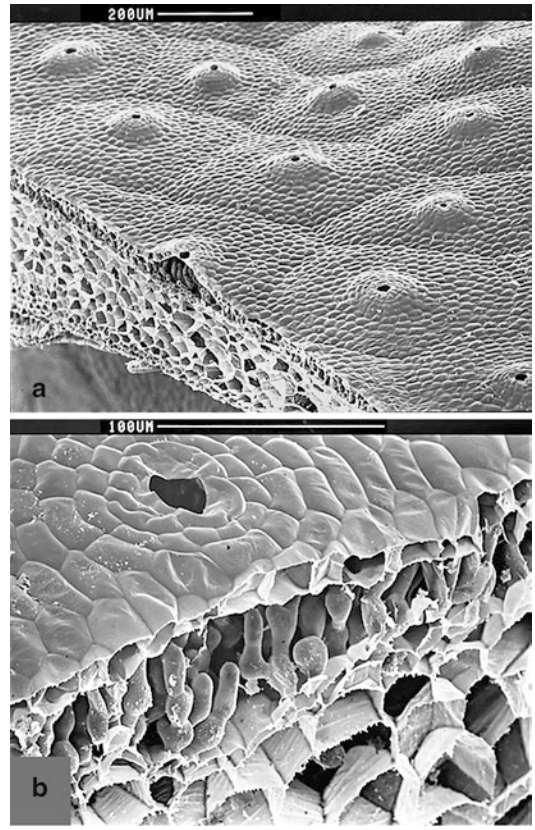


Fig. 4.1. Scanning electron micrographs of a vertical section of the thallus of the marchantialean liverwort *Lunularia cruciata*. The Marchantiales typically have a ventilated photosynthetic tissue, a "pseudo-mesophyll", analogous to a vascular-plant leaf, but evolved independently. The photosynthetic filaments occupy chambers within the upper surface of the thallus, protected from waterlogging and mechanical damage by an epidermis and opening to the exterior by pores. These allow access of CO_2 but not liquid water; they have sharp water-repellent margins but do not regulate water loss. Most of the thickness of the thallus is colorless parenchyma. (**a**) General view showing the pattern of air-chambers and pores on the surface of the thallus. (**b**) A closer view of an individual air-chamber and pore showing the photosynthetic filaments lining the chamber floor.

of vascular plants (Ligrone et al. 2000; Edwards et al. 2003). A polarized cytoplasmic organization with a distinctive axial system of microtubules characterizes the food-conducting cells of polytrichaceous mosses (Pressel et al. 2006). A similar organization probably with the same function occurs in other parts of the plant in mosses, including *Sphagnum*, and in thallus parenchyma of

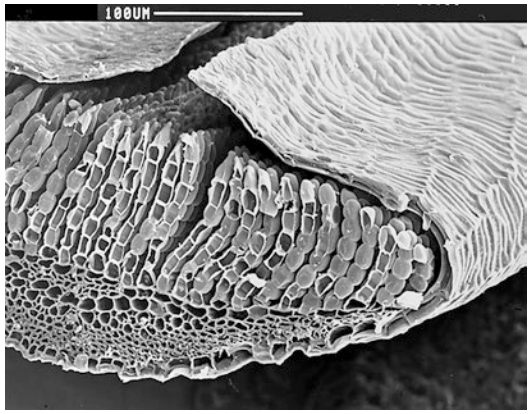


Fig. 4.2. Scanning electron micrograph of a leaf of the moss *Polytrichum piliferum*. Another "pseudo-mesophyll" of radically different structure and origin. In the Polytrichales the photosynthetic tissue consists of closely-spaced lamellae on the upper side of the mid-rib; the unistratose leaf lamina is reduced to a narrow colorless border. The marginal cells of the lamellae are thickened and water-repellent, and serve as a protective "epidermis", in many species with prominent epicuticular wax. In this species and its close relatives the colorless leaf margins are inflexed over the lamellae adding still more protection to the photosynthetic lamellae.

liverworts. The distinctive structure of these food-conducting cells of bryophytes precludes any homology with the phloem of vascular plants (Ligrone et al. 2000). However, the apparent simplicity of the early land-plant fossils conceals a surprising diversity of structure in their conducting elements, as SEM studies of coalified fossils has shown (Edwards et al. 2003).

B. The Importance of Scale

Modern bryophytes and vascular plants differ in size by around two orders of magnitude. This difference in scale brings with it major differences in physiology and responses to the environment; the scale-dependence of heat and mass transfer through the boundary-layer has been indicated already. Other things being equal, a plant a tenth of the linear dimensions of another has a hundredth of the surface area, and a thousandth of the volume and mass (and its root system, if it had one, could exploit a thousandth of the volume of soil). The force of gravity depends on mass (proportional to volume), so it is important at our scale and a limiting factor for tall trees, but trivial for bryophytes. Surface tension, which works on linear interfaces, is trivial for us, but important physiologically for bryophytes, and life or death to small insects. The demands of tissues are proportional to volume, so the need of a plant for specialized transport systems increases with size. Volume is important in itself; there would simply not be room for the elaboration of vascular-plant structure in the bryophyte body. Particular scaling considerations apply to rates of uptake of nutrients by roots from soil, and rates of flow through water-conducting channels (Raven and Edwards 2001). The vascular pattern of adaptation is unquestionably optimal for a large plant, but there is much reason to believe that the poikilohydric strategy is optimal for one less than a few centimeters high (Proctor and Tuba 2002; Proctor 2009). The two strategies overlap, and both are viable, only in a limited "window" of scale from about 1 cm to about 10 cm, and it is in this size range that we should look for transitions between them – and for the earliest vascular plants.

Difference in scale brings profound differences in physiology between vascular plants and bryophytes, especially in relation to water (Proctor and Tuba 2002; Proctor 2009, 2011). Bryophytes are *poikilohydric*; vascular plants are *homoiohydric*. The basic cell biology of poikilohydric and homoiohydric plants is the same. Both need to be near full turgor for normal metabolism to take place. The difference is that the poikilohydric plant metabolizes when water is available, and goes into a state of suspended metabolism when it is not. In a Höfler diagram (Fig. 4.3), a vascular plant operates between about 30 % relative water content (RWC) and full turgor. Much of interest in the corresponding diagram for a poikilohydric plant lies in the regions below 30 % and above 100 % RWC (Proctor et al. 1998; Proctor 1999, 2009). Below c. 30 % RWC metabolism is slow or ceases altogether.

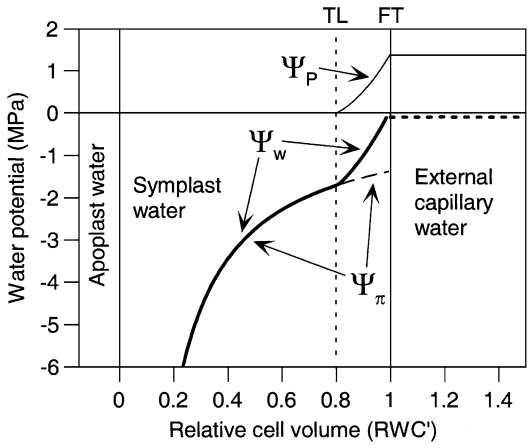


Fig. 4.3. Höfler diagram for a typical bryophyte, based on thermocouple psychrometer measurements on the leafy liverwort *Porella platyphylla* (Proctor 1999). The body of the diagram shows the relation of relative water content (RWC, strictly relative cell volume, RWC′) to water potential (Ψ), and its components: Ψ_W water potential of the cell, Ψ_π osmotic potential of the cell sap, Ψ_P turgor pressure. The water potential of the cell (Ψ_W) is zero at full turgor (*FT*); the cell is in equilibrium with liquid water in its environment. As the cell loses water, turgor pressure (Ψ_P) falls, and becomes zero at the point of turgor loss (*TL*). The tissue then becomes flaccid, and Ψ_W becomes equal to $\Psi\pi$. Bryophytes share this much of the diagram with vascular plants. But in addition to water inside the cell, turgid metabolically-active bryophytes have *external capillary water* held in spaces at near-zero water-potential outside the cells, and this water is physiologically important too. The external capillary water is physically continuous with the *apoplast* water in the cell-walls, spanning a range of water potentials from zero to negative values far outside the limits of metabolism.

Most poikilohydric plants dry out to 5–10 % RWC or less, and in the dry state they can survive for weeks or months, and revive when they are remoistened. Respiration recovers more rapidly than photosynthesis (Proctor et al. 2007a); it commonly takes from a few minutes to a few hours for the plant to return to a positive carbon balance. Vascular plants are also *endohydric* – their physiologically important free water is inside the plant's vascular system, separated from the surroundings by the cuticle and the stomata. Most poikilohydric plants are *ectohydric* – for them, the free water outside the plant body is physiologically important. Many bryophyte structures provide capillary spaces in which water can be stored or moved freely from one part of the plant to another. The needs for water storage and movement may conflict with the needs of gas exchange. Bryophytes often have epicuticular waxes on the leaf surfaces; these usually have much more to do with controlling the distribution of water over the leaf surfaces than with reducing the rate of water loss (Proctor 1979a, b). The role of external water storage in bryophyte carbon balance is well illustrated by Alpert (1988), Zotz et al. (2000) and Zotz and Rottenberger (2001) and the data of Proctor (2004). Poikilohydry and desiccation tolerance is, in a sense, a drought avoidance strategy (Proctor 2000). For poikilohydric plants, partial hydration is a transient state between full turgor and desiccation, so they may spend less of their metabolically active time at sub-optimal RWC than drought-tolerant vascular plants.

In open, sun-exposed situations not only is there full exposure to near-UV, but diffusion limitation of CO_2 uptake may mean that there is an excess of excitation energy, with the attendant hazard of generating damaging reactive oxygen species (ROS; Smirnoff 2005; Chapter 7). For a poikilohydric plant, the periods of drying out and recovering from desiccation are particularly hazardous. This leads to strong selection pressure for photoprotection (Heber et al. 2006). The xanthophyll cycle is typically very active in these plants; chlorophyll fluorescence generally shows high, but fast-relaxing, non-photochemical quenching (Marschall and Proctor 1999, 2004; Proctor MCF and Smirnoff N, unpublished data). In mosses that have been investigated, CO_2 and O_2 act as alternative electron sinks (Proctor and Smirnoff 2011), probably by the Mehler reaction (Asada 1999, 2006).

C. The Vascular-Plant Package

It is inconceivable that a vascular plant could have evolved *de novo* as an integrated whole (Raven 1984). All the ingredients of the "vascular-plant package" exist in small poikilohydric modern plants, as models of

potential Palaeozoic precursors, but never all together. Which part of the vascular-plant package evolved first, roots, a vascular system, ventilated photosynthetic tissue, a waterproof cuticle, or stomata? All of the major extant groups adopted a different course.

The liverworts (Marchantiophyta) remained faithful to the gametophyte, and the limitations of boundary-layer life. The sporophyte passes most of its existence protected by gametophyte structures (involucre, perianth, perichaetial leaves) until the spores are mature, when rapid elongation of the seta, capsule dehiscence and spore liberation and dispersal, all generally take place within a day or two. Seta elongation occurs only if the plant is turgid, and capsule dehiscence takes place only if the air is dry. The sporophyte confines its venture into the dangerous world away from its substratum and protective gametophyte to a sacrificial few hours before shedding its spores and dying. The liverwort life-cycle seems to offer no insights into the origin of vascular plants.

Mosses (Bryophyta sensu stricto) have coped better with evolving a long-lived sporophyte capable of life outside the boundary layer, but have failed to make the critical breakthrough to full independence of the sporophyte. The embryonic sporophyte develops an apical cell at both ends (Campbell 1918; Smith 1938), the growing apex at the lower end forming the bottom part of the seta and the foot, that at the top end forming the upper part of the seta and the capsule. The developing sporophyte depends entirely on the gametophyte for water and mineral nutrients, and to a large but varying extent for photosynthate as well (Proctor 1977). By its small diameter and by growing away from the surface it speeds convective heat transfer with the surrounding air. Moss capsules are typically cuticularized and resistant to water *loss* but, in species with sporophyte development spanning a dry summer period, probably less *desiccation* tolerant than the gametophyte (Stark et al. 2007). The moss sporophyte has solved part of the heat-balance problem, and possesses ventilated photosynthetic tissue with cuticle and stomata, and a conducting strand in the seta. It lacks one crucial ingredient of the vascular-plant package. It has no root system, and it is probably not nearly big enough to develop one that is viable. So it remains locked into dependence on the gametophyte. Nevertheless, moss (and hornwort) sporophytes do provide (small scale) models for a credible stage in the evolution of the first vascular plants (Ligrone et al. 2012).

D. Possible Scenarios for the Evolution of Vascular Plants

An impermeable cuticle would make no sense for a liverwort or moss growing on the ground; when hydrated and photosynthesizing in bright sun they would need to absorb CO_2 freely, and they would need evaporative cooling to keep temperature within tolerable limits. There are no obvious preconditions (apart from a multicellular plant body) for the evolution of a conducting system. Stomata only make sense in the context of a cutinized epidermis and ventilated photosynthetic tissue, and an efficient conducting system to support a transpiration stream, so the ventilated photosynthetic tissue has to come first (Edwards et al. 1998a). Roots, or parts of the shoot system serving the same function, are the last crucial innovation that made independent orthotropic growth possible, and paved the way for exploitation by plants of the third dimension – height. Roots with an anatomy distinct from stems, appear in the fossil record some 15 million years after the first evidence of vascular plants. The evolution of true roots heralded the increasingly rapid escalation of plant size, diversity and complexity from herbaceous dimensions to tall forest trees during the Devonian.

Early fossil vascular-plant floras are all from low (palaeo)latitudes, so they were probably all from environments free from seasonal extremes. As a possible place of origin of homoiohydric vascular plants, we may consider a constantly-watered spot, with rainfall distributed round the year and comfortably exceeding annual evapo-transpiration. Nowadays

such a place can support communities of bryophytes, in which gametophytes make up the bulk of the plant cover. Some species grow to a few centimeters above the general level and are able to maintain turgor at their apices by either external capillary or internal conduction. Sufficient heat is lost by the latent heat of evaporation and keeps the temperature well within tolerable limits. Sporophytes of mosses and hornworts grow a few centimeters taller and both have air spaces and stomata. A modern marsh of this kind often includes rushes (*Juncus* spp.), or other plants of similar erect terete growth form. These have roots, ventilated photosynthetic tissue, cuticle and stomata. They also have a morphology which offers a small target for solar radiation, a large area in contact with air and a small diameter perpendicular to the airflow, hence thin boundary-layers, low resistance to heat transfer, and close coupling to air temperature. Rushes can close their stomata and still have enough convective cooling to keep their temperature from rising to lethal levels. This is an environment in which we can visualize the evolution of first, air spaces in the photosynthetic tissue, then cuticle and stomata evolving hand in hand, and simultaneously with these, basal parts of the shoot system increasingly devoted to uptake of water and nutrients. "Roots" no doubt evolved more than once, and various lines of evidence suggest roots evolved at least two and possibly three times (Raven and Edwards 2001).

Fossil cooksonioid axes span a range of diameters from slender examples with conducting strands, which could have borne aloft sporangia but could not have been photosynthetically self-supporting (much like modern moss setae), to axes wide enough to have contained not only a conducting strand, but sufficient photosynthetic tissue to be self-sufficient for carbon nutrition (Boyce 2008). Modern hornwort sporophytes (Anthocerophyta) provide suggestive models of a transitional stage – with a slender conducting strand, ventilated photosynthetic tissue, and stomata. But any successful breakthrough into homoiohydry would be expected soon to have been massively outnumbered in the fossil record by diverse and numerous fast-evolving progeny – a besetting problem of the search for "missing links"!

Much of the argument of the preceding paragraphs could be read as favoring the *antithetic* or "rise of the sporophyte" model of vascular plant evolution first suggested in the 1870s (Bower 1890, 1935; Hemsley 1994), which saw the sporophyte as an intercalation into a basically haploid life-cycle. An alternative *homologous* model, also dating from the 1870s, saw the origins of the gametophyte and sporophyte as the two phases of an isomorphic alternation of generations, exemplified by some marine algae (Eames 1936; John 1994). The finding of gametophyte axes apparently anatomically similar to known fossil sporophytes in the Rhynie Chert (Remy et al. 1993; Remy and Hass 1996), seem to support the homologous model (Kenrick 1994; Taylor et al. 2005). With the discovery of apospory and diploid gametophytes, and developing concepts in genetics, morphological differences between the generations of the life cycle can now be seen as less fundamental than they were perceived to be a century ago. The scenario sketched above need not have been a unique event; different groups of vascular plants may well have evolved independently, many lineages sooner or later becoming extinct (Crane et al. 2004; Palmer et al. 2004).

E. Why Did Vascular Plants Not Supersede Bryophytes?

In the favourable habitats just envisaged for the origin of vascular plants, vascular plants may have superseded bryophytes. However, wide expanses of the Earth's surface must have been less favourable. There would always have been rocky places impenetrable to roots, and places intermittently too dry. At high latitudes and altitudes conditions would have been too cool for growth except close the ground during the day, especially in sunshine; Davey and Rothery (1997)

measured midday temperatures up to 11–12 °C at 10 mm depth on Signy Island in the sub-Antarctic. In cold climates, the temperature gradients at the surface of the Earth would have been not a potential hazard, but a prerequisite of plant growth. At the present day we take for granted a low vegetation, in which bryophytes and lichens are prominent, in polar regions and on high mountains.

Vascular plants opened up a new dimension. They did not simply replace the smaller poikilohydric plants. Rather, they created their own new ecological niches, and by increasing the complexity of the landscape created a new range of microhabitats for smaller plants to colonize. The small poikilohydric plants continued to evolve *at their own scale.* And who would argue that the bryophytes were unsuccessful with around 20,000 species distributed in almost every habitat from the Equator to the Polar regions?

F. Physiological Consequences of the "Vascular-Plant Package"

This had physiological consequences for the vascular plants themselves. The mesophyll cells found themselves in a constantly-humid environment with a regular water supply, relieved of the selection-pressure to tolerate intermittent desiccation. Growth to overtop neighbors was the new imperative. There was still a need for desiccation tolerance at particular points in the life cycle; almost all vascular plants have desiccation-tolerant spores or pollen grains, and many have desiccation-tolerant seeds. Accordingly vascular plants have retained genes for desiccation tolerance, but they are only switched on during sporogenesis and seed development, and vegetative tissues are generally sensitive to desiccation.

The vascular-plant package also had implications for photoprotection. As we have already seen, poikilohydry and CO_2 limitation both tend to lead to intermittent production of excess excitation energy, which needs to be degraded harmlessly to heat if it is not to generate damaging free-radicals. Vascular-plant leaves have a larger, and more constant, photosynthetic capacity. They have less need for photoprotection; glycolate photorespiration can be seen as the principal vascular-plant answer to what need they have. Vascular plants, with their large complex bodies and conducting systems, also have the option of exporting excess photosynthetic products to non-photosynthesizing storage organs.

We take these vascular-plant traits for granted, and tend to regard them as fundamental, but they are derived consequences of the evolution of the vascular-plant package.

VI. The Post-palaeozoic Scene: Complex Habitats

A. The Close of the Palaeozoic Era

In the earlier part of the Palaeozoic temperatures were c. 4–6 °C higher, and atmospheric CO_2 levels some 15 times higher than at the present day. By the late Devonian (c. 360 mya) lycophytes, ferns, Equisetales and pteridosperms were in existence, some of them large trees; atmospheric CO_2 had declined to around five times present levels, and temperature was falling too. By the close of the Carboniferous period (300 mya), complex phyletically-rich tropical forests had been in existence for 50 million years, with considerable habitat and regional diversification. Atmospheric CO_2 had dropped to no more than 500–600 ppm (some measurements put it lower than that), the concentration of oxygen in the air was about 30 %, and temperatures were similar to the present day. The evolution of large (megaphyll) leaves probably had more to with mutual shelter as vegetation increased in height and closure than with declining CO_2 in the air, despite the arguments of Beerling et al. (2001). It is more likely that the scale of photosynthesis, which large leaves made possible, drove the fall in atmospheric CO_2 than vice versa. The rise in O_2 was reflected by the increasing

frequency of fire in the later Palaeozoic (Scott and Glasspool 2006).

Ecologically, in the course of 150 million years, the Earth had become an incomparably richer, more complex and more diverse place. Forests created a whole new set of niches for bryophytes, on the shady forest floor, on fallen wood, and probably as epiphytes on trunks and branches. The shade meant that light, not CO_2, became the limiting factor for growth. This was less of a constraint on bryophytes, with their largely unistratose leaves and poikilohydric habit, than on vascular plants. Bryophytes could draw nutrients from throughfall, the rain penetrating the canopy, and dripping from the leaves, and from stemflow, the rainwater running down the trunks. Microhabitats on bark were largely inaccessible to vascular plants because there was nowhere for roots to penetrate.

The great Carboniferous coal-forming rainforests declined abruptly (within a few thousand years) to a fraction of their former extent about 315 mya. The causes of this (geologically) sudden collapse are not certain, but probably the onset of a cycle of glaciations, coupled with a long-term trend to drier climate, was a major factor (Montañez et al. 2007; DiMichelle et al. 2010). The tall lycophytes that had dominated the rainforests declined with them and became extinct by the end of the Permian, and the tree-ferns and pteridosperms declined with them. Cordaitales and ferns had long occupied the drier uplands, and had spread into the lowlands during drier climatic phases. These, together with early conifers, expanded to dominate the increasingly dry and continental landscape, until by the end of the Permian (250 mya) primitive conifers and the maidenhair trees (Ginkgoales) were the predominant trees.

From the evidence of molecular phylogenies, combined with such fossil evidence as we have, all the major backbone lineages of bryophytes were in place by the end of the Palaeozoic. The Marchantiopsida (complex thalloid liverworts) had probably split from the remaining liverworts (Jugermanniopsida) in the late Devonian (c. 370 mya), and the simple thalloid and leafy liverworts probably diverged in the late Carboniferous (c. 310 mya; Heinrichs et al. 2007). The split of the line leading to Porellineae, Radulineae and Lepidolaeninae (all largely epiphytic) from the remaining leafy liverworts is dated by Heinrichs et al. at about 280 mya in the Permian. Among the mosses, *Takakia*, *Sphagnum*, and *Andreaea* diverged earliest, and the Oedopodiaceae, Tetraphidaceae, Polytrichaceae, Buxbaumiaceae, Diphysciaceae, Timmiaceae and the Funariidae, Dicranidae and the Bryidae/Hypnidae lineages were probably in place by the opening of the Mesozoic (Goffinet and Buck 2004; Bell and Newton 2004; Newton et al. 2007), having evolved in the late Devonian and Permo-Carboniferous forests.

B. The Mesozoic Era: Continuing Evolution of Bryophytes

About 250 mya there was the greatest mass extinction in the history of the planet; 96 % of marine species died out. The extinction bore less heavily on land life, some 50 % of plant species may have become extinct, but this figure is very uncertain (McElwain and Punyasena 2007). The tree lycophytes and sphenophytes and the Cordaitales disappeared from the fossil record at the end of the Permian, as did most of the Palaeozoic families of ferns. The Permian–Triassic boundary marks a dramatic change from a primitive Palaeozoic flora and vegetation to one of essentially modern aspect in the Mesozoic. The causes of the extinction are obscure, but vulcanism, the releasing of methane clathrates, ‘greenhouse’ warming, and anoxia in the oceans with emission of hydrogen sulfide, may all have played a part.

It appears to have taken some millions of years before a substantial forest cover was regained. How the extinction affected bryophytes we can only conjecture. Heinrichs et al. (2007) locate two dated nodes of their phylogeny in the Permian, two in the Triassic, and four in the Jurassic – periods of roughly

equal length. Evidently the Triassic was not a period of very active radiation in liverworts. The early Triassic climate was warm and arid, becoming cooler towards the end of the period. Atmospheric CO_2 was some six times and O_2 rather below present atmospheric levels. Recognizably modern conifers and the cycads and cycad-like plants that were to be so characteristic of Mesozoic vegetation began to appear in the Upper Triassic, joining the still-abundant Ginkgoales. Liverworts continued to differentiate, though slowly. The Triassic–Jurassic boundary was marked by another major extinction event, probably caused by widespread flood-basalt eruptions associated with the imminent break-up of Pangaea (McElwain et al. 1999; Whiteside et al. 2010).

The ensuing Jurassic period (c. 200–146 mya) was a time of rising temperature, rising O_2 levels (~25 %), high CO_2 (peaking at ~2,000 vpm) – and renewed evolution. Pangaea broke up, the northern continents (Laurasia) separating from the southern continents (Gondwana), and Laurasia itself began to break up before the end of the period. Gymnosperms dominated the forests. They included cycads and the cycad-like Bennettitales, Ginkgoales (especially in temperate northern latitudes), and varied conifers including Pinaceae, Taxaceae, Taxodiaceae and Podocarpaceae (the last particularly in the southern hemisphere). Some of the conifers had broad leaves, as does the extant *Phyllocladus* (the celery-top pine of Tasmania, and the toatoa and tanekaha of New Zealand) and the Ginkgoales, so the Mesozoic forests would have been more varied than their modern coniferous counterparts. A few modern bryophyte families seem to go back to the Jurassic, Frullaniaceae, Radulaceae, Porellaceae and Metzgeriaceae among them. The pleurocarpous mosses first appeared early in the Jurassic and the major pleurocarpous families diverged later in this period or early in the Cretaceous (Newton et al. 2007).

The Cretaceous Period (c.146–66 mya) followed on from the Jurassic, and was the longest period of the Mesozoic. The break-up of Laurasia continued, but what were to become North America, Greenland and northern Europe remained close together, with the North Atlantic a broadening triangle between and south of them. Gondwana broke up during the Cretaceous, South America, Africa, Madagascar/India, Australia and Antarctica becoming discrete continents. The last links between the southern continents, between Antarctica and Australia and between Antarctica and South America, were not broken until the Cenozoic. Climatically, the trends set in the Jurassic continued. Temperature continued to rise, approaching early Triassic levels. Carbon dioxide remained high (~2,000 ppm) declining toward the end of the period, and oxygen peaked at levels(~30 %) not reached since the Carboniferous. Vegetationally, the early part of the period was essentially a continuation of the Jurassic, dominated by conifers, Ginkgoales, cycads and Bennettitales; these last became extinct in the mid-Cretaceous. The fossil record shows that coniferous forests extended to both northern and southern polar regions in the Mesozoic (Beerling and Osborne 2002). Dinosaurs peaked in diversity in the mid-Cretaceous. Flowering plants (Angiosperms) first appeared as pollen early in the Cretaceous (Soltis and Soltis 2004). At first they expanded slowly, but in the mid-Cretaceous (Albian–Cenomanian) they diversified rapidly, and by the end of the period 70 % of known terrestrial plant species were angiosperms. They seem to have evolved in open upland situations, and they may not have been generally dominant until the Cenozoic (Wing and Boucher 1998).

The Cretaceous was also a time of active diversification amongst bryophytes. Many of the major bryophyte families (at least in a broad sense) trace their origins back to the Cretaceous, including Scapaniaceae, Lophocoleaceae, Plagiochilaceae, and Lepidoziaceae amongst liverworts. Many of the better-circumscribed genera are of similar age.

The close of the Cretaceous was marked by a mass extinction (the "K–T boundary"), probably primarily due to an asteroid impact

66.5 mya, though the eruption of the Deccan Traps (flood basalts) which spanned the same period may have contributed. Groups becoming extinct included the dinosaurs (except the ancestral birds), plesiosaurs, pterosaurs, ammonites and belemnites. Corals, coccolithophorids and foraminifera suffered heavy losses, but fish, amphibians, turtles and crocodiles were relatively unaffected. There was major global disruption in terrestrial vegetation at the K–T boundary. In North America (relatively close to the postulated impact site) more than half the plant species became extinct, but in New Zealand and Antarctica the mass death of vegetation seems to have caused no significant turnover in species. In the immediate aftermath, open habitats seem to have been widely colonized by ferns, leading to a brief 'fern spike' in the geological record before closed forests re-established (Nichols and Johnson 2008).

C. The Cenozoic Era: The Modern World

The Cenozoic era (66 mya onwards) brings us to the modern world. From the outset, plant cover was predominantly of angiosperms, mostly belonging to modern genera, or to closely related taxa. In the Paleocene and Eocene dense tropical, sub-tropical and deciduous forests covered the globe; polar regions were ice-free, and occupied by temperate forest of deciduous and coniferous trees. Average global temperature peaked at c. 6 °C above present levels around the opening of the Eocene, a little over 55 mya, but (as in the Jurassic and Cretaceous) the difference between equatorial and polar regions was only half that at the present day, so the tropical regions were little or no warmer than now. By the Eocene, the mammals had evolved to occupy most of the ecological niches left vacant by the demise of the dinosaurs. From mid-Eocene onwards temperature had begun the slow decline that was to culminate in the Pleistocene glaciations, and by the end of the period (34 mya) Antarctica was an expanse of tundra fringed by deciduous forests. Collisions between tectonic plates following the fragmentation of Pangaea led to mountain-building in western North America, Asia (Himalayas etc.) and Europe (Alps etc.) that was to continue into the following Oligocene and Miocene periods and beyond; the Andes came later starting in the Miocene. Early in the Oligocene the first permanent ice-sheets appeared in Antarctica. A trend towards cooler and drier climate, coupled with continuing evolution of grazing mammals, saw an expansion of grasslands at the expense of forest, trends continued in the Miocene (23–5.3 mya) and Pliocene (5.3–2.6 mya). Declining temperatures and atmospheric CO_2 levels culminated in the Pleistocene glaciations, which are still with us in Greenland and Antarctica.

The Cenozoic has been an eventful period, of mountain building, climatic change, and vigorous evolutionary radiation of angiosperms, mammals, birds and insects. It has also been an eventful period for bryophyte evolution. We know that the Cenozoic has been a time of active speciation in both mosses and liverworts, in response to the diversity of angiosperm forests and the mountain building that has characterized the era. The polar-alpine bryoflora is a creation of Cenozoic evolution as surely as the angiosperm arctic-alpine flora (Hultén 1937).

D. Phyletic Conservatism and Life-Strategy Correlations

Evolution has left us with four major clades of plants whose fossil record goes back to the Paleozoic; from molecular evidence they have been independent lines at least since that time. Two of these are of large vascular plants; the Lycophyta, once prominent as large trees in Palaeozoic forests but since reduced to a minor role, and the fern–horsetail–pteridosperm line with its later offshoots the conifers and flowering plants. Two groups, the mosses and liverworts, are of small poikilohydric plants; these we traditionally lump together as "bryophytes". A third bryophyte group, the hornworts (Anthocerophyta) of which the fossil record is sparse, must from molecular evidence be equally old. These taxonomic groups have retained their

phyletic independence, and their characteristic ecophysiological adaptation, since those early days.

There are evidently just two basic strategies of adaptation for plant life on land, each optimal at a particular scale. They overlap at the scale of the largest bryophytes, and the smallest vascular plants, around 1–10 cm. It is in this range that the earliest vascular plants probably arose. Crossovers between the two strategies seem to be very rare. The filmy ferns are one of the few examples. Their sporophytes function ecologically as "bryophytes"; they are the right size, they are poikilohydric, and they grow in company with mosses and liverworts in shady humid situations (Proctor 2012). The ferns in fact have a foot in both camps. Their sporophytes are in general unequivocal vascular plants, but their gametophytes share the "bryophyte" strategy of adaptation (Watkins et al. 2007; Proctor 2007). "Why are desiccation-tolerant organisms so small or rare?" (Alpert 2006). Small poikilohydric plants are numerous, because they are optimal at that scale. Large desiccation-tolerant plants are rare because they are basically mainstream vascular plants, which only later evolved (or re-evolved) desiccation tolerance in response to seasonally-desiccated habitats which, globally, are themselves rare. The molecular evidence speaks for a common origin for bryophytes and vascular plants. However, it is clear that the vascular plants, and the bryophytes have evolved independently, facing different selection pressures at the cellular level, since the mid-Palaeozoic – in round figures some 400 million years.

VII. Overview

Bryophytes thus have a long evolutionary history of era-by-era diversification. The major divisions into mosses (Bryophyta), liverworts (Marchantiophyta) and hornworts (Anthocerotophyta) were probably in place by the end of the Ordovician, and these (and perhaps other bryophyte groups) took their place alongside early vascular plants. Since then bryophytes have evolved with vascular plants as part of their environment. The main backbone lineages of both the mosses and the liverworts probably diverged in the late Devonian and Carboniferous landscape, which must have included many habitats for (and some dominated by) small poikilohydric plants, in addition to the forests of which we are most aware from palaeobotany. Cladistic analyses of molecular data give every reason to suppose that Andreaeales, Sphagnales, Polytrichales, Dicranales, Grimmiales and others go back to the Palaeozoic. The Sphagnales are a particularly interesting case; presumably their limited but important ecological niche has existed since that time. The Carboniferous forests would have provided terrestrial habitats for a range of acrocarpous mosses and thalloid liverworts, but many of the dominant vascular plants might not have provided extensive habitat for epiphytes. Nevertheless, the "leafy I" clade of liverworts (Davis 2004), which now include many epiphytes, appears to have diverged in the late Palaeozoic. I have emphasized forests because these provide a wide range of ecological niches for bryophytes; it is noteworthy that most of the largest bryophytes grow in the shade and shelter of forests. The dendroid life-form, surely a response to conditions on the forest floor, has evolved repeatedly amongst unrelated mosses, e.g. *Dendroligotrichum*, Hypnodendraceae, Climaciaceae, *Thamnobryum*. Many of our modern families and genera diverged and diversified in the Mesozoic, in the gymnosperm-dominated Jurassic and (especially) Cretaceous periods. The major diversification of modern families and genera, such as the majority of the leafy liverworts (Davis 2004; Heinrichs et al. 2007) and pleurocarpous mosses (Bell and Newton 2004; Newton et al. 2007) which are so prominent in the present-day world came, along with the flowering plants, in the late Mesozoic and Cenozoic. Forests have been important in the evolution of bryophytes, but we should never forget that non-forested habitats have been ever present and important too.

Some genera and many species date from the Cenozoic, and active speciation (Shaw 2009) has been going on throughout the Cenozoic and still continues in "difficult" groups (*Calypogeia*, Lejeuneaceae, *Marchantia*, *Pellia endiviifolia* complex, *Bryum*, *Philonotis*, *Plagiothecium*, *Schistidium*, *Sphagnum*, and many others). As we approach the present day we are most aware of speciation. That is a consequence of the limited time frame afforded by the human life-span! Were we able to go back and bryologize the Mesozoic or Palaeozoic world, we should encounter a diverse and fascinating bryoflora, of which we might be able to assign a proportion to families (and even genera) we knew, but the species would be different – and evolving. The "present" is merely the point at which we step into the "river of time".

Acknowledgements

Many people over the years have contributed to the ideas presented in this chapter, from Kenneth Sporne, Paul Richards and Harold Whitehouse who introduced me to palaeobotany and bryophytes in my student days. Many others have influenced my thoughts about bryophyte ecophysiology and evolution since, among them Tim Dilks, Janet Jenkins, Tom Revesz, Mike Patrick and Nick Smirnoff in Exeter, and John Raven, Dianne Edwards, Derek Bewley, Mel Oliver, Zoltán Tuba, Zoltán Nagy, Zsolt Csintalan, Jeff Duckett, Roberto Ligrone and Silvia Pressel elsewhere. I haven't always agreed with them but they have all made me think. If I needed a challenge to think-through the big picture, it was the invitation to write this chapter, for which I am grateful to our two editors.

References

Alpert P (1988) Survival of a desiccation-tolerant moss, *Grimmia laevigata*, beyond its observed microdistributional limit. J Bryol 15:219–227

Alpert P (2005) The limits and frontiers of desiccation tolerant life. Integr Comp Biol 45:685–695

Alpert P (2006) Constraints of tolerance: why are desiccation-tolerant organisms so small or rare? J Exp Biol 209:1575–1584

Asada K (1999) The water–water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. Annu Rev Plant Physiol Plant Mol Biol 50:601–639

Asada K (2006) Production and scavenging of reactive oxygen species in chloroplasts and their functions. Plant Physiol 141:391–396

Beerling DJ, Osborne CP (2002) Physiological ecology of Mesozoic polar forests in a high CO_2 environment. Ann Bot 89:329–339

Beerling DJ, Osborne CP, Challoner WG (2001) Evolution of leaf-form in land plants linked to atmospheric CO_2 decline in the Late Palaeozoic era. Nature 410:352–354

Bell NE, Newton AE (2004) Systematic studies of non-hypnean pleurocarps: establishing a phylogenetic framework for investigating the origins of pleurocarpy. In: Goffinet B, Hollowell V, Magill R (eds) Molecular systematics of bryophytes. Missouri Botanical Garden Press, St. Louis, pp 290–319

Belnap J, Lange OL (2001) Biological soil crusts: structure, function and management. In: Ecological studies, vol 150. Springer, Berlin

Bergman NM, Lenton TM, Watson AJ (2004) COPSE: a new model of biogeochemical cycling over Phanerozoic time. Am J Sci 304:397–437

Berner RA, Kothavala Z (2001) GEOCARB III: a revised model of atmospheric CO_2 over Phanerozoic time. Am J Sci 301:182–204

Bower FO (1890) On antithetic as distinct from homologous alternation of generations in plants. Ann Bot 4:347–370

Bower FO (1935) Primitive land plants. Macmillan, London

Boyce CK (2008) How green was *Cooksonia*? The importance of size in understanding the early evolution of physiology in the vascular plant lineage. Paleobiology 34:179–194

Campbell DH (1918) The structure and development of the mosses and ferns. Macmillan, New York

Campbell GS, Norman JM (1998) Introduction to environmental biophysics. 2nd ed. Springer, New York

Crane PR, Herendeen P, Friis EM (2004) Fossils and plant phylogeny. Am J Bot 91:1683–1699

Davey MC, Rothery P (1997) Interspecific variation in respiratory and photosynthetic parameters in Antarctic bryophytes. New Phytol 137:231–240

Davis EC (2004) A molecular phylogeny of leafy liverworts (Jungermannineae: Marchntiophyta). In: Goffinet B, Hollowell V, Magill R (eds) Molecular systematics of bryophytes. Missouri Botanical Garden Press, St. Louis, pp 61–86

DiMichelle W, Cecil CB, Montañez IP, Falcon-Lang HJ (2010) Cyclic changes in Pennsylvanian paleoclimate and effects on floristic dynamics in tropical Pangaea. J Coal Geol 83:329–344

Duckett JG, Pressel S, P'ng KMY, Renzaglia KS (2009) Exploding a myth; the capsule dehiscence mechanism and the function of pseudostomata in *Sphagnum*. New Phytol 183:1053–1063

Eames AJ (1936) Morphology of vascular plants. McGraw-Hill, New York

Edwards D (1996) New insight into early land ecosystems: a glimpse of a Liliputian world. Rev Palaeobot Palynol 90:159–174

Edwards D (2000) The role of Mid-Paleozoic mesofossils in the detection of early bryophytes. Phil Trans Roy Soc Lond B 355:733–755

Edwards D, Duckett JG, Richardson JB (1995) Hepatic characters in the earliest land plants. Nature 374:635–636

Edwards D, Kerp H, Hass H (1998a) Stomata in early land plants: an anatomical and ecophysiological approach. J Exp Bot 49:255–278

Edwards D, Wellman CH, Axe L (1998b) The fossil record of early land plants and interrelationships between primitive embryophytes: too little and too late? In: Bates JW, Ashton NW, Duckett JG (eds) Bryology for the twenty-first century. Maney Publishing and British Bryological Society, Leeds

Edwards D, Axe L, Duckett JG (2003) Diversity in conducting cells in early land plants and comparisons with extant bryophytes. Bot J Linn Soc 141:297–347

Frey W, Stech M (2005) A morpho-molecular classification of the liverworts (Hepaticophytina, Bryophyta). Nova Hedwigia 81:55–78

Gates DM (1980) Biophysical ecology. Springer, New York

Goffinet B, Buck W (2004) Systematics of the Bryophyta (Mosses): from molecules to a revised classification. In: Goffinet B, Hollowell V, Magill R (eds) Molecular systematics of bryophytes. Missouri Botanical Garden Press, St. Louis, pp 205–239

Hearnshaw GF, Proctor MCF (1982) The effect of temperature on the survival of dry bryophytes. New Phytol 90:221–228

Heber U, Lange OL, Shuvalov VA (2006) Conservation and dissipation of light energy as complementary processes: homoiohydric and poikilohydric autotrophs. J Exp Bot 57:1211–1223

Heinrichs J, Hentschel J, Wilson R, Feldberg K, Schneider H (2007) Evolution of leafy liverworts (Jungermanniidae, Marchantiophyta): estimating divergence times from chloroplast DNA sequences using penalized likelihood with integrated fossil evidence. Taxon 56:31–44

Hemsley AR (1994) The origin of the land plant sporophyte: an interpolation scenario. Biol Rev 69:263–273

Hultén E (1937) Outline of the history of arctic and boreal biota during the quaternary period: their evolution during and after the glacial period as indicated by the equiformal progressive areas of present plant species. Thule, Stockholm

Ingold CT (1965) Spore liberation. Clarendon, Oxford

John DM (1994) Alternation of generations in algae: its complexity, maintenance and evolution. Biol Rev 69:275–291

Jones HG (1992) Plants and microclimate, 2nd edn. Cambridge University Press, Cambridge

Kenrick P (1994) Alternation of generation in land plants: new phylogenetic and palaeobotanical evidence. Biol Rev 69:293–330

Ligrone R, Duckett JG, Renzaglia KS (2000) Conducting tissues and phyletic relationships of bryophytes. Phil Trans R Soc Lond B 355:795–813

Ligrone R, Duckett JG, Rezaglia KS (2012) Major transitions in the evolution of early land plants: a bryological perspective. Ann Bot 109:851–871

Marschall M, Proctor MCF (1999) Desiccation tolerance and recovery of the leafy liverwort *Porella platyphylla* (L.) Pfeiff.: chlorophyll-fluorescence measurements. J Bryol 21:261–267

Marschall M, Proctor MCF (2004) Are bryophytes shade plants? Photosynthetic light responses and proportions of chlorophyll *a*, chlorophyll *b* and total carotenoids. Ann Bot 94:593–603

McElwain JC, Punyasena SW (2007) Mass extinction events and the plant fossil record. Trends Ecol Evol 22:548–557

McElwain JC, Beerling DJ, Woodward FI (1999) Fossil plants and global warming at the Triassic-Jurassic boundary. Science 285:1386–1390

Montañez IP, Tabor NJ, Niemeier D, DiMichelle W, Frank TD, Fielding CR, Isbell JL, Birgenheier LP, Rygel MC (2007) CO_2-forced climate and vegetation instability during late Palaeozoic deglaciation. Science 315:87–91

Monteith JL, Unsworth MH (1990) Principles of environmental physics, 2nd edn. Edward Arnold, London

Newton AE, Wikström N, Bell N, Forrest LL, Ignatov M (2007) Dating the diversification of pleurocarpous mosses. In: Newton AE, Tangney R (eds) Pleurocarpous mosses. Taylor and Francis, Boca Raton, pp 337–366

Nichols DJ, Johnson KR (2008) Plants and the K–T boundary. Cambridge University Press, New York

Nobel PS (1977) Internal leaf area and cellular CO_2 resistance: photosynthetic implications of variations with growth conditions and plant species. Physiol Plant 40:137–144

Oliver MJ, Velten J, Mishler BD (2005) Desiccation tolerance in bryophytes: a reflection of the primitive strategy for plant survival in dehydrating habitats. Integr Comp Biol 45:788–799

Palmer JD, Soltis DE, Chase MW (2004) The plant tree of life: an overview and some points of view. Am J Bot 91:1437–1445

Penman HL (1948) Natural evaporation from open water, bare soil and grass. Proc R Soc Lond A194:120–145

Pressel S, Ligrone R, Duckett JG (2006) Effects of de- and rehydration on food-conducting cells in the moss *Polytrichum formosum*: a cytological study. Ann Bot 98:67–76

Pressel S, P'ng KMY, Duckett JG (2010) A cryo-scanning electron microscope study of the water relations of the remarkable cell wall in the moss *Rhacocarpus purpurascens* (Rhacocarpaceae, Bryophyta). Nova Hedwigia 91:289–299

Proctor MCF (1977) Evidence on the carbon nutrition of moss sporophytes from $^{14}CO_2$ uptake and the subsequent movement of labelled assimilate. J Bryol 9:375–386

Proctor MCF (1979a) Structure and eco-physiological adaptation in bryophytes. In: Clarke GCS, Duckett JG (eds) Bryophyte systematics. Academic, London, pp 479–509

Proctor MCF (1979b) Surface wax on the leaves of some mosses. J Bryol 10:531–538

Proctor MCF (1999) Water-relations parameters of some bryophytes evaluated by thermocouple psychrometry. J Bryol 21:269–277

Proctor MCF (2000) The bryophyte paradox: tolerance of desiccation, evasion of drought. Plant Ecol 151:41–49

Proctor MCF (2004) How long must a desiccation-tolerant moss tolerate desiccation? Some results of two years' data logging on *Grimmia pulvinata*. Physiol Plant 122:21–27

Proctor MCF (2005) Why do Polytrichaceae have lamellae? J Bryol 27:221–229

Proctor MCF (2007) Ferns, evolution, scale and intellectual impedimenta. New Phytol 176:504–506

Proctor MCF (2009) Physiological ecology. In: Goffinet B, Shaw AJ (eds) Bryophyte biology, 2nd edn. Cambridge University Press, Cambridge, pp 237–268

Proctor MCF (2010) Trait correlations in bryophytes: exploring an alternative world. New Phytol 185:1–3

Proctor MCF (2011) Climatic responses and limits of bryophytes: comparisons and contrasts with vascular plants. In: Slack N, Tuba Z, Stark LR (eds) Bryophyte ecology and climatic change. Cambridge University Press, Cambridge, pp 35–54

Proctor MCF (2012) Light and desiccation responses of some Hymenophyllaceae (filmy ferns) from Trinidad, Venezuela and New Zealand: poikilohydry in a light-limited but low evaporation ecological niche. Ann Bot 109:1019–1026

Proctor MCF, Smirnoff N (2011) Ecophysiology of photosynthesis in bryophytes: major roles for oxygen photoreduction and non-photochemical quenching at high irradiance in mosses with unistratose leaves? Physiol Plant 141:130–140

Proctor MCF, Tuba Z (2002) Poikilohydry and homoiohydry: antithesis or spectrum of possibilities? New Phytol 156:327–349

Proctor MCF, Nagy Z, Csintalan ZS, Takács Z (1998) Water-content components in bryophytes: analysis of pressure – volume relationships. J Exp Bot 49:1845–1854

Proctor MCF, Ligrone R, Duckett JG (2007a) Desiccation tolerance in the moss *Polytrichum formosum*: physiological and fine-structural changes during desiccation and recovery. Ann Bot 99:75–93

Proctor MCF, Oliver MJ, Wood AJ, Alpert P, Stark LR, Cleavitt NL, Mishler BD (2007b) Desiccation tolerance in bryophytes: a review. Bryologist 110:595–621

Qiu Y-L, Li LB, Wang B, Chen ZD, Knoop V, Groth-Malonek M, Dombrovska O, Lee J, Kent L, Rest J, Estabrook GF, Hendry TA, Taylor DW, Testa CM, Ambros M, Crandall-Stotler B, Duff RJ, Stech M, Frey W, Quandt D, Davis CC (2006) The deepest divergences in land plants inferred from phylogenomic evidence. Proc Natl Acad Sci USA 103:15511–15516

Raven JA (1977) The evolution of vascular land plants in relation to supracellular transport processes. Adv Bot Res 5:153–219

Raven JA (1984) Physiological correlates of the morphology of early vascular plants. Bot J Linn Soc 88:105–126

Raven JA (1996) Into the voids: the distribution, function, development and maintenance of gas spaces in plants. Ann Bot 78:137–142

Raven JA, Edwards D (2001) Roots: evolutionary origins and biogeochemical significance. J Exp Bot 52:381–401

Raven JA, Handley LL (1987) Transport processes and water relations. New Phytol 106:217–233

Remy W, Hass H (1996) New information on gametophytes and sporophytes of *Aglaophyton major* and inferences about possible environmental adaptations. Rev Palaeobot Palynol 90:175–193

Remy W, Gensel PG, Hass H (1993) The gametophyte generation of some early Devonian land plants. Int J Plant Sci 154:35–58

Scott AC, Glasspool IJ (2006) The diversification of Paleozoic fire systems and fluctuations in atmospheric oxygen concentration. Proc Natl Acad Sci USA 103:10861–10865

Shaw AJ (2009) Bryophyte species and speciation. In: Goffinet B, Shaw AJ (eds) Bryophyte biology, 2nd edn. Cambridge University Press, Cambridge, pp 445–485

Smirnoff N (ed) (2005) Antioxidants and reactive oxygen species in plants. Blackwell Publishing Ltd., Oxford

Smith GM (1938) Cryptogamic botany, vol 2, Bryophytes and pteridophytes. McGraw-Hill, New York

Soltis PS, Soltis DE (2004) The origin and diversification of angiosperms. Am J Bot 91:1614–1626

Stark LR, Oliver MJ, Mishler BD, McLetchie DN (2007) Generational differences in response to desiccation stress in the desert moss *Tortula inermis*. Ann Bot 99:53–60

Steemans P, Le Hérissé A, Melvin J, Miller MA, Paris F, Verniers J, Wellman CH (2009) Origin and radiation of the earliest vascular land plants. Science 324:353

Taylor TN, Kerp H, Hass H (2005) Life history biology of early land plants: deciphering the gametophyte phase. Proc Natl Acad Sci USA 102:5892–5897

Watkins JE, Mack MC, Sinclair TR, Mulkey SS (2007) Ecological and evolutionary consequences of desiccation tolerance in tropical fern gametophytes. New Phytol 176:708–717

Whiteside JH, Olsen PE, Eglinton T, Brookfield ME, Sambrotto RN (2010) Compound-specific carbon isotopes from Earth's largest flood basalt eruptions directly linked to the end-Triassic mass extinction. Proc Nat Acad Science USA 107:6721–6725

Whittaker DL, Edwards J (2010) *Sphagnum* moss disperses spores with vortex rings. Science 329:406

Wing SL, Boucher LD (1998) Ecological aspects of the cretaceous flowering plant radiation. Annu Rev Earth Planet Sci 26:379–421

Zotz G, Rottenberger S (2001) Seasonal changes in diel CO_2 exchange of three Central European moss species: a one-year field study. Plant Biol 3:661–669

Zotz G, Schweikert A, Jetz W, Westerman H (2000) Water relations and carbon gain are closely related to cushion size in the moss *Grimmia pulvinata*. New Phytol 148:59–67

Chapter 5

Best Practices for Measuring Photosynthesis at Multiple Scales

Steven K. Rice*
Department of Biological Sciences, Union College, Schenectady, NY 12308, USA

J. Hans C. Cornelissen
Systems Ecology, Department of Ecological Science, VU University, Amsterdam, The Netherlands

Summary

Studies of bryophyte photosynthetic performance have generally adapted techniques developed for use in vascular plants and relied on underlying vascular plant functional models as guides. Within this context, bryophytes present intellectual and methodological challenges, but also opportunities relative to their vascular plant counterparts. For example, although the leaf is clearly a functional unit for vascular plants, the comparable bryophyte structure may or may not serve a similar purpose. Instead, shoot systems and their organization into canopies are often employed as the functional equivalent. Unfortunately, due to issues of scale and alternative functional demands on bryophyte shoots like external transport and nutrient

*Author for correspondence, e-mail: rices@union.edu

D.T. Hanson and S.K. Rice (eds.), Photosynthesis in Bryophytes and Early Land Plants, Advances in Photosynthesis and Respiration 37, pp. 79–93, DOI 10.1007/978-94-007-6988-5_5,

uptake, neither the methodologies nor the underlying models that lead to an integrated understanding of photosynthesis in vascular plants apply well to bryophytes. This chapter will consider the appropriate functional units for studies of bryophyte photosynthesis and relate it to the growth form and life form literature. Methods to characterize photosynthetic "leaf" area, water content, and canopy structure will be evaluated relative to their use in characterizing rates of photosynthesis. In addition, various methods are used to study photosynthetic function and these will be considered in light of their appropriate spatial and temporal domains.

I. Introduction

Technological advances have allowed organismal plant physiologists to shift focus from leaves to canopies to ecosystems, from the lab to the field to remote sensing from space and from seconds to seasons or longer and into deep time using fossils and environmental reconstruction. Different methods to evaluate photosynthetic function apply across such broad spatial and temporal scales (Fig. 5.1) and many of these have been recently developed, improved, and/or made more widely available.

Investigators studying bryophyte function have often adapted techniques developed for use in vascular plants. Within this context, bryophytes present challenges caused by their size and slow rate of photosynthetic tissues (Martin and Adamson 2001), by dramatic dependence of photosynthesis on plant water status, and by lack of accepted standard practices. However, bryophytes also present opportunities not only as contributors to carbon dynamics of widespread ecosystems, but also as subjects to study the integration of leaf, shoot and canopy processes. This chapter will review the organization of bryophyte photosynthetic systems as it relates to photosynthetic function and propose standards that can guide measurement and reporting of photosynthetic rates.

Abbreviations: LAI – Leaf area index; P_{max} – Maximum rate of net photosynthesis; ϕ_{PSII} – Quantum yield of photosystem II; SAI – Shoot area index; STAR – Shoot silhouette to needle leaf area ratio

II. The Photosynthetic Organ in Bryophytes

A. Life Forms and Photosynthesis

Growth, development and organization of bryophyte shoot systems is modular and hierarchical (Fig. 5.2). In leafy forms, which comprise the vast majority of bryophyte species (100 % of >10,000 mosses, 85 % of 6,000–8,000 liverworts, although 0 % of 300 hornworts; Buck and Goffinet 2000; Crandall-Stotler and Stotler 2000; Vanderpoorten and Goffinet 2009), normally unistratose leaves (i.e., phyllids) are arranged on branches and stems, which in turn, organize into shoots and shoot systems by characteristic cell division at apices and/or by growth from subapical buds. Although developed from variants of a common plan, the morphological patterns that result differ considerably and have important functional consequences (Gimingham and Birse 1957; Scholfield 1981; Hedderson and Longton 1996; Kürschner et al. 1999; Cornelissen et al. 2007; Rice et al. 2008; Waite and Sack 2010; Elumeeva et al. 2011). The bryophyte canopy, affected by the size, density and arrangement of leaves, branches, shoots and shoot systems, is generally accepted as the primary functional unit of bryophytes as it relates to carbon and water dynamics (During 1992; Proctor 1990, 2000; Bates 1998; Cornelissen et al. 2007; Zotz and Kahler 2007; Waite and Sack 2010). Although the bryophyte canopy has served as the primary focus of functional studies, there lack standard methodologies that allow for easy comparison among studies.

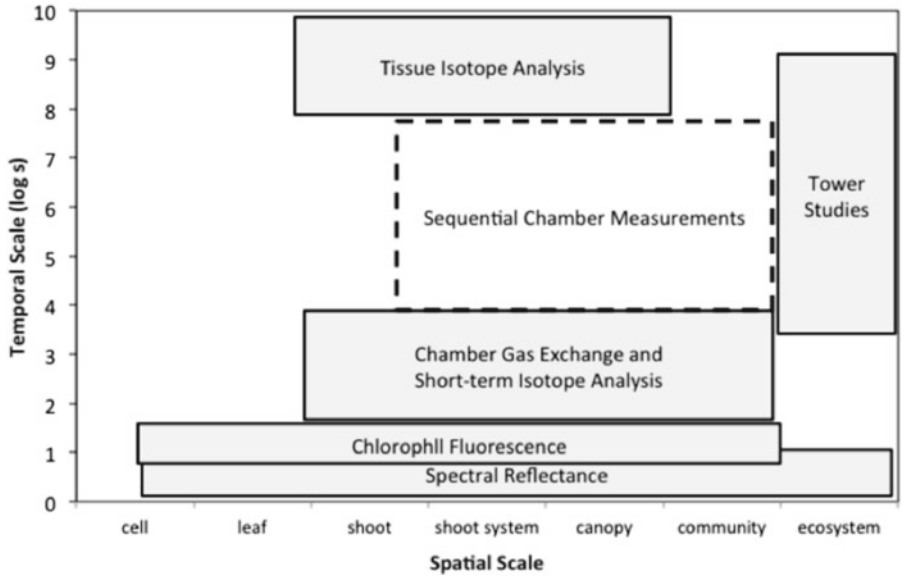


Fig. 5.1. Scale of photosynthesis measurements. Each set of techniques used to evaluate photosynthetic function occupies limited spatial and temporal domains. Most studies utilize chamber gas exchange in small diameter (<10 cm diameter) samples or chlorophyll fluorescence probes, which mainly evaluate <1 cm diameter regions. Used sequentially, or with larger chambers or imaging techniques, these may have extended application in space and time (dashed line), although see Bader et al. (2009) for limitations of temporal scaling in poikilohydric organisms. To evaluate photosynthesis at larger temporal and spatial scale, functional performance gets integrated over space and time, with a loss in resolution within those domains.

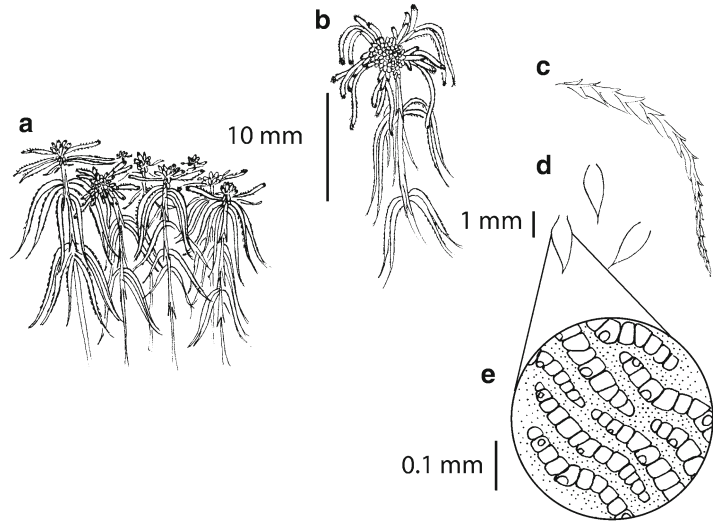


Fig. 5.2. Organization of Bryophyte photosynthetic systems. Variation in photosynthesis may be caused by differences in the structure and organization of units at many scales. The hierarchical arrangement of photosynthetic units shown for a *Sphagnum* species at decreasing scale from (**a**) canopy; (**b**) shoot; (**c**) branch; (**d**) leaf; and (**e**) cell (Drawing by S. Webb, adapted from Rice (2009)).

The desire to characterize shoot system organization has arisen from two arenas. One focus emerged from interest in identifying taxonomic characters and establishing homology for use in classification and systematics (Hedenäs 2002; Newton 2007). Although often considered in a functional context, it is clear that similar functional states can arise from different branching architectures and morphologies, although there is evident conservatism at the level of genera or family (Hedderson and Longton 1996; Hedenäs 2002). Alternatively, canopy structure has been considered in more functional terms in the discussion of life forms. This concept emerged from the notion of growth forms (Gimingham and Birse 1957; Gimingham and Smith 1971) that sought to characterize different canopy structures that related to their function, although with specific reference to the underlying patterns of growth and branching that create them. Life forms developed from this idea with a greater emphasis on function (Magdefrau 1982; During 1992; Bates 1998). In studies of polar (Gimingham and Smith 1971; Fowbert 1996), temperate (Gimingham and Birse 1957) and tropical (Kürschner et al. 1999) species, variation in growth or life form classifications associates significantly with environmental conditions, especially factors that affect water relations. Given the poikilohydric nature of bryophytes, plant water status, particularly the length of time plants remain hydrated, controls long-term carbon gain in many environments (Proctor 2000; Zotz et al. 2000; Rice and Schneider 2004; Mishler and Oliver 2009). Consequently, life forms may provide a suitable, general scheme for considering production. However, short-term dynamics affected by light interception and carbon exchange may not be adequately differentiated by the life form groupings, or if they are, there has been little research aimed at understanding these relationships (Bates 1998). If life forms are inadequate for quantifying canopy variation as it relates directly to photosynthetic processes, what alternatives are available? Recent research has explored the use of quantitative, continuous traits in the place of life form groupings to understand and predict canopy-level physiological function (Rice et al. 2008, 2011b; Cornelissen et al. 2007; Waite and Sack 2010; Elumeeva et al. 2011). In vascular plants, this approach has led to the development of broadly applicable models that link plant traits to photosynthetic function (Wright et al. 2004) and offers promise for the study of bryophytes.

Carbon and water dynamics of thalloid forms such as those in liverworts and hornworts have often been considered analogous to vascular plant leaf function. In complex, ventilated thalli like those in some genera in the Marchantiaceae, internal compartments increase the internal surface area relative to that of the leaf surface, thereby increasing maximal rates of photosynthesis (Proctor 1980; Green and Lange 1995; Meyer et al. 2008). With epicuticular waxes impeding water and CO_2 movement, pores on the thallus surface restrict, but do not exert short-term control over water and carbon diffusion. Species with simple, solid thalli, experience higher diffusion resistances and have lower rates of photosynthesis (Meyer et al. 2008), although carbon concentration mechanisms may overcome this limitation (Griffiths et al. 2004; see Chapter 6). When expressed on a chlorophyll basis, rates of net photosynthesis for complex thalli are comparable with vascular plant leaves, although they are much lower when expressed relative to dry mass (Green and Lange 1995). This difference is partly caused by the multiple functions of the thallus as it serves as the primary organ for water and nutrient uptake and storage, in addition to photosynthesis, a constraint shared with leafy bryophytes as well.

B. Functional Trait Relationships in Bryophytes

Although often considered analogous with vascular plant leaves as a photosynthetic unit with bryophyte leaves performing the role of mesophyll, recent studies have shown that many functional trait relationships observed in vascular plant leaves are not found in

bryophyte canopies. For example, the robust relationship observed in vascular plants between leaf maximum rates of photosynthesis and nitrogen (Hikosaka 2004; Wright et al. 2004) has not been observed in multi-species comparisons in either Hawaiian forest mosses (Waite and Sack 2010) or in a multi-species comparison of *Sphagnum* (Rice et al. 2008). However, bryophyte canopies show some similar trait relationships with both studies indicating strong negative relationships between canopy mass per area and maximum rates of photosynthesis expressed on a mass basis, a similar pattern found when comparing leaf mass per area and maximum assimilation on a mass basis for vascular plant leaves (Wright et al. 2004). In the forest mosses, low rates of maximum photosynthesis were associated with increased costa length and width, which correlate with increased structural support and plant height, characteristics that influence photosynthetic efficiency, the former by the allocation of non-photosynthetic tissues and the latter by decreasing the light efficiency of photosynthesis through self-shading (Waite and Sack 2010). In *Sphagnum*, allocation to non-photosynthetic hyaline cells, which contribute to enhanced water holding capacity, reduces photosynthetic efficiency on a mass basis. In *Sphagnum*, the distribution of mass within the canopy exerts primary influence on photosynthetic assimilation on a mass basis—species that concentrate mass in the upper-canopy achieve higher rates of maximal assimilation (Rice et al. 2008).

These traits that associate with biomass allocation patterns and affect support or water storage also have vascular plant leaf analogues. However, some shoot functions in bryophytes like nutrient uptake are more important than in vascular plants and these create alternative trait relationships. For example in *Sphagnum*, cell wall polyuronic acids, which are involved in ion exchange and sequestration, are responsible for up to 30 % of shoot dry weight (Clymo 1963; Popper and Fry 2003; Kremer et al. 2004) and shoot water storage is strongly and negatively correlated with maximum assimilation (Rice et al. 2008), relationships that will not affect the leaf economics spectrum of vascular plant leaves. Consequently, although they share some similarities, the bryophyte canopy represents a unique functional type.

C. Photosynthesis-Related Traits and the Carbon Balance of Bryophytes

While photosynthesis is obviously the key pathway for carbon sequestration by bryophytes, it is only one of the processes that determines the overall carbon gain of individual living bryophytes. Their net carbon gain will also depend on the allocation of photosynthates to (1) compounds and tissues promoting further photoassimilation versus those (2) promoting longer tissue lifespan through protective chemistry, including anti-herbivore defense (Coley 1988; Glime 2006; Cornelissen et al. 2007); or those (3) supporting organs for vegetative or generative reproduction (During 1979). Actual losses of tissues to physical damage, pathogens or herbivore attack will have direct negative effects on net carbon gain of individual bryophytes. At the ecosystem scale, the carbon balance of the bryophyte compartment depends on the balance between net carbon gains of living tissues and carbon losses from dead bryophyte tissues (Clymo and Hayward 1982; Gorham 1991; Clark et al. 1998; Cornelissen et al. 2007; Limpens et al. 2008). Microbial decomposition and fire (Kuhry 1994) are the predominant pathways for such losses. As for fire, a preliminary screening in a fire laboratory (methods in van Altena et al. 2012) indicated that some moss species were more flammable than others in the Dutch flora (NA Soudzilovskaia and JHC Cornelissen, in preparation); *Pleurozium schreberi* was more flammable in terms of rate of fire spread and fire temperatures and also continued to ignite at higher moisture content than *Hypnum jutlandicum* and *Polytrichum commune*, respectively. However, investigations on the differential effects of bryophyte species on fire regimes are still in their very infancy.

We know a bit more about bryophyte species and decomposition. It is now well established that bryophyte litter generally decomposes slowly compared to that of vascular plants, even in given environmental regimes (Hobbie 1996; Lang et al. 2009). But also within bryophytes as a group great variation in litter decomposition rate has been reported among higher clades and species (Lang et al. 2009). *In situ* decomposition rates of bryophyte litter of different species are strongly driven by both environmental (biotic and abiotic) conditions of their actual habitats and species traits, and their interactions (Clymo and Hayward 1982; Limpens and Berendse 2003; Turetsky et al. 2008; Lang et al. 2009). However, different bryophyte species also show consistent and large variation in litter decomposability at given environmental regime (Lang et al. 2009). For instance, *Sphagnum* species are generally among the most recalcitrant bryophytes around worldwide (Clymo and Hayward 1982; Scheffer et al. 2001; Dorrepaal et al. 2005; Lang et al. 2009) and this has been attributed to their anti-microbial phenolic chemistry (Verhoeven and Liefveld 1997) as well as to polysaccharide deposits in cell walls (Hajek et al. 2011). It is important to recognize that 'a *Sphagnum* is not a *Sphagnum*' as even within this genus ten-fold trait-driven variation in decomposition rates has been reported between different species, with hummock species tending to be more recalcitrant than hollow species (Johnson and Damman 1991; Rydin et al. 2006; Lang et al. 2009). Such differences have been attributed to chemical traits as well. Turetsky et al. (2008) pinpointed the ratio between structural and non-structural carbohydrates as a good predictor of interspecific variation in *Sphagnum* decomposition. Lang et al. (2009) also simultaneously compared multiple subarctic non-*Sphagnum* bryophyte species for litter decomposability in standard outdoor litter matrices. They found a comparable five-to six-fold range of litter mass loss rates both among moss species and among liverwort species. Such strong inherent variation in traits that drive litter decomposability has implications for the consequences of environmentally driven shifts in bryophyte species composition for ecosystem carbon budgets. However, the critical issue is ultimately how the concomitant shifts in carbon release play out relative to the species' productivity responses. In theory, if there were perfect one to one correspondence of productivity and decomposability across species, the net species effect on the carbon balance should be nil. This is still a virtually blank field of research as, to our knowledge, there are no multispecies studies that compare patterns of variation between photosynthetic rates, growth rates and decomposabilities. However, we do have a few preliminary pointers from combining different literatures based on high-latitude experiments with bryophytes. Skre and Oechel (1981) screened five boreal moss species for photosynthetic rates under a range of environmental conditions to derive P_{max}, under the assumption that at least one of the experimental environmental regimes would be close to the optimum for a given species. *Polytrichum commune* had the highest P_{max} (2.65 mg CO_2 g^{-1} h^{-1}) and this species was also the fastest decomposing moss species in the mentioned subarctic multispecies litter decomposability screening, where all species were exposed simultaneously to the same environment for microbial decomposition (Lang et al. 2009). *Hylocomium spendens* and *Pleurozium schreberi* had intermediate P_{max} (1.39 and 1.20 CO_2 g^{-1} h^{-1}) and these two species also had intermediate to high decomposability in the study by Lang et al. The two *Sphagnum* species tested by Skre and Oechel (1981), *S. nemoreum* and *S. subsecundum*, had particularly low P_{max} (0.25 and 0.57 CO_2 g^{-1} h^{-1}). While these species were not included in the decomposability screening, it is likely based on the *Sphagnum* evidence described above that these two species would have been very recalcitrant to decomposition compared to the other three species. This indirect comparison suggests a positive relationship between potential

photosynthetic rates and potential litter decomposition rates among bryophytes, which would match the evidence for photosynthesis-related traits and decomposability among vascular plants (e.g. Cornelissen and Thompson 1997; Cornwell et al. 2008). Also, Furness and Grime (1982) screened multiple bryophyte species in the NW European flora for relative growth rates (RGR) in biomass terms, in a standardized greenhouse setup. Four of their species were common with the litter decomposability screening of Lang et al. (2009) and broadly the RGR ranking corresponded with the litter decomposability ranking: *Racomitrium lanuginosum* < *Aulacomnium palustre* < *Hylocomium splendens* = *Polytrichum commune*. In contrast, within the genus *Sphagnum* four species (*S. balticum*, *S. fuscum*, *S. teres*, *S. riparium*) measured for productivity by Gunnarsson (2005) did not match in rank with decomposability measured by Lang et al. (2009). To sum up, it is obvious from these poorly matched combinations of studies that much work needs to be done before we can make any robust linkages between interspecific variation in photosynthesis and growth related traits of bryophytes on one side and their litter decomposabilities and flammabilities on the other; and on the interactions of these linkages with vascular plants and their litters. Progress in this field would greatly improve our predictive power of consequences of species shifts for the carbon balance of bryophyte-dominated ecosystems.

III. Standardizing Photosynthetic Measurements

The bryophyte canopy represents a unique functional type as it relates to carbon and water dynamics. In this section, we review tissue and canopy characteristics that may serve as appropriate standards to develop a better understanding of the mechanisms that cause variation in bryophyte photosynthesis.

A. Surface Roughness

Important differences in canopy structure that relate to boundary layer properties and, hence, water loss, have been summarized by measuring variation in shoot height in intact canopies. Hayward and Clymo (1983) calculated the variance in canopy height measurements obtained using a contact probe at 8 mm increments to parameterize an evaporation model for *Sphagnum* colonies. More recently, Rice et al. (2005) developed a non-contact laser scanning technique that provides fine-scale canopy height measurements that they use to calculate a surface roughness parameter based on semivariance analysis. This analysis provides a measure of the variance of canopy height measurements at the scale of canopy exchange elements (leaf, shoot or shoot system, depending on the species) and is less likely to be influenced by the spacing of canopy sampling. Krumnikl et al. (2010) demonstrate that even greater resolution can be obtained using stereoscopic imaging. Surface roughness obtained using scanning methods indirectly relates to the thickness of external boundary layers, but directly to conductance of water from the bryophyte surface (Rice et al. 2000). However, surface roughness has been shown to be unrelated to differences in canopy light dynamics as summarized by light extinction coefficients or to variation in canopy photosynthetic characteristics in a multiple species comparison of *Sphagnum* (Rice et al. 2008) or in intraspecific studies of gas exchange in *Pleurozium schreberi* (Rice et al. 2010). Although it is likely that surface roughness affects light capture, particularly at low angles of directional light, thereby influencing daily production, it presently remains of limited use in studies of photosynthesis.

B. Area- and Mass-Based Measurements

Depending on the purpose of study, rates of photosynthesis in bryophytes have been expressed relative to leaf area, shoot area, projected canopy area, canopy dry mass and/or chlorophyll concentrations. The distribution

within the canopy of leaf area, shoot area, or dry mass also can be used to characterize canopy structure in a way that relates meaningfully to function.

Although leaf area based rates of photosynthesis allow for functional comparisons with vascular plant leaves (Nobel 1977) or among different bryophyte leaf types (Krupa 1984), they have only been performed on species with large, non-overlapping leaves (e.g., *Mnium* spp., *Polytrichum* spp.). Given that leaves are not independent functional units for most species and that leaf area is sufficiently difficult to obtain, leaf area has not been a common metric to standardize or compare rates of photosynthesis in bryophytes. Most investigators, instead, employ either projected canopy area (i.e., ground area) if they are interested in ecological questions that have a spatial component (e.g., community interactions or ecosystem fluxes) or they utilize mass based measurements as this standardizes values relative to plant carbon. Recognizing that bryophyte shoots (i.e., stems and leaves) can serve as appropriate functional units, recently researchers have used shoot area as a standard as well. Below, we discuss these various measures as summaries of canopy traits and comment on their utility in studies of bryophyte photosynthesis.

In broad-leaved, vascular plant canopies, carbon exchange is often expressed relative to or compared with total canopy leaf area. Canopy leaf area is often summarized using the leaf area index (LAI, m^2/m^2), the total single-sided leaf area relative to the ground area. When expressed in this manner, the canopy photosynthetic rate is a function not only of leaf-level photosynthetic response, but of canopy properties that affect light availability (e.g., self-shading, leaf angle) and the distribution of physiological characteristics of leaves throughout the canopy, properties that may vary due to differences in leaf age, to acclimation to light levels within the canopy, and/or to allocation of resources like N differentially within the canopy (Chap. 9).

Given their dense, often overlapping needle-like leaves, conifers, perhaps, present a more appropriate model for understanding how to estimate leaf area for photosynthetic studies of bryophytes. In conifers, clustering of leaves with non-uniform orientation causes self-shading, but also allows deeper light penetration (Thérézein et al. 2007). Due to the interaction of light and leaves within conifer shoots, projected area of shoots insufficiently characterizes light dynamics. Instead, the shoot silhouette area to total needle area ratio (STAR) has been developed to better characterize shoot—light dynamics (Stenberg et al. 2001; Thérézein et al. 2007; Smith and Hughes 2009). In conifers, shoots that have a higher density of leaves as those grown in open conditions, have low values of STAR, whereas flattened or low density needles on branches lead to higher values. In addition, variation in STAR associates strongly ($r = 0.99$) with light interception efficiency in samples of Scots pine, *Pinus sylvestris*, grown in different light environments (Stenberg et al. 2001). Presently, there are no studies of bryophytes where STAR has been calculated, although it may be very useful to standardize across species or studies. Measurements require projected silhouette areas, which can be obtained on excised bryophyte canopy samples, together with whole canopy leaf area, which is possible, although difficult to measure as described above.

In bryophytes, leaf area is difficult to obtain although modern photographic and scanning methods have made it easier (see Bond-Lamberty and Gower 2007 for method). In general, LAI measurements range from 6 to over 140 (Simon 1987; Vitt 1990; Proctor 2000), with generally lower values associate with acrocarpous species with low leaf densities. Except for the low reported values, these are much greater than the leaf area of vascular plant canopies (range 1 to over 20, Barnes et al. 1998). Indeed, bryophyte LAI values correspond more closely with the mesophyll area in vascular plants, where ratio of mesophyll area to leaf area is normally between 10 and 40 (Nobel and Walker 1985). These considerations have led to the suggestion that bryophyte

canopies and vascular leaf mesophyll are functionally analogous. Unfortunately, differences in the scale of the exchange surface and stomatal control in vascular plant mesophyll limits the usefulness of this analogy for development of unified models of function. To our knowledge, there are few studies of photosynthesis that have reported leaf area or report results on a leaf area basis (for exceptions, see Nobel 1977; Krupa 1984). Given that bryophyte leaves often significantly overlap and do not function independently from adjacent leaves, leaf area is not normally a useful way to characterize bryophyte canopies.

Instead, area based measurements normally focus on ground area. Given that community (e.g. species colonization or replacement) and ecosystem (e.g., fluxes of H_2O or CO_2) processes have important spatial components where ground based measures relate to biological function, these are often the most ecologically relevant. However, these measurements do not allow for the development of an understanding about how the organization of the primary functional unit, the canopy, affects physiological function. In other words, this focus does not provide adequate information about mechanisms that link organismal form or within-canopy physiological variation to whole-organism function that would further our understanding of bryophyte photosynthesis.

Occupying the scale between leaf area and ground area is the area of the shoot system. In many species with small, overlapping leaves including most pleurocarps and many acrocarps, shoots represent a relevant unit for exchange of water and energy. Consequently, the shoot area index (SAI; shoot area per ground area) has been used to summarize light dynamics in studies of light attenuation within bryophyte canopies (van der Hoeven et al. 1993; Williams and Flanagan 1998; Rice et al. 2011a, b) as well as serving as a way to standardize rates of photosynthesis (Williams and Flanagan 1998; Rice et al. 2011a, b). The distribution of shoot area can also be measured vertically within the canopy and help lead to a mechanistic understanding of light and photosynthetic function within canopies. However, rapid light attenuation and senescence instead of acclimation of shoots to low light within the canopy limits the contribution of the canopy interior to whole-plant photosynthesis, which has been shown in *Tortula* (=*Syntrichia*) *ruralis* (Zotz and Kahler 2007), in *Pleurozium schreberi* (Tobias and Niinemets 2010) and in *Sphagnum balticum* and *S. fuscum* (Johansson and Linder 1980). However, with greater recognition of photoinhibitory processes that are localized in the upper canopy (Chap. 7), studies relating the vertical stratification of shoot area will be a valuable component of understanding canopy carbon dynamics. In addition, in many ectohydric species, SAI also varies directly with water holding capacity as shoots serve an important water storage function. This leads to their use as an important parameter in bryophyte production models that seek to integrate bryophyte carbon and water dynamics (Rice et al. 2010).

Shoot area can be obtained with similar techniques as leaf area using fine resolution scanners or imaging microscopy. Shoot area measurements are used to calculate a shoot area to dry weight ratio and SAI is estimated using canopy dry weight. The measure is normally the projected shoot area, not the sum of leaf area of a shoot, although conversions to reflect the surface area of non-flat shoots have been employed (Bond-Lamberty and Gower 2007). Expressed for green tissue relative to the ground area, SAI provides an indicator that is easily comparable among species, which can be expressed in an index relative to ground area and that can be measured at different depths within the canopy. Although this measure has been used to model light dynamics and as a unit measure for photosynthesis, comparative data are few. Van der Hoeven et al. (1993) found SAI values that ranged from 4 to 7 for *Calliergonella cuspidata*, *Rhytidiadelphus squarrosus* and *Ctenidium molluscum* (estimated for green tissue from data presented), although total canopy SAI including brown

tissue could be greater than 20. For *Pleurozium schreberi*, Williams and Flanagan (1998) and Rice et al. (2011a, b) obtained SAI values in the range of 1.6–4.8. In the latter study using 25 field collected samples, SAI was the strongest predictor of light saturated rates of photosynthesis, which were expressed on a ground area basis ($R^2 = 0.41$).

In many bryophytes including cushion forming and some acrocarpous mosses, SAI may be difficult to obtain due to the high density of shoots. Instead of converting to shoot area, canopy dry weight expressed alone or per unit ground area is a reasonable unit for species comparisons (Alpert and Oechel 1987; Zotz and Rottenberger 2001; Rice et al. 2008; Waite and Sack 2010) as well as for understanding physiological dynamics within canopies (Zotz and Kahler 2007). Mass based measures correlate well with SAI (Rice et al. 2011a, b).

C. Chlorophyll

In addition to characterizing variation in the amount or distribution of photosynthetic tissues, the photosynthetic efficiency of these tissues also affects whole plant carbon dynamics. Expressing photosynthetic rates relative to concentrations of light harvesting pigments provides an indication of the efficiency of light capture. Although vascular plants have approximately two to tenfold higher rates of photosynthesis expressed on a weight basis compared with bryophytes, the rates are much more similar when standardized using chlorophyll (Green and Lange 1995). In comparative studies, differences in total chlorophyll (a + b) concentrations show high positive correlations with mass or area based measures of photosynthesis (Rice et al. 2008; Waite and Sack 2010; Rice et al. 2011b). Chlorophyll also varies vertically within canopies and Tobias and Niinmets (2010) suggest patterns of decreasing chlorophyll concentrations they observed within *Pleurozium schreberi* canopies reflect senescence of photosynthetic tissues in older shoots within the canopy interior. Similar patterns were found in *Tortula* (=*Syntrichia*) *ruralis* (Zotz and Kahler 2007).

D. Effects of Water

At saturating light, photosynthesis in bryophytes shows strong dependence on water content. The response is typically unimodal, with decreased rates of photosynthesis at higher water contents due to additional external water films that increase diffusion resistance and at low water contents due to biochemical changes that accompany tissue desiccation (Dilks and Proctor 1979; Proctor 1980). Although the response curve is typically asymmetrical about the maximum, the details about the curve vary from one species to the next. Indeed, in *Sphagnum* optimal water contents varied from 12 to 26 g H_2O/g dry weight among ten species (Rice, unpublished data, 2008). Given that maximal rates of photosynthesis are often two to over three times higher than those at full water content, measurements of photosynthesis can be quite sensitive to plant water status.

Although some studies that focus on the physiological effects of water content report full or partial response curves, many studies typically report that they remove excess water from the plant surface using a drip-dry or blotting technique. In our experience, these treatments can satisfactorily place plants near a water content optimal for photosynthesis. However, given the sensitivity described above doing this alone is insufficient. Instead, full or partial water content curves should be performed to establish the water content where maximal photosynthesis occurs and this water content should be replicated in the pre-treatments. If situations where non-optimal water contents are preferred (e.g., ecological conditions where high or low water contents are found), the photosynthetic—water content relationships will establish a context for the particular measurements. This will aid in better comparisons among studies and species. (note: see Chap. 13 for recommendations to deal with rapid drying in photosynthetic chambers).

As a measure of plant water status, plant water content does not allow for useful comparisons among species. Indeed, plant water contents vary considerably among bryophytes and this variation can be caused by differences in cell wall thickness, specialized water-holding cells, organs like paraphyllia, leaf size, shape or arrangement, or by other aspects of shoot organization. These differ in regards to their effect on water in the apoplast, in the symplast or held externally. Consequently, water content as a measure of plant water status is not adequately comparative across species. Instead, techniques have been developed to quantify plant water potential and determine the water content where physiologically important states like cell turgor loss point are achieved (Proctor et al. 1998; Proctor 1999). For example, Hájek and Beckett (2008) performed photosynthetic drying curves on five *Sphagnum* species and evaluated photosynthetic activity using the chlorophyll fluorescence parameter ϕ_{PSII}. The water content where cell turgor was lost, represented the point where ϕ_{PSII} began to decline and there was a strong quantitative relationship between these parameters. The relative water content (relative to the water content when external water has fully evaporated) at turgor loss varied by almost a factor of two (0.36–0.62) among the species. Consequently, relative water content is a coarse measure of plant water status, at least in how it relates to physiological state.

E. Sampling

In terms of their morphology and physiology, bryophytes display high levels of phenotypic plasticity that can alter photosynthetic dynamics (Tobias and Niinemets 2010; Rice et al. 2011a, b). In addition, their response to desiccation or other physiological stress may lead to prolonged recovery that needs to be considered when evaluating photosynthesis, especially when most studies of photosynthesis in bryophytes use field collected material for evaluation. This approach combines environmental and genetic variation and provides insight into the behavior of plants acclimated to the conditions where they grow, conditions that are ecologically relevant. However, investigators need to be careful to ensure that samples are fully recovered from transient stress, unless of course, it is the recovery that is of particular interest. Species express different recovery times in relation to full or partial desiccation stress and the recovery may be affected by the duration and intensity of the stress (Proctor 2000; Proctor et al. 2007). Since the recovery times vary, it is prudent to perform preliminary trials with the study species to determine an appropriate pre-treatment.

Alternatively, there have been recent studies that utilize common garden conditions for physiological studies (Rice et al. 2008). Following adequate periods to allow for plant growth responses, these studies allow investigators to discriminate genetic differences in physiological performance. It is important that these studies focus on new tissue that developed following transplant or initiation of the environmental treatment. This might mean 4–8 weeks for some species like *Sphagnum* grown in optimum conditions to one or more growing seasons in bryophytes with slow growth rates.

IV. Best Practices for Studies of Photosynthesis

1. Employ the canopy as primary unit of study. If technical restrictions prevent this (e.g., using a chlorophyll fluorescence probe on a leaf or shoot), provide information on variation of the measure and its distribution within the canopy that would allow for scaling to canopy-level (see Chap. 9).
2. Provide sufficient information that would allow the conversion of measurements to be expressed on ground-area, dry weight of green tissue and chlorophyll bases. Also, when appropriate, report shoot area as this represents a useful comparative exchange unit for bryophytes.

3. Characterize plant water status and photosynthetic responses adequately. Studies should complete photosynthetic drying curves and report how water content during measurements relates to optimal water content. It is also worthwhile to perform more detailed analyses on the physiological water status by measuring plant water potentials and relating relative water contents to state transitions like the turgor loss point. Finally, it is useful to report on the recovery phase of photosynthesis from desiccation as this has important effects on plant carbon gain.
4. When scaling from short-term field measurements to seasonal or annual measurements, perform adequate sampling within days as well as over many days during the year. This will help overcome problems caused by variation in plant water content or other environmental factors such as light availability and temperature. Although this has not been explored quantitatively for bryophytes, suggestions made by Bader et al. (2009) for lichens should be considered.
5. If using field-collected samples in the lab, allow for sufficient acclimation and recovery from short-term physiological stress in the field. We have found some species require 4–6 day to achieve maximum rates of photosynthesis in mesic forest species. When genetic and not environmental variation is the study focus, employ a common garden approach.
6. Identify specimens to species, when possible. There remain too many studies that use ecological or generic groupings.

Acknowledgements

The authors thank Heinjo During, Nadia Soudzilovskaia, Tanya Elumeeva and Simone Lang for discussions and collaboration and acknowledge support of NWO (Dutch Science Foundation) through grants ALW 852.00.070 and N-047.018.003 to HCC, National Science Foundation grant 0922883 to SKR and to Union College for sabbatical leave for SKR to complete this project.

References

Alpert P, Oechel WC (1987) Comparative patterns of net photosynthesis in an assemblage of mosses with contrasting distributions. Am J Bot 74:1787–1796

Bader MY, Zotz G, Lange OL (2009) How to minimize the sampling effort for obtaining reliable estimates of diel and annual CO_2 budgets in lichens. Lichenologist 42:97–111

Barnes BV, Zak DR, Denton SR, Spurr SH (1998) Forest ecology, 4th edn. Wiley, New York

Bates JW (1998) Is "life-form" a useful concept in bryophyte ecology? Oikos 82:223–237

Benscoter BW, Vitt DH (2007) Evaluating feathermoss growth: a challenge to traditional methods and implications for the boreal carbon budget. J Ecol 95:151–158

Bond-Lamberty B, Gower ST (2007) Estimation of stand-level leaf area for boreal bryophytes. Oecologia 151:584–592

Buck WR, Goffinet B (2000) Morphology and classification of mosses. In: Shaw AJ, Goffinet G (eds) Bryophyte biology. Cambridge University Press, Cambridge, pp 71–123

Clark DL, Nadkarni NM, Gholz HL (1998) Growth, net production, litter decomposition, and net nitrogen accumulation by epiphytic bryophytes in a tropical montane forest. Biotropica 30:12–23

Clymo RS (1963) Ion exchange in *Sphagnum* and its relation to bog ecology. Ann Bot 27:309–324

Clymo RS, Hayward PM (1982) The ecology of *Sphagnum*. In: Smith AJE (ed) Bryophyte ecology. Chapman and Hall, New York, pp 229–289

Coley PD (1988) Effects of plant growth rate and leaf lifetime on the amount and type of antiherbivore defense. Oecologia 74:531–536

Cornelissen JHC, Thompson K (1997) Functional leaf attributes predict litter decomposition rate in herbaceous plants. New Phytol 135:109–114

Cornelissen JHC, Lang SI, Soudzilovskaia NA, During HJ (2007) Comparative cryptogam ecology: a review of bryophyte and lichen traits that drive biogeochemistry. Ann Bot 99:987–1001

Cornwell WK, Cornelissen JHC, Amatangelo K, Dorrepaal E, Eviner VT, Godoy O, Hobbie SE, Hoorens B, Kurokawa H, Pérez-Harguindeguy N, Quested HM, Santiago LS, Wardle DA, Wright IJ, Aerts R, Allison SD, van Bodegom P, Brovkin V, Chatain A, Callaghan TV, Díaz S, Garnier E, Gurvich DE, Kazakou E, Klein JA, Read J, Reich PB, Soudzilovskaia NA, Vaieretti MV, Westoby M (2008) Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. Ecol Lett 11:1065–1071

Crandall-Stotler B, Stotler RE (2000) Morphology and classification of the Marchantiophyta. In: Shaw AJ, Goffinet G (eds) Bryophyte biology. Cambridge University Press, Cambridge, pp 21–70

Dilks TJK, Proctor MCF (1979) Photosynthesis, respiration and water content in bryophytes. New Phytol 82:97–114

Dorrepaal E, Cornelissen JHC, Aerts R, Wallen B, Van Logtestijn RSP (2005) Are growth forms consistent predictors of leaf litter quality and decomposability across peatlands along a latitudinal gradient? J Ecol 93:817–828

During HJ (1979) Life strategies of bryophytes – preliminary review. Lindbergia 5:2–18

During HJ (1992) Ecological classification of bryophytes and lichens. In: Bates JW, Farmer AM (eds) Bryophytes and lichens in a changing environment. Clarendon Press, Oxford, pp 1–31

Elumeeva TG, Soudzilovskaia NA, During HJ, Cornelissen JHC (2011) The importance of colony structure versus shoot morphology for the water balance of 22 subarctic bryophyte species. J Veg Sci 22:152–164

Fowbert JA (1996) An experimental study of growth in relation to morphology and shoot water content in maritime Antarctic mosses. New Phytol 133:363–373

Furness SB, Grime JP (1982) Growth rate and temperature responses in bryophytes II. A comparative study of species of contrasted ecology. J Ecol 70:525–536

Gimingham CH, Birse EM (1957) Ecological studies on growth-form in bryophytes: I. Correlation between growth-form and habitat. J Ecol 45:533–545

Gimingham CH, Smith RIL (1971) Growth form and water relations of mosses in the maritime Antarctic. Br Antarct Surv Bull 25:1–21. http://www.antarctica.ac.uk/documents/bas_bulletins/bulletin25_02.pdf

Glime JM (2006) Bryophytes and herbivory. Cryptogam Bryol 27:191–203

Gorham E (1991) Northern peatlands: role in the carbon cycle and probable responses to climatic warming. Ecol Appl 1:182–195

Green TGA, Lange OL (1995) Photosynthesis in poikilohydric plants: a comparison of lichens and bryophytes. In: Schulze E-D, Caldwell MM (eds) Ecophysiology of photosynthesis. Springer, Berlin

Griffiths H, Maxwell K, Richardson D, Robe W (2004) Turning the land green: inferring photosynthetic physiology and diffusive limitations in early bryophytes. In: Hemsley AR, Poole I (eds) The evolution of plant physiology: from whole plants to ecosystems, chapter 1. Elsevier Academic Press, Amsterdam

Gunnarsson U (2005) Global patterns of *Sphagnum* productivity. J Bryol 27:269–279

Hájek T, Beckett RP (2008) Water content components on desiccation and recovery in *Sphagnum*. Ann Bot 101:165–173

Hajek T, Ballance T, Limpens J, Zijlstra M, Verhoeven JTA (2011) Cell-wall polysaccharides play an important role in decay resistance of *Sphagnum* and actively depressed decomposition in vitro. Biogeochemistry 103:1–3

Hayward PM, Clymo RS (1983) The growth of *Sphagnum*: experiments on, and simulation of, some effects of light flux and water-table depth. J Ecol 71:845–863

Hedderson TA, Longton RE (1996) Life history variation in mosses: water relations, size and phylogeny. Oikos 77:31–43

Hedenäs L (2002) Important complexes of intercorrelated character states in pleurocarpous mosses. Lindbergia 27:104–121

Hikosaka K (2004) Interspecific difference in the photosynthetic-nitrogen relationship: patterns, physiological causes, and ecological consequences. J Plant Res 117:481–494

Hobbie SE (1996) Temperature and plant species control over litter decomposition in Alaskan tundra. Ecol Monogr 66:503–522

Johansson LG, Linder S (1980) Photosynthesis in *Sphagnum* in different microhabitats on a Subarctic mire. In: Sonesson M (ed) Ecology of a subarctic mire, vol 30, Ecol bull., pp 181–190

Johnson LC, Damman AWH (1991) Species-controlled *Sphagnum* decay on a South Swedish raised bog. Oikos 61:234–242

Kremer C, Pettolino F, Bacic A, Drinnan A (2004) Distribution of cell wall components in *Sphagnum* hyaline cells and in liverwort and hornwort elaters. Planta 219:1023–1035

Krumnikl M, Sojka E, Gaura J, Motyka O (2010) Three-dimensional reconstruction of macroscopic features in biological materials. Comm Com Inf Sc 52:225–234

Krupa J (1984) Anatomical structure of moss leaves and their photosynthetic rate. Acta Soc Bot Pol 53:43–51

Kuhry P (1994) The role of fire in the development of *Sphagnum*-dominated peatlands in western boreal Canada. J Ecol 82:899–910

Kürschner H, Frey W, Parolly G (1999) Patterns and adaptive trends of life forms, life strategies and ecomorphological structures in tropical epiphytic bryophytes—a pantropical synopsis. Nova Hedwigia 69:73–99

Lang SI, Cornelissen JHC, Klahn T, van Logtestijn RSP, Broekman R, Schweikert W, Aerts R (2009) An experimental comparison of chemical traits and litter decomposition rates in a diverse range of

subarctic bryophyte, lichen and vascular plant species. J Ecol 97:886–900

Limpens J, Berendse F (2003) How litter quality affects mass loss and N loss from decomposing *Sphagnum*. Oikos 103:537–547

Limpens J, Berendse F, Blodau C, Canadell JG, Freeman C, Holden J, Roulet N, Rydin H, Schaepman-Strub G (2008) Peatlands and the carbon cycle: from local processes to global implications – a synthesis. Biogeosciences 5:1475–1491

Magdefrau K (1982) Life-forms of bryophytes. In: Smith I (ed) Bryophyte ecology. Chapman and Hall, New York, pp 45–57

Martin CE, Adamson VJ (2001) Photosynthetic capacity of mosses relative to vascular plants. J Bryol 23:319–323

Meyer M, Seibt U, Griffiths H (2008) To concentrate or ventilate? Carbon acquisition, isotope discrimination and physiological ecology of early land plant life forms. Philos T Roy Soc B 363:2767–2778

Mishler BD, Oliver MJ (2009) Putting Physcomitrella patens on the tree of life: the evolution and ecology of mosses. Ann Plant Rev 36:1–15

Newton AE (2007) Branching architecture in pleurocarpous mosses. In: Newton AE, Tangney S (eds) Pleurocarpous mosses: systematics and evolution. CRC Press, Boca Raton, pp 287–306

Nobel PS (1977) Internal leaf area and cellular CO_2 resistance: photosynthetic implications of variations with growth conditions and plant species. Physiol Plant 40:137–144

Nobel PS, Walker DB (1985) Structure of leaf photosynthetic tissue. In: Barber J, Baker NR (eds) Photosynthetic mechanisms and the environment. Elsevier Science Publishers BV, Amsterdam, pp 501–531

Popper ZA, Fry SC (2003) Primary cell wall composition of bryophytes and charophytes. Ann Bot 91:1–12

Proctor MCF (1980) Diffusion resistance in bryophytes. In: Grace J, Ford ED, Jarvis PG (eds) Plants and their atmospheric environment. Blackwell Scientific, Oxford, pp 219–229

Proctor MCF (1990) The physiological basis of bryophyte production. Bot J Linn Soc 104:61–77

Proctor MCF (1999) Water-relations parameters of some bryophytes evaluated by thermocouple psychrometry. J Bryol 21:263–270

Proctor MCF (2000) Physiological ecology. In: Shaw AJ, Goffinet B (eds) Bryophyte biology. Cambridge University Press, Cambridge, pp 225–247

Proctor MCF, Nagy Z, Csintalan Z, Tuba Z (1998) Water-content components in bryophytes: analysis of pressure-volume relationships. J Exp Bot 49:1845–1854

Proctor MCF, Oliver MJ, Wood AJ, Alpert P, Stark LR, Cleavitt NL, Mishler BD (2007) Desiccation-tolerance in bryophytes: a review. Bryologist 110:595–621

Rice SK (2009) Mosses (Bryophytes). In: Likens GE (ed) Encyclopedia of inland waters, vol 1. Elsevier, Oxford, pp 88–96

Rice SK, Schneider N (2004) Cushion size, surface roughness, and the control of water balance and carbon flux in the cushion moss *Leucobryum glaucum* (Leucobryaceae). Am J Bot 91:1164–1172

Rice SK, Gutman C, Krouglicof N (2005) Laser scanning reveals bryophyte canopy structure. New Phytol 166:695–704

Rice SK, Aclander L, Hanson DT (2008) Do bryophyte shoot systems function like vascular plant leaves or canopies? Functional trait relationships in *Sphagnum* mosses (Sphagnaceae). Am J Bot 95:1366–1374

Rice SK, Neal N, Mango J, Black K (2011a) Modeling bryophyte productivity across gradients of water availability using canopy form-function relationships. In: Tuba Z, Slack NG, Stark LR (eds) Bryophyte ecology and global change. Cambridge University Press, Cambridge, pp 441–457

Rice SK, Neal N, Mango J, Black K (2011b) Relationships among shoot tissue, canopy and photosynthetic characteristics in the feathermoss *Pleurozium schreberi*. Bryologist 114:367–378

Rydin H, Gunnarsson U, Sundberg S (2006) The role of *Sphagnum* in peatland development and persistence. In: Wieder RK, Vitt DH (eds) Boreal peatland ecosystems, Ecological Studies Series. Springer, Berlin, pp 47–65

Scheffer RA, van Logtestijn RSP, Verhoeven JTA (2001) Decomposition of *Carex* and *Sphagnum* litter in two mesotrophic fens differing in dominant plant species. Oikos 92:44–54

Scholfield WB (1981) Ecological significance of morphological characters in the moss gametophyte. Bryologist 84:149–165

Simon T (1987) The leaf-area index of three moss species (*Tortula ruralis*, *Ceratodon purpureus*, and *Hypnum cupressiforme*). Symp Biol Hung 35:699–706

Skre O, Oechel WC (1981) Moss functioning in different taiga ecosystems in interior Alaska. I. Seasonal, phenotypic, and drought effects on photosynthesis and response patterns. Oecologia 48:50–59

Smith WK, Hughes NM (2009) Progress in coupling plant form and photosynthetic function. Castanea 74:1–26

Stenberg P, Palmroth S, Bond BJ, Sprugel DG, Smolander H (2001) Shoot structure and photosynthetic efficiency along the light gradient in a Scots pine canopy. Tree Physiol 21:805–814

Thérézein M, Palmroth S, Brady R, Oren R (2007) Estimation of light interception properties of conifer shoots by an improved photographic method and a 3D model of shoot structure. Tree Physiol 27:1375–1387

Tobias M, Niinemets Ü (2010) Acclimation of photosynthetic characteristics of the moss *Pleurozium schreberi* to among-habitat and within-canopy light gradients. Plant Biol 12:743–754

Turetsky MR, Crow SE, Evans RJ, Vitt DH, Wieder RK (2008) Trade-offs in resource allocation among moss species control decomposition in boreal peatlands. J Ecol 96:1297–1305

Van Altena C, van Logtestijn RSP, Cornwell WK, Cornelissen JHC (2012) Species composition and fire: non-additive mixture effects on ground fuel flammability. Front Funct Plant Ecol 3:1–10

Van der Hoeven EC, Huynen CIJ, During HJ (1993) Vertical profiles of biomass, light intercepting area and light intensity in chalk grassland mosses. J Hattori Bot Lab 74:261–270

Vanderpoorten A, Goffinet B (2009) Introduction to bryophytes. Cambridge University Press, Cambridge

Verhoeven JTA, Liefveld WM (1997) The ecological significance of organochemical compounds in *Sphagnum*. Acta Bot Neerl 46:117–130

Vitt DH (1990) Growth and production dynamics of boreal mosses over climatic, chemical and topographic gradients. Bot J Linn Soc 104:35–59

Waite M, Sack L (2010) How does moss photosynthesis relate to leaf and canopy structure? Trait relationships for 10 Hawaiian species of contrasting light habitats. New Phytol 185:156–172

Williams TG, Flanagan LB (1998) Measuring and modeling environmental influences on photosynthetic gas exchange in *Sphagnum* and *Pleurozium*. Plant Cell Environ 21:555–564

Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J et al (2004) The worldwide leaf economics spectrum. Nature 428:821–827

Zotz G, Kahler H (2007) A moss "canopy"—small scale differences in microclimate and physiological traits in *Tortula ruralis*. Flora 202:661–666

Zotz G, Rottenberger S (2001) Seasonal changes in Diel CO_2 exchange of three central European moss species: a one-year field study. Plant Biol 3:661–669

Zotz G, Schweikert A, Jetz W, Westerman H (2000) Water relations and carbon gain are closely related to cushion size in the moss *Grimmia pulvinata*. New Phytol 148:59–67

Chapter 6

Diffusion Limitation and CO_2 Concentrating Mechanisms in Bryophytes

David T. Hanson*
Department of Biology, University of New Mexico, Albuquerque, NM 87131, USA

Karen Renzaglia
Department of Plant Biology, Southern Illinois University, Carbondale, IL 62901, USA

Juan Carlos Villarreal
Biology Department, Ludwig-Maximilians-Universität München, 80638 München, Germany

Summary

This chapter explores how the diffusion of CO_2 into photosynthetic tissues is affected by the morphology and biochemistry of bryophytes from the sub-cellular level to that of leaf-like structures, with an emphasis on the most ancient form of a land plant CO_2 concentrating mechanism, the hornwort pyrenoid. Interest in the control of CO_2 diffusion has increased dramatically over the past 5–10 years due to the discovery of CO_2 transporting aquaporins in

*Author for correspondence, e-mail: dthanson@unm.edu

D.T. Hanson and S.K. Rice (eds.), Photosynthesis in Bryophytes and Early Land Plants, Advances in Photosynthesis and Respiration 37, pp. 95–111, DOI 10.1007/978-94-007-6988-5_6,

chloroplast membranes and the ever-increasing interest in photosynthetic carbon fixation as a source of food and biologically generated fuels. The diffusion of CO_2 is of critical importance to our understanding of photosynthesis in land plants because it is inextricably linked to water loss. Photosynthetic tissues need to be well hydrated to function properly, but must lose water in order to capture CO_2 since water vapor can diffuse out of photosynthetic tissues through any pore large enough to allow CO_2 in. At the same time, too much water also limits photosynthesis because even thin films of liquid water present significant barriers to CO_2 diffusion. Furthermore, the partial pressure of CO_2 reaching the sites of carboxylation in the chloroplast is what inherently controls the efficiency of photosynthetic carbon assimilation. The amazing variation in bryophyte morphology provides a broad palette for sampling how plants have balanced these structural and biochemical trade-offs. Here we discuss how studying this variability can generate invaluable insight into both the limitations and opportunities for enhancing land plant photosynthesis.

I. Introduction

The transition to land from aquatic environments presented several challenges for photosynthetic organisms to overcome (Chaps. 2, 3 and 4), but getting CO_2 to the surface of photosynthetic tissues was not one of them. On land, access to CO_2 improved relative to that in aquatic environments due to the faster diffusion of CO_2 through air than water. The problem was, and is, that getting the CO_2 from the surface to the chloroplast generally requires features that also increase water loss. This trade-off has led to the evolution of a wide range of canopy, shoot, organ, and cellular structures in bryophytes that have improved CO_2 transport to the chloroplast with some of that diversity still present today. The impacts of canopy- and shoot-level variation on photosynthesis are discussed in Chaps. 5, 9 and 10. Here we focus on the photosynthetic organ and cellular structures that enhance CO_2 uptake in bryophytes.

The morphology of the photosynthetic organ where the trade-off between CO_2 uptake and water-loss occurs has a large and fundamental effect on the diffusive pathways encountered. There are three generalized forms of photosynthetic, leaf-like, organs that are repeatedly encountered in bryophytes, and they provide insight into the biophysical constraints that influenced the evolution of true leaves. The first form is a simple thallus found in some liverworts and all hornworts. This is a relatively uniform, poorly differentiated, flattened plant body that is closely attached to the substrate and a few to several cells thick with all cells photosynthetic. Second, is a complex thallus of some liverworts where cells have differentiated into several types, providing much more structure to the photosynthetic tissue, including epidermal pores opening into internal air spaces. The third is a simple leaf-like structure attached to a stem that may or may not contain photosynthetically active cells and is found in mosses and most liverworts. These leaf-like structures, or phyllids, differ from true leaves because they are generally one cell layer thick over most of the lamina, may or may not have a midrib, and lack well-developed vascular tissue. However, their form and arrangement is quite diverse and can be aggregated along branches or stems into larger functional units, or in the Polytrichaceae, can have complex structure including upward unistratose extensions and in-rolled margins.

Abbreviations: CA – Carbonic anhydrase; CCM – CO_2 concentrating mechanism; $\delta^{13}C$ – Isotopic composition of carbon ^{13}C and ^{12}C; Δ – Isotopic discrimination; Rubisco – Ribulose-1,5-bisphosphate carboxylase/oxygenase

In addition to the gross morphology of photosynthetic tissues, cellular properties also influence the diffusion of CO_2. In this chapter, we discuss the pyrenoid, a unique structure within the chloroplast that is responsible for the hornwort CO_2 concentrating mechanism (CCM). We also discuss morphological aspects of complex thalli, leaf-like photosynthetic organs, and the impacts on diffusion of CO_2, with particular attention to features that impact diffusion from the cell wall inwardly to the chloroplast.

II. Tissue Structure and CO_2 Diffusion

A. Simple Thallus

There have been very few studies of photosynthetic organisms with simple thalli (Smith and Griffiths 1996a, b, 2000; Hanson et al. 2002; Griffiths et al. 2004; Meyer et al. 2008), especially within liverworts. Most of this research has focused on the hornworts, a morphologically and phylogenetically isolated, species-poor land plant lineage. Two studies have compared simple thalloid liverwort CO_2 diffusion with that of hornworts lacking a CCM and found similar limitations between groups (Griffiths et al. 2004; Meyer et al. 2008). All simple thalli that lack CCMs appear to be diffusion limited, especially when covered by a film of water. Aside from slow growth in conditions that are unfavorable to other plants, this limitation would provide a strong selective force for one or more of the following: the development of a CCM, improved Rubisco kinetics, and/or structural features that could reduce the resistance between the atmosphere and the chloroplast stroma.

As discussed later in this chapter, a pyrenoid-style CCM did evolve in the hornworts although it is important to realize that like other CCMs, it requires additional electron transport (Hanson et al. 2002; Griffiths et al. 2004; Meyer et al. 2008) making a CCM less favorable in low-light environments often occupied by bryophytes. Rubisco kinetics in bryophytes have been poorly studied, and more investigations are desperately needed. One study found that CCM-lacking hornworts had intermediate properties between CCM containing hornworts and the C_3 liverwort *Marchantia polymorpha,* which has a complex thallus (Hanson et al. 2002). The net result was a lower investment in Rubisco per chlorophyll and a little more CO_2 assimilation per active site. Additional research is greatly needed to determine if this pattern is common to all organisms forming simple thalli or if it is a generalizable response among all CO_2 limited bryophytes. Lastly, it is unknown if liverworts and CCM-lacking hornworts with simple thalli consistently have structural modifications such as chloroplast (Chap. 8) or mitochondrial positioning within cells to maximize CO_2 recycling, functional CO_2-porins (Uehlein et al. 2008), thallus surface properties to regulate water films, or growth habits to maximize capture of CO_2 respired from soil.

The extent that CO_2 diffusion is limiting photosynthesis and the operation of a CCM can be examined by measuring the differential usage of the isotopologues (^{13}C and ^{12}C forms) of CO_2. This approach is predicated on the biochemical preference of Rubisco for the lighter isotopologue of CO_2, and the faster diffusion of the lighter isotopologue into and through photosynthetic tissues. The combined effect of the biochemical and physical fractionations is referred to as a discrimination (Δ) against the heavier forms of CO_2 and assessed through measurements of the isotopic composition of carbon in samples (Farquhar et al. 1982, 1989; Brugnoli and Farquhar 2000). Isotopic composition ($\delta^{13}C$) is reported in the unit-less notation of per mil (‰), that describes the ratio of ^{13}C content to ^{12}C relative to an international standard ($\delta^{13}C = [(^{13}C/^{12}C)_{sample}/(^{13}C/^{12}C)_{standard} - 1] \times 1{,}000$). Online isotopic gas exchange studies measure the isotopic composition and total CO_2 of air entering and exiting a leaf gas exchange chamber along with the water exchange. The total CO_2 uptake and water loss are used to model an expected Δ caused by diffusion in to the intercellular spaces of leaves (or to the surface of photosynthetic cells).

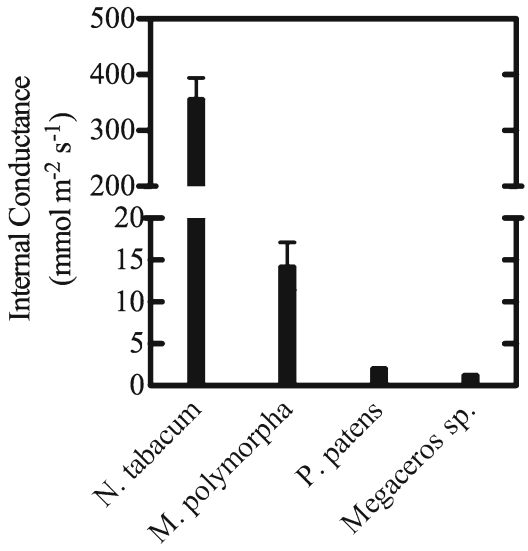


Fig. 6.1. Internal conductance to CO_2 in photosynthetic tissues. Data are presented for the angiosperm leaf *Nicotiana tabacum*, the complex thallus liverwort *Marchantia polymorpha*, the moss *Physcomitrella patens* with unistratose leaf-like tissues, and an unknown species of the hornwort genus *Megaceros* that has a simple thallus and no CCM (Hanson 2004, unpublished). Both *P. patens* and *Megaceros* were measured using tissue-cultured material that had been lightly blotted dry. Internal conductance is often referred to as mesophyll conductance with units of mol m^{-2} s^{-1} in plants with complex photosynthetic tissues that contain mesophyll cells. The more general term, internal conductance, and the smaller units used here are more suited for the anatomical variability and low conductance found in bryophytes.

The modeled Δ is compared with the measured Δ and the difference provides an estimate of the resistance to diffusion from the surface of photosynthetic cells into the chloroplast stroma (Evans et al. 1986).

Measurements of online Δ have shown that organisms with simple thalli have a much smaller Δ than predicted if CO_2 could diffuse through photosynthetic tissues as easily as in angiosperms and gymnosperms with true leaves (Meyer et al. 2008). A simple explanation for this difference is that this "internal" conductance of CO_2 (the inverse of resistance) through simple photosynthetic thalli is two orders of magnitude lower than the conductance through thin leaves with intricate air spaces (Fig. 6.1 Hanson 2004, unpublished). One must also account for changes in "external" conductance because it drops rapidly as surface water film thickness increases (Meyer et al. 2008). Even after accounting for water film effects, and estimating the effect of stomata and intercellular air spaces in true leaves, the difference between leaves and simple thalli for internal conductance is still greater than would be expected based on known structural changes. As discussed in section "Evolutionary trade-off between cell wall structure and CO_2 diffusion", it is possible that the low conductance of simple thalli is due to low cell wall permeability to CO_2. It is also possible, that some of the difference in Δ is not due to internal conductance but instead due to different amounts of cellular respiration and recycling relative to what is found in true leaves. Respired and recycled CO_2 would decrease the apparent rate of photosynthesis and also deplete the isotopic composition of air around a leaf making Δ appear to be less than it is.

B. Complex Thallus

The addition of epidermal pores and internal air spaces to thalloid photosynthetic tissues clearly reduces diffusion limitation for photosynthesis when expressed on either tissue area (Meyer et al. 2008) or chlorophyll content (Hanson et al. 2002). Several lines of evidence, including stable isotopes, have been employed to show that improvements in photosynthesis are achieved without a CCM in *Marchantia* and other liverworts with complex thalli (see Fig. 6.2 Hanson 2004, unpublished) (Smith and Griffiths 1996b, 2000; Hanson et al. 2002; Griffiths et al. 2004; Meyer et al. 2008). There is also no evidence that major changes in Rubisco properties, aside from an increase in content, accompanied the development of more complex photosynthetic thalli (Hanson et al. 2002), although as mentioned above, this is an area where little research has been published. It appears that the roughly two-fold increase in maximal photosynthesis between simple and complex thalli correlates with

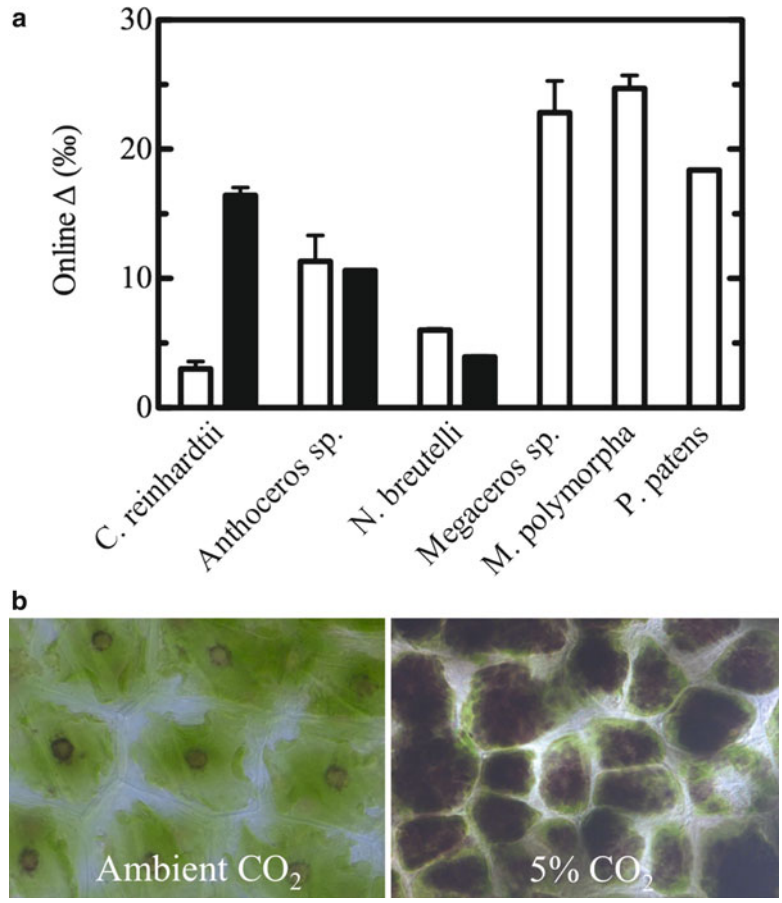


Fig. 6.2. Online photosynthetic ^{13}C discrimination (Δ) measured and the impact of high CO_2 for cultured algae and bryophytes (Hanson 2004, unpublished). *(***a***)* Δ was measured using a combined tunable diode laser/infra-red gas analyzer system (Barbour et al. 2007) for *Chlamydomonas reinhartdtii*, *Anthoceros* sp. (hornwort with pyrenoid), *Notothylas breutelli* (hornwort with pyrenoid), *Megaceros* sp. (hornwort without pyrenoid), *Marchantia polymorpha* (complex thallus liverwort), and *Physcomitrella patens* (moss). *Black columns* represent measurements on organisms growing at 0.7–5% CO_2, which is high enough to down-regulate the CCM and increase *Δ* in *C. reinhardtii*. Hornworts were transferred to ambient CO_2 for the measurement, but all data were collected within 15 min of exposure to the lower concentration. (**b**) Light microscopy showing hornworts grown at ambient and 5% CO_2 that were collected immediately prior to dawn and stained with potassium iodide to show starch grains.

about a five to tenfold increase in internal CO_2 conductance (Fig. 6.1) (Meyer et al. 2008). However, it is interesting to note that plants with true leaves have internal conductances that can be significantly higher; conifers like juniper range from the same magnitude as bryophytes to >100 fold higher (Bickford et al. 2009) and many seed plants like tobacco have an internal conductance >200 fold higher (Fig. 6.1). This higher conductance facilitates 10–20 fold higher photosynthetic rates in plants with true leaves when expressed on an area or mass basis (see Waite and Sack 2010), though the increases are not as evident on a per chlorophyll basis (Martin and Adamson 2001). The combination of this information suggests that internal conductance may be more important in spatial organization of photosynthesis within photosynthetic tissues, impacting how much Rubisco and chlorophyll can be utilized in a given volume more than the inherent relationship between light harvesting and carbon capture.

C. Phyllid

At first glance, the evolutionary progression from multistratose thalli to mostly unistratose leaf-like structures (phyllids) should have greatly reduced the importance of internal conductance as each photosynthetic cell is directly exposed to the atmosphere. However, the fundamental terrestrial problem of water-loss prevents such a simple solution from being a panacea. Unistratose tissues lose water rapidly, and in order to sustain high enough water contents for biological activity they must spend a significant amount of time covered with a film of water. Unistratose tissues also need to tolerate desiccation when water is not readily available. As the classical isotopic gas exchange studies of Rice and Giles (1996) and Williams and Flanagan (1996, 1998) have shown, this water film has a large impact on the diffusion of CO_2 to the surface of photosynthetic cells. The complex three dimensional structures and canopies facilitated by a stem- and leaf-style architecture of phyllid containing species requires balancing water loss with CO_2 diffusion and light penetration rather than optimization for CO_2 diffusion alone (see canopy structure discussion in Chaps. 9 and 10).

The limited extent to which CO_2 diffusion has been maximized in bryophytes is especially evident when examining internal conductance to CO_2. There have only been a few measurements of online Δ for mosses (Fig. 6.2) (Rice and Giles 1996; Williams and Flanagan 1996, 1998). These data show that internal conductance in *P. patens* may be as low as that found in simple thalli of CCM-lacking hornworts (Fig. 6.1), similar between *Pleurozium* and complex thalli, or 50% higher than complex thalli for *Sphagnum* (Williams and Flanagan 1998; Meyer et al. 2008). More data, explicitly separating internal and surface liquid conductance to CO_2 are needed to develop a robust understanding of how bryophytes regulate CO_2 diffusion. However, it appears that the maximum internal conductance in bryophytes is *at least* one order of magnitude lower than maximum internal conductance found in species with true leaves (see Fig. 6.1 for this comparison).

III. Evolutionary Trade-off Between Cell Wall Structure and CO_2 Diffusion

The realization that photosynthetic tissues in bryophytes have a much lower internal CO_2 conductance than has been achieved by angiosperms in particular, leads one to wonder why? What is so different between the mesophyll cells of a tobacco leaf and that of *Marchantia* or the single layer of cells making up most moss phyllids? The relatively recent demonstration of the presence of CO_2 pores in higher plant chloroplast membranes (Uehlein et al. 2008) is one candidate explanation if these are lacking in bryophytes. However, the internal conductance measured for antisense knockouts versus over-expression of CO_2 pores only accounts for a twofold difference at most (Flexas et al. 2006), not 10 or 100 fold. Similarly, chloroplast size and position, carbonic anhydrase activity and location, and cell wall properties all impact CO_2 diffusion. Recently, the potential relative contribution of internal conductance components has been modeled in three dimensions showing that the chloroplast envelope and the cell wall are the largest resistances (Tholen and Zhu 2011). This is of particular interest for bryophytes because cell wall properties of photosynthetic tissues are under very strong selection for properties other than photosynthesis, especially tortuosity associated with desiccation and UV tolerance (see Chaps. 7 and 16). The toughness of bryophyte cell walls has been clearly demonstrated through acid hydrolysis studies (Graham et al. 2004 and see Chap. 2). In some extreme examples, this would be similar to conducting photosynthesis in xylem cells of seed plants. Furthermore, Tholen and Zhu (2011) calculated that the importance of the cell wall resistance in the limitation of photosynthesis by CO_2 diffusion is greatly diminished at elevated CO_2 (60 Pa). Since many bryophytes grow close to the soil, they func-

tion in a high CO_2 environment due to soil respiration. In such an environment, the penalty for developing mechanically tough, diffusion-limiting cell walls in photosynthetic tissues would be small.

The concept that cell wall thickness and/or composition, possibly in combination with the lack of CO_2 pores in chloroplast envelopes are the primary reasons for low bryophyte internal conductance remains to be tested. Historically, the measurement of internal conductance in mosses has been difficult since the online Δ measurements have been time consuming and water content varies rapidly with time unless carefully maintained. However, the recent development of high-frequency, semi-automated, laser-based measurements of isotopic gas exchange for studying internal conductance (Flexas et al. 2006; Barbour et al. 2007; Bickford et al. 2009) and its application for bryophytes (Figs. 6.1 and 6.2) makes this research much more practical. In addition, the necessary instrumentation has become available in over a dozen labs world-wide, which should spur a new era of investigation. Hopefully, the ensuing years will answer the fundamental question of how evolution has influenced CO_2 diffusion limitation of photosynthesis.

IV. The Carbon Concentrating Mechanism (CCM) of Bryophytes

Plants growing under extreme conditions often have CCMs, enabling them to accumulate CO_2 and thereby enhance their ability to fix energy and grow (Badger et al. 1998; Giordano et al. 2005; Raven et al. 2008). For example, many C_4 plants concentrate CO_2 in specialized cells within an elaborate leaf organization known as Kranz anatomy. The C_4 photosynthetic pathway occurs in many grasses and is being explored as an efficient strategy to improve carbon fixation in crops with C_3 photosynthesis (e.g. rice) (Hibberd et al. 2008; Burnell 2011; Whitney et al. 2011; von Caemmerer et al. 2012). In recent years, some non-Kranz C_4 plants have been discovered where single cells maintain two separate populations of chloroplasts (Voznesenskaya et al. 2001; Edwards and Voznesenskaya 2011). Some algae are also thought to have a single-cell C_4 metabolism facilitated by the co-location of Rubisco and phosphoenolpyruvate carboxykinase in the chloroplast pyrenoid (McGinn and Morel 2008). There have also been suggestions of an inducible C_4-like CCM in the moss *Fissidens* (see Chap. 12), a supposition that requires additional research, so as of yet no single-cell C_4 plants have been identified in the bryophytes. The lack of single-cell C_4 in bryophytes could be due to the difficulty of maintaining the large cells with intricately sub-divided cytoplasm (found in single-cell C_4 flowering plants) through dehydration and re-hydration cycles that bryophytes commonly experience. The proposed diatom C_4 system could conceivably work in hornworts as both have pyrenoids, but to date there is no evidence for C_4 metabolism in hornworts and no other land plants have pyrenoids. Even without C_4 metabolism there is substantial diversity in pyrenoid and chloroplast morphology among bryophytes with consequences for photosynthesis that may have impacted their adaptation to life on land.

A. Chloroplast Structure and CO_2 Diffusion

The transition to land from aquatic algal ancestors involved a suite of adaptations to terrestrial life. One important transformation was in the structure and physiology of chloroplasts. Most green algae have few, large chloroplasts with one to several pyrenoids, 90 % of which are composed of the CO_2 – fixing enzyme Rubisco (Vaughn et al. 1990; Badger et al. 1998; Borkhsenious et al. 1998). The pyrenoid functions as an essential component of the CCM as a location where CO_2 can be elevated around Rubisco (McKay and Gibbs 1991; Badger and Price 1992). In pyrenoid containing species, CO_2 and HCO_3^- are actively taken-up into the cell and into the chloroplast where they can diffuse into the pyrenoid. Any CO_2 reaching the pyrenoid

can be directly utilized by Rubisco, however, the high pH of stroma in the light makes it likely that much of the CO_2 will be converted to HCO_3^-. When HCO_3^- arrives at the pyrenoid, it is thought to be transported into the low pH lumen, which would favor the conversion to CO_2 for Rubisco. Although a thylakoid-localized HCO_3^- transporter has not been identified, a lumen-localized carbonic anhydrase has been characterized and shown to be essential for CCM function (Spalding et al. 1983; Karlsson et al. 1998; Hanson et al. 2003). It is also not clear how leakage of CO_2 not captured by Rubisco is limited as leakage would lead to a futile cycle of CO_2 pumping and loss. Pyrenoid and/or chloroplast morphology appears to be important for reducing leakage as species with more compact pyrenoids leak less (Hanson et al. 2002). In algae, the pyrenoid provides an efficient CCM that increases CO_2 levels up to 180 times that in the rest of the cell, enhancing photosynthesis in aquatic environments where CO_2 diffusion is limited (Badger et al. 1998; Giordano et al. 2005). Hornworts are the only land plants that possess a pyrenoid and exhibit this type of CCM (Smith and Griffiths 1996a, b, 2000; Hanson et al. 2002), although the mechanics of its operation are primarily drawn by analogy to algal structures.

The pyrenoid-style CCM in algae is generally down-regulated within a few hours of exposure to high CO_2 ranging from 0.5 to 5% (Badger et al. 1980; Beardall and Raven 1981; Fukuzawa et al. 2001; Vance and Spalding 2005). In general, the regulation of CCM function has been shown via physiological adjustments in photosynthesis and gene expression, but changes in CCM activity based on the differential usage of the isotopologues of CO_2 and/or HCO_3^- were also hypothesized and a simple screen showing these changes was developed in the early 1980s (Beardall et al. 1982; Sharkey and Berry 1985). When a CCM is active at ambient and sub-ambient levels of inorganic carbon (CO_2 and HCO_3^-), the heavier inorganic carbon cannot readily escape fixation by Rubisco and a relatively high proportion is incorporated, resulting in a low Δ. The effect can be seen by comparing Δ calculated from online ^{13}C gas exchange for hornworts containing and lacking pyrenoid-style CCMs (Fig. 6.2 Hanson 2004, unpublished) (Meyer et al. 2008). It is clear that pyrenoid containing species have low Δ values (consistent with CCM activity) relative to pyrenoid lacking species of *Nothoceros, Megaceros* and mosses (Smith and Griffiths 1996b).

Suppression of algal CCMs under conditions of high inorganic carbon availability increases the leakiness of cells to CO_2 (Badger et al. 1980; Beardall and Raven 1981; Raven and Beardall 2003). This facilitates the escape of all inorganic carbon including the heavier forms, allowing Rubisco to exert its high Δ (preference for the lighter forms). Using this approach our data (Fig. 6.2a) show that, unlike most algae (Badger et al. 1998), at least some hornworts are unable to down-regulate their CCM in response to high CO_2 as they have a low Δ under both ambient and high CO_2. Interestingly, we also found a hyper-accumulation of starch grains at high CO_2 in pyrenoid-containing hornworts (Fig. 6.2b), and a small suppression of maximum photosynthesis (data not shown). Thus, even at high CO_2 when daily carbon assimilation greatly exceeds the ability of the cell to utilize assimilated carbon, photosynthesis is only mildly down-regulated.

In addition to online ^{13}C gas exchange, the isotopic effect of CCM activity can also be seen via analyses of the isotopic composition of tissues. In contrast to the measurement of instantaneous ^{13}C usage provided by online gas exchange, tissue analyses integrate the relative accumulation and retention of ^{13}C over the lifetime of a tissue, where species with CCMs incorporate relatively more $^{13}CO_2$ than species lacking CCMs. An integrated lifetime Δ can be calculated from the difference between the $\delta^{13}C$ of the atmosphere (source CO_2) and the $\delta^{13}C$ of the tissue, $\Delta_{lifetime} = [(\delta^{13}C_{atmosphere} - \delta^{13}C_{tissue})/(1{,}000+\delta^{13}C_{tissue})] \times 1{,}000$ (Farquhar et al. 1989). Since $\delta^{13}C_{atmosphere}$ is relatively constant and negative in sign, and since Δ is generally

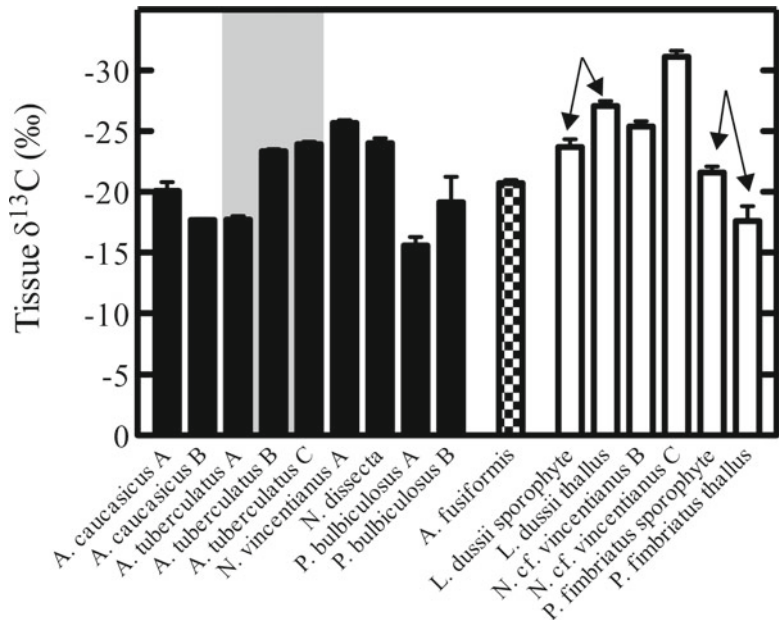


Fig. 6.3. Isotopic composition of ^{13}C of field collected hornwort tissues (Hanson and Villarreal 2008, unpublished). Solid columns are the pyrenoid containing species *Anthoceros caucasicus, Anthoceros tuberculatus, Nothoceros vincentianus, Notothylas dissecta*, and *Phymatoceros bulbiculosus.* Open columns are the pyrenoid-lacking species *Leiosporoceros dussii, Nothoceros c.f. vincentianus*, and *Phaeomegaceros fimbriatus*. The *checkered column is Anthoceros fusiformis*, which has a starch-free area located where a traditional pyrenoid would be expected. The letters A, B, and C following species designations denote that the same species was collected at different locations or times of year. The shaded solid columns highlight that *A. tuberculatus* collections from different locations can differ substantially in their isotopic composition. The paired arrows denote where sporophyte and gametophyte (thallus) tissue were analyzed from the same collected material.

positive in sign, a plant with a small Δ (active CCM) will generate tissues with a less negative $\delta^{13}C$ than a plant with a large Δ (inactive CCM).

We have compiled a figure of unpublished (D. Hanson and J.C. Villarreal 2008) tissue $\delta^{13}C$ measurements to assess the frequency of CCM function among hornworts (Fig. 6.3). Among seed plants, C_3 species tend to have $\delta^{13}C$ values ranging from −22 to −30‰, whereas CCM containing C_4 seed plants range from −10 to −14‰ (Cerling et al. 1997; Osborne 2011). A similar division has been seen within bryophytes, where hornworts with a pyrenoid-based CCM ranged from −13 to −20‰ and C_3 mosses, liverworts, and hornworts ranged from −21 to −35‰ (Griffiths et al. 2004). Overall, our data support the pattern that pyrenoid-containing hornworts have a $\delta^{13}C$ that is −20‰ or less negative, whereas those lacking a pyrenoid are more negative than −20‰. This division is consistent with all existing physiological data that clearly demonstrate an active CCM for hornworts containing pyrenoids and no CCM activity in pyrenoid-lacking species. However, Fig. 6.3 also shows that the pyrenoid-containing species *Anthoceros tuberculatus* can be both above and below the −20‰ threshold depending on collection time and locality. In addition, we found that sporophyte tissue from *Phaeomegaceros fimbriatus* was C_3-like (more negative than −20‰), while the gametophyte thallus $\delta^{13}C$ suggested the presence of a CCM. Since other factors such as water content (Rice and Giles 1996; Williams and Flanagan 1996, 1998; Meyer et al. 2008) and fixation of soil respired CO_2 (which is much more negative than $\delta^{13}C_{atmosphere}$, the normal presumed CO_2

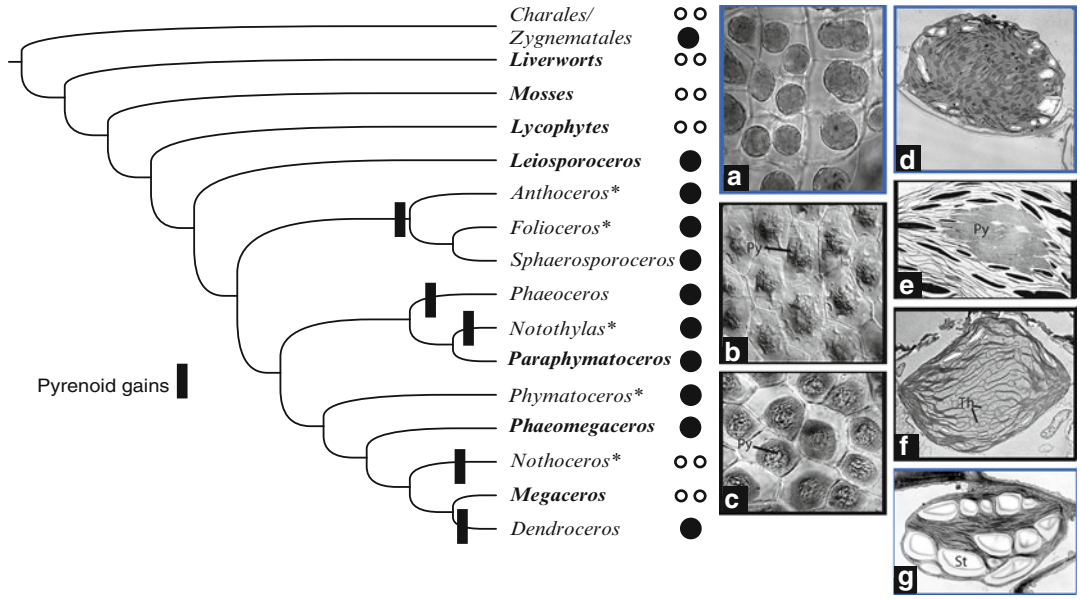


Fig. 6.4. Skeleton phylogeny of hornworts based on three genes (see Duff et al. 2007) with inferences on the evolution of the pyrenoid. Under either scenario of Zygnematales (pyrenoid bearing) or Charales (pyrenoid-less) sister to land plants, hornworts may have re-evolved the pyrenoid after it was lost in liverworts, mosses and tracheophytes. *Leiosporoceros*, the sister taxon to all hornworts, is pyrenoidless. Genera with pyrenoids and without pyrenoids (*bold*) are shown. Pyrenoid-bearing genera with one or more species lacking pyrenoids have an asterisk (*). *Megaceros* and *Phaeomegaceros* are the only genera in which all species lack a pyrenoid, while in *Phymatoceros* (two species) one species has a pyrenoid (*P. bulbiculosus*) and one lacks pyrenoids (*P. phymatodes*). Taxa with multiple plastids per cell are represented by *open circles* and those that are typically uniplastidic are shown with *solid circles*. More sampling would further clarify the evolution of pyrenoids within hornworts. Collage of hornwort chloroplasts: (**a**) *Leiosporoceros dussii* chloroplast without pyrenoids from the *upper* epidermis, (**b**) *Anthoceros* sp. light microscopy of upper epidermis of gametophyte, each cell contains single plastid with pyrenoids (Py), (**c**) *Nothoceros vincentianus* light microscopy of upper epidermal cells of gametophyte, each with modified central pyrenoid (Py) with abundant starch granules, (**d**) *Leiosporoceros dussii* transmission electron micrograph (TEM) of chloroplast without pyrenoid, (**e**) *Phaeoceros carolinianus* TEM of subunits of the multiple pyrenoid (Py) are more electron-opaque than the surrounding stroma, (**f**) *Nothoceros canaliculatus* TEM of an electron translucent pyrenoid area traversed by numerous channel thylakoids (Th), (**g**) *Phaeomegaceros aff. fimbriatus* TEM of a pyrenoidless chloroplast in the *upper cell*s of the gametophyte with abundant central grana and mostly peripheral starch (St).

source) can have large impacts on the $\delta^{13}C$ of tissues, these data are not proof of an exception to the CCM activity of pyrenoids. However, within-species and within-plant variability in $\delta^{13}C$ does open up the possibility that CCM activity is facultative in some species. Definitive resolution of this question will require more physiological and online Δ measurements under controlled conditions.

The presence of pyrenoids is more variable among hornworts than traditionally thought (see Fig. 6.4 and the evolution of pyrenoids section below). Interestingly, species and even cells within a thallus lacking pyrenoids have more and smaller chloroplasts than those containing pyrenoids. Re-examination of chloroplast morphology and their effect on CO_2 diffusion suggests that large chloroplasts hinder diffusion more than small ones (Terashima et al. 2011; Tholen and Zhu 2011), although it is unknown if expression of chloroplast-localized CO_2-transporting aquaporins (Uehlein et al. 2003, 2008; Flexas et al. 2006) increases with chloroplast size to compensate for the slower diffusion. Therefore, there could be a mechanistic linkage between chloroplast size and functioning of a pyrenoid-style CCM.

If CO_2 diffusion is sufficiently limited by chloroplast size, then species with large chloroplasts may require a CCM for rapid growth. However, this slowed diffusion would also reduce the rate of CO_2 leakage from a centrally located pyrenoid where HCO_3^- is presumably converted to CO_2 for Rubisco. This reduced leakage would be a result of the larger distance to escape the chloroplast coupled with increased time to reach equilibrium with HCO_3^- in the high pH stroma of illuminated chloroplasts. So, it is also possible that large chloroplasts are required for terrestrial pyrenoid-based CCMs to function as there is no other known mechanism to reduce the futile cycle caused by CO_2 leakage from the pyrenoid.

B. Evolution of Pyrenoids in Land Plants

Characteristics of hornworts appear intermediate between algal and land plant traits, providing insights into selective pressures that may have faced plants as they colonized the land c. 400 million years ago (Kenrick and Crane 1997; Renzaglia et al. 2000; Qiu et al. 2006). Despite morphological simplicity and homogeneity they have endured through severe and prolonged climatic change. Hornworts exhibit "ancestral" traits, such as poikilohydry (the ability to survive desiccation) (Proctor 2009) and simple, thalloid gametophytes (the gamete-bearing life phase) with 'derived' traits, such as stomata in sporophytes (the spore-producing phase) (Renzaglia et al. 2009) and a pyrenoid (Smith and Griffiths 1996a, b, 2000; Hanson et al. 2002).

Reconstructing the evolution of pyrenoids in hornworts is not a trivial task. First of all, data on chloroplast ultrastructure has been collected in less than 20 % of the taxa and these results indicate great variability across species and within plants. The extensive geological history of this ancient lineage, spanning multiple extinction events, has resulted in ambiguous phylogenetic reconstruction and large genetic distances between hornworts and other land plants. Current phylogenetic hypotheses suggest that the closest relative to land plants is either a member of the Charales (pyrenoidless) or Zygnematales (pyrenoid bearing) (Turmel et al. 2005; Graham et al. 2009). These two outgroups to land plants result in dramatically different reconstructions of ancestral characters in pyrenoids of hornworts. In addition, hornworts are nested among liverworts, mosses and lycophytes, all of which do not have pyrenoids. The appearance of the trait may have been triggered by specific environmental factors when hornworts originated. Pyrenoid presence appears to have evolved at least five times independently within hornworts, although it is lost in several clades (Fig. 6.4) (Villarreal and Renner 2012).

A large drop in atmospheric CO_2 concentration in the Late Devonian is thought to have promoted the evolution of the leaf blade, which allowed multiplication of the number of stomata per mm^2 and increased CO_2 sequestration (Beerling et al. 2001). The evolution of pyrenoids in green algae and carboxysomes in cyanobacteria has also been hypothesized to be a response to this Devonian drop in CO_2 (Raven 1997; Badger et al. 2002). Our recent insight on hornwort pyrenoids suggests that the ancestor of all hornworts about 300 mya lacked pyrenoids (Villarreal and Renner 2012). Therefore, alternative micro-habitat conditions may have influenced the evolution of pyrenoids within some hornwort clades. One study examined Rubisco content in hornworts and found that a species without a pyrenoid had a higher Rubisco content than two species containing pyrenoids, leading to more efficient CO_2 capture in at least one of the pyrenoid containing species (Hanson et al. 2002). Since Rubsico is a major sink for nitrogen in plants, a reduction in Rubisco content without a loss in CO_2 uptake would generate significant savings in the nitrogen budget. Therefore, fluctuations in the available nitrogen could also affect the evolution of pyrenoids. Interestingly, all hornworts are thought to form symbioses with cyanobacteria, though in the basal genus *Leisporoceros* the symbiosis differs morphologically from all others (Villarreal and Renzaglia 2006).

This symbiosis has been shown to provide nitrogen and is under the genetic control of the hornwort in the genus *Anthoceros* (Meeks et al. 1983; Campbell and Meeks 1992; Meeks and Elhai 2002), though the variability and effectiveness of this association is not well quantified in other genera. If environmental conditions reduced the formation of cyanobacterial-hornwort symbioses or their effectiveness, this would increase the benefit of having a pyrenoid CCM irrespective of CO_2 concentrations in the atmosphere.

Ancestral reconstruction of pyrenoid evolution in hornworts would benefit from a solid and time-explicit phylogeny of the group, and, of course, ultrastructural and physiological examination of more taxa. As it stands today, evolution of pyrenoids within hornworts appears to be an excellent example of atavism, involving multiple losses and gains that mirror the situation in green algae (Nozaki et al. 2002; Villarreal and Renner 2012). The sister taxon to all other hornworts, *Leiosporoceros,* lacks pyrenoids. If plant cells containing single chloroplasts without pyrenoids (i.e. the condition in *Leiosporoceros*) are plesiomorphic in hornworts, pyrenoids have been gained independently at least five times (Villarreal and Renner 2012). Additional losses are seen in the five pyrenoid-bearing genera that have one or more species that lack pyrenoids (Fig. 6.4, genera denoted by *). Lack of a pyrenoid typically is associated with reduction in plastid size and increase in plastid number per cell. The pyrenoid was lost in the crown hornwort group that includes *Phaeomegaceros*, *Megaceros*, and most *Nothoceros*, and appears again in *Dendroceros* and two species of *Nothoceros* (Fig. 6.4). Thus, pyrenoidless plastids are ubiquitous in only two genera, *Phaeomegaceros* and *Megaceros*. *Megaceros* has a lower CCM activity than taxa with pyrenoids such as *Anthoceros*, *Notothylas* and *Phaeoceros* (Hanson et al. 2002; Meyer et al. 2008). This rather simple interpretation of pyrenoid evolution in hornworts does not take into consideration that chloroplast microanatomy is highly variable, especially in those plants that do not possess well-defined pyrenoids. Indeed, starch-free or pyrenoid-like zones occupy the central region of chloroplasts in some of these plants and their tissue $\delta^{13}C$ is on the borderline between species with and without CCM activity (Fig. 6.3). If these organisms have functional CCMs, then an even more sophisticated CCM may have evolved. Localizations of *rbc*L and other photosynthetic enzymes within this microanatomy may provide clues to the nature of these evolutionary changes. Moreover, in *Anthoceros,* the largest hornwort genus with ca. 80 species (Villarreal et al. 2010), less than 20 % of species have been examined at the light microscope level, limiting accurate reconstruction of pyrenoid evolution in hornworts.

The advantage of a pyrenoid-style CCM in hornworts is puzzling since pyrenoid containing and lacking species occur in a range of terrestrial environments. Some hornworts are semi-aquatic (e.g. the pyrenoidless *Nothoceros aenigmaticus,* a few *Anthoceros* species and occasionally *Phaeoceros carolinianus*); in these the CCM would provide the same costs and benefits as they do in freshwater microalgae. However, the vast majority of hornwort species are terrestrial, growing on soil banks together with liverworts and mosses. In the case of the epiphytic genera *Dendroceros,* the presence of a pyrenoid may relate to desiccation-tolerance in its habitat (Schuette and Renzaglia 2010). Thus, numerous questions remain in regards to structural and physiological modifications within the unique chloroplast of hornworts. With the diversity and pattern of chloroplast evolution, continued research on pyrenoids in hornworts will yield a wealth of information that will inform the development of crop plants with efficient CCM capabilities.

C. Engineering A Crop Plant Pyrenoid

The increased efficiency of CO_2 utilization and the concomitant savings in nitrogen and water loss achieved by CCM containing organisms suggests that it could be beneficial to engineer a CCM into a C_3 crop. A large consortium of scientists are already working to engineer C_4 metabolism into C_3 crops

(Hibberd et al. 2008; Burnell 2011; von Caemmerer et al. 2012), but some have begun to explore engineering a pyrenoid-style CCM into crops as mechanisms of its assembly are uncovered (Meyer et al. 2012). The pyrenoid CCM is intriguing because it does not require multiple cell or plastid types needed for C_4, so it may be easier to engineer. However, little is known about what is needed to make a pyrenoid-style CCM.

The green alga *Chlamydomonas* is currently the primary candidate donor to implement a single cell CCM in higher plants because of the availability of nuclear genome data, ongoing research on its CCM, and ease of mutagenesis (Spreitzer et al. 1985; Merchant et al. 2007; Genkov et al. 2010; Meyer 2010; Meyer et al. 2012, http://cambridgecapp.wordpress.com/). The enzyme Rubisco is partitioned into a large subunit (*rbc*L, in the chloroplast) and a small subunit (*rbc*S, in the nucleus). A major recent development is the discovery that a specific portion of *rbc*S is responsible for the presence of pyrenoids in *Chlamydomonas* where an engineered *Chlamydomonas* mutant that combined an angiosperm *rbc*S with *Chlamydomonas rbc*L lacked a pyrenoid (Genkov et al. 2010; Meyer et al. 2012). Further essential components of the CCM in *Chlamydomonas* are carbonic anhydrases (CA), inorganic carbon transporters and regulatory factors, though there are a range of other potential genes that are expressed when CCMs are stimulated but that have not been fully characterized (Fujiwara et al. 1990; Spalding 2008; Yamano et al. 2008; Ohnishi et al. 2010; Ma et al. 2011; Brueggeman et al. 2012). Carbonic anyhdrases are part of a gene family and their activity is central to the concentrating mechanism because they maintain equilibrium levels of CO_2 to support assimilation by Rubisco and to facilitate diffusion of CO_2 across membranes (Spalding et al. 1983; Badger and Price 1992; Borkhsenious et al. 1998; Hanson et al. 2003; Giordano et al. 2005; Spalding 2008).

Another potentially crucial hornwort feature, absent from most green algae, is a stacked arrangement of thylakoid membranes (grana) involved in light capture (Mullineaux 2005). Grana result in the spatial separation of photosystems and increase the efficiency of light capturing systems in terrestrial environments (Mullineaux 2005; Cardon et al. 2008). In hornworts, grana consist of stacks of short thylakoids and lack end membranes. Therefore, unlike in other land plants, hornwort grana are devoid of the membrane 'sacs' that fully enclose intra-thylakoid spaces. Presumably the unique channel thylakoid system in hornwort chloroplasts assumes the role of isolating biochemical processes necessary for photosynthesis. Because hornworts are the only group to combine pyrenoids, like green algae, with channel thylakoids and grana that resemble those of other land plants but that lack end membranes, the internal architecture of chloroplasts in this group is unparalleled (Vaughn et al. 1992). Multilocus molecular studies place hornworts sister to vascular plants, thus they are separated from algae by two pyrenoid-less lineages (liverworts and mosses).

Arguably hornworts are better donors than algae for genetic engineering of pyrenoids in crop plants because they are phylogenetically closer, and shared features of chloroplast organization with flowering plants may result in a more efficient way to implement a fully functional CCM in crops. Hornworts offer a promising opportunity to study chloroplast evolution, and in particular, the acquisition and maintenance of an alternative way to concentrate carbon in terrestrial environments. However, it is critical to ascertain if hornwort pyrenoids form in a homologous fashion to those in *Chlamydomonas* and whether features not seen in seed plants such as large chloroplasts and channel thylakoids are important to the operation of terrestrial pyrenoid-based CCMs. A hornwort nuclear genome project is reportedly underway, and a chloroplast and mitochondrial genome from a pyrenoid containing species are already available (Kugita et al. 2003; Li et al. 2009) making it more possible to perform the transcriptomic and mutagenic work that will be essential for unveiling the components of the hornwort CCM. Finally, there is some

suggestion that environmental conditions will control expression based on variability of pyrenoid expression between populations of the same species and between tissues in a single plant. A thorough study of chloroplast diversity in different tissues and structures within a carefully selected taxon would reveal the answer to this question.

Acknowledgements

This work was possible through support of the National Science Foundation (IOS 0719118 and DEB 0531751) and the University of New Mexico.

References

Badger MR, Price GD (1992) The CO_2 concentrating mechanism in cyanobacteria and microalgae. Physiol Plant 84:606–615

Badger MR, Kaplan A, Berry JA (1980) The internal inorganic C pool of *Chlamydomonas reinhardtii*: evidence for a CO_2 concentrating mechanism. Plant Physiol (Rockv) 66:407–413

Badger MR, Andrews TJ, Whitney SM, Ludwig M, Yellowlees DC, Leggat W, Price GD (1998) The diversity and coevolution of Rubisco, plastids, pyrenoids, and chloroplast-based CO_2-concentrating mechanisms in algae. Can J Bot 76:1052–1071

Badger MR, Hanson DT, Price GD (2002) Evolution and diversity of CO_2 concentrating mechanisms in cyanobacteria. Funct Plant Biol 29:161–173

Barbour MM, McDowell NG, Tcherkez G, Bickford CP, Hanson DT (2007) A new measurement technique reveals rapid post-illumination changes in the carbon isotope composition of leaf-respired CO_2. Plant Cell Environ 30:469–482

Beardall J, Raven JA (1981) Transport of inorganic carbon and the "CO_2 concentrating mechanism" in *Chlorella emersonii* (Chlorophyceae). J Phycol 92:1–20

Beardall J, Griffiths H, Raven JA (1982) Carbon isotope discrimination and the CO_2 accumulating mechanism in *Chlorella* emersonii. J Exp Bot 33:729–737

Beerling DJ, Osborne CP, Chaloner WG (2001) Evolution of leaf-form in land plants linked to atmospheric CO_2 decline in the Late Palaeozoic era. Nature 410:352–354

Bickford CP, McDowell NG, Erhardt EB, Hanson DT (2009) High-frequency field measurements of diurnal carbon isotope discrimination and internal conductance in a semi-arid species, *Juniperus monosperma*. Plant Cell Environ 32:796–810

Borkhsenious ON, Mason CB, Moroney JV (1998) The intracellular localization of ribulose-1,5-bisphosphate carboxylase/oxygenase in *Chlamydomonas reinhardtii*. Plant Physiol (Rockv) 116:1585–1591

Brueggeman AJ, Gangadharaiah DS, Cserhati MF, Casero D, Weeks DP, Ladunga I (2012) Activation of the carbon concentrating mechanism by CO_2 deprivation coincides with massive transcriptional restructuring in *Chlamydomonas reinhardtii*. Plant Cell 24:1860–1875

Brugnoli E, Farquhar GD (2000) Photosynthetic fractionation of carbon isotopes. In: Leegood RC, Sharkey TD, von Caemmerer S (eds) Photosynthesis: physiology and metabolism. Kluwer, Norwell, pp 399–434

Burnell JN (2011) Hurdles to engineering greater photosynthetic rates in crop plants: C_4 rice. In: Raghavendra AS, Sage RF (eds) C_4 Photosynthesis and related CO_2 concentrating mechanisms. Springer, Dordrecht, pp 361–378

Campbell EL, Meeks JC (1992) Evidence for plant-mediated regulation of nitrogenase expression in the *Anthoceros-Nostoc* symbiotic association. Microbiology 138:473–480

Cardon ZG, Gray DW, Lewis LA (2008) The green algal underground: evolutionary secrets of desert cells. Bioscience 58:114

Cerling TE, Harris JM, Macfadden BJ, Leakey MG, Quadek J, Eisenmann V, Ehleringer JR (1997) Global vegetation change through the Miocene/Pliocene boundary. Nature 389:153–158

Duff RJ, Villarreal JC, Cargill DC, Renzaglia KS (2007) Progress and challenges toward developing a phylogeny and classification of the hornworts. Bryologist 110:214–243

Edward EE, Voznesenskaya EV (2011) C_4 photosynthesis: Kranz forms and single-cell C_4 in terrestrial plants. In: Raghavendra AS, Sage RF (eds) C_4 Photosynthesis and related CO_2 concentrating mechanisms. Springer, Dordrecht, pp 29–61

Evans JR, Sharkey TD, Berry JA, Farquhar GD (1986) Carbon isotope discrimination measured concurrently with gas exchange to investigate CO_2 diffusion in leaves of higher plants. Aust J Plant Physiol 13:281–292

Farquhar GD, Leary MHO, Berry JA (1982) On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. Aust J Plant Physiol 9:121–137

Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. Annu Rev Plant Physiol Plant Mol Biol 40:503–537

Flexas J, Ribas-Carbó M, Hanson DT, Bota J, Otto B, Cifre J, McDowell N, Medrano H, Kaldenhoff R (2006) Tobacco aquaporin NtAQP1 is involved in mesophyll conductance to CO_2 in vivo. Plant J 48:427–439

Fujiwara S, Fukuzawa H, Tachiki A, Miyachi S (1990) Structure and differential expression of two genes encoding carbonic anhydrase in *Chlamydomonas reinhardtii*. Proc Natl Acad Sci U S A 87:9779–9783

Fukuzawa H, Miura K, Ishizaki K, Kucho K, Saito T, Kohinata T (2001) Ccm1, a regulatory gene controlling the induction of a carbon-concentrating mechanism in *Chlamydomonas reinhardtii* by sensing CO_2 availability. Proc Natl Acad Sci U S A 98:5347–5352

Genkov T, Meyer M, Griffiths H, Spreitzer RJ (2010) Functional hybrid rubisco enzymes with plant small subunits and algal large subunits: engineered rbcS cDNA for expression in *Chlamydomonas*. J Biol Chem 285:19833–19841

Giordano M, Beardall J, Raven JA (2005) CO_2 concentrating mechanisms in algae: mechanisms, environmental modulation, and evolution. Annu Rev Plant Biol 56:99–131

Graham LE, Kodner RG, Fisher MM, Graham JM, Wilcox LE, Hackney JM, Obst J, Bilkey PS, Hanson DT, Cook ME (2004) Early land plant adaptations to terrestrial stress: a focus on phenolics. In: Hemsley A, Poole I (eds) The evolution of plant physiology. From whole plants to ecosystems, Linnean society symposium series number 21. Elsevier Academic Press, London, pp 155–169

Graham LE, Graham JM, Wilcox LW (2009) Algae. Benjamin Cummings/Pearson, San Francisco

Griffiths H, Maxwell K, Richardson D, Robe W (2004) Turning the land green: inferring photosynthetic and diffusive limitations in early bryophytes. In: Hemsley A, Poole I (eds) The evolution of plant physiology. From whole plants to ecosystems, Linnean society symposium series number 21. Elsevier Academic Press, London, pp 3–16

Hanson DT, Andrews TJ, Badger MR (2002) Variability of the pyrenoid-based CO_2 concentrating mechanism in hornworts (Anthocerotophyta). Funct Plant Biol 29:407–416

Hanson DT, Franklin LA, Samuelsson G, Badger MR (2003) The *Chlamydomonas reinhardtii* cia3 mutant lacking a thylakoid lumen-localized carbonic anhydrase is limited by CO_2 supply to Rubisco and not Photosystem II function *in vivo*. Plant Physiol (Rockv) 132:2267–2275

Hibberd JM, Sheehy JE, Langdale JA (2008) Using C_4 photosynthesis to increase the yield of rice-rationale and feasibility. Curr Opin Plant Biol 11:228–231

Karlsson J, Clarke AK, Chen ZY, Hugghins SY, Park YI, Husic H, Moroney JV, Samuelsson G (1998) Novel alpha-type carbonic anhydrase associated with the thylakoid membrane in *Chlamydomonas reinhardtii* is required for growth at ambient CO_2. EMBO Eur Mol Biol Organ J 17:1208–1216

Kenrick P, Crane P (1997) The origin and early diversification of land plants: a cladistic study. Smithsonian Institution Press, Washington, DC, 441 pp

Kugita M, Kaneko A, Yamamoto Y, Takeya Y, Matsumoto T, Yoshinaga K (2003) The complete nucleotide sequence of the hornwort (*Anthoceros formosae*) chloroplast genome: insight into the earliest land plants. Nucleic Acids Res 31:716–721

Li L, Wang B, Liu Y, Qiu Y-L (2009) The complete mitochondrial genome sequence of the hornwort *Megaceros aenigmaticus* shows a mixed mode of conservative yet dynamic evolution in early land plant mitochondrial genomes. J Mol Evol 68:665–678

Ma Y, Pollock SV, Xiao Y, Cunnusamy K, Moroney JV (2011) Identification of a novel gene, CIA6, required for normal pyrenoid formation in *Chlamydomonas reinhardtii*. Plant Physiol (Rockv) 156:884–896

Martin CE, Adamson VJ (2001) Photosynthetic capacity of mosses relative to vascular plants. J Bryol 23:319–323

McGinn PJ, Morel FMM (2008) Expression and inhibition of the carboxylating and decarboxylating enzymes in the photosynthetic C_4 pathway of marine diatoms. Plant Physiol (Rockv) 146:300–309

McKay RML, Gibbs SP (1991) Composition and function of pyrenoids: cytochemical and immuncytochemical approaches. Can J Bot 69:1040–1052

Meeks JC, Elhai J (2002) Regulation of cellular differentiation in filamentous cyanobacteria in free-living and plant-associated symbiotic growth states. Microbiol Mol Biol Rev 66:94–121

Meeks JC, Enderlin CS, Wycoff KL, Chapman JS, Joseph CM (1983) Assimilation of $^{13}NH_4^+$ by *Anthoceros* grown with and without symbiotic *Nostoc*. Planta 158:384–391

Merchant SS, Prochnik SE, Vallon O, Harris EH, Karpowicz SJ et al (2007) The *Chlamydomonas* genome reveals the evolution of key animal and plant functions. Science 318:245–250

Meyer M (2010) Physiological and molecular determinants of the *Chlamydomonas reinhardtii* pyrenoid. Ph.D. thesis. University of Cambridge, Cambridge, pp 1–183

Meyer MT, Seibt U, Griffiths H (2008) To concentrate or ventilate? Carbon acquisition, isotope discrimination and physiological ecology of early land plant life. Proc R Soc Biol Sci Ser B 363:2767–2778

Meyer MT, Genkov T, Skepper JN, Jouhet J, Mitchell MC, Spreitzer RJ, Griffiths H (2012) Rubisco small-subunit α-helices control pyrenoid formation in *Chlamydomonas*. Proc Natl Acad Sci U S A 1–6

Mullineaux CW (2005) Function and evolution of grana. Trends Plant Sci 10:521–525

Nozaki H, Onishi K, Morita E (2002) Differences in pyrenoid morphology are correlated with differences in the rbcL genes of members of the *Chloromonas* lineage (Volvocales, Chlorophyceae). J Mol Evol 55:414–430

Ohnishi N, Mukherjee B, Tsujikawa T, Yanase M, Nakano H, Moroney JV, Fukuzawa H (2010) Expression of a low CO_2-inducible protein, LCI1, increases inorganic carbon uptake in the green alga Chlamydomonas reinhardtii. Plant Cell 22:3105–3117

Osborne CP (2011) The geologic history of C_4 plants. In: Raghavendra AS, Sage RF (eds) C_4 Photosynthesis and related CO_2 concentrating mechanisms. Springer, Dordrecht, pp 339–357

Proctor MCF (2009) Physiological ecology. In: Goffinet B, Shaw AJ (eds) Bryophyte biology, 2nd edn. Cambridge University Press, Cambridge, pp 237–268

Qiu Y-L, Li L, Wang B, Chen Z, Knoop V, Groth-Malonek M, Dombrovska O, Lee J, Kent L, Rest J, Estabrook GF, Hendry TA Taylor DW, Testa CM, Ambros M, Crandall-Stotler B, Duff RJ, Stech M, Frey W, Quandt D, Davis CC (2006) The deepest divergences in land plants inferred from phylogenomic evidence. Proc Natl Acad Sci U S A 103:5511–15516

Raven JA (1997) The role of marine biota in the evolution of terrestrial atmospheric composition and evolution of terrestrial biota: gases and genes. Biogeochemistry 39:139–164

Raven JA, Beardall J (2003) Carbon acquisition mechanisms of algae: carbon dioxide diffusion and carbon dioxide concentrating mechanisms. In: Larkum AWD, Douglas SE, Raven JA (eds) Photosynthesis in algae. Kluwer, Dordrecht, pp 225–244

Raven JA, Cockell CA, de La Rocha C (2008) The evolution of inorganic carbon concentrating mechanisms in photosynthesis. Proc R Soc Biol Sci Ser B 363:2641–2650

Renzaglia KS, Duff RJ, Nickrent DL, Garbary D (2000) Vegetative and reproductive innovations of early land plants: implications for a unified phylogeny. Proc R Soc Biol Sci Ser B 355:769–793

Renzaglia KS, Villarreal JC, Duff RJ (2009) New insights into morphology, anatomy, and systematics of hornworts. In: Goffinet B, Shaw AJ (eds) Bryophyte biology, 2nd edn. Cambridge University Press, Cambridge, pp 139–172

Rice SK, Giles L (1996) The influence of water content and leaf anatomy on carbon isotope discrimination and photosynthesis in *Sphagnum*. Plant Cell Environ 19:118–124

Schuette S, Renzaglia KS (2010) Development of multicellular spores in the hornwort genus *Dendroceros* (Dendrocerotaceae, Anthocerotophyta) and the occurrence of endospory in bryophytes. Nova Hedwigia 91:301–316

Sharkey TD, Berry JA (1985) Carbon isotope fractionation of algae as influenced by an inducible CO_2 concentrating mechanism. In: Lucas WJ, Berry JA (eds) Inorganic carbon uptake by aquatic photosynthetic organisms. American Society of Plant Physiologists, Rockville, pp 389–401

Smith EC, Griffiths H (1996a) A pyrenoid-based carbon-concentrating mechanism is present in terrestrial bryophytes of the class Anthocerotae. Planta 200:203–212

Smith EC, Griffiths H (1996b) The occurrence of the chloroplast pyrenoid is correlated with the activity of a CO_2-concentrating mechanism and carbon isotope discrimination in lichens and bryophytes. Planta 198:6–16

Smith EC, Griffiths H (2000) The role of carbonic anhydrase in photosynthesis and the activity of the carbon-concentrating-mechanism in bryophytes of the class Anthocerotae. New Phytol 145:29–37

Spalding MH (2008) Microalgal carbon-dioxide-concentrating mechanisms: *Chlamydomonas* inorganic carbon transporters. J Exp Bot 59:1463–1473

Spalding MH, Spreitzer RJ, Ogren WL (1983) Carbonic anhydrase-deficient mutant of *Chlamydomonas reinhardtii* requires elevated carbon-dioxide concentration for photoautotrophic growth. Plant Physiol (Rockv) 73:268–272

Spreitzer RJ, Goldschmidt-Clermont M, Rahire M, Rochaix JD (1985) Nonsense mutations in the *Chlamydomonas* chloroplast gene that codes for the large subunit of ribulosebisphosphate carboxylase/oxygenase. Proc Natl Acad Sci U S A 82:5460–5464

Terashima I, Hanba YT, Tholen D, Niinemets Ü (2011) Leaf functional anatomy in relation to photosynthesis. Plant Physiol (Rockv) 155:108–116

Tholen D, Zhu X-G (2011) The mechanistic basis of internal conductance: a theoretical analysis of mesophyll cell photosynthesis and CO_2 diffusion. Plant Physiol (Rockv) 156:90–105

Turmel M, Otis C, Lemieux C (2005) The complete chloroplast DNA sequences of the charophycean green algae *Staurastrum* and *Zygnema* reveal that the chloroplast genome underwent extensive changes during the evolution of the Zygnematales. BMC Biol 3:22

Uehlein N, Lovisolo C, Siefritz F, Kaldenhoff R (2003) The tobacco aquaporin NtAQP1 is a membrane CO_2 pore with physiological functions. Nature 425:734–737

Uehlein N, Otto B, Hanson DT, Fischer M, McDowell N, Kaldenhoff R (2008) Function of *Nicotiana tabacum* aquaporins as chloroplast gas pores challenges the concept of membrane CO_2 permeability. Plant Cell 20:648–657

Vance P, Spalding MH (2005) Growth, photosynthesis, and gene expression in Chlamydomonas over a range of CO2 concentrations and CO2/O2 ratios: CO2 regulates multiple acclimation states. Can J Bot 809:796–809

Vaughn KC, Campbell EO, Hasegawa J, Owen HA, Renzaglia KS (1990) The pyrenoid is the site of ribulose-1,5-bisphosphate carboxylase/oxygenase accumulation in the hornwort (Bryophyta: Anthocerotae) chloroplast. Protoplasma 156:117–129

Vaughn KC, Ligrone R, Owen HA, Hasegawa J, Campbell EO, Renzaglia KS, Mongenajera J (1992) The Anthocerote chloroplast – a review. New Phytol 120:169–190

Villarreal JC, Renner SS (2012) Hornwort pyrenoids, carbon-concentrating structures, evolved and were lost at least five times during the last 100 million years. Proc Natl Acad Sci U S A 109(46):18873–18878

Villarreal JC, Renzaglia KS (2006) Structure and development of *Nostoc* strands in *Leiosporoceros dussii* (Anthocerotophyta): a novel symbiosis in land plants. Am J Bot 93:693–705

Villarreal JC, Cargill DC, Hagborg A, Söderström L, Renzaglia KS (2010) Hornwort diversity: patterns, causes and future work. Phytotaxa 9:150–166

von Caemmerer S, Quick WP, Furbank RT (2012) The development of C_4 rice: current progress and future challenges. Science 336:1671–1672

Voznesenskaya EV, Franceschi VR, Kiirats O, Freitag H, Edwards GE (2001) Kranz anatomy is not essential for terrestrial C_4 plant photosynthesis. Nature 414:543–546

Waite M, Sack L (2010) How does moss photosynthesis relate to leaf and canopy structure? Trait relationships for 10 Hawaiian species of contrasting light habitats. New Phytol 185:156–172

Whitney SM, Houtz RL, Alonso N (2011) Advancing our understanding and capacity to engineer nature's CO_2 sequestering enzyme, Rubisco. Plant Physiol (Rockv) 155:27–35

Williams TG, Flanagan LB (1996) Effect of changes in water content on photosynthesis, transpiration and discrimination against $^{13}CO_2$ and $C^{18}O^{16}O$ in *Pleurozium* and *Sphagnum*. Oecologia 108:38–46

Williams TG, Flanagan LB (1998) Measuring and modelling environmental influences on photosynthetic gas exchange in *Sphagnum* and *Pleurozium*. Plant Cell Environ 21:555–564

Yamano T, Miura K, Fukuzawa H (2008) Expression analysis of genes associated with the induction of the carbon-concentrating mechanism in *Chlamydomonas reinhardtii*. Plant Physiol (Rockv) 147:340–354

Chapter 7

Sunsafe Bryophytes: Photoprotection from Excess and Damaging Solar Radiation

Sharon A. Robinson* and Melinda J. Waterman
Institute for Conservation Biology and Environmental Management, University of Wollongong, Wollongong 2522, NSW, Australia

Summary

Whilst light is essential for photosynthesis and development of plants, both excess photosynthetically active radiation and certain wavelengths (e.g. high energy ultraviolet-B) radiation can be damaging. Plants in general possess a suite of mechanisms that act to either prevent absorption of damaging and excess radiation or to mitigate against the damage that such radiation can cause once it is absorbed. Whilst bryophytes share many of these photoprotective mechanisms with the vascular plants, there are key differences in the photoprotection available to bryophytes. Some of these differences pertain to structural features, such as protective epidermal layers, that are available to vascular plants but not generally to bryophytes. Bryophytes thus have to invest more in cellular level photoprotection than vascular plants. In other respects bryophytes may retain mechanisms found in algal ancestors (e.g. thermal energy dissipation associated with the LHCSR protein) that have been lost during the evolution of vascular plants. Many bryophytes are able to manage light absorption during desiccation and rehydration and freezing and thawing, resulting in potentially novel mechanisms of energy dissipation. Given the high stress environments that many bryophytes inhabit, from hot or frozen deserts to alpine habitats with high incident UV-B radiation, it is unsurprising that they have a suite of photoprotective strategies.

*Author for correspondence, e-mail: sharonr@uow.edu.au

D.T. Hanson and S.K. Rice (eds.), Photosynthesis in Bryophytes and Early Land Plants, Advances in Photosynthesis and Respiration 37, pp. 113–130, DOI 10.1007/978-94-007-6988-5_7,

I. Introduction

Light provides the energy source for photosynthesis and is essential for all plants, however, certain wavelengths, especially ultraviolet-B (UV-B) radiation can cause direct damage to the photosynthetic apparatus especially photosystem II (PSII). The challenge facing photosynthetic organisms is therefore to optimize light absorption for photosynthesis while avoiding damage. Plants have evolved a number of strategies to tailor light absorption to the capacity for utilization by photosynthesis and to either protect themselves from photodamage or repair any that occurs (Takahashi and Badger 2011). Photoprotection occurs at a range of scales from processes at the molecular level such as dissipation of absorbed light energy as heat (Demmig-Adams and Adams 1992; Niyogi 1999; Nichol et al. 2012) to organ level mechanisms e.g. leaf movements and shading of radiation by waxes and hairs and screening pigments (Robberecht and Caldwell 1978; Ehleringer and Cook 1987; Robinson et al. 1993; Barker et al. 1997; Karabourniotis and Bornman 1999).

The energy to drive photosynthesis comes mainly from the visible spectrum (400–700 nm). However, solar radiation also contains ultraviolet (UV) radiation, which is absorbed by plants and can damage a range of biomolecules including DNA, RNA, proteins and PSII. Ultraviolet radiation increases naturally with altitude and decreases with latitude but has also been anthopogenically increased in polar regions, as a result of the ozone hole (McKenzie et al. 2007). Bryophytes are the dominant plant species in many of these high UV environments (alpine and polar regions; see Chap. 17) and appear to be generally well protected from the damaging effects of UV-B radiation (Newsham and Robinson 2009).

Abbreviations: PSII – photosystem II; UVAC – ultraviolet-B absorbing compound; UV-B – ultraviolet-B; L/Lx – lutein/lutein epoxide; NPQ – non-photochemical quenching; ROS – reactive oxygen species; PAR – photosynthetically active radiation

Recent work suggests that primary photodamage to the photosynthetic apparatus occurs through direct absorption of light by the manganese cluster in the oxygen-evolving complex of PSII, with UV wavelengths followed by yellow wavelengths being most damaging (Takahashi et al. 2010). Primary photodamage to PSII is thus prevented by avoiding exposure to the damaging wavelengths, rather than dissipating the excess energy once it has been absorbed. Excess photosynthetically active radiation (PAR) absorbed by the light-harvesting complexes can still lead to production of reactive oxygen species (ROS) and so mechanisms that prevent ROS accumulation also play a role in photoprotection (reviewed in Takahashi and Badger 2011). Whilst some photoprotective mechanisms offer cross protection by screening both visible and UV radiation, terrestrial plants also have a range of specific strategies to protect themselves from UV radiation.

As photosynthetic organisms, bryophytes therefore need to optimize light utilization but also protect their photosynthetic apparatus from damage. Although, many bryophytes can avoid damage by virtue of their environmental niche, for example those that grow in shady forests and other low light environments, some exist in open environments that combine high radiation with other potential stressors such as high temperatures and desiccation. Even shady environments can have a variable light regime, with sunflecks potentially supplying excess light to the chloroplasts (Watling et al. 1997). The absolute quantity of excess light depends on the photosynthetic capacity of the plant. Plants adapted to growth in high radiation environments will have high photosynthetic capacities, and thresholds for excess light will be greater than in plants adapted to low light, with correspondingly low photosynthetic capacities. In addition plants are usually able to cope with normal, diurnal fluctuations in light levels and can adapt to seasonal changes over time. Sudden increases present the greatest challenge to plants, for example the low- to high-light transition that occurs when a treefall gap is created in a

rainforest (Lovelock et al. 1994). Often plants experience excess light because an additional environmental or biotic stress reduces their photosynthetic rate and therefore the threshold for excess light is reduced. Whilst drought and temperature stress can impact photosynthetic rates in any plant species, many bryophytes have unusual physiological properties that could increase their risk of exposure to excess light. For example as water is lost from a desiccation tolerant moss the photosynthetic rate will decline (see Chaps. 15 and 16) and this will often coincide with exposure to high radiation, potentially increasing the requirement for photoprotection (Proctor and Smirnoff 2011). Not surprisingly, tolerance to UV radiation exposure is often correlated with desiccation tolerance in bryophyte species (Csintalan et al. 1999). Phototolerance has also been shown to develop seasonally in desiccation-tolerant mosses, for example *Rhytidiadelphus squarrosus* shows greater tolerance to high light during dry summers than during the more humid winter and this tolerance can be simulated under laboratory conditions (Heber et al. 2006).

Plant protective strategies can be divided into those that operate to reduce light absorption and those that act within the leaf or photosynthetic organ to prevent absorbed light causing damage within the chloroplast.

II. Avoiding Absorption of Excessive or Damaging Radiation

Bryophytes differ greatly from vascular plants in their morphology as they lack a protective cuticle and tissue differentiation (Gehrke 1999) consequently leaving them more susceptible to photoinhibition and UV-induced damage (Fig. 7.1). Many external photoprotective mechanisms rely on structural features found in leaves of higher plants but not mosses, for example, external or epidermal screening through coatings or structures (e.g. wax and hairs; Ehleringer and Björkman 1978; Robinson et al. 1993) or the ability of thick leaves to self shade lower cell layers (Robinson and Osmond 1994). Avoidance type photoprotective mechanisms that could be employed by mosses include leaf orientation, self shading within the canopy, chloroplast movement and specific screening compounds.

A. Generic Screening Mechanisms in Bryophytes

Surface reflectance of moss turfs varies between species (Lovelock and Robinson 2002) and also within species depending on the exposure to incident PAR and UV radiation (Robinson et al. 2005). Light attenuation through moss canopies varied six fold in *Pleurozium schreberi* collected from a range of habitats, showing that transmission characteristics are also plastic (Rice et al. 2011). Reflectance from the moss canopy also increases as mosses desiccate reducing the quantity of light that can be absorbed and therefore lowering the potential for photodamage (Van Gaalen et al. 2007). Curling of stems of the desiccation tolerant pteridophyte *Selaginella lepidophylla* has been shown to reduce photoinhibition (Lebkuecher and Eickmeier 1991) and this mechanism could also operate in mosses where drying and curling of leaves allows light to penetrate deeper into the canopy as less is intercepted by the top layer (Davey and Ellis-Evans 1996; Zotz and Kahler 2007; Rice et al. 2011). Chloroplasts can move within the cell to optimize light interception, as has been shown in the moss *Physcomitrella patens* (reviewed in Wada et al. 2003; Suetsugu and Wada 2007, see Chap. 8).

Compounds which act to screen specific wavelengths particularly UV-B radiation can be located within the photosynthetic cell itself or in the exposed epidermal layers. Within the typical leaf of vascular plants these sunscreens are often located in the epidermal layers but since most bryophytes lack such differentiation they will mainly occur within the photosynthetic cell (Lovelock and Robinson 2002; Newsham et al. 2002, 2005; Dunn and Robinson 2006; Newsham 2011). In some plants (Semerdjieva et al. 2003) and

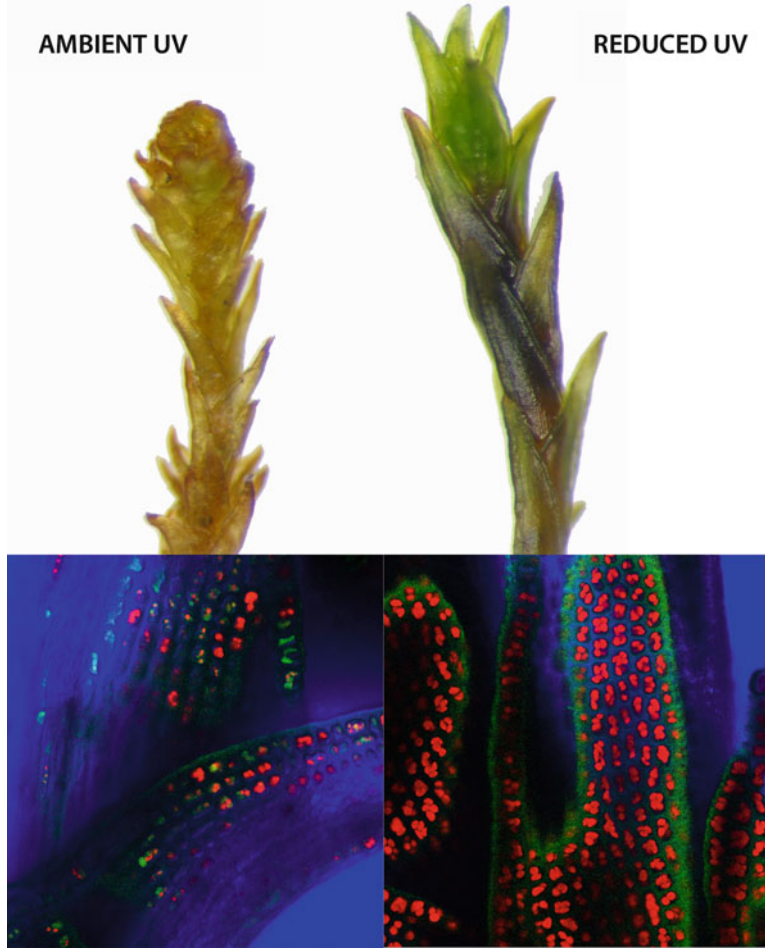


Fig. 7.1. Comparison of *Schistidium antarctici* gametophytes showing the normal leaf morphology and green coloration under reduced levels of UV exposure (*top right*) in contrast to the atypical leaf morphology and loss of chlorophyll in moss exposed to near-ambient UV radiation levels (*top left*). Below confocal images of the same leaves show brightly fluorescing chloroplasts in moss grown under reduced UV light and negligible chloroplasts under near-ambient UV (Partly reproduced with permission from Robinson et al. (2005); Images produced by Johanna Turnbull and Andrew Netherwood).

certain moss species they also accumulate in the cell walls (Fig. 7.2; Semerdjieva et al. 2003; Clarke and Robinson 2008). Since most experiments concerned with the accumulation of UV absorbing compounds (UVAC) focus on methanol soluble compounds, accumulation of such compounds in the cell walls maybe seriously underreported. Since UVAC should also reduce damage to PSII their accumulation, location and effectiveness in screening the photosynthetic apparatus is an important aspect of photoprotection (Takahashi and Badger 2011).

B. Production of Specific UV Absorbing Compounds in Bryophytes

Both vascular and non-vascular plants produce secondary metabolites that can specifically screen out damaging ultraviolet radiation. A range of compounds with UV-absorbing properties including flavonoids,

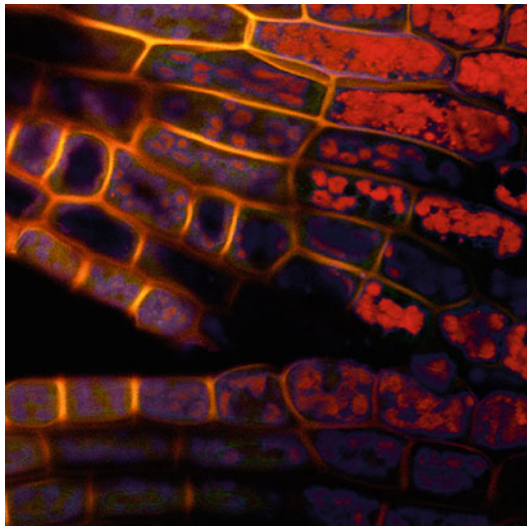


Fig. 7.2. Confocal images of *Ceratodon purpureus* leaves stained with stained with Naturstoffreagenz A to show the location of UV-B absorbing compounds. Yellow/orange fluorescence indicates the presence of phenolic compounds and chlorophyll autofluorescence is colored in *red* (Image produced by Laurence Clarke and Andrew Netherwood).

mycosporine-like amino acids, carotenoids, simple phenolics and hydroxycinnamic esters have been extracted and isolated from various organisms including several vascular plants, mosses, liverworts, phytoplankton, algae and cyanobacteria. Not only can these photoprotective compounds absorb UV light reducing the levels of harmful solar radiation reaching the photosynthetic apparatus and UV sensitive molecules (Fig. 7.3) some, such as carotenoids and flavonoids, can also scavenge reactive oxygen species generated by UV radiation (Cash et al. 2007) preventing further UV-induced damage to DNA, proteins, membranes and PSII (section IIIB). The composition of UVAC differs between organisms (Cooper-Driver and Bhattacharya 1998; Rozema et al. 2002; Bjorn 2007). Whilst flavonoids are the most common UVAC found in plants, and are ubiquitous in vascular plants, less than half of the bryophytes studied contain flavonoids (Markham 1990; Cooper-Driver and Bhattacharya 1998).

Comparison of studies into the impact of UV-B radiation on plants in general are often compounded by the methodology used; e.g. location (controlled laboratory conditions or field experiments) and sources of radiation whether natural fluctuating UV, solar radiation filtered through various screens or artificially produced using lamps that enhanced UV-B radiation levels (e.g. Caldwell and Flint 1997; Newsham and Robinson 2009). Whilst the synthesis of UV photoprotective compounds is less studied in bryophytes than vascular plants, it still appears to be one of the most common plant responses to elevated UV-B exposure (Searles et al. 2001, 2002; Newsham and Robinson 2009). Accumulation of photoprotective compounds in response to elevated UV-B radiation occurs in many mosses including *P. schreberi* (Lappalainen et al. 2008), *Bryum argenteum* (Markham 1990), *Polytrichastrum alpinum*, *Funaria hygrometrica* and three Sphagnum species (Huttunen et al. 2005) as well as the Antarctic species *Bryum pseudotriquetrum* (Dunn and Robinson 2006), *Andreaea regularis* (Newsham 2003) and *Sanionia uncinata* (Newsham et al. 2002). Liverworts that showed similar trends include *Jungermannia exsertifolia* subsp. *cordifolia* (Arroniz-Crespo et al. 2011) and *Cephaloziella varians* (*exiliflora*) (Snell et al. 2009).

However, the synthesis of UVAC did not increase with increasing UV-B light in all moss species studied e.g. *Polytrichum commune* (Barsig et al. 1998; Gehrke 1999), *Schistidium antarctici* (Dunn and Robinson 2006), *Hylocomium splendens* (Gehrke 1999; Taipale and Huttunen 2002), *S. uncinata* (temperate species; Lud et al. 2002), *Polytrichum juniperinum* (Lappalainen et al. 2009), and *Sphagnum balticum* and *Sphagnum papillosum* (Niemi et al. 2002). The lack of UV absorbing pigments detected in some or all of these species may reflect the methodology used, which commonly only extracts the intracellular UVAC (Section IIC; Semerdjieva et al. 2003; Clarke et al. 2008). It is also possible that some species maintain a high level of UVAC compounds constitutively rather than producing them only in response to elevated UV-B radiation.

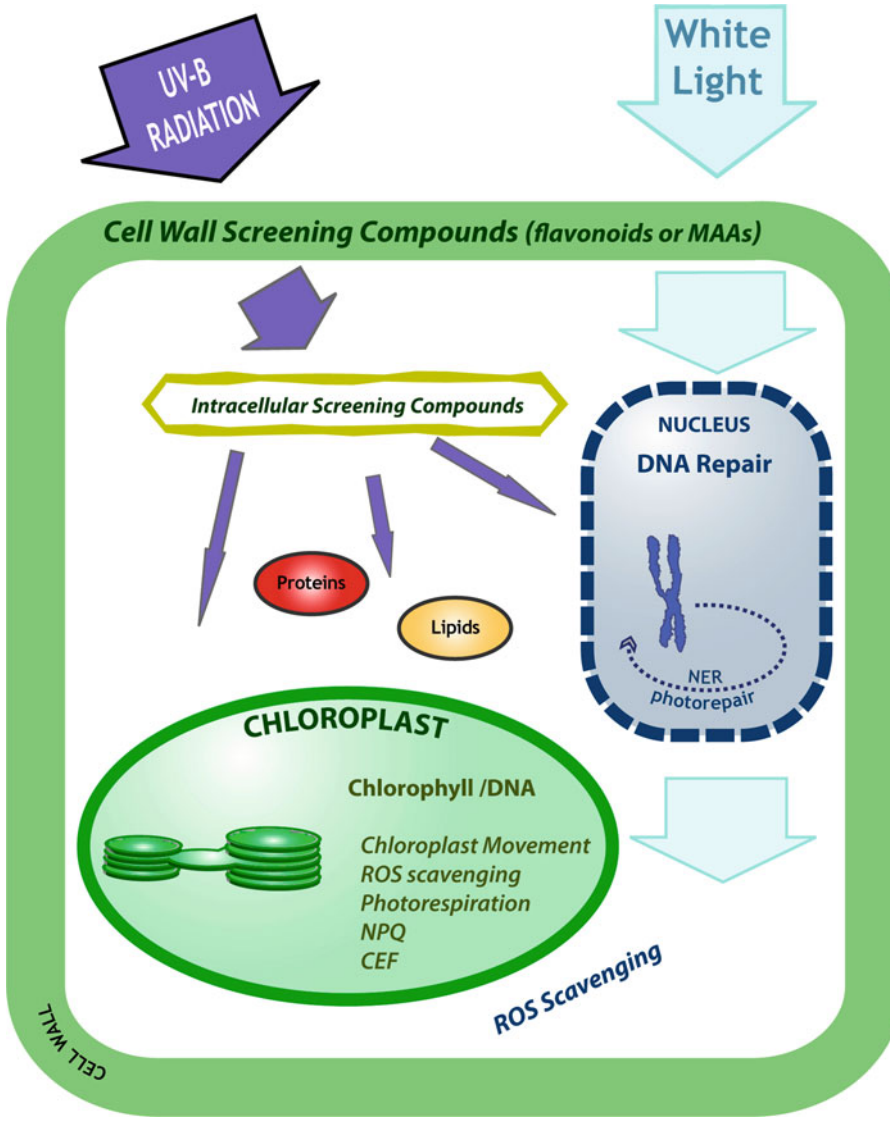


Fig. 7.3. The location of various mechanisms of photoprotection at the cellular level. Biological molecules susceptible to damage are shown in *bold* and mechanisms that provide photoprotection are displayed in *italics*. Since screens are selective to UV photosynthetically active radiation is unaffected (Image produced by Andrew Netherwood). Abbreviations: *CEF* cyclic electron flow, *NER* nuclear excision repair, *NPQ* non-photochemical quenching, *MAA* mycosporine-like amino acids, *ROS* reactive oxygen species.

Few studies have actually quantified the metabolic cost to bryophytes of UVAC production, a study of the liverwort *C. varians* in Antarctica suggests the cost maybe relatively low (<2 %; Snell et al. 2009).

Whether photoprotective compounds are induced by elevated levels of UV or are constitutively produced (Bornman 1998), their presence in bryophytes is usually effective in maintaining optimal photosynthetic efficiency measured by chlorophyll fluorescence (Fv/Fm, a measure of plant stress). This is demonstrated in the photoprotection exhibited in Antarctic mosses *B. argentum* and *Ceratodon purpureus* (Green et al. 2005) and in the temperate mosses *H. splendens* and *P. commune* (Arroniz-Crespo et al. 2011). Similarly multiple regression analysis

of the response of two Antarctic bryophytes (*S. uncinata* and *C. varians*) suggests that UV-B screening pigments protect against UV-B induced lowering of Fv/Fm in these species (Newsham et al. 2002). In contrast low concentrations of UVAC were found in the endemic Antarctic moss species *S. antarctici* (Dunn and Robinson 2006; Clarke and Robinson 2008) resulting in a lack of protection to PSII that could be causing photoinhibition when this moss is exposed to high UV-B radiation levels (Adamson et al. 1988) and contributing to its susceptibility to the ozone hole increased, UV environment (Turnbull et al. 2009; Turnbull and Robinson 2009). An UV-B specific decline in Fv/Fm (under PAR + UVA + UVB as compared to PAR and PAR + UVA treatments) was also observed in two aquatic bryophytes, the moss *Fontinalis antipretica* and the liverwort *J. exsertifolia*, for the duration of a 36 day experiment (Martinez-Abaigar et al. 2003) possibly demonstrating direct UV induced photoinhibition of PSII as described by Takahashi et al. (2010).

Some bryophytes exhibit naturally green and red forms that change in response to differing UV environments. Generally, the red forms are found in exposed and drier sites and the morphologically similar green form grows in naturally shaded and wetter sites. Red forms of bryophytes appear more resistant to the damaging effects of UV radiation (Post 1990; Post and Vesk 1992; Hooijmaijers and Gould 2007). For example, the red form of the liverwort *Jamesoniella colorata* maintained greater Fv/Fm, photochemical quenching (qP) and non-photochemical quenching (NPQ) than its green counterpart when exposed to UV-B radiation (Hooijmaijers and Gould 2007). The red pigment in this liverwort was found to be tightly associated with the cell wall but has not yet been identified. Similarly, red anthocyanic pigmentation is evident within the Antarctic liverwort *C. varians* (Post and Vesk 1992; Newsham 2010) and the cell walls of red *C. purpureus* (Post 1990; Green et al. 2005) and may contribute to the greater resistance to UV-induced effects of the red rather than the green forms of these species.

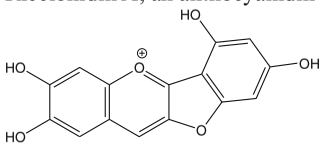
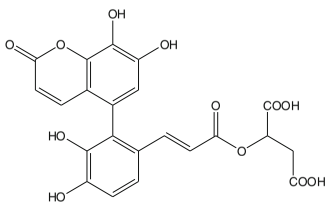
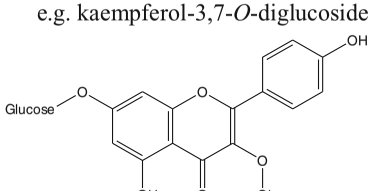
C. Structure of UV Absorbing Compounds in Bryophytes

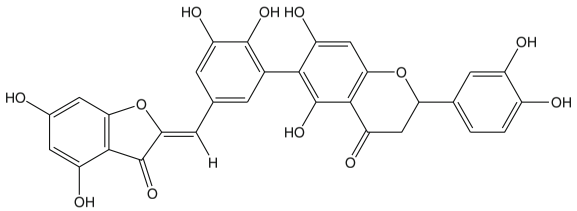
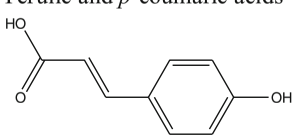
The ability of flavonoids, hydroxycinnamic acids and other photoprotective compounds to absorb within the UV-B range (280–315 nm) is based on their aromatic structures. The majority of these compounds are phenolics containing at least one aromatic ring, usually in the form of benzene, which allows high absorption in the UV range (Cockell 1998). This absorption range is completely dependent on the structure and does not include photosynthetically active radiation (Schnitzler et al. 1996; Cove et al. 1997). Simple phenolics have one absorption peak in the UV region and more complex phenolics, like flavonoids, have two or more (Meijkamp et al. 1999). Peaks of absorbance are not only determined by the aromatic rings but also by the nature and position of any substituents.

Flavonoids, which are commonly found in plants including many bryophytes, have a backbone consisting of 15 carbons that form aromatic rings connected by a three carbon bridge (Swain 1976; Koes et al. 1994). Flavonoids are divided into four prominent groups consisting of flavones, flavonols, isoflavones and anthocyanins. Various derivatives of these, hydroxycinnamic acids and other UV absorbing compounds have been found in polar and temperate bryophytes (Table 7.1).

Complex phenolics like flavonoids are derived from a combination of the shikimate and phenylpropanoid pathways (Koes et al. 1994). The phenylpropanoid pathway begins with the conversion of phenylalanine to cinnamic acid by phenylalanine ammonia-lyase (PAL; Fig. 7.4) Further catalysis by two other enzymes in the pathway leads to the formation of *p*-coumaroyl coenzyme A (CoA). The general flavonoid biosynthesis pathway in plants begins with chalcone synthase (CHS), an enzyme which catalyses the reaction between *p*-coumaroyl CoA (from the phenylpropanoid pathway) and three units of malonyl CoA (a product of the shikimate pathway). Cyclization results in the formation of a

Table 7.1. Some examples of the diverse range of UV absorbing compounds extracted, isolated and characterized from various bryophytes.

	Species	Types of UV absorbing compounds	Location	Region	Reference
Liverworts	*Cephaloziella varians*	Riccionidin A, an anthocyanidin 	Cell wall	Polar (Antarctica)	Snell et al. (2009)
	Jungermannia cordifolia	Hydroxycinnamic acid derivatives e.g. 5″-(7″,8″-dihydroxycoumaroyl)-2-caffeoylmalic acid 	Intracellular	Temperate (Spain)	Arroniz-Crespo et al. (2008)
	Marchantia polymorpha (*aquatica)*	Apigenin and luteolin glucuronides (major) and apigenin and luteolin (minor)	Intracellular	Temperate	Markham and Porter (1974)
	Tylimanthus renifolius	Flavone and flavanone derivatives e.g. 6-hydroxy-5,7-dimethoxyflavone	Intracellular	Temperate (Argentina)	Feld et al. (2003)
Mosses	*Bartramia pomiformis*	Bartramia flavone	Intracellular	Temperate	Basile et al. (1999)
	Brachythecium rutabulum	Ferulic and *p*-coumaric acids	Cell wall	Temperate	Davidson et al. (1989)
	Bryum argenteum	Luteolin and apigenin aglycones	Intracellular	Polar (Antarctic)	Ryan et al. (2009)
	Bryum algens	A total of 28 flavonoids found including many flavone *C*- and *O*-glycosides and flavonols e.g. kaempferol-3,7-*O*-diglucoside 	Intracellular	Polar (Antarctic)	Webby et al. (1996)

Bryum capillare	Isoflavones orobol and pratensein and their 7-*O*-glucosides	Intracellular	Temperate	Anhut et al. (1984)
Bryum spp. Incl. *Bryum argenteum*	Apigenin and luteolin glucosides and their 6″-malonyl esters, and 7-*O*-glucosides of 8-hydroxyapigenin and 8-hydroxyluteolin	Intracellular	Polar (Antarctica)	Markham and Given (1988)
Campylopus clavatus	Three biflavonoids e.g. campylopusaurone 	Intracellular	Temperate (New Zealand)	Geiger and Markham (1992)
Campylopus holomitrium	Same three biflavonoids as *C. clavatus* (above)	Intracellular	Temperate (New Zealand)	Geiger and Markham (1992)
Dicranium scoparium	Apigenin-7-*O*-triglycoside and luteolin-7-*O*-neohesperidoside	Intracellular	Temperate	Basile et al. (1999)
Hedwigia ciliate	Lucenin-2	Intracellular	Temperate	Basile et al. (1999)
Mnium hornum	Three biflavonoids, 6 4,2′-epoxy-3-phenyl-coumarin derivatives (isoflavone related) and a sugar cafeate	Intracellular	Temperate (Germany)	Brinkmeier et al. (1999)
Mnium hornum	Ferulic and *p*-coumaric acids  *p*-coumaric acid	Cell wall	Temperate	Davidson et al. (1989)
Plagiomnium affine	Apigenin and vitexin	Intracellular	Temperate	Basile et al. (1999)
Plagiomnium cuspidatum	Saponarine	Intracellular	Temperate	Basile et al. (1999)

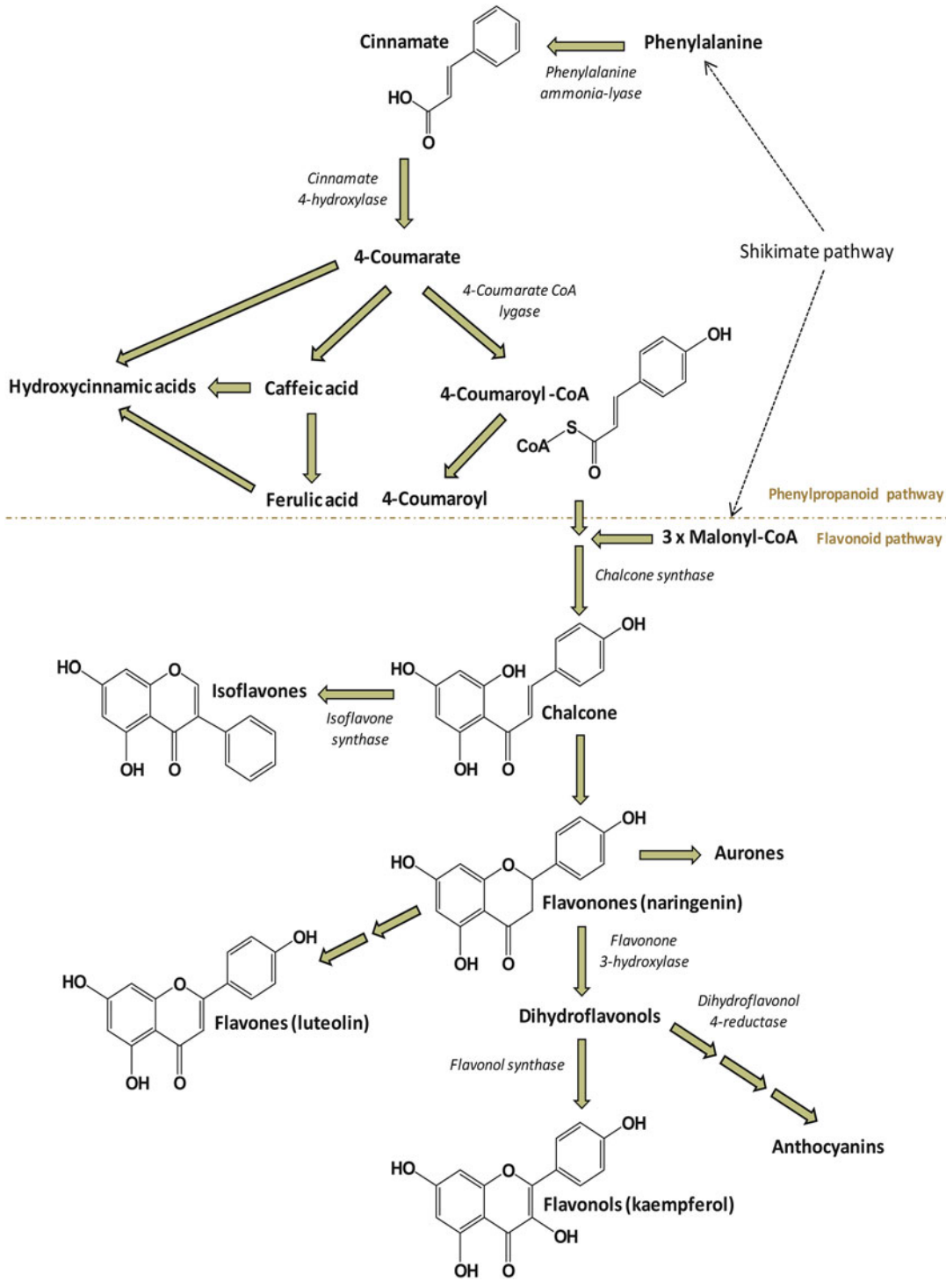


Fig. 7.4. The general phenylpropanoid and flavonoid pathway for the biosynthesis of various UV absorbing compounds.

chalcone (naringenin chalcone). This initiates the development of complex phenolic compounds including flavonoids and lignin (Boelen et al. 2006). Whilst there is limited information regarding biosynthesis of flavonoids and other UV absorbing compounds in bryophytes specifically, genome sequences confirm that a CHS multigene family exists in *Physcomitrella patens* and there are similarities between the enzymatic properties of CHS from this moss species and that of higher plants (Jiang et al. 2006).

The accumulation of flavonoids and other photoprotective compounds is most likely activated due to PAL, CHS and other enzymes involved in their production being stimulated by UV-B (Rozema et al. 2002; Rizzini et al. 2011; United Nations Environment Programme 2012). There is also evidence suggesting that the genes encoding these enzymes can be up-regulated by UV radiation (Cooper-Driver and Bhattacharya 1998; Ballare et al. 2011) as has been demonstrated in the moss *P. patens* (Wolf et al. 2010).

Within vascular plant cells, flavonoids are located in the cytoplasm, plastid membranes, vacuoles, nuclei and cell walls (Swain 1976; Schnitzler et al. 1996; Agati et al. 2007). The majority of studies of UVAC in bryophytes have focused on the methanol-extractable or intracellular compounds. These are the most accessible for extraction and subsequent isolation and characterization. However, recent studies showing the presence of cell wall photoprotective compounds within bryophytes potentially indicates a more effective protective barrier against UV-B radiation. The UV tolerant *C. purpureus* is one such bryophyte that localizes the majority of its UVAC within its cell walls (Fig. 7.2; Clarke and Robinson 2008). Although reports of photoprotective compounds bound to the cell walls of bryophytes or other plant species are rare (Semerdjieva et al. 2003; Clarke and Robinson 2008) this may reflect the lack of studies that have used alkaline digestion to extract these wall bound pigments rather than the absence of UVAC in these locations. Cell wall UVAC would function as a first defense barrier to UV radiation in bryophytes and could prove to be a more effective UV screen than intracellular UV absorbing compounds (Turnbull et al. 2009; Turnbull and Robinson 2009). Two intermediates in the phenylpropanoid pathway, ferulic and coumaric acids have been isolated from the cell walls of *Mnium hornum* (Davidson et al. 1989). These compounds are acetylated within the cell to form polymers that can then be bound within the cell wall.

III. Dealing with Excess Light Absorbed Within the Chloroplast

If excess or damaging light is not absorbed by screening compounds in the cell wall or intracellularly there are mechanisms within the chloroplast that can also protect against photodamage. Absorption of excess PAR radiation could lead to accumulation of ROS, which in turn inhibits the repair of damaged PSII. Prevention of ROS accumulation occurs through both dissipation of the energy prior to ROS formation and scavenging of any ROS that are produced. Photoprotective mechanisms that can reduce the production of ROS include thermal dissipation of light energy (Nichol et al. 2012), as well as pathways that consume the excess light energy such as cyclic electron flow and photorespiration (reviewed in Takahashi and Badger 2011). The discrepancy between relatively low carbon fixation rates and the often non-saturating electron transport rates (measured by chlorophyll fluorescence) suggest that alternative electron sinks are an important component of photoprotection in many bryophytes (Proctor and Smirnoff 2011).

A. Dissipating Excess Energy as Heat, Non Photochemical Quenching and the Xanthophyll Cycles

If excess light is absorbed by the light-harvesting complexes (LHC) of PSII it can be dissipated as harmless heat energy (thermal energy dissipation; qE or non photochemical

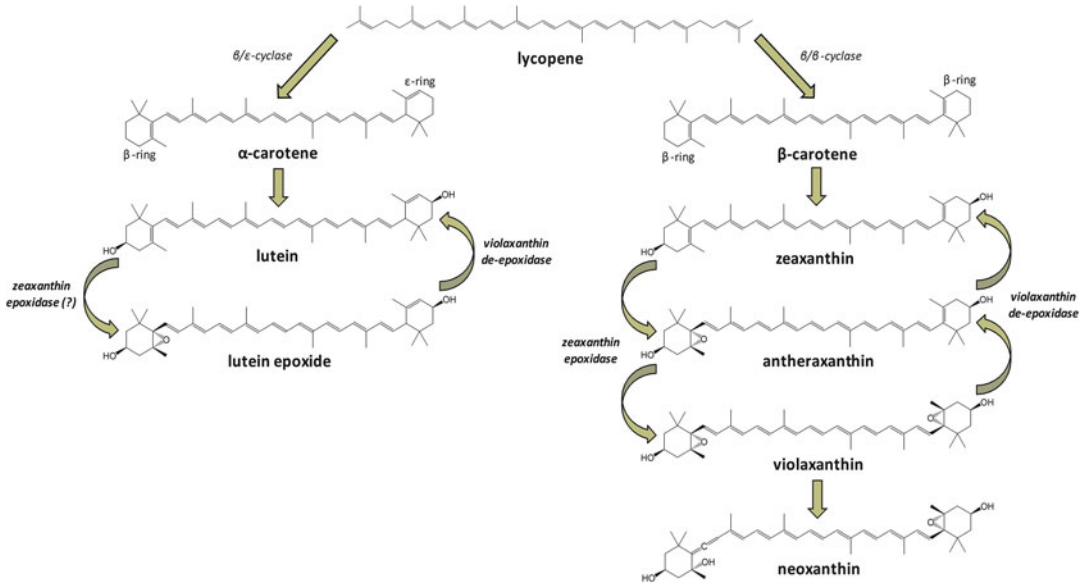


Fig. 7.5. The synthesis and interconversion of the pigments that comprise the two plant xanthophyll cycles (VAZ and L/Lx).

quenching NPQ). Thermal energy dissipation is associated with the activity of one or more xanthophyll cycles (reviewed in Nichol et al. 2012). The first of these involves the light dependent conversion of violaxanthin (V) to zeaxanthin (Z) via antheraxanthin (A) (Demmig-Adams and W. W. Adams 1992); whilst the second involves the direct interconversion of lutein to lutein epoxidase (Fig. 7.5; Bungard et al. 1999; García-Plazaola et al. 2007). These conversions are catalyzed by the enzyme violaxanthin de-epoxidase and, in addition to the involvement of lutein or zeaxanthin, qE also requires protonation of the PSII protein subunit PsbS (an ortholog of this protein is present in mosses Alboresi et al. 2008). A low pH in the chloroplast lumen also enhances both the interconversion to the photoprotective form (L or Z) and the potential for thermal dissipation, which enables subtle switching of qE activity to correspond with the need for photoprotection (Niyogi 1999). Recent work with Arabidopsis mutants suggests that qE acts to prevent photoinhibition by suppressing the formation of ROS, which would otherwise impair the processes that repair damaged PSII (Takahashi and Badger 2011).

Sequence analysis of the antenna protein multigene family in *P. patens*, has shown that some antenna polypeptides, such as Lhcb6, are present only in land plants, suggesting they play a role in adaptation to the subaerial environment and more particularly in the formation of NPQ (Alboresi et al. 2008). In addition to PsbS, *P. patens* produces isoforms of another protein (LHCSR), which is involved in formation of NPQ in algae (Alboresia et al. 2010; Gerotto et al. 2011). The presence of these two NPQ related proteins in *P. patens* suggests that the PsbS-dependent NPQ of plants evolved before the LHCSR based mechanism typical of the algal ancestor was lost. LHCSR was subsequently lost, in vascular plants, presumably as the newly evolved PsbS-dependent mechanism ensured a sufficient level of photoprotection (Alboresia et al. 2010).

Acclimation of *P. patens* to either high light or low temperature is accompanied by the ability to produce a strong, fast NPQ response associated with overexpression of both PsbS and LHCSR proteins (Gerotto et al. 2011). Mutants depleted of PsbS and/or LHCSR confirm that the NPQ response is associated with presence of these proteins

and show enhanced photosensitivity when exposed to either high light or low temperature. Different isoforms of LHCSR appear to be involved in acclimation to either high light (LHCSR1) or low temperatures (LHCSR2, Gerotto et al. 2011).

Whilst the VAZ xanthophyll cycle has been shown to be present in many bryophytes (Deltoro et al. 1998; Lovelock and Robinson 2002; Newsham et al. 2002; Robinson et al. 2005; Arroniz-Crespo et al. 2011), until recently the L/Lx cycle has received less attention. Lutein epoxide was found in leaves of 62 % of the species specifically examined for this carotenoid (García-Plazaola et al. 2007) and its prevalence in shade plants and co-occurrence with ∝-carotene (another pigment associated with shade leaves) suggests it should be present in most shade inhabiting bryophytes (Matsubara et al. 2009). In a survey of 14 species of bryophytes using thin layer chromatography (TLC), Czeczuga and coworkers (2006) found the gametophytes contained up to 25 carotenoids, with β-carotene, β-cryptoxanthin, lutein, and lutein epoxide found in all species examined. Quantification and studies into the involvement of the L/Lx xanthophyll cycle in bryophytes will require the adoption of modified methods of high performance liquid chromatography (HPLC; Förster et al. 2009) since the shorter HPLC runs normally used to analyze the VAZ xanthophyll cycle pigments, tend to cause co-elution of pigments and can mask the presence of Lx.

Strong NPQ, often associated with de-epoxidation of V to Z, is common in bryophytes, especially those from sun-adapted habitats (Marschall and Proctor 2004), and under desiccating (Deltoro et al. 1998) or freezing conditions (Deltoro et al. 1999) suggesting that they can dissipate excess light energy effectively. The epoxidation of Z back to V can also be slow leading to sustained high levels of Z and the potential for fast activation of NPQ dependent on the ΔpH (Lovelock et al. 1995a; Deltoro et al. 1998). The constitutive presence of Z is likely to be particularly important in those bryophytes that go through repeated cycles of desiccation and rehydration or freezing and thawing. A good example of priming of the xanthophyll cycle in the protective form has been demonstrated in desiccation tolerant species from a range of plant forms including mosses and liverworts (Fernandez-Marin et al. 2011). Slow desiccation, of paired desiccation sensitive (*Lunularia cruciata* and *Palustriella* sp.) and desiccation tolerant (*Frullania dilatata* and *Syntrichia ruralis*) species produced de-epoxidation of the xanthophyll cycle pigments in darkness, accompanied by a reduction in Fv/Fm. After re-wetting in darkness, the pigments were converted back to V in parallel with the recovery of Fv/Fm in both mosses and the desiccation tolerant liverwort, with the desiccation tolerant bryophytes both showing full recovery of initial Fv/Fm. The stability of the β-carotene pool confirmed that Z was produced from V and not by *de novo* synthesis. This ability to produce Z in the dark during dehydration presumably offers potential protection when bryophytes face sudden rehydration in the light.

Several groups (Heber et al. 2006; Heber et al. 2007; Nabe et al. 2007) have proposed that during slow desiccation another thermal energy dissipation mechanism is activated which requires neither protonation nor Z but acts alongside Z-dependent energy dissipation, providing desiccation occurs in the light. They attribute this to the formation of quenching PSII reaction centers in desiccated poikilohydric autotrophs (Heber et al. 2006). Such quenching centers might explain similar findings in the Antarctic moss *S. antarctici* during freezing (Lovelock et al. 1995a, b). The extent to which this is related to LHCSR proteins remains to be elucidated (Gerotto et al. 2011).

B. Consuming Excess Energy in the Chloroplasts: Cyclic Electron Flow, Photorespiration and the Mehler Reaction

Processes that consume energy in the chloroplast effectively prevent the formation of ROS. Cyclic electron flow around PSI

enhances the development of ΔpH across the thylakoid membrane and has been shown to play a role photoprotection via at least two mechanisms (reviewed in Shikanai 2007; Takahashi and Badger 2011).

Photorespiration, the oxygenation of ribulose -1,5-bisphospate (RuBP) by ribulose -1,5-bisphospate carboylase-oygenase (Rubisco) maintains energy utilization and can thus have a photoprotective function when carboxylation is limited by low CO_2 concentration. This could be particularly important in bryophytes since diffusion of CO_2 into leaves maybe limited by relatively unventilated leaf surfaces (compared to higher plant leaves) (Marschall and Proctor 2004). Studies with sun exposed *Schistidium apocarpum* indicate a very high capacity for oxygen photoreduction when CO_2 assimilation is limited but suggest this is not photorespiratory in nature but more likely the Mehler-peroxidase reaction (water-water cycle; Asada 2006; Proctor and Smirnoff 2011). Since the Mehler reaction causes photoreduction of oxygen to hydrogen peroxide (H_2O_2) in Photosystem I this reaction depends on an effective ROS scavenging system (Asada 2006).

If all these photoprotective mechanisms fail or the Mehler reaction is occurring and ROS are produced within the chloroplasts, oxidative stress can still be avoided if the ROS are effectively scavenged. Multiple enzymes, including superoxide dismutase and ascorbate peroxidase (and peroxiredoxin) and antioxidant compounds (e.g. ascorbate, ∝-tocopherol and carotenoids such as zeaxanthin, lutein and β-carotene) act as scavenging systems. These ROS scavenging systems have been demonstrated in mosses as in other plants (Dhindsa 1991; Seel et al. 1992).

IV. Conclusions

Despite being commonly associated with low light environments bryophytes generally show an impressive suite of photoprotective mechanisms most but not all of which are also common to vascular plants. Areas that stand out as requiring further study include; an assessment of the role of UV radiation in causing specific damage to PSII, analysis of the role of cell wall UVAC in screening damaging UV-B radiation, clarification of the roles of the PsbS and LHCSR proteins in nonphotochemical quenching and an investigation of the role of the L/Lx cycle in photoprotection in bryophytes. Determination of sequences for additional bryophyte species, such as *C. purpureus*, combined with targeted physiological and biochemical studies should ensure improved understanding of the evolution of photoprotective strategies in the land plants.

Acknowledgements

Melinda Waterman is in receipt of an Australian Postgraduate Award and an Australian Institute of Nuclear Science and Engineering postgraduate award. Funding was provided by Australian Research Council DP110101714 and Antarctic Science Grants 3129 & 3042. We would like to thank Diana King for her support in preparing this review; and Johanna Turnbull, Laurence Clarke and Andrew Netherwood for production of figures.

References

Adamson H, Wilson M, Selkirk PM, Seppelt RD (1988) Photoinhibition in Antarctic mosses. Polarforschung 58:103–111

Agati G, Matteini P, Goti A, Tattini M (2007) Chloroplast-located flavonoids can scavenge singlet oxygen. New Phytol 174:77–89

Alboresi A, Caffarri S, Nogue F, Bassi R, Morosinotto T (2008) In silico and biochemical analysis of *Physcomitrella patens* photosynthetic antenna: identification of subunits which evolved upon land adaptation. PLoS One 3:e2033

Alboresia A, Gerottob C, Giacomettib GM, Bassia R, Morosinotto T (2010) *Physcomitrella patens* mutants affected on heat dissipation clarify the evolution of photoprotection mechanisms upon land colonization. Proc Natl Acad Sci U S A 107:11128–11133

Anhut S, Zinsmeister HD, Mues R, Barz W, Mackenbrock K, Koster J, Markham KR (1984) The first identification of isoflavones from a bryophyte. Phytochemistry 23:1073–1075

Arroniz-Crespo M, Nunez-Olivera E, Martinez-Abaigar J (2008) Hydroxycinnamic acid derivatives in an aquatic liverwort as possible bioindicators of enhanced UV radiation. Environ Pollut 151:8–16

Arroniz-Crespo M, Gwynn-Jones D, Callaghan TV, Nunez-Olivera E, Martinez-Abaigar J, Horton P, Phoenix GK (2011) Impacts of long-term enhanced UV-B radiation on bryophytes in two sub-Arctic heathland sites of contrasting water availability. Ann Bot 108:557–565

Asada K (2006) Production and scavenging of reactive oxygen species in chloroplasts and their functions. Plant Physiol 141:391–396

Ballare CL, Caldwell MM, Flint SD, Robinson SA, Bornman JF (2011) Effects of solar ultraviolet radiation on terrestrial ecosystems. Patterns, mechanisms, and interactions with climate change. Photochem Photobiol Sci 10:226–241

Barker DH, Seaton GGR, Robinson SA (1997) Internal and external photoprotection in developing leaves of the CAM plant *Cotyledon orbiculata*. Plant Cell Environ 20:617–624

Barsig M, Schneider K, Gehrke C (1998) Effects of UV-B radiation on fine structure, carbohydrates, and pigments in *Polytrichum commune*. Bryologist 101:357–365

Basile A, Giordano S, Lopez-Saez JA, Cobianchi RC (1999) Antibacterial activity of pure flavonoids isolated from mosses. Phytochemistry 52:1479–1482

Bjorn LO (2007) Stratospheric ozone, ultraviolet radiation, and cryptogams. Biol Conserv 135:326–333

Boelen P, De Boer MK, De Bakker NVJ, Rozema J (2006) Outdoor studies on the effects of solar UV-B on bryophytes: overview and methodology. Plant Ecol 182:137–152

Bornman J (1998) Protective strategies in plants exposed to high visible and UV-B (280–315 nm) radiation. Photosynth Mech Eff V:3973–3978

Brinkmeier E, Geiger H, Zinsmeister HD (1999) Biflavonoids and 4,2′-epoxy-3-phenylcoumarins from the moss *Mnium hornum*. Phytochemistry 52:297–302

Bungard RA, Ruban AV, Hibberd JM, Press MC, Horton P, Scholes JD (1999) Unusual carotenoid composition and a new type of xanthophyll cycle in plants. Proc Natl Acad Sci U S A 96:1135–1139

Caldwell MM, Flint SD (1997) Uses of biological spectral weighting functions and the need of scaling for the ozone reduction problem. Plant Ecol 128:66–76

Cash TP, Pan Y, Simon MC (2007) Reactive oxygen species and cellular oxygen sensing. Free Radical Biol Med 43:1219–1225

Clarke LJ, Robinson SA (2008) Cell wall-bound ultraviolet-screening compounds explain the high ultraviolet tolerance of the Antarctic moss, *Ceratodon purpureus*. New Phytol 179:776–783

Clarke LJ, Ayre DJ, Robinson SA (2008) Somatic mutation and the Antarctic ozone hole. J Ecol 96:378–385

Cockell CS (1998) Ultraviolet-radiation, evolution and the Pi-electron system. Biol J Linn Soc 63:449–457

Cooper-Driver G, Bhattacharya M (1998) Role of phenolics in plant evolution. Phytochemistry 49: 1165–1174

Cove DJ, Knight CD, Lamparter T (1997) Mosses as model systems. Trends Plant Sci 2:99–105

Csintalan Z, Proctor MCF, Tuba Z (1999) Chlorophyll fluorescence during drying and rehydration in the mosses *Rhytidiadelphus loreus* (Hedw.) Warnst., *Anomodon viticulosus* (Hedw.) Hook. & Tayl. and *Grimmia pulvinata* (Hedw.) Sm. Ann Bot 84: 235–244

Czeczuga B, Czeczuga-Semeniuk E, Semeniuk A (2006) Effect of light quality on the content of chlorophylls, carotenoids and phytochrome in bryophytes. Trends Photochem Photobiol 11:105–116

Davey MC, Ellis-Evans JC (1996) The influence of water content on the light climate within Antarctic mosses characterized using an optical microprobe. J Bryol 19:235–242

Davidson AJ, Harborne JB, Longton RE (1989) Identification of hydroxycinnamic acid and phenolic acids in *Mnium hornum* and *Brachythecium rutabulum* and their possible role in protection against herbivory. J Hattori Bot Lab 67:413–422

Deltoro VI, Calatayud A, Gimeno C, Abadia A, Barreno E (1998) Changes in chlorophyll *a* fluorescence, photosynthetic CO_2 assimilation and xanthophyll cycle interconversions during dehydration in desiccation-tolerant and intolerant liverworts. Planta 207:224–228

Deltoro V, Calatayud A, Morales F, Abadia A, Barreno E (1999) Changes in net photosynthesis, chlorophyll fluorescence and xanthophyll cycle interconversions during freeze-thaw cycles in the Mediterranean moss *Leucodon sciuroides*. Oecologia 120:499–505

Demmig-Adams B, Adams I WW (1992) Photoprotection and other responses of plants to high light stress. Annu Rev Plant Physiol Plant Mol Biol 43:599–626

Dhindsa RS (1991) Drought stress, enzymes of glutathione metabolism, oxidation injury, and protein-synthesis in *Tortula-Ruralis*. Plant Physiol 95:648–651

Dunn JL, Robinson SA (2006) Ultraviolet B screening potential is higher in two cosmopolitan moss species than in a co-occurring Antarctic endemic moss: implications of continuing ozone depletion. Global Change Biol 12:2282–2296

Ehleringer J, Björkman O (1978) Pubescence and leaf spectral characteristics in a desert shrub *Encelia farinosa*. Oecologia 36:151–162

Ehleringer J, Cook C (1987) Leaf hairs in *Encelia* (Asteraceae). Am J Bot 74:1532–1540

Feld H, Zapp J, Becker H (2003) Secondary metabolites from the liverwort *Tylimanthus renifolius*. Phytochemistry 64:1335–1340

Fernandez-Marin B, Miguez F, Becerril JM, Garcia-Plazaola JI (2011) Dehydration-mediated activation of the xanthophyll cycle in darkness: is it related to desiccation tolerance? Planta 234:579–588

Förster B, Osmond C, Pogson B (2009) De-novo synthesis and degradation of Lx and V cycle pigments during shade and sun acclimation in avocado leaves (*P. americana*). Plant Physiol 149:1179–1195

García-Plazaola JI, Matsubara S, Osmond CB (2007) The lutein epoxide cycle in higher plants: its relationships to other xanthophyll cycles and possible functions. Funct Plant Biol 34:759–773

Gehrke C (1999) Impacts of enhanced ultraviolet-B radiation on mosses in a subarctic heath ecosystem. Ecology 80:1844–1851

Geiger H, Markham K (1992) Campylopusaurone, an auronoflavanone biflavonoid from the mosses *Campylopus clavayus* and *Campylopus holomitrium*. Phytochemistry 31:4325–4328

Gerotto C, Alboresi A, Giacometti GM, Bassi R, Morosinotto T (2011) Role of PSBS and LHCSR in *Physcomitrella patens* acclimation to high light and low temperature. Plant Cell Environ 34:922–932

Green TGA, Kulle D, Pannewitz S, Sancho LG, Schroeter B (2005) UV-A protection in mosses growing in continental Antarctica. Polar Biol 28:822–827

Heber U, Lange OL, Shuvalov VA (2006) Conservation and dissipation of light energy as complementary processes: homoiohydric and poikilohydric autotrophs. J Exp Bot 57:1211–1223

Heber U, Azarkovich M, Shuvalov V (2007) Activation of mechanisms of photoprotection by desiccation and by light: poikilohydric photoautotrophs. J Exp Bot 58:2745–2759

Hooijmaijers CAM, Gould KS (2007) Photoprotective pigments in red and green gametophytes of two New Zealand liverworts. N Z J Bot 45:451–461

Huttunen S, Lappalainen NM, Turunen J (2005) UV-absorbing compounds in subarctic herbarium bryophytes. Environ Pollut 133:303–314

Jiang CG, Schommer CK, Kim SY, Suh DY (2006) Cloning and characterization of chalcone synthase from the moss, *Physcomitrella patens*. Phytochemistry 67:2531–2540

Karabourniotis G, Bornman JF (1999) Penetration of UV-A, UV-B and blue light through the leaf trichome layers of two xeromorphic plants, olive and oak, measured by optical fibre microprobes. Physiol Plant 105:655–661

Koes R, Quattrocchio F, Mol J (1994) The flavonoid biosynthetic pathway in plants: function and evolution. Bioessays 16:123–132

Lappalainen NM, Huttunen S, Suokanerva H (2008) Acclimation of a pleurocarpous moss *Pleurozium schreberi* (Britt.) Mitt. to enhanced ultraviolet radiation in situ. Global Change Biol 14:321–333

Lappalainen NM, Huttunen S, Suokanerva H, Lakkala K (2009) Seasonal acclimation of the moss *Polytrichum juniperinum* Hedw. to natural and enhanced ultraviolet radiation. Environ Pollut 158: 891–900

Lebkuecher JG, Eickmeier WG (1991) Reduction of high irradiance damage with stem curling in *Selaginella lepidophylla*: a field experiment. Oecologia 88:597–604

Lovelock CE, Robinson SA (2002) Surface reflectance properties of Antarctic moss and their relationship to plant species, pigment composition and photosynthetic function. Plant Cell Environ 25: 1239–1250

Lovelock CE, Jebb M, Osmond CB (1994) Photoinhibition and recovery in tropical plant species: response to disturbance. Oecologia 97:297–307

Lovelock CE, Jackson AE, Melick DR, Seppelt RD (1995a) Reversible photoinhibition in Antarctic moss during freezing and thawing. Plant Physiol 109:955–961

Lovelock CE, Osmond CB, Seppelt RD (1995b) Photoinhibition in the Antarctic moss *Grimmia antarctici* Card. when exposed to cycles of freezing and thawing. Plant Cell Environ 18:1395–1402

Lud D, Moerdijk TCW, Van De Poll WH, Buma AGJ, Huiskes AHL (2002) DNA damage and photosynthesis in Antarctic and Arctic *Sanionia uncinata* (Hedw.) Loeske under ambient and enhanced levels of UV-B radiation. Plant Cell Environ 25: 1579–1589

Markham K (1990) Bryophyte flavonoids, their structures, distribution, and evolutionary significance. In: Zinsmeister H, Mues R (eds) Bryophytes: their chemistry and chemical taxonomy. Oxford University Press, Oxford, pp 143–159

Markham KR, Given DR (1988) The major flavonoids of an Antarctic Bryum. Phytochemistry 27:2843–2845

Markham KR, Porter IJ (1974) Flavonoids of the liverwort *Marchantia polymorpha*. Phytochemistry 13:1937–1942

Marschall M, Proctor MCF (2004) Are bryophytes shade plants? Photosynthetic light responses and proportions of chlorophyll *a*, chlorophyll *b* and total carotenoids. Ann Bot 94:593–603

Martinez-Abaigar J, Nunez-Olivera E, Beaucourt N, Garcia-Alvaro MA, Tomás R, Arróniz M (2003) Different physiological responses of two aquatic bryophytes to enhanced ultraviolet-B radiation. J Bryol 25:17–30

Matsubara S, Krause GH, Aranda J, Virgo A, Beisel KG, Jahns P, Winter K (2009) Sun-shade patterns of leaf carotenoid composition in 86 species of neotropical forest plants. Funct Plant Biol 36:20–36

McKenzie RL, Aucamp PJ, Bais AF, Björn LO, Ilyasd M (2007) Changes in biologically-active ultraviolet radiation reaching the earth's surface. Photochem Photobiol Sci 6:218–231

Meijkamp B, Aerts R, Staaij J, Tosserams M, Ernst W, Rozema J (1999) Effects of UV-B on secondary metabolites in plants. In: Rozema J (ed) Stratospheric ozone depletion: the effects of enhanced UV-B radiation on terrestrial ecosystems. Backhuys Publishers, Leiden, pp 71–99

Nabe H, Funabiki R, Kashino Y, Koike H, Satoh K (2007) Responses to desiccation stress in bryophytes and an important role of dithiothreitol-insensitive non-photochemical quenching against photoinhibition in dehydrated states. Plant Cell Physiol 48:1548–1557

Newsham KK (2003) UV-B radiation arising from stratospheric ozone depletion influences the pigmentation of the Antarctic moss *Andreaea regularis*. Oecologia 135:327–331

Newsham KK (2010) The biology and ecology of the liverwort *Cephaloziella varians* in Antarctica. Antarct Sci 22:131–143

Newsham KK (2011) A meta-analysis of plant responses to dark septate root endophytes. New Phytol 190:783–793

Newsham KK, Robinson SA (2009) Responses of plants in polar regions to UVB exposure: a meta-analysis. Global Change Biol 15:2574–2589

Newsham KK, Hodgson DA, Murray AWA, Peat HJ, Smith RIL (2002) Response of two Antarctic bryophytes to stratospheric ozone depletion. Global Change Biol 8:972–983

Newsham KK, Geissler P, Nicolson M, Peat HJ, Lewis Smith RI (2005) Sequential reduction of UV-B radiation in the field alters the pigmentation of an Antarctic leafy liverwort. Environ Exp Bot 54:22–32

Nichol CJ, Pieruschka P, Takayama K, Förster B, Kolber Z, Rascher U, Grace J, Robinson S, Pogson B, Osmond B (2012) Canopy conundrums: exploring the envelope of reversible down-regulation of photosynthesis in the outer and inner canopy to scale observations from the leaf to the landscape. Funct Plant Biol 39:1–24

Niemi R, Martikainen PJ, Silvola J, Sonninen E, Wulff A, Holopainen T (2002) Responses of two *Sphagnum* moss species and *Eriophorum vaginatum* to enhanced UV-B in a summer of low UV intensity. New Phytol 156:509–515

Niyogi KK (1999) Photoprotection revisited: genetic and molecular approaches. Annu Rev Plant Physiol Plant Mol Biol 50:333–359

Post A (1990) Photoprotective pigment as an adaptive strategy in the Antarctic moss *Ceratodon purpureus*. Polar Biol 10:241–245

Post A, Vesk M (1992) Photosynthesis, pigments and chloroplast ultrastructure of an Antarctic liverwort from sun-exposed and shaded sites. Can J Bot 70:2259–2264

Proctor MCF, Smirnoff N (2011) Ecophysiology of photosynthesis in bryophytes: major roles for oxygen photoreduction and non-photochemical quenching? Physiol Plant 141:130–140

Rice SK, Neal N, Mango J, Black K (2011) Relationships among shoot tissue, canopy and photosynthetic characteristics in the feathermoss *Pleurozium schreberi*. Bryologist 114:367–378

Rizzini L, Favory JJ, Cloix C, Faggionato D, O'Hara A, Kaiserli E, Baumeister R, Schäfer E, Nagy F, Jenkins G, Ulm R (2011) Perception of UV-B by the Arabidopsis UVR8 protein. Science 332:103–106

Robberecht R, Caldwell MM (1978) Leaf epidermal transmittance of ultraviolet radiation and its implications for plant sensitivity to ultraviolet-radiation induced injury. Oecologia 32:277–287

Robinson SA, Osmond CB (1994) Internal gradients of chlorophyll and carotenoid pigments in relation to photoprotection in thick leaves of plants with Crassulacean acid metabolism. Aust J Plant Physiol 21:497–506

Robinson SA, Lovelock CE, Osmond CB (1993) Wax as a mechanism for protection against photoinhibition: a study of *Cotyledon orbiculata*. Botanica Acta 106:307–312

Robinson SA, Turnbull JD, Lovelock CE (2005) Impact of changes in natural ultraviolet radiation on pigment composition, physiological and morphological characteristics of the Antarctic moss, *Grimmia antarctici*. Global Change Biol 11: 476–489

Rozema J, Bjorn LO, Bornman JF, Gaberscik A, Hader DP, Trost T, Germ M, Klisch M, Groniger A, Sinha RP, Lebert M, He YY, Buffoni-Hall R, de Bakker NVJ, van de Staaij J, Meijkamp BB (2002) The role of UV-B radiation in aquatic and terrestrial ecosystems – an experimental and functional analysis of the evolution of UV-absorbing compounds. J Photochem Photobiol B Biol 66:2–12

Ryan KG, Burne A, Seppelt RD (2009) Historical ozone concentrations and flavonoid levels in herbarium specimens of the Antarctic moss *Bryum argenteum*. Global Change Biol 15:1694–1702

Schnitzler J-P, Jungblut T, Heller W, Kofferlein M, Hutzler P, Heinzmann U, Schmelzer E, Ernst D, Langebartels C, Sandermann H Jr (1996) Tissue localisation of UV-B screening pigments and of chalcone synthase mRNA in needles of Scots pine seedlings. New Phytol 132:247–258

Searles PS, Flint SD, Caldwell MM (2001) A meta-analysis of plant field studies simulating stratospheric ozone depletion. Oecologia 127:1–10

Searles PS, Flint SD, Diaz SB, Rousseaux MC, Ballare CL, Caldwell MM (2002) Plant response to solar ultraviolet-B radiation in a southern South American *Sphagnum* peatland. J Ecol 90:704–713

Seel WE, Hendry GAF, Lee JA (1992) Effects of desiccation on some activated oxygen processing enzymes and anti-oxidants in mosses. J Exp Bot 43:1031–1037

Semerdjieva SI, Sheffield E, Phoenix GK, Gwynn-Jones D, Callaghan TV, Johnson GN (2003) Contrasting strategies for UV-B screening in sub-Arctic dwarf shrubs. Plant Cell Environ 26:957–964

Shikanai T (2007) Cyclic electron transport around photosystem I: genetic approaches. Annu Rev Plant Biol 58:199–217

Snell KRS, Kokubun T, Griffiths H, Convey P, Hodgson DA, Newsham KK (2009) Quantifying the metabolic cost to an Antarctic liverwort of responding to an abrupt increase in UVB radiation exposure. Global Change Biol 15:2563–2573

Suetsugu N, Wada M (2007) Chloroplast photorelocation movement mediated by phototropin family proteins in green plants. Biol Chem 388:927–935

Swain T (1976) Nature and properties of flavonoids. In: Goodwin TW (ed) Chemistry and biochemistry of plant pigments. Academic Press, London, pp 425–463

Taipale T, Huttunen S (2002) Moss flavonoids and their ultrastructural localisation under enhanced UV-B radiation. Polar Rec 38:211–218

Takahashi S, Badger MR (2011) Photoprotection in plants: a new light on photosystem II damage. Trends Plant Sci 16:53–60

Takahashi S, Milward SE, Yamori W, Evans JR, Hillier W, Badger MR (2010) The solar action spectrum of photosystem II damage. Plant Physiol 153:988–993

Turnbull JD, Robinson SA (2009) Accumulation of DNA damage in Antarctic mosses: correlations with ultraviolet-B radiation, temperature and turf water content vary among species. Global Change Biol 15:319–329

Turnbull JD, Leslie SJ, Robinson SA (2009) Desiccation protects two Antarctic mosses from ultraviolet-B induced DNA damage. Funct Plant Biol 36:214–221

United Nations Environment Programme (2012) Environmental Effects Assessment Panel. Photochem Photobiol Sci 11:13–27. doi:10.1039/C1PP90033A

Van Gaalen KE, Flanagan LB, Peddle DR (2007) Photosynthesis, chlorophyll fluorescence and spectral reflectance in *Sphagnum* moss at varying water contents. Oecologia 153:19–28

Wada M, Kagawa T, Sato Y (2003) Chloroplast movement. Annu Rev Plant Biol 54:455–468

Watling JR, Robinson SA, Woodrow IE, Osmond CB (1997) Responses of rainforest understorey plants to excess light during sunflecks. Aust J Plant Physiol 24:17–25

Webby RF, Markham KR, Lewis Smith RI (1996) Chemotypes of the Antarctic moss *Bryum algens* delineated by their flavonoid constituents. Biochem Syst Ecol 24:469–475

Wolf L, Rizzini L, Stracke R, Ulm R, Rensing SA (2010) The molecular and physiological responses of *Physcomitrella patens* to ultraviolet-B radiation. Plant Physiol 153:1123–1134

Zotz G, Kahler H (2007) A moss "canopy" – small-scale differences in microclimate and physiological traits in *Tortula ruralis*. Flora 202:661–666

Chapter 8

Chloroplast Movement in Higher Plants, Ferns and Bryophytes: A Comparative Point of View

Martina Königer*
Department of Biological Sciences, Wellesley College, Wellesley, MA 02481, USA

Summary

It is well known that chloroplasts move in response to changes in blue light intensity. Under low light conditions chloroplasts spread out in a so-called accumulation response and maximize light interception. Under high light they move to the anticlinal sides of cells, in a so-called avoidance reaction, minimizing light interception. In recent years tremendous progress has been made in our understanding of chloroplast movement due to a combination of new approaches and model systems. Mutant screens in *Arabidopsis thaliana* revealed a considerable number of new players, which modify the speed and the degree of the blue light driven movement of chloroplasts. In addition, better microscopy technologies revealed a fascinating picture of highly dynamic changes in chloroplast associated actin filaments that are essential for chloroplast movement. Our understanding has been further enhanced by studies of the gametophytes of the moss *Physcomitrella patens* and the fern *Adiantum capillus-veneris.* Using a microbeam that illuminates part of a cell, these microscopy studies gave insights into differences and similarities in photoreception and the mechanics of chloroplast movement comparing angiosperms and cryptogams. In addition by studying the behavior of individual chloroplasts within cells, information was gained on the speed and duration with which light signal information travels. Despite advances on the molecular level, our understanding of the species-specific variability and ecological importance of chloroplast movement is still rudimentary. This review will give an overview of our current understanding of chloroplast movement and will point out similarities and differences in behavior among higher plants, ferns and bryophytes.

*Author for correspondence, e-mail: mkoniger@wellesley.edu

D.T. Hanson and S.K. Rice (eds.), Photosynthesis in Bryophytes and Early Land Plants, Advances in Photosynthesis and Respiration 37, pp. 131–150, DOI 10.1007/978-94-007-6988-5_8,

I. Introduction

Photosynthesis is of central importance to all plants, but light which drives photosynthesis is one of the most challenging and variable environmental factors that plants have to content with. In environments such as the understory, plants are limited by light and need to maximize light interception, while canopy leaves have to protect themselves from excess light and the danger of photoinhibition. In addition, light intensities can vary greatly within minutes, which poses great challenges if plants are to optimize their photosynthetic behavior. Not surprisingly, plants have evolved a wide range of sophisticated mechanisms that allow them to deal with ever changing light intensities. Those mechanisms range from acclimation via altered gene expression to physiological processes that act on a time scale of minutes (Li et al. 2009). One such mechanism is the ability of plants to move their chloroplasts into regions of more desirable light intensities within minutes. Under low light intensities chloroplasts spread out within a cell in a so-called accumulation response, thereby maximizing light interception (Zurzycki 1955), while under high light they move to the anticlinal cell walls in a so-called avoidance response thereby minimizing the exposure to light and the likelihood of photoinhibition (Kasahara et al. 2002; Königer et al. 2008). This ability of chloroplasts to move within cells was first documented over a century ago in studies on algae, mosses, ferns and higher plants (Senn 1908). For an example, showing the chloroplast distribution in the model species *Arabidopsis thaliana*, *Adiantum capillus-veneris* and *Physcomitrella patens* see Fig. 8.1. Clever experimental setups laid the groundwork for our understanding of the phenomenon, but it has been only during the past 10 years that some of the key players involved in chloroplast movement and anchoring have been discovered (for reviews see Wada et al. 2003; Takagi et al. 2009).

II. Photoreceptors

Significant progress has been made characterizing the key components involved in perceiving the light signals that induce chloroplast movement in various species. Blue light exclusively induces accumulation and avoidance movements in all terrestrial higher plants, most fern (e.g., *Pteris vittata, Pteris cretica, Adiantum caudatum, Adiantum diaphanum, Cyrtomium fortunei, Microsorum pustulatum*) and moss species (e.g., *Funaria hygrometrica, Ceratodon purpureus*) studied so far (Zurzycki 1967; Inoue and Shibata 1974; Kadota et al. 1989; Kagawa et al. 1997; Augustynowicz and Gabryś 1999; Königer and Bollinger 2012). In these species blue light is perceived by phototropin1 and phototropin2, plasma membrane-associated serine/threonine protein kinases that undergo autophosphorylation in response to blue light. Both phototropins contain two LOV domains, which sense light through the cofactor flavin mononucleotide. Light stimulation leads to the autophosphorylation of the kinase domain, which then phosphorylates other yet unknown targets (Kagawa et al. 2001; Jarillo et al. 2001; Christie 2007). In *Arabidopsis thaliana* the accumulation response is triggered by signals from phot1 and phot2, which operate redundantly, but through distinct pathways. The phot2 mediated avoidance response overrides the phot1 mediated accumulation response under high light intensities. The dark positioning of chloroplasts is also controlled by phot2 (Kagawa et al. 2001; Sakai et al. 2001; Suetsugu et al. 2005a; Luesse et al. 2010). A study on the distinct functions of the various regions and domains of the phototropin receptors showed that in *A. thaliana* the N-terminal end mainly determines the light sensitivity of the phototropins, while the specific combination of the N- and C-terminal regions of phot1 suppresses the avoidance response. Part of the N-terminus of phot2 is required for the proper dark positioning of chloroplasts (Aihara et al. 2008). Using a GFP-phot1 fusion in *A. thaliana* showed that phot1 localizes to the plasma membrane

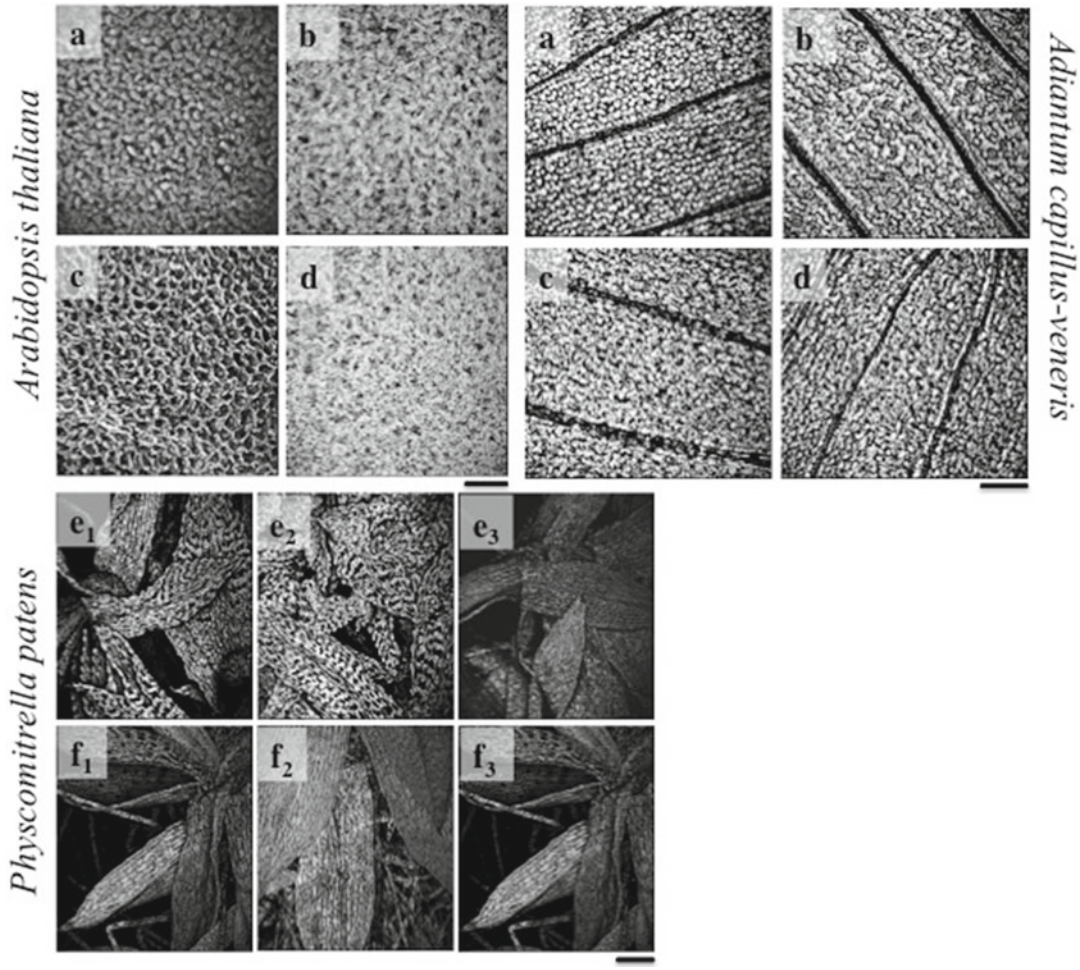


Fig. 8.1. Chloroplast distribution in three model species under low and high light intensities. In *A. thaliana* chloroplasts assume very clear accumulation and avoidance positions, while changes in chloroplast positioning are not as obvious in the other two species. The chloroplast positioning on the adaxial (**a**, **c**) and abaxial (**b**, **d**) leaf sides of *Arabidopsis thaliana* and *Adiantum capillus-veneris* after a 1 h exposure of leaves to white light of 1 (**a**, **b**) or 1,000 µmol photons m^{-2} s^{-1} (**c**, **d**). For *Physcomitrella patens* leaflets and protonemata (cultured on plates) were exposed for a 1 h to white light of 1 ($\mathbf{e}_{1-3}$) or 1,000 µmol photons m^{-2} s^{-1} ($\mathbf{f}_{1-3}$). Samples were fixed in 2 % glutaraldehyde and chlorophyll a fluorescence was used to image the chloroplasts. Confocal images are maximum projections of optical sections spaced at 1 µm. *Scale bar* = 200 µm.

regions of leaf epidermal and mesophyll cells in the dark (Sakamoto and Briggs 2002), but moves to unidentified cytosolic structures in the light in a response that is dependent on phot1 phosphorylation status and kinase activation (Kaiserli et al. 2009; Sullivan et al. 2010). Phot2 localizes mainly to the plasma membrane in the dark, but a fraction of the receptor pool moves to the Golgi apparatus in response to blue light (Kong et al. 2006).

The phototropins and their functions are well conserved among higher plants, ferns and mosses (Christie 2007; Suetsugu and Wada 2007). There are however, a few plant species in which chloroplast movement is induced by red light in addition to blue light. For example, in the aquatic monocot *Vallisneria*

gigantea chloroplasts in the epidermis move into an accumulation position in response to dim red light, while they move to an avoidance position in response to elevated red and strong blue light (Izutani et al. 1990; Dong et al. 1995; Takagi 2003). The accumulation response of chloroplasts in these epidermal cells is affected by illumination with far-red light, pointing towards a phytochrome-dependent mechanism. In addition, DCMU, an inhibitor of photosynthesis adversely affected the ability of chloroplasts to reach the accumulation position (Takagi 2003). In gametophytes and sporophytes of some fern species such as *Adiantum capillus-veneris* and *Dryopteris sparsa* both blue and red light can cause chloroplasts to move (Yatsuhashi et al. 1985; Yatsuhashi and Kobayashi 1993; Augustynowicz and Gabryś 1999). Blue light induced chloroplast avoidance movement in *A. capillus-veneris* was observed under ten-fold lower blue light intensities than under red light (Yatsuhashi et al. 1985). In addition to phot1 and phot2, *A. capillus-veneris* has a chimeric photoreceptor (NEOCHROME1) made up of the chromophore-binding domain of phytochrome3 and nearly full-length phot1. Neochrome functions both as a red and a blue light receptor. Interestingly phot1 and NEO1 are responsible for the accumulation response, while phot2 is responsible exclusively for the avoidance response and proper dark positioning (Nozue et al. 1998; Kagawa et al. 2004; Suetsugu et al. 2005b; Tsuboi et al. 2009). NEO1-like sequences have been found also in some other polypodiaceous ferns (*Dryopteris filix-max, Hypolepis punctata, Onoclea sensibilis*), but not in more primitive ferns (*Osmunda japonica, Lygodium japonicum*) (Kawai et al. 2003; Suetsugu et al. 2005b).

Both red and blue light also can induce chloroplast movement in protonemal cells of the moss *Physcomitrella patents* (Kadota et al. 2000), as long as the cells were cultured in red, not white light (Kadota et al. 2000). *P. patens* has a complex set of receptors with four phototropins (photA1, photA2, photB1, photB2) which are responsible for the blue light induced chloroplast movement. Interestingly, both photA and photB groups are involved in the avoidance response. The primary photoreceptor for the red light induced chloroplast movement is phytochrome, but phototropins may act downstream since the triple phototropin mutant *photA2photB1photB2* showed reduced red light induced chloroplast movement. No neochrome-like protein has been found in the mosses *P. patens* and *Ceratodon purpureus* (Kasahara et al. 2004; Suetsugu et al. 2005b). In *P. patens* the canonical PHY1-3, which are localized in the cytoplasm, are involved in the avoidance response in protoplasts (Uenaka and Kadota 2007), while PHY4 seems to be involved in the avoidance response in protonemal cells (Mittmann et al. 2004). It is not clear why these mutant studies showed conflicting results. Interestingly, the intensities at which the accumulation and avoidance responses occurred were different depending on the light quality (Kadota et al. 2000).

Little is known about the signaling pathways involved in phototropin mediated chloroplast movement. However, it is clear that blue light leads to characteristic changes in internal calcium concentrations in species ranging from higher plants and ferns to mosses. Insights into the role of calcium were gained using a variety of approaches ranging from inhibiting different types of calcium channels with chemicals, measuring calcium channel activities, and determining localized calcium levels with aequorin Ca^{2+} reporter systems. The details that are emerging show calcium changes to be species specific. For example, in *A. thaliana* phot1 and phot2 mediated the blue light induced activation of Ca^{2+} channels in the plasma membrane of mesophyll protoplasts, which in turn led to specific transient increases in cytoplasmic Ca^{2+} levels. Phot2 also induced the release of Ca^{2+} from internal storage compartments (Baum et al. 1999; Harada et al. 2003; Stoelzle et al. 2003). In contrast, the influx of external Ca^{2+} seemed unimportant for blue light induced chloroplast movement in the aquatic angiosperm *Lemna trisulca*

(Tlalka and Gabryś 1993; Tlalka and Fricker 1999) and the fern *A. capillus-veneris* (Sato et al. 2001a, b). In the moss *P. patens* external calcium influx plays an important role in chloroplast movement under blue, but not red light conditions (Russell et al. 1998). In some cases it was difficult to pinpoint if changes of calcium concentration were early or late components of the phototropin signaling pathways, but at least in a few species Ca^{2+} seems to be acting downstream of pathways that involve phosphoinositide-3-kinases, as experiments with wortmannin in *Nicotiana tabacum* (Anielska-Mazur et al. 2009) and *Lemna trisulca* (Grabalska and Malec 2004) showed. These results suggested that the directionality of chloroplast movement was not influenced by calcium, but by the phosphoinositides. Ca^{2+} may affect chloroplast movement through its influence on actin filament integrity or motor molecule activity (Kadota and Wada 1992b; Grabalska and Malec 2004; Anielska-Mazur et al. 2009).

Studies using microbeams that illuminate only a small fraction of an individual cell in *A. thaliana* or *A. capillus-veneris* have shown that whatever the signaling cascade, the light signal does not travel to neighboring cells. Interestingly chloroplasts that were positioned in the area that was illuminated with the high light microbeam, moved away from the light, while those outside of the microbeam moved towards it but stopped before entering the high light area. This indicates that there may exist a gradient in signaling molecules that can reach chloroplasts at a fair distance, but when chloroplasts get close to excessively high light they stop moving. Studies in which only a brief pulse of light was given indicated that high light signals lasted for a shorter period of time (three-fold difference) than low intensity signals and hence could induce movement on different time scales after the microbeam was turned off (Kagawa and Wada 1994, 1999). The distances from which chloroplasts could be attracted towards the light were greater the higher the light intensity of the microbeam (Kagawa and Wada 1996).

III. The Role of the Cytoskeleton

It has been long suggested that chloroplasts in higher plants, ferns and mosses move along actin cables. Numerous studies have shown that chloroplasts are surrounded by a basket of actin filaments, or honeycomb-like actin structures (e.g., in *A. thaliana, Nicotiana tabacum, Spinacia oleraceae, Vallisneria gigantea, A. capillus-veneris, Selaginella helvetica, P. patens*) and that they are localized in close proximity to larger actin cables that transverse the cells. In addition there is evidence in many species that actin polymerization inhibitors prevent chloroplast movement (Cox et al. 1987; Kadota and Wada 1992b; Dong et al. 1996; Kandasamy and Meagher 1999; Sato et al. 2001a, b; Takagi 2003; Kumatani et al. 2006; Anielska-Mazur et al. 2009). Several studies also documented a reorganization of the actin cytoskeleton in response to strong light. For example, in the epidermal cells of the aquatic monocot *Vallisneria gigantea*, blue light led to the reorganization of the actin cytoskeleton. Thick bundles that surrounded and anchored the chloroplasts in the dark, disappeared under strong blue light, and instead straight, aggregated actin bundles appeared (Sakurai et al. 2005). Under weak red light actin cables formed honeycomb like structures, which trapped the chloroplasts of *V. gigantea* (Takagi 2003). In *A. thaliana* red light had more significant effects on the F-actin filaments than blue light and high light intensities appeared to lead to more fragile actin filaments (Krzeszowiec et al. 2007). In *Nicotiana tabacum,* leaves exposure to strong red or blue light led to diffuser and wider actin cables. However, these changes did not correlate with the directionality of chloroplast movement (Anielska-Mazur et al. 2009). In the fern *A. capillus-veneris* it had been shown that arrays of actin filaments appeared when chloroplasts reached their final destination, while they disappeared before movement started (Kadota and Wada 1992b). Using GFP-mTalin constructs that allowed the visualization of very fine actin filaments in

combination with a microcopy system in which part of a cell could be illuminated with a microbeam to induce chloroplast movement, finally led to a significant breakthrough. *A. thaliana* chloroplasts were shown to be surrounded by very small actin filaments covering the entire surface of the chloroplasts when they were anchored to the plasma membrane. When high intensity blue light was shining on them, these filaments first disappeared and then formed on the leading edge of the chloroplasts, meaning on the side facing the direction of chloroplast movement. Hence, chloroplasts did not utilize preexisting or newly formed large cables, but depended on small, newly polymerized actin filaments that formed on the leading edge of chloroplasts (cp-actin filaments) in response to blue light in a phototropin dependent fashion (Kadota et al. 2009).

In the moss *P. patens* chloroplast movement depends both on microtubules and microfilaments. In the absence of light, chloroplasts moved quickly back and forth along microtubules in a longitudinal direction, while actin cables allowed for slow movement in any direction. Red light induced movement via the photoreceptor phytochrome occurred only along microtubules, while blue-light induced movement could take place either along microtubules or actin filaments. Interestingly, red light caused chloroplasts to move in a fairly inefficient way towards their final destination, while blue light caused chloroplasts to move along the shortest way. This specific system may be an intermediate between the algal motility system, which relies mainly on microtubules and that of higher plants, which relies exclusively on microfilaments (Sato et al. 2001b). A recent study using GFP-labeled actin and microtubules revealed that irradiation with a blue microbeam induced changes in actin filaments but not microtubules. High blue light intensities led to the disappearance of actin filaments in the high light area, while low and high blue light led to the appearance of actin filaments in the areas to which the chloroplasts migrated. These short actin filaments (cp-actin) seemed to emerge from the center part of chloroplasts and soon extended to the area of the chloroplast surface facing the plasma membrane. Just like in *A. thaliana* these cp-actin filaments seemed to form in many cases on that side of the chloroplasts that was leading the directional movement. In contrast to *A. thaliana* the cp-actin filaments in *P. patens* were not present in the dark, hence they may not be involved in anchoring the chloroplasts under these conditions (Yamashita et al. 2011). For an overview of our understanding of the light receptors and the cytoskeletal elements in movement in a range of species see Table 8.1 and Fig. 8.2.

It is not known how the force is generated that allows the chloroplasts to move, as there is contradictory evidence for the involvement of myosins. While some, but not all inhibitor studies pointed towards a role of myosin in the accumulation response of higher plants and ferns (Liebe and Menzel 1995; Paves and Truve 2007), there had been limited success localizing specific myosins to the chloroplasts of higher plants (Malec et al. 1996; Wang and Pesacreta 2004; Reisen and Hanson 2007) and none of the myosin mutant lines in higher plants investigated so far have shown any deficits in chloroplast movement (Peremyslov et al. 2008). A recent study which used transient RNA silencing and YFP::myosin XI fusions in tobacco plants found evidence of myosin XI-F involvement in chloroplast dark positioning (Sattarzadeh et al. 2009). This indicates that while myosins may be involved in chloroplast movement, there is either considerable redundancy between the members of this large gene family found in plants and/or they are only partially responsible for the movement of chloroplasts. There is also evidence that myosins change their localization in a blue-light and phot2 dependent fashion in *A. thaliana*. Under weak blue light antibodies detected the presence of myosin associated with the chloroplast envelope, while in strong light very few patches of myosin could be detected on the chloroplasts (Krzeszowiec and Gabryś 2007). Hence myosin relocation may be essential in chloroplast movement

Table 8.1. Some characteristics of chloroplast movement comparing higher plants, ferns and mosses.

Species	Tissue/cells in which light dependent movement has been shown	Quality of light that induces movement	Photoreceptors involved in movement	cp-actin involved	Microtubules involved
Higher plants					
A. thaliana	Mesophyll, guard cells	Blue	Phot1, phot2	Yes	No
Other terrestrial plants (C_3)	Mesophyll	Blue	Phot1, phot2	nd	No
Terrestrial plants (C_4)	Mesophyll, not bundle sheath cells	Blue	Phot1, phot2	nd	No
Aquatic submerged plants (C_3)	Epidermis	Red, blue	phy	nd	No
Ferns					
A. capillus-veneris and other polypodiaceous ferns	Protonema, prothallus, sporophyte	Red, blue	Phot1, phot2, neo1	nd	No
Other ferns	Protonema, sporophyte	Blue	Phot1, phot2	nd	nd
Mosses					
P. patens	Protonema, gametophyte	Red, blue	PhotA1, photA2, photB1, photB2, phy	Yes	Yes[a]
Other mosses	Protonema, gametophyte	Blue	nd	nd	No

nd not determined
[a]In movement under dark, red and blue light

and may play a role in signaling rather than the movement itself. Alternatively, myosins may only be involved in certain circumstances such as in anchoring of chloroplasts in the dark and positioning them under low light, but not in the avoidance response. Clearly more work is needed to elucidate the precise role of myosin in chloroplast movement and its behavior across various species.

An important player in the actin mediated chloroplast movement and anchoring to the plasma membrane is the protein CHUP1 (chloroplast unusual positioning), which localizes to the outer chloroplast envelope via its hydrophobic N-terminus. CHUP1 contains a coiled-coil domain, an actin-binding domain that allows it to interact with G- and F-actin, a proline-rich motif and two leucin-zipper domains (Oikawa et al. 2003; Oikawa et al. 2008). CHUP1 cannot polymerize G-actin, but interacts with the actin-binding protein profilin (Schmidt von Braun and Schleiff 2008a, b). Recent evidence suggests that the leucine zipper motifs in the N- and C-terminal regions of CHUP1 are important for an intramolecular fold that may help to bring the actin- and profilin-binding domains together (Lehmann et al. 2011). Three other proteins have been shown to affect chloroplast movement by influencing cp-actin filament formation: Two kinesin-like proteins, KAC1 and KAC2, with a C-terminus that can interact with F-actin are crucial for chloroplast movement and anchoring and seem to be involved in the generation or stability of cp-actin filaments (Suetsugu et al. 2010a, b). THRUMIN1, an actin-bundling protein that localizes to the plasma membrane in a light- and phototropin-dependent fashion, plays an important role in chloroplast movement under low and high

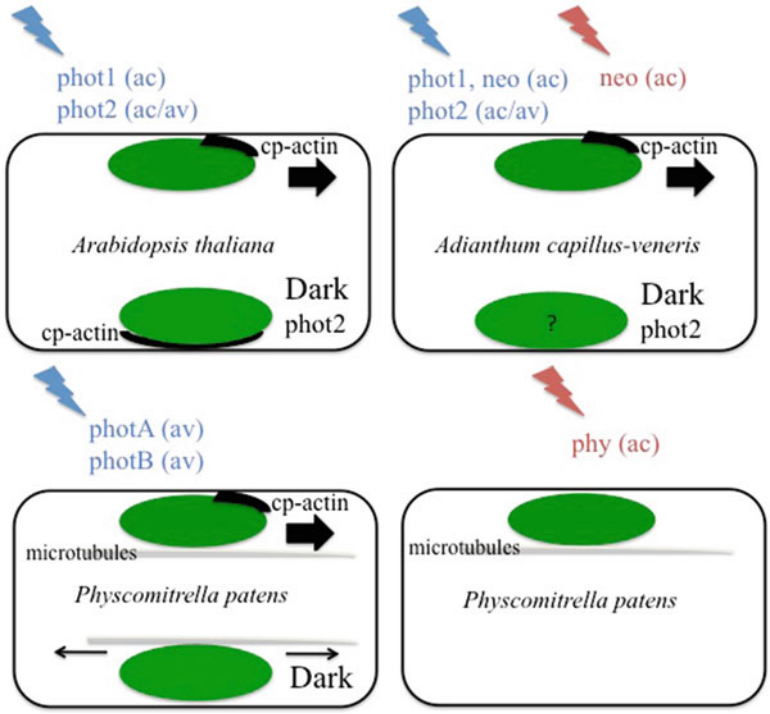


Fig. 8.2. Overview of factors driving chloroplast movement in three model species. In *Arabidopsis thaliana* blue light drives the accumulation response through phot1 and phot2, and the avoidance response through phot2. Through a signaling cascade that is not yet indentified cp-actin filaments form on the leading edge of chloroplasts, allowing chloroplasts to be pulled either into or out of the light. In the dark chloroplasts are anchored via cp-actin filaments to the plasma membrane. A similar model is proposed for *Adiantum capillus-veneris*, however in this species red and blue light can also induce an accumulation response through the photoreceptor neo1. In *Physcomitrella patens* the situation is more complicated and less well understood. Blue light causes an accumulation response in protonemal cells via photA and photB through the biased formation of cp-actin filaments. In addition blue light and red light (via phytochrome) can induce chloroplast movement along microtubules. Chloroplasts are not anchored in the dark, but move along microtubules and possibly actin filaments.

light intensities (Whippo et al. 2011). More studies are needed to investigate how KAC1, KAC2, CHUP1 and THRUMIN1 interact and if they play similarly important roles in other species. So far CHUP1 orthologues have been reported in *Zea mays* and *Eleusine coracana* (Kobayashi et al. 2009), but no mutants are available as of yet to test if they are functionally equivalent.

Not only is it important to move chloroplasts into the correct position to optimize light interception, it is equally important to ensure the anchoring of chloroplasts once they have reached the appropriate position. It has been shown in *A. thaliana* that chloroplasts seem to be anchored to the plasma membrane through CHUP1 and actin cables, as chloroplasts cluster together in *chup1* mutants or after application of actin depolymerization agents such as cytochalasin B. If chloroplasts were not anchored cytoplasmic streaming would displace them (Takagi et al. 2009). Chloroplast anchoring plays an especially important for chloroplasts in the bundle sheath cells of C_4 plants such as *Eleusine coracana*, where they are organized in a centripetal fashion, supposedly to allow for the efficient exchange of metabolites between mesophyll and bundle sheath cells. In young leaves chloroplasts are distributed evenly along the cell walls and only achieve the centripetal arrangement as the leaves mature (Miyake and Yamamoto 1987; Miyake and Nakamura 1993). The

actin cytoskeleton and cytosolic protein synthesis seem to be crucial for chloroplast movement and anchoring after disturbance (through centrifugation). Interestingly, the bundle sheath chloroplasts do not move in response to changes in blue light (Kobayashi et al. 2009).

IV. Chloroplast Movement Speed

Several methods have been employed to characterize chloroplast movement behavior in plants. On a leaf level one can determine the changes in transmission to red light through the leaf in response to various blue light intensity. If the chloroplasts within the cells are spread out in a typical accumulation response then the transmission value will be low, as most of the light will be absorbed by the chloroplasts. On the other hand, if the chloroplasts are arranged along the anticlinal cell walls, as is typical in an avoidance response, the transmission through the leaf will be high (Walczak and Gabryś 1980; DeBlasio et al. 2005; Berg et al. 2006). One can determine the speed of movement as a change in transmission per unit time when the light intensity is changed and chloroplasts move in order to achieve a more favorable positioning (Königer and Bollinger 2012). For an example showing transmission changes in the model organisms *A. thaliana*, *A. capillus-veneris* and *P. patens* see Fig. 8.3. Alternatively, one can follow the movement of individual chloroplasts within a cell that is partially illuminated by a microbeam that induces chloroplasts to move (e.g., Kadota and Wada 1992a, 1999). Both methods have been used to characterize the behavior of various species and mutants under control and experimental conditions.

A study comparing four fern species (*Pteris cretica, Adiantum caudatum, Adiantum diaphanum, Adiantum capillus-veneris*) found considerable differences in overall movement speeds as determined by changes in transmission values per unit time. Since the two species that exhibited the fastest speed came from environments with variable light intensities,

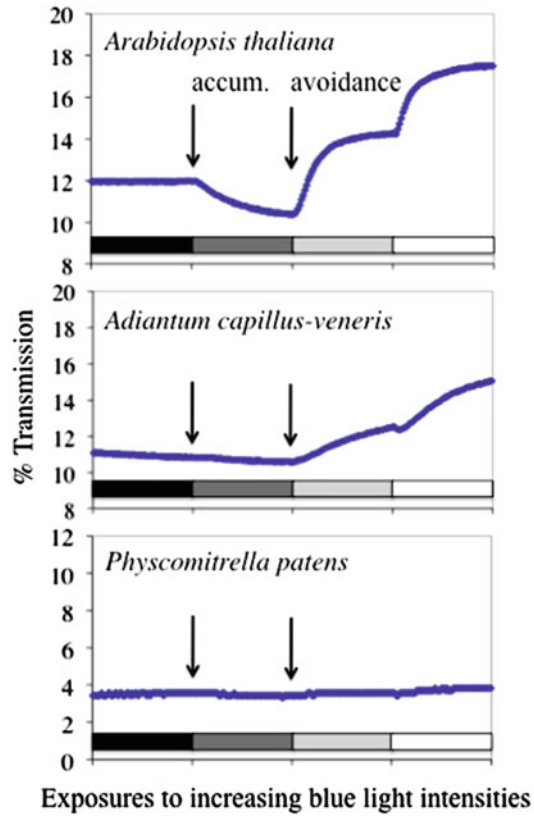


Fig. 8.3. Chloroplast movement behavior in the model species of higher plants, ferns and mosses, measured as the percentage of red light transmission through leaves or pieces of moss. Plants were dark-adapted overnight before leaves or pieces of moss (leaflets and protonemata grown on agar plates) were placed in a photometer measuring the red light transmission under increasing blue light intensities (1 h exposures to 0, 0.1, 40 and 100 µmol photons m^{-2} s^{-1}). While *Arabidopsis thaliana* showed both strong and fast accumulation and avoidance responses, *Adiantum capillus-veneris* showed no accumulation response and a slow avoidance response. No net change in transmission could be observed in *Physcomitrella patens*.

the authors speculated that environmental flexibility rather than growth light conditions determine chloroplast movement speed (Augustynowicz and Gabryś 1999). However, a more recent study that included ten species ranging from ferns to monocots and eudicots found no support for this idea. In this study the plants that preferred higher light intensities during growth exhibited on average higher speeds of movement during both accumulation

and avoidance responses than those that preferred shade environments (Königer and Bollinger 2012). Clearly the question regarding the ecological pressures that lead to the selection of different chloroplast movement speeds needs further elucidation.

Among the higher plant and fern species that have been investigated by measuring transmission changes through the leaves, the speed during the avoidance response was consistently about three-times faster than the accumulation response (Augustynowicz and Gabryś 1999; Königer and Bollinger 2012). Studies in *A. thaliana* indicate that reasons for the differences in speed seem to be related mainly to factors that influence the formation of cp-actin filaments. Both the accumulation and the avoidance speeds of individual chloroplasts correlated with the difference in amounts of cp-actin filaments comparing the front and rear ends of chloroplasts: the larger the difference, the faster the chloroplasts moved. Increasing light intensities also led to increasing movement speeds through this mechanism (Kadota et al. 2009). Mutant screens in *A. thaliana* identified four proteins (PMI2, WEB1, KAC1 and PHOT2), which all modified the speed of movement via their effects on cp-actin filaments. PMI2 (plastid movement impaired 2), a protein with a long coiled-coil domain (Luesse et al. 2006), interacts with WEB1 (weak chloroplast movement under blue light 1) in the cytosol. A mutation in either protein impaired both the accumulation and the avoidance speeds of individual chloroplasts (Kodama et al. 2010). The kinesin-like protein KAC1 was shown to be essential for rapid avoidance speeds also through its effects on the dynamics of cp-actin filaments (Suetsugu et al. 2010a, b). It is not known if mutations in the sequences or different protein concentrations of WEB1, PMI2 and KAC1 can explain the variation in chloroplast movement speed observed between different species, however in *A. thaliana* it has been shown that PHOT2 has a concentration-dependent effect on movement speed. Heterozygous PHOT2/*phot2* mutant plants moved their chloroplasts at half the speed as wild type *A. thaliana* (Kagawa and Wada 2004), and *PHOT2 overexpressor* lines showed increasing speed with elevated PHOT2 concentrations, but saturated at PHOT2 concentrations more than five-times higher than those of wild-type (Kimura and Kagawa 2009). Further evidence for the involvement of PHOT2 comes from a study that showed that prolonged exposure to sucrose or glucose reduced the speed of accumulation and avoidance movement in *A. thaliana* and *Lemmna trisulca*. This effect was less severe in *PHOT2 overexpressors* than in wild-type, pointing towards the involvement of the phot2-signaling pathway (Banaś and Gabryś 2007).

Microbeam studies have greatly enhanced our understanding of the behavior of individual chloroplasts, but are mostly limited to organisms with a single layer of cells like gametophytes. In higher plants such as *A. thaliana* it is possible to use a microbeam, but since the light has to traverse the epidermis, the beam is not as focused as when applied to gametophytes and *A. thaliana* chloroplasts are not as sensitive to increases in blue light in this system. In general, chloroplasts in all species moved away from the area illuminated by a strong blue light microbeam, but those situated outside of the beam moved towards it without entering it (e.g., Kagawa and Wada 2000; Sato et al. 2001b). The speed with which individual chloroplasts move seemed comparable across species, as individual chloroplasts in *A. thaliana* moved along actin cables at about the same speed as those of *P. patens* and *A. capillus-veneris* (Sato et al. 2003). In *A. thaliana* and *A. capillus-veneris* the velocity of individual chloroplasts during an avoidance response increased with increasing blue light intensities, while the speed during the accumulation response was unaffected by light intensities. As a consequence the avoidance movement was faster than the accumulation movement (Kagawa and Wada 2004; Tsuboi and Wada 2010b). In contrast, light intensity had no effect on speed in *P. patens*, resulting in similar speeds when comparing accumulation and avoidance responses (Sato et al. 2001b).

The specific behavior of individual chloroplasts and the influence of light quality was also species specific. In *A. thaliana* it was necessary to apply background red light illumination, which increased cytoplasmic motility, to achieve significant blue light induced chloroplast movement during microbeam irradiation (Kagawa and Wada 2000). In *A. capillus-veneris* the speed of movement of individual chloroplasts was the same in red and blue light, but the further the chloroplasts were away from the microbeam the faster they moved towards it (Kagawa and Wada 1996; Tsuboi and Wada 2010a). In the moss *P. patens* fast chloroplast movement was observed along microtubules and slower movement along actin cables (Sato et al. 2001b). With the recent breakthroughs in microscopy technology, it should be possible to further investigate the dynamic changes in cp-actin filaments in different species and under different light qualities and quantities.

V. Degrees of Movement

By determining the transmission levels at maximum accumulation and avoidance relative to the dark values one can quantify the degree or amplitude of movement in a given species. It is necessary to normalize transmission levels relative to the dark level, since chloroplast arrangements in dark-adapted leaves vary greatly among species, as does leaf thickness. For example, in dark-adapted leaves of *Tradescantia*, chloroplasts distribute themselves evenly along all cell walls (Zurzycki 1980), while in *A. thaliana* they assume a position similar to the avoidance response in the palisade cells, but a position similar to an accumulation response in the spongy mesophyll cells (Berg et al. 2006). Dark level transmission values are species specific (Königer and Bollinger 2012) and are influenced by the light conditions during growth. For example, *A. thaliana* leaves exhibited dark transmission values nearly twice as high when grown under very low versus high light due to differences in chloroplast positioning and leaf thickness (Trojan and Gabryś 1996). Mutant screen in *A. thaliana* have identified two proteins, namely JAC1 (J-domain accumulation response 1) and PHOT2, as important players in the proper dark positioning of mesophyll chloroplasts, but it is not clear how they mediate their effects (Suetsugu et al. 2005a). In dark-adapted *A. capillus-veneris* and *P. patens* protonemal cells, the chloroplasts are spread out evenly along the entire cell periphery (Sato et al. 2001b; Kadota and Wada 1992a), in prothallial cells of *A. capillus-veneris* the chloroplasts are localized along the anticlinal wall excluding the upper surface (Kagawa and Wada 1999), and in sporophytes the chloroplasts are randomly distributed within the cells (Kawai et al. 2003). It is interesting that the dark positioning in *A. capillus-veneris* was so distinctly different depending on the developmental state of the plant. Interestingly, in the protonemal cells of *P. patens* chloroplasts were not anchored to the plasma membrane in the dark, but exhibited a back and forth motion along the longitudinal axis probably along microtubules (Sato et al. 2001b). Is not known if the same proteins are responsible for the dark positioning in ferns and mosses as in *A. thaliana* and if the developmental state also changes chloroplast distribution in mosses. Clearly, more research is needed to understand the physiological importance of distinct dark positions in various species and to identify the components that determine the proper dark positioning in various species.

Species also differ greatly with regard to the degree of their accumulation and avoidance responses. In general, the species-specific variations observed in the avoidance response were smaller than those in the accumulation response. Interestingly, growth light preferences seemed to influence the degree of accumulation responses. For example, while some shade plants such as the fern *Cyrtomium fortunei*, and the monocot *Alocasia odora* showed barely a decrease in transmission after the change from dark to low blue light, sun plants such as *Taraxacum officinale* and *Digitaria sanguinalis* showed very distinct accumulation responses

(Königer and Bollinger 2012). It is not well understood how various molecular factors influence the amplitude of accumulation and avoidance responses. Interestingly some proteins only influence the accumulation or the avoidance response, while others influence both. The amplitude of the accumulation response was adversely affected by mutations in JAC1 (Suetsugu et al. 2010a, b), the amplitude of the avoidance response was reduced by knockouts of PMI2 (Luesse et al. 2006; Kodama et al. 2010), while THRUMIN1, PMI1, and WEB1 influenced both the degree of the accumulation and the avoidance response (DeBlasio et al. 2005; Kodama et al. 2010; Whippo et al. 2011). Taken together these results point towards separate mechanisms and signaling pathways for dark positioning, accumulation and avoidance responses. Careful studies on chloroplast movement in *A. capillus-veneris* prothallial cells supported this idea of separate signaling pathways as it was shown that the red light signal was transferred over longer distances the higher the light intensity (Kagawa and Wada 1996). For blue light the signals were transferred over longer distances and lasted longer for low light intensities than for the high intensities (Kagawa and Wada 1994, 1999).

VI. Effects of Other Environmental Factors on Chloroplast Positioning

Certainly light seems to be the most important factor inducing chloroplast movement through the phototropin, neochrome or phytochrome pathways. However, several other environmental stressors can modify or induce chloroplast movement, at least in some species. These studies clearly show that our picture of light induced chloroplast movement is too simplistic. The effects of other environmental factors and drastic differences in the behavior of various species need to be included in our models of chloroplast movement and may yield helpful information on the signaling pathways that trigger movement.

For example, chloroplast movement in the epidermal cells of *Vallisneria gigantea* is known to be red light induced, in a phytochrome-mediated manner, but red light also acts through its effects on photosynthesis in some unknown way (Dong et al. 1995, 1996). A recent study in a different system, namely the prothallial cells of *A. capillus-veneris,* also showed that red light induced chloroplast movement through its effects on photosynthesis in *neo1* mutants, which could be eliminated by treating the cells with inhibitors of photosynthesis (Sugiyama and Kadota 2011). It is unclear how these light receptor independent pathways mediate their effects, but possibly photosynthetic rates affect Ca^{2+} concentrations outside of the chloroplasts. Alternatively depending on the amount of light, zeaxanthin concentrations within the chloroplasts change (Demmig-Adams and Adams III 2006) and may modulate chloroplast movement behavior (Tlalka et al. 1999).

In addition to light, low temperatures have been shown to induce chloroplast movement in a variety of species ranging from higher plants to ferns. For example temperatures below 10 °C induced an avoidance movement in prothallial cells of *A. capillus-veneris*. Interestingly the movement was enhanced by high light and not observed in *phot2* indicating that temperature also mediated its effect through phototropin (Kodama et al. 2008). Low temperatures affected chloroplast movement in the tropical evergreen higher plant *Tradescantia albiflora* and the conifer *Taxus cuspidata*, but not in herbaceous plants such as *A. thaliana, Nicotiana tabacum, Viola odorata* and *Taraxacum officinale*. Low temperature induced chloroplast positioning was further observed in several evergreen ferns (*Pteris cretica, Pteris vittata, Crepidomanes amabile, Hymenophyllum wrighii*), but not in summer-green ferns (*Lygodium japonicum, Pteridium aquilinum*; Haberlandt 1876; Gabryś and Konopacka 1980; Tanaka 2007; Kodama et al. 2008). Hence temperature induced movement may be a mechanism important for plants with overwintering leaves or plants that need to

protect their chloroplasts from photoinhibition induced by a combination of unfavorable temperatures and high light.

In addition to cold temperatures, water stress also seems to affect chloroplast positioning in the mesophyll cells of some C_4 plants. Studies in *Eleusine coracana* and *Zea mays* showed that mesophyll chloroplasts aggregated in response to severe drought or after application of the water stress hormone ABA in the presence of moderate blue light (Yamada et al. 2009; Maai et al. 2011). Similarly, high light stress in combination with water stress or a treatment with ABA induced clumping of chloroplasts in the CAM plants *Zygocactus truncatus*, *Kalanchoe fedtschenkoi* and *K. blossfeldiana* (Kondo et al. 2004). ABA also influenced the chloroplast positioning in guard cells of the C_3 plant *A. thaliana,* causing them to cluster in the center of the guard cells (Königer et al. 2010). Clumping of chloroplasts has been also observed in the submerged seagrass *Halophila stipulacea*, but was certainly not caused by water stress (Sharon and Beer 2008).

Given that various environmental stressors can influence chloroplast positioning it is not surprising that hydrogen peroxide, a reactive oxygen species that is formed in response to stress, can affect chloroplast movement. Studies in *A. thaliana* showed that elevated levels of hydrogen peroxide induce an avoidance response at lower light intensities and caused an increased degree of avoidance movement. The increase in hydrogen peroxide was PHOT2 dependent and DCMU, an inhibitor of the photosynthetic electron transport chain, prevented in part the blue light induced generation of hydrogen peroxide (Wen et al. 2008). In contrast, in the C_4 plants *Eleusine coracana* and *Zea mays*, application of hydrogen peroxide did not alter the chloroplast distribution in mesophyll cells and did not induce an aggregation of chloroplasts in the dark (Maai et al. 2011).

In the bryophyte *P. patens* and the fern *A. capillus-veneris* chloroplast movement can also be induced by mechanical stress e.g., by touching the protenemal cells with a microcapillary. In *A. capillus-veneris* and *P. patens* this response is dependent on Ca^{2+} influx via the plasma membrane and the mechano-movement is dominant over light induced chloroplast movement. Interestingly in other respects there are species-specific differences: in the mosses *P. patens, Ceratodon purpureus* and *Marchantia polymorpha* chloroplasts move towards the stimulus, while they move away from the stimulus in the ferns *A. capillus-veneris, Dryopteris filix-mas, Onoclea sensibilis,* and *Matteucia struthiopteris.* Chloroplasts also move along different systems with mosses using microtubules, while ferns employ actin for the mechano-relocation (Sato et al. 1999, 2001a, b, 2003).

VII. Chloroplast Movement in Different Cellular Locations

Nearly all studies on chloroplast movement of terrestrial higher plants focus on the behavior of chloroplasts in palisade mesophyll cells, however several studies show the behavior of chloroplasts is greatly influenced by their cellular location.

For example, when leaves of a wide range of species were illuminated with high light, the chloroplasts in the cells on the adaxial and abaxial leaf surfaces did not always behave in a uniform way. In some species, such as *A. thaliana*, chloroplasts in both palisade and spongy mesophyll cells responded by retracting to the anticlinal walls, but in other species such as *Taraxacum officinale* and *Eichhornia crassipes*, only the palisade cell chloroplasts showed an avoidance response. In the shade plant *Hosta*, the chloroplasts in the cells on the lower leaf surface even spread out more in high light than low light (Königer and Bollinger 2012).

Even more extreme is the situation in C_4 plants in which the chloroplasts in the mesophyll and bundle sheath cells exhibit vastly different behavior. Chloroplasts in bundle sheath cells are either centrifugally or centripetally arranged and do not move in response to light, while those in the mesophyll

do. In some of the C_4 species investigated, like *Zea mays,* mesophyll chloroplasts behave similarly to those in C_3 species by exhibiting accumulation and avoidance responses. However, in *Eleusine coracana* mesophyll chloroplasts behaved differently in that they moved during an avoidance response mainly towards the anticlinal cell walls close to the bundle sheath cells. They also moved very slowly, over the course of hours rather than minutes, and only did so in response to light intensities higher than full sunlight. When environmental stressors such as water stress acted on C_4 plants in addition to the high light stress then an aggregation of the mesophyll chloroplasts was observed (Yamada et al. 2009).

Chloroplasts in submerged aquatic plants such as *Vallisneria gigantea* and *Halophila stipulacea* are present in epidermal cells and move in response to light (Takagi 2003; Sharon and Beer 2008). Chloroplast movement in the epidermis has also been observed in a couple of fern species (Königer and Bollinger 2012) and in the guard cells of *A. thaliana* where chloroplasts moved horizontally towards the pore under high light conditions and exhibited a behavior that was in part similar to an avoidance response, but clearly had other qualities (Königer et al. 2010).

As mentioned earlier the dark positioning of chloroplasts in *A. capillus-veneris* was different in protonemal cells than prothallial cells than sporophytes (Kadota and Wada 1992a; Kagawa and Wada 1999; Kawai et al. 2003). Clearly, the question of how the cellular environment mediates its effects on chloroplast positioning needs to be addressed.

VIII. Ecological Importance

It has long been suggested that chloroplast movement serves as a means to optimize light interception (Zurzycki 1955) and the amazing ability of species to fine-tune their chloroplast positioning even after small changes in light intensity certainly speaks to its importance (Gorton et al. 1999; Williams et al. 2003; Königer and Bollinger 2012). Particular importance has been given to the avoidance response as a photoprotective mechanism under conditions of excess light. Several studies provide clear evidence for this in *A. thaliana*, as *phot2* and *chup1* plants were shown to be more sensitive to high light stress treatments than WT and *phot1* plants (Kasahara et al. 2002; Sztatelman et al. 2010; Königer and Bollinger 2012).

However, little is known about how different movement behaviors affect stress tolerance across different species. As discussed earlier, species vary greatly in their chloroplast movement behavior in terms of light qualities that trigger it, the cytoskeletal elements that provide the tracks, and especially the degree and speed with which their chloroplasts move. Only a few studies have compared species with regard to their chloroplast movement behavior and their stress tolerance. A comparison of a wide range of species including ferns, monocots and eudicots showed that there was no correlation between the speed or the degree of chloroplast avoidance responses and high light stress tolerance of these species (Königer and Bollinger 2012). Clearly, plants utilize various mechanisms to deal with high light stress and an analysis of the relative importance of chloroplast movement is complicated by the extent to which other photoprotective mechanisms are employed. Interestingly, two studies indicated that the avoidance movement probably is more important for shade than sun plants. For example, the shade plant *T. albicans* exhibited greater high light stress tolerance than the sun plant *P. sativum* due its superior chloroplast movement behavior despite a lower ability to utilize light for photosynthesis and to repair damage to the D1 protein (Park et al. 1996). Another study showed that on average sun plants showed a lower degree of avoidance response than shade acclimated plants (Königer and Bollinger 2012). Maybe chloroplast movement is not as important for most sun loving species, since they have higher photosynthetic capacities and a larger potential for non-photochemical dissipation

of excess light via a zeaxanthin-dependent mechanism (Königer et al. 1995; Demmig-Adams 1998).

It has been suggested that the avoidance response may not only be important for minimizing high light stress, but that it allows light to penetrate deeper into leaves thereby increasing photosynthesis in more light-limited tissue layers (Brugnoli and Björkman 1992; Terashima and Hikosaka 1995; Gorton et al. 1999). This is certainly possible, as in many species investigated few chloroplasts were found in the periclinal position of the cells on the adaxial leaf surface under high light intensities, allowing more light to reach the chloroplasts in the layer below under high light intensities (Königer and Bollinger 2012).

Chloroplasts need not only light but also CO_2 for photosynthesis and hence there has been interest in understanding if chloroplasts also position themselves to influence leaf mesophyll conductance for CO_2. In moderate to high light chloroplasts move to the anticlinal cell walls, hence closer to intercellular airspaces where CO_2 concentrations are supposedly higher (Terashima and Hikosaka 1995). Studies in *Alocasia brisbanensis*, and *A. thaliana* wild-type and mutant plants indicated that when the chloroplasts were in their avoidance response they were not enhancing or in some cases even limiting CO_2 diffusion within the leaf. This was the consequence of the fact that this position on the anticlinal cell walls reduced the surface area of chloroplasts bordering intercellular airspaces and hence decreased internal conductance for CO_2 through the mesophyll, which in turn limited photosynthesis. This reduction in conductance was not observed in *phot2* mutants, which do not assume an avoidance positioning. However, the conductance was always low in *chup1* plants because of their clustered arrangement (Gorton et al. 2003; Tholen et al. 2008). However, it seems that the reduction in conductance after blue light irradiation was not exclusively caused by the avoidance response since part of it still occurred after treatment with cytochalasin B which inhibits chloroplast movement, and since the kinetics with which conductance changed and chloroplasts moved did not match up (Loreto et al. 2009).

IX. Conclusions

The last decade has brought exciting new insights into the mechanism and importance of chloroplast movement. Mutant screens have revealed some of the proteins that are involved in chloroplast movement and anchoring, while other technological advances have allowed us to study the behavior of individual chloroplasts in more detail. Most of this work has been done in the model species *A. thaliana*, *A. capillus-veneris* and *P. patens*. It will be crucial to continue working on these model species in order to gain a better understanding of how the different players that have been identified interact. In addition we will need a broader approach comparing mechanisms and the importance of chloroplast movement behavior in a range of species in order to understand the ecological importance of this behavior.

Acknowledgements

I would like to thank Wellesley College for granting me a sabbatical leave and Prof. Schleiff for providing me with a ‘home’ in his lab to think and write.

References

Aihara Y, Tabata R, Suzuki T, Shimazaki K-I, Nagatani A (2008) Molecular basis of the functional specificities of phototropin 1 and 2. Plant J 56:364–375

Anielska-Mazur A, Bernaś T, Gabryś H (2009) In vivo reorganization of the actin cytoskeleton in leaves of *Nicotiana tabacum* L. transformed with plastin-GFP. Correlation with light-activated chloroplast responses. BMC Plant Biol 9:64

Augustynowicz J, Gabryś H (1999) Chloroplast movements in fern leaves: correlation of movement dynamics and environmental flexibility of the species. Plant Cell Environ 22:1239–1248

Banaś AK, Gabryś H (2007) Influence of sugars on blue light-induced chloroplast relocations. Plant Signal Behav 2(4):221–230

Baum G, Long JC, Jenkins GI, Trewavas AJ (1999) Stimulation of the blue light phototropic receptor NPH1 causes a transient increase in cytosolic Ca^{2+}. Proc Natl Acad Sci USA 96:13554–13559

Berg R, Königer M, Schjeide B-M, Dikmak G, Kohler S, Harris GC (2006) A simple low-cost microcontroller-based photometric instrument for monitoring chloroplast movement. Photosynth Res 87:303–311

Brugnoli E, Björkman O (1992) Chloroplast movements in leaves: influence on chlorophyll fluorescence and measurements of light-induced absorbance changes related to ΔpH and zeaxanthin formation. Photosynth Res 32:23–35

Christie JM (2007) Phototropin blue-light receptors. Annu Rev Plant Biol 58:21–45

Cox G, Hawes CR, Van Der Lubbe L, Juniper BE (1987) High-voltage electron microscopy of whole, critical-point dried plant cells 2. Cytoskeletal structures and plastid motility in Selaginella. Protoplasma 140(2–3):173–186

DeBlasio SL, Luesse DL, Hangarter RP (2005) A plant-specific protein essential for blue-light-induced chloroplast movements. Plant Physiol 139:101–114

Demmig-Adams B (1998) Survey of thermal energy dissipation and pigment composition in sun and shade leaves. Plant Cell Physiol 39(5):474–482

Demmig-Adams B, Adams WW III (2006) Photoprotection in an ecological context: the remarkable complexity of thermal energy dissipation. New Phytol 172:11–21

Dong X-J, Takagi S, Nagai R (1995) Regulation of the orientation movement of chloroplasts in epidermal cells of *Vallisneria*: cooperation of phytochrome with photosynthetic pigment under low-fluence-rate light. Planta 197:257–263

Dong X-J, Ryu J-H, Takagi S, Nagai R (1996) Dynamic changes in the organization of microfilaments associated with the photocontrolled motility of chloroplasts in epidermal cells of *Vallisneria*. Protoplasma 195:18–24

Gabryś H, Konopacka M (1980) The effect of temperature on chloroplast phototranslocations in *Tradescantia albiflora* leaves. Acta Physiol Plant 2(4):291–297

Gorton HL, Williams WE, Vogelmann TC (1999) Chloroplast movement in *Alocasia macrorrhiza*. Physiol Plant 106:421–428

Gorton HL, Herbert SK, Vogelmann TC (2003) Photoacoustic analysis indicates that chloroplast movement does not alter liquid-phase CO_2 diffusion in leaves of *Alocasia brisbanensis*. Plant Physiol 132:1529–1539

Grabalska M, Malec P (2004) Blue light-induced chloroplast reorientations in *Lemna trisulca* L. (duckweed) are controlled by two separable cellular mechanisms as suggested by different sensitivity to wortmannin. Photochem Photobiol 79(4):343–348

Haberlandt G (1876) Über den Einfluss des Frostes auf the Chlorophyllkörner. Österreichische Botanische Zeitschrift 26:249–255

Harada A, Sakai T, Okada K (2003) Phot1 and phot2 mediate blue light-induced transient increases in cytosolic Ca^{2+} differently in *Arabidopsis* leaves. Proc Natl Acad Sci USA 100(14):8583–8588

Inoue Y, Shibata K (1974) Comparative examination of terrestrial plant leaves in terms of light-induced absorption changes due to chloroplast rearrangements. Plant Cell Physiol 15(4):717–721

Izutani Y, Takagi S, Nagai R (1990) Orientation movements of chloroplasts in *Vallisneria* epidermal cells: different effects of light at low-and high-fluence rate. Photochem Photobiol 51:105–111

Jarillo JA, Gabryś H, Capel J, Alonso JM, Ecker JR, Cashmore AR (2001) Phototropin-related NPL1 controls chloroplast relocation induced by blue light. Nature 410:952–954

Kadota A, Wada M (1992a) Photoorientation of chloroplasts in protonemal cells of the fern *Adiantum* as analyzed by use of a video-tracking system. Bot Mag 105:265–279

Kadota A, Wada M (1992b) Photoinduction of formation of circular structures by microfilaments on chloroplasts during intracellular orientation in protonemal cells of the fern *Adiantum capillus-veneris*. Protoplasma 167:97–107

Kadota A, Kohyama I, Wada M (1989) Polarotropism and photomovement of chloroplasts in the protonemata of the ferns *Pteris* and *Adiantum*. Evidence for the possible lack of dichroic phytochrome in Pteris. Plant Cell Physiol 30(4):523–531

Kadota A, Sato Y, Wada M (2000) Intracellular chloroplast photorelocation in the moss *Physcomitrella patens* is mediated by phytochrome as well as a blue-light receptor. Planta 210:932–937

Kadota A, Yamada N, Suetsugu N, Hirose M, Saito C, Shoda K, Ichikawa S, Kagawa T, Nakano A, Wada M (2009) Short actin-based mechanism for light-directed chloroplast movement in *Arabidopsis*. Proc Natl Acad Sci USA 106(31):13106–13111

Kagawa T, Wada M (1994) Brief irradiation with red or blue light induces orientational movement of chloroplasts in dark-adapted prothallial cells of the fern *Adiantum*. J Plant Res 107:389–398

Kagawa T, Wada M (1996) Phytochrome- and blue light-absorbing pigment-mediated directional movement of chloroplasts in dark-adapted prothallial cells of fern *Adiantum* as analyzed by microbeam irradiation. Planta 198:488–493

Kagawa T, Wada M (1999) Chloroplast-avoidance response induced by high-fluence blue light in prothallial cells of the fern *Adiantum capillus-veneris* as analyzed by microbeam irradiation. Plant Physiol 119:917–923

Kagawa T, Wada M (2000) Blue light-induced chloroplast relocation in *Arabidopsis thaliana* as analyzed by microbeam irradiation. Plant Cell Physiol 41(1):84–93

Kagawa T, Wada M (2004) Velocity of chloroplast avoidance movement is fluence rate dependent. Photochem Photobiol Sci 3:592–595

Kagawa T, Lamparter T, Hartman E, Wada M (1997) Phytochrome-mediated branch formation in protonemata of the moss *Ceratodon purpureus*. J Plant Res 110:363–370

Kagawa T, Sakai T, Suetsugu N, Oikawa K, Ishiguro S, Kato T, Tabata S, Okada K, Wada M (2001) *Arabidopsis* NPL1: a phototropin homolog controlling the chloroplast high-light avoidance response. Science 291:2138–2141

Kagawa T, Kasahara M, Abe T, Yoshida S, Wada M (2004) Function analysis of Phototropin2 using fern mutants deficient in blue light-induced chloroplast avoidance movement. Plant Cell Physiol 45(4): 416–426

Kaiserli E, Sullivan S, Jones MA, Feeney KA, Christie JM (2009) Domain swapping to assess the mechanistic basis of *Arabidopsis* phototropin1 receptor kinase activation and endocytosis by blue light. Plant Cell 21:3226–3244

Kandasamy MK, Meagher RB (1999) Actin-organelle interaction: association with chloroplast in *Arabidopsis* leaf mesophyll cells. Cell Motil Cytoskeleton 44:110–118

Kasahara M, Kagawa T, Oikawa K, Suetsugu N, Miyao M, Wada M (2002) Chloroplast avoidance movement reduces photodamage in plants. Nature 420:829–832

Kasahara M, Kagawa T, Sato Y, Kiyosue T, Wada M (2004) Phototropins mediate blue and red light-induced chloroplast movements in *Physcomitrella patens*. Plant Physiol 135:1388–1397

Kawai H, Kanegae T, Christensen S, Kiyosue T, Sato Y, Imalzumi T, Kadota A, Wada M (2003) Responses of ferns to red light are mediated by an unconventional photoreceptor. Nature 421:287–290

Kimura M, Kagawa T (2009) Blue light-induced chloroplast avoidance and phototropic responses exhibit distinct dose dependency of PHOTOTROPIN2 in Arabidopsis thaliana. Photochem Photobiol 85(5): 1260–1264

Kobayashi H, Yamada M, Taniguchi M, Kawasaki M, Sugiyama T, Miyake H (2009) Differential positioning of C_4 mesophyll and bundle sheath chloroplasts: recovery of chloroplast positioning requires the actomyosin system. Plant Cell Physiol 50(1):129–140

Kodama Y, Tsuboi H, Kagawa T, Wada M (2008) Low temperature-induced chloroplast relocation mediated by a blue light receptor, phototropin 2, in fern gametophytes. J Plant Res 121:441–448

Kodama Y, Suetsugu N, Kong S-G, Wada M (2010) Two interacting coiled-coil proteins, WEB1 and PMI2, maintain the chloroplast photorelocation movement velocity in Arabidopsis. Proc Natl Acad Sci USA 107(45):19591–19596

Kondo A, Kaikawa J, Funaguma T, Ueno O (2004) Clumping and dispersal of chloroplasts in succulent plants. Planta 219:500–506

Kong S-G, Suzuki T, Tamura K, Mochizuki N, Hara-Nishimura I, Nagatani A (2006) Blue light-induced association of phototropin 2 with the Golgi apparatus. Plant J 45(6):994–1005

Königer M, Bollinger N (2012) Chloroplast movement behavior varies widely among species and does not correlate with high light stress tolerance. Planta 236(2):411–426

Königer M, Harris GC, Virgo A, Winter K (1995) Xanthophyll-cycle pigments and photosynthetic capacity in tropical forest species: a comparative field study on canopy, gap and understory plants. Oecologia 104:280–290

Königer M, Delamaide JA, Marlow ED, Harris GC (2008) *Arabidopsis thaliana* leaves with altered chloroplast numbers and chloroplast movement exhibit impaired adjustments to both low and high light. J Exp Bot 59(9):2285–2297

Königer M, Jessen B, Yang R, Sittler D, Harris GC (2010) Light, genotype, and abscisic acid affect chloroplast positioning in guard cells of *Arabidopsis thaliana* leaves in distinct ways. Photosynth Res 105(3):213–227

Krzeszowiec W, Gabryś H (2007) Phototropin mediated relocation of myosins in *Arabidopsis thaliana*. Plant Signal Behav 2(5):333–336

Krzeszowiec W, Rajwa B, Dobrucki J, Gabryś H (2007) Actin cytoskeleton in *Arabidopsis thaliana* under blue and red light. Biol Cell 99:251–260

Kumatani T, Sakurai-Ozato N, Miyawaki N, Yokota E, Shimmen T, Terashima I, Takagi S (2006) Possible association of actin filaments with chloroplasts of spinach mesophyll cells in vivo and in vitro. Protoplasma 229:45–52

Lehmann P, Bohnsack MT, Schleiff E (2011) The functional domains of the chloroplast unusual positioning protein 1. Plant Sci 180:650–654

Li Z, Wakao S, Fischer BB, Niyogi KK (2009) Sensing and responding to excess light. Annu Rev Plant Biol 60:239–260

Liebe S, Menzel D (1995) Actomyosin-based motility of endoplasmic reticulum and chloroplasts in *Vallisneria* mesophyll cells. Biol Cell 85:207–222

Loreto F, Tsonev T, Centritto M (2009) The impact of blue light on leaf mesophyll conductance. J Exp Bot 60(8):2283–2290

Luesse DR, DeBlasio SL, Hangarter RP (2006) Plastid movement impaired 2, a new gene involved in normal blue-light-induced chloroplast movements in *Arabidopsis*. Plant Physiol 141:1328–1337

Luesse DR, DeBlasio SL, Hangarter RP (2010) Integration of phot1, phot2, and phyB signalling in light-induced chloroplast movements. J Exp Bot 61(15):4387–4397

Maai E, Shimada S, Yamada M, Sugiyama T, Miyake H, Taniguchi M (2011) The avoidance and aggregative movements of mesophyll chloroplasts in C_4 monocots in response to blue light and abscisic acid. J Exp Bot 62(9):3213–3221

Malec P, Rinaldi RA, Gabryś H (1996) Light-induced chloroplast movements in *Lemna trisulca.* Identification of the motile system. Plant Sci 120:127–137

Mittmann F, Brücker G, Zeidler M, Repp A, Abts T, Hartmann E, Hughes J (2004) Targeted knockout in *Physcomitrella* reveals direct actions of phytochrome in the cytoplasm. Proc Natl Acad Sci USA 101(38):13939–13944

Miyake H, Nakamura M (1993) Some factors concerning the centripetal disposition of bundle sheath chloroplasts during the leaf development of *Eleusine coracana*. Ann Bot 72:205–211

Miyake H, Yamamoto Y (1987) Centripetal disposition of bundle sheath chloroplasts during the leaf development of *Eleusine coracana*. Ann Bot 60:641–647

Nozue K, Kanegae T, Imaizumi T, Fukuda S, Okamoto H, Yeh K-C, Lagarias JC, Wada M (1998) A phytochrome from the fern *Adiantum* with features of the putative photoreceptor NPH1. Proc Natl Acad Sci USA 95:15826–15830

Oikawa K, Kasahara M, Kiyosue T, Kagawa T, Suetsugu N, Takahashi F, Kanegae T, Niwa Y, Kadota A, Wada M (2003) Chloroplast unusual positioning1 is essential for proper chloroplast positioning. Plant Cell 15:2805–2815

Oikawa K, Yamasato A, Kong S-G, Kasahara M, Nakai M, Takahashi F, Ogura Y, Kagawa T, Wada M (2008) Chloroplast outer envelope protein CHUP1 is essential for chloroplast anchorage to the plasma membrane and chloroplast movement. Plant Physiol 148:829–842

Park Y-I, Chow WS, Anderson JM (1996) Chloroplast movement in the shade plant *Tradescantia albiflora* helps protect photosystem II against light stress. Plant Physiol 111:867–875

Paves H, Truve E (2007) Myosin inhibitors block accumulation movement of chloroplasts in *Arabidopsis thaliana* leaf cells. Protoplasma 230:165–169

Peremyslov VV, Prokhnevsky AI, Avisar D, Dolja VV (2008) Two class XI myosins function in organelle trafficking and root hair development in *Arabidopsis*. Plant Physiol 146:1109–1116

Reisen D, Hanson MR (2007) Association of six YFP-myosin XI-tail fusions with mobile plant cell organelles. BMC Plant Biol 7:6

Russell AJ, Cove DJ, Trewavas AJ, Wang TL (1998) Blue light but not red light induces a calcium transient in the moss *Physcomitrella patens* (Hedw) B, S & G. Planta 206:278–283

Sakai T, Kagawa T, Kasahara M, Swartz TE, Christie JM, Briggs WR, Wada M, Okada K (2001) *Arabidopsis* nph1 and npl1: blue light receptors that mediate both phototropism and chloroplast relocation. Proc Natl Acad Sci USA 98(12):6969–6974

Sakamoto K, Briggs WR (2002) Cellular and subcellular localization of phototropin 1. Plant Cell 14:1723–1735

Sakurai N, Domoto K, Takagi S (2005) Blue-light-induced reorganization of the actin cytoskeleton and the avoidance response of chloroplasts in epidermal cells of *Vallisneria gigantea*. Planta 221:66–74

Sato Y, Kadota A, Wada M (1999) Mechanically induced avoidance response of chloroplasts in fern protonemal cells. Plant Physiol 121:37–44

Sato Y, Wada M, Kadota A (2001a) External Ca^{2+} is essential for chloroplast movement induced by mechanical stimulation but not by light stimulation. Plant Physiol 127:497–504

Sato Y, Wada M, Kadota A (2001b) Choice of tracks, microtubules and/or actin filaments for chloroplast photo-movement is differentially controlled by phytochrome and a blue light receptor. J Cell Sci 114:269–279

Sato Y, Kadota A, Wada M (2003) Chloroplast movement: dissection of events downstream of photo- and mechano-perception. J Plant Res 116:1–5

Sattarzadeh A, Krahmer J, Germain AD, Hanson MR (2009) A myosin XI tail domain homologous to the yeast myosin vacuole-binding domain interacts with plastids and stromules in *Nicotiana benthamiana*. Mol Plant 2(6):1351–1358

Schmidt von Braun S, Schleiff E (2008a) Moving the Green. CHUP1 and chloroplast movement – an obvious relationship? Plant Signal Behav 3:488–489

Schmidt von Braun S, Schleiff E (2008b) The chloroplast outer membrane protein CHUP1 interacts with actin and profilin. Planta 227:1151–1159

Senn G (1908) Die Gestalts- und Lageveränderung der pflanzenchromatophoren. Engelmann, Leipzig

Sharon Y, Beer S (2008) Diurnal movements of chloroplasts in *Halophila stipulacea* and their effect on PAM fluorometric measurements of photosynthetic rates. Aquat Bot 88:273–276

Stoelzle S, Kagawa T, Wada M, Hedrich R, Dietrich P (2003) Blue light activates calcium-permeable channels in *Arabidopsis* mesophyll cells via the phototropin signaling pathway. Proc Natl Acad Sci USA 100(3):1456–1461

Suetsugu N, Wada M (2007) Chloroplast photorelocation movement mediated by phototropin family proteins in green plants. Biol Chem 388:927–935

Suetsugu N, Kagawa T, Wada M (2005a) An auxilin-like J-domain protein, JAC1, regulates phototropin-mediated chloroplast movement in *Arabidopsis*. Plant Physiol 139:151–162

Suetsugu N, Mittmann F, Wagner G, Hughes J, Wada M (2005b) A chimeric photoreceptor gene, NEOCHROME, has arisen twice during plant evolution. Proc Natl Acad Sci USA 102:13705–13709

Suetsugu N, Takano A, Kohda D, Wada M (2010a) Structure and activity of JAC1 J-domain implicate the involvement of the cochaperone activity with HSC70 in chloroplast photorelocation movement. Plant Signal Behav 5(12):1602–1606

Suetsugu N, Yamada N, Kagawa T, Yonekura H, Uyeda TQP, Kadota A, Wada M (2010b) Two kinesin-like proteins mediate actin-based chloroplast movement in *Arabidopsis thaliana*. Proc Natl Acad Sci USA 107(19):8860–8865

Sugiyama Y, Kadota A (2011) Photosynthesis-dependent but neochrome1-independent light positioning of chloroplasts and nuclei in the fern *Adiantum capillus-veneris*. Plant Physiol 155:1205–1213

Sullivan S, Kaiserli E, Tseng T-S, Christie JM (2010) Subcellular localization and turnover of *Arabidopsis* phototropin 1. Plant Signal Behav 5(2):184–186

Sztatelman O, Waloszek A, Banas AK, Gabryś H (2010) Photoprotective function of chloroplast avoidance movement: in vivo chlorophyll fluorescence study. J Plant Physiol 167:709–716

Takagi S (2003) Actin-based photo-orientation movement of chloroplasts in plant cells. J Exp Biol 206:1963–1969

Takagi S, Takamatsu H, Sakurai-Ozato N (2009) Chloroplast anchoring: its implications for the regulation of intracellular chloroplast distribution. J Exp Bot 60(12):3301–3310

Tanaka A (2007) Photosynthetic activity in winter needles of the evergreen tree *Taxus cuspidata* at low temperatures. Tree Physiol 27:641–648

Terashima I, Hikosaka K (1995) Comparative ecophysiology of leaf and canopy photosynthesis. Plant Cell Environ 18:1111–1128

Tholen D, Boom C, Noguchi K, Ueda S, Katase T, Terashima I (2008) The chloroplast avoidance response decreases internal conductance to CO_2 diffusion in *Arabidopsis thaliana* leaves. Plant Cell Environ 31:1688–1700

Tlalka M, Fricker M (1999) The role of calcium in blue-light-dependent chloroplast movement in *Lemna trisulca* L. Plant J 20(4):461–473

Tlalka M, Gabryś H (1993) Influence of calcium on blue-light-induced chloroplast movement in *Lemna trisulca* L. Planta 189:491–498

Tlalka M, Runquist M, Fricker M (1999) Light perception and the role of the xanthophyll cycle in blue-light-dependent chloroplast movements in *Lemna trisulca* L. Plant J 20(4):447–459

Trojan A, Gabryś H (1996) Chloroplast distribution in *Arabidopsis thaliana* (L.) depends on light conditions during growth. Plant Physiol 111:419–425

Tsuboi H, Wada M (2010a) Speed of signal transfer in the chloroplast accumulation response. J Plant Res 123(3):381–390

Tsuboi H, Wada M (2010b) The speed of intracellular signal transfer for chloroplast movement. Plant Signal Behav 5(4):433–435

Tsuboi H, Yamashita H, Wada M (2009) Chloroplasts do not have a polarity for light-induced accumulation movement. J Plant Res 122:131–140

Uenaka H, Kadota A (2007) Functional analyses of the *Physcomitrella patens* phytochromes in regulating chloroplast avoidance movement. Plant J 51: 1050–1061

Wada M, Kagawa T, Sato Y (2003) Chloroplast movement. Annu Rev Plant Biol 54:455–468

Walczak T, Gabryś H (1980) New type of photometer for measurements of transmission changes corresponding to chloroplast movements in leaves. Photosynthetica 14:65–72

Wang Z, Pesacreta TC (2004) A subclass of myosin XI is associated with mitochondria, plastids and the molecular chaperone subunit TCP-1a in maize. Cell Motil Cytoskeleton 57:218–232

Wen F, Xing D, Zhang L (2008) Hydrogen peroxide is involved in high blue light-induced chloroplast avoidance movements in *Arabidopsis*. J Exp Biol 59(10):2891–2901

Whippo CW, Khurana P, Davis PA, DeBlasio SL, DeSloover D, Staiger CJ, Hangarter RP (2011) THRUMIN1 is a light-regulated actin-bundling protein involved in chloroplast motility. Curr Biol 21:59–64

Williams WE, Gorton HL, Witiak SM (2003) Chloroplast movements in the field. Plant Cell Environ 26:2005–2014

Yamada M, Kawasaki M, Sugiyama T, Miyake H, Taniguchi M (2009) Differential positioning of C4 mesophyll and bundle sheath chloroplasts: aggregative movement of C4 mesophyll chloroplasts in response to environmental stresses. Plant Cell Physiol 50(10):1736–1749

Yamashita H, Sato Y, Kanegae T, Kagawa T, Wada M, Kadota A (2011) Chloroplast actin filaments organize meshwork on the photorelocated chloroplasts in the moss *Physcomitrella patens*. Planta 233(2):357–368

Yatsuhashi H, Kobayashi H (1993) Dual involvement of phytochrome in light-oriented chloroplast movement in *Dryopteris sparsa* protonemata. J Photochem Photobiol B 19:25–31

Yatsuhashi H, Kadota A, Wada M (1985) Blue- red-light action in photoorientation of chloroplasts in *Adiantum* protonemata. Planta 165:43–50

Zurzycki J (1955) Chloroplast arrangement as a factor in photosynthesis. Acta Soc Bot Pol 24:27–63

Zurzycki J (1967) Properties and localization of the photoreceptor active in displacements of chloroplasts in *Funaria hygrometrica*. I. Action spectrum. Acta Soc Bot Pol 36:133–142

Zurzycki J (1980) Blue light-induced intracellular movements. In: Senger H (ed) The Blue light syndrome. Springer, New York, pp 50–68

Chapter 9

Scaling Light Harvesting from Moss "Leaves" to Canopies

Ülo Niinemets* and Mari Tobias
Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Kreutzwaldi 1, Tartu 51014, Estonia

Summary

This review provides an overview of chemical, anatomical and morphological changes in bryophytes in response to changes in light availability and assesses the role of these changes in altering bryophyte canopy performance. As a key chemical change, the concentration of chlorophyll increases in response to reduced light availability. Apart from light, within-canopy patterns in chlorophyll are importantly driven by the age of foliage that increases with decreasing light availability, resulting in reduced foliage chlorophyll contents in lower light. In addition, foliage is less strongly aggregated and the density of plants decreases in lower light resulting in greater efficiency of light interception per unit leaf area formed. There is large species variability in canopy architecture, accompanied by species differences in light gradients. Species also differ in structural acclimation to within-canopy light gradients. The species forming new leaves and branches from lateral buds and extending existing lateral branches, in particular, pleurocarpous mosses, can structurally adapt to reductions in light during moss growth, while non-branching, in particular, acrocarpous mosses,

*Author for correspondence, e-mail: ylo@emu.ee

D.T. Hanson and S.K. Rice (eds.), Photosynthesis in Bryophytes and Early Land Plants, Advances in Photosynthesis and Respiration 37, pp. 151–171, DOI 10.1007/978-94-007-6988-5_9,

are inherently less plastic in their acclimation to light. The degree of aggregation also depends importantly on moss water content with greater degree of aggregation under low water availability, suggesting that changes in aggregation play a dual role in enhancing light interception under wet conditions and decreasing light harvesting under dry conditions.

I. Introduction

Light strongly varies within and along plant communities, and foliage photosynthetic function acclimates to prevailing light conditions resulting in enhanced whole canopy light interception and photosynthetic production (Hirose and Werger 1987; Gutschick and Wiegel 1988; Sands 1995; Niinemets and Anten 2009). So far, studies on plant canopies have focused mainly on distribution of light and resources, and photosynthetic acclimation in vascular plants, but the variation in light availability can be particularly large in bryophytes, many species of which colonize the most deeply shaded understory habitats, and are often particularly densely aggregated.

Dense aggregation of mosses can be explained by their inability for active control of water loss by stomata. Individual bryophyte shoots are especially vulnerable to water loss (Dilks and Proctor 1979; Proctor 1984; Green and Lange 1994; Chap. 4). Aggregation of moss foliage elements and shoots to form dense moss cushions significantly increases the moss boundary layer resistance, and reduces the evaporation rate and improved hydration status of entire moss clumps (Proctor 1984; Rice et al. 2001; Rice and Schneider 2004; Chap. 4). Higher degree of moss shoot packing also increases moss water storage capacity and enhances water transport by generating capillary spaces among stems, modifications that altogether significantly extend the period of photosynthetic activity (Proctor 1990; Pedersen et al. 2001).

The trade-off of formation of a "canopy" is a strong reduction of light availability within the moss cushions (Skré et al. 1983; van der Hoeven et al. 1993; Zotz and Kahler 2007; Tobias and Niinemets 2010). In fact, extremely high stem densities and leaf area indices have been reported for mosses in general (Simon 1987; van der Hoeven et al. 1993; Waite and Sack 2010). On the other hand, there is large interspecific variability in moss architecture, leaf area supported and pigment content (Simon 1987; van der Hoeven et al. 1993; Waite and Sack 2010), and the question is how such variation is reflected in within-canopy light gradients. In fact, studies do demonstrate interspecific variation in light gradients among moss species (Skré et al. 1983; van der Hoeven et al. 1993), but the underlying functional traits have not been routinely explored.

Given the extensive light gradients and capacity of many moss species to colonize a range of understory light environments, it is also pertinent to ask to what extent moss foliage and canopy architecture are able to acclimate to such huge variations in light. A number of recent studies demonstrate important variations in moss structural, chemical and photosynthetic characteristics within the moss canopy (Tobias and Niinemets 2005, 2010; Zotz and Kahler 2007;

Abbreviations: $\overline{A}_L$ – average leaf area; A_M – moss leaf area per shoot section mass; A_S – leaf area on the shoot; $\overline{F}_N$ – average number of leaves on the stem; h – depth in the canopy; $k(\theta)$ – canopy extinction coefficient (Eq. 9.1); k_{depth} – apparent light extinction coefficient characterizing reduction in R_Q with canopy depth (h); L – canopy leaf area index; L_C – cumulative leaf area index from the canopy top to given location in the canopy; N_S – number of shoots per area (shoot density); Q – photosynthetic quantum flux density at given location in the canopy; Q_0 – Q at canopy top; R_Q – relative quantum flux density (transmittance of light from canopy top to given position in the canopy, Q/Q_0); S – shoot area index; S_c – cumulative shoot area index; Ω – clumping index (Eq. 9.2); ζ – leaf absorptance; θ – solar zenith angle; χ_A – chlorophyll content per leaf area; χ_M – chlorophyll content per leaf dry mass

Rice et al. 2011). Although light gradients in moss canopies strongly interact with gradients in leaf senescence, limiting acclimation of foliage to low light and thereby partly deviating from patterns in vascular plants, leaves in the lower canopy still possess a certain photosynthetic activity (Zotz and Kahler 2007; Tobias and Niinemets 2010). This photosynthetic activity likely moderately contributes to carbon gain of the entire canopy when upper canopy leaves are fully active and lower canopy receives extremely low light, but it can be fully employed during dry periods when upper canopy photosynthetic activity is reduced, and leaves become rolled around stem, resulting in enhanced penetration of light deeper into the moister canopy interior (Davey and Ellis-Evans 1996). Such variations in moss water status can occur during the day (Hamerlynck et al. 2000), among the days and during the season (Vitt 1990; Harris 2008) Thus, understanding of within-canopy variations in moss functional traits and dynamics in canopy architecture can play a major role in estimating moss carbon gain over days to seasons.

In this review, we first analyze basic functional attributes determining moss light interception, then summarize the variation patterns in moss canopy traits, study within-canopy variation in light, and how these patterns are related to plant architectural traits and pigment contents, and finally analyze within canopy variation in pigments and photosynthetic activity. Overall, this review demonstrates large variation in moss functional traits, emphasizes the richness of within-canopy variation patterns and suggests that consideration of basic modes of acclimation in different functional traits is needed to scale from moss leaves to canopies.

II. Light Interception in Mosses

A. Basics of Light Interception

Quantum flux density within plant community (Q) decreases from canopy top to bottom with increasing cumulative leaf area index (L_C). Traditionally, the Lambert-Beer law has been applied to simulate reduction of light intensity within vegetation (Monsi and Saeki 1953). For direct beam solar radiation,

$$Q = Q_0 e^{\frac{-k(\theta)L_C}{cos\theta}} \tag{9.1}$$

where Q_0 is the quantum flux density incident to the vegetation, θ is the solar zenith angle and $k(\theta)$ is the canopy extinction coefficient that depends on leaf inclination angle distribution (Ross 1981). As Q_0 varies during and between days, it may be often more convenient to analyze variation in relative incident light intensity (transmittance to given position in the canopy), $R_Q = Q/Q_0$, especially when studying implications of differences in canopy structure on light harvesting.

There are two important assumptions in Eq. 9.1: (1), foliage is randomly dispersed, and (2), leaves are optically black. As regards to the assumption of random dispersion of foliage elements, foliage in real canopies tends to be often aggregated (clumped). Foliage aggregation can occur at shoot, branch and canopy scales, and reduces light interception at given L_C. Markov models or negative binomial models have been employed to simulate light interception by aggregated foliage elements (Nilson 1971; Baldocchi and Bowling 2003; Cescatti and Niinemets 2004; Niinemets and Anten 2009). In simple Markov approximation, the transmittance of light is given as:

$$R_Q = e^{\frac{-k(\theta)L_C\Omega}{\cos\theta}}, \tag{9.2}$$

where Ω is the clumping index, and for aggregated canopies $1 > \Omega \geq 0$. Thus, aggregated canopies with given foliage inclination angle distribution and L_C transmit more light than random canopies (Fig. 9.1). In fact, in canopies with large leaf area indices, random dispersion would result in too strong light interception and very dark lower canopies. From Eq. 9.1 it follows that a canopy with random dispersion of foliage and uniform leaf inclination angle distribution (no preferential foliage orientation in space, $k = 0.5$) is

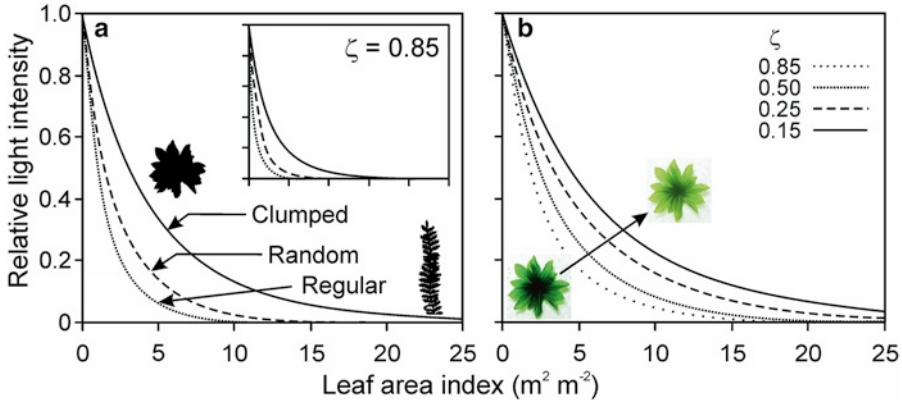


Fig. 9.1. Theoretical dependencies of relative incident quantum flux density at different canopy layers (canopy transmittance) in dependence on cumulative leaf area index (L_C) from canopy *top* to *bottom* for (**a**) hypothetical canopies with clumped, random and regular dispersion, and (**b**) for clumped canopies with varying leaf absorptance (ζ). The simulations were conducted for diffuse light conditions using uniformly overcast sky model. Leaf inclination angle distribution was considered spherical (no preferential orientation in space). For clumped canopy in (**a**) and in (**b**), the clumping index, Ω (Eqs. 9.2 and 9.3), was taken as 0.5, while $\Omega = 1.5$ was used for regular dispersion. Leaf absorptance was taken as 0.3 in the main panel of (**a**) assumed to correspond to typical values in mosses (see section "Moss pigment content and light harvesting"). In the inset of (**a**) $\zeta = 0.85$, corresponding to typical values in vascular plants (Vogelmann et al. 1996b). For further details in simulating canopy light profiles see Cescatti and Niinemets (2004). The insets in (**a**) demonstrate shoot silhouettes for moss *Rhodobryum roseum* that has clumped foliage, and in liverwort *Plagiochila deflexa* that has regularly dispersed foliage. The insets in (**b**) show *Rhodobryum roseum* shoots of varying ζ (greenness).

absorbing 95 % of light for a L_C of ca. 6 m^2 m^{-2}. Natural plant stands, including moss canopies (Table 9.1), often support much higher leaf area indices (Niinemets 2010; Waite and Sack 2010), further underscoring that spatial aggregation is common in nature (Asner and Wessman 1997; Cescatti and Niinemets 2004; Duursma et al. 2012).

On the other hand, foliage can also be regularly dispersed, especially in understory where foliage of low-light acclimated plants is filling up the gaps among neighboring foliage elements, resulting in planar canopies with very high efficiency of light interception (Niinemets 2010 for a review). Regular dispersions can be fitted by a positive binomial model or with the Markov model (Nilson 1971; Cescatti and Niinemets 2004). In the case of the Markov model for regular canopies, Ω in Eq. 9.2 is >1, implying that canopies with regular dispersion of foliage transmit less light relative to canopies with randomly dispersed leaves (Fig. 9.1). Overall, we conclude that random dispersion of foliage elements (Eq. 9.1) is frequently not the case in natural canopies, signifying that it is highly relevant to consider foliage aggregation in modeling canopy light climate.

The assumption of optically black leaves that is applied by default in vegetation models (Eq. 9.1) rests on the assumption that leaf absorptance (ζ) for Q is generally so large that transmitted and reflected radiation fluxes (scattering) are relatively small. Precise consideration of scattered radiation fluxes requires complex models (Brakke 1994; Nilson and Ross 1997; Cescatti and Niinemets 2004), but a simplified way of consideration of scattering effects has been suggested by Goudriaan (1977). According to Goudriaan (1977), the effect of scattering scales with $\sqrt{\zeta}$, and thus, Eq. 9.2 becomes

$$R_Q = e^{\frac{-k(\theta)L_C\Omega}{\cos\theta}\sqrt{\zeta}}. \qquad (9.3)$$

This form of canopy transmittance is currently widely used in modeling light

Table 9.1. Estimates of moss leaf area index (L) and shoot area index (S)

Species[a]	Life form[b]	L (m^2 m^{-2})	S (m^2 m^{-2})	Reference
Acroporium fuscoflavum	Large cushion	11.2		Waite and Sack (2010)
Calliergonella cuspidata	Tall turf/weft		11.9–23.6	van der Hoeven et al. (1993)
Campylopus hawaiicus	Large cushion	14.4		Waite and Sack (2010)
Ceratodon purpureus	Short turf	129		Simon (1987)
Ctenidium molluscum	Weft		11.8–12.0	van der Hoeven et al. (1993)
Distichophyllum freycinetii	Rough mat	8.4		Waite and Sack (2010)
Drummondia prorepens	Rough mat	19.6 (15.0)[c]		Vitt (1990)
Fissidens pacificus	Short turf	4.1		Waite and Sack (2010)
Holomitrium seticalycinum	Short turf	6.1		Waite and Sack (2010)
Hookeria acutifolia	Rough mat	6.5		Waite and Sack (2010)
Hypnum cupressiforme	Smooth mat	103		Simon (1987)
Leucobryum seemannii	Large cushion	11.8		Waite and Sack (2010)
Macromitrium microstomum	Short turf	9.6		Waite and Sack (2010)
Macromitrium piliferum	Short turf	9.6		Waite and Sack (2010)
Mnium hornum	Tall turf	18.0		Proctor (1979)
Pleurozium schreberi	Weft	13.0		Tobias and Niinemets (2005), Tobias and Niinemets, unpublished
Pleurozium schreberi	Weft		1–5	Rice et al. (2011)
Pyrrhobryum pungens	Tall turf	4.7		Waite and Sack (2010)
Rhytidiadelphus squarrosus	Tall turf/weft		8.8–20.6	van der Hoeven et al. (1993)
Scleropodium purum	Weft	22.5		Proctor (1979)
Tortula ruralis	Small cushion	6.0		Proctor (1979)
Tortula ruralis	Small cushion	44		Simon (1987)

[a]The species taxonomy follows *Integrated Taxonomic Information System* (ITIS, http://www.itis.gov) and checklist of Hawaiian mosses (Staples et al. 2004)
[b]Life forms according to (Bates 1998; Hill et al. 2007)
[c]The number in parentheses refers to green foliage without hyaline parts

transmission of natural stands as a simple approximation including both variation in foliage aggregation and light scattering (Anten and Hirose 1999, 2003; Aan et al. 2006). Typically, leaves of vascular plants have leaf absorptances (ζ) between 0.8 and 0.9, and thus, the scattering correction, $\sqrt{\zeta}$, is relatively small. However, for thin moss leaves, often consisting of only single-layered cells, the scattering effect can be substantial (Fig. 9.1), and need to be included in simulating light climate in moss canopies.

B. Moss Leaf Area Index

A key player in radiative transfer models is the leaf area index (L, Eqs. 9.1, 9.2, and 9.3), but moss leaf area indices are not routinely determined. The situation with L is simple in thalloid liverworts such as e.g., *Marchantia polymorpha*, *Conocephalum conicum* or *Monoclea forsteri*, where L is close to 1 m^2 m^{-2} or somewhat higher (Green and Lange 1994). However, due to small size of foliage elements, determination of L in non-thalloid mosses and liverworts with more complex canopies is not a trivial task, requiring microscopy techniques (Fig. 9.2, Simon 1987).

In mosses, leaf area index can be expressed as the product of leaf area on the shoot (A_S) and the number of shoots per sampled area (shoot density, N_S):

$$L = A_S N_S. \tag{9.4}$$

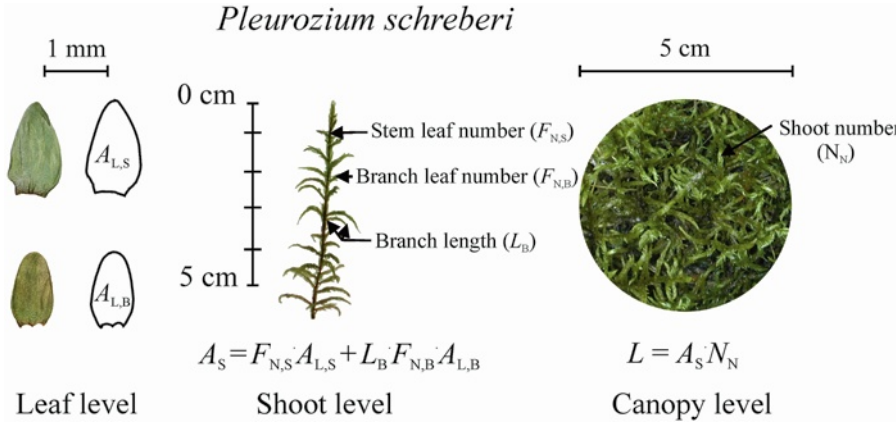


Fig. 9.2. Illustration of estimation of leaf area index (L) in the moss *Pleurozium schreberi*. Leaf area index is estimated from the measurements of branch ($A_{L,B}$) and stem ($A_{L,S}$) leaf area, number, branch length and stem density as shown in the figure (Adapted from Tobias and Niinemets 2005).

In the case of non-branched mosses, the shoot leaf area is given as the product of average leaf area, $\overline{A}_L$ and the average number of leaves on the stem $\overline{F}_N$:

$$A_S = \overline{A}_L \overline{F}_N. \quad (9.5)$$

The situation is more complex for branched mosses, where both stems and branches support the leaves, especially given that stem and branch leaves can differ in size (Fig. 9.2). Thus, for branched mosses, A_S is the sum of the leaves carried by stems and branches (Fig. 9.2 for calculation of L in branched moss *Pleurozium schreberi*).

In practice, for studying light vs. L_C relationships, L has to be determined separately for leaf layers of 0.2–1 cm depth, depending on the size of the moss canopy, thereby significantly increasing the effort needed to characterize moss canopy architecture.

To our knowledge, L for non-thalloid mosses has been so far determined only for 17 species (Table 9.1). Overall, compared with vascular plants (Niinemets 2010; Waite and Sack 2010), L is large in mosses, with most published values between 10 and 20 $m^2\ m^{-2}$ (Table 9.1). However, the published values of L differ largely from values as high as 129 $m^2\ m^{-2}$ in *Ceratodon purpureus* (Simon 1987) to as low as 4.1 $m^2\ m^{-2}$ in *Fissidens pacificus* (Waite and Sack 2010). The question is whether this large range of variation among published values of L corresponds to true variation in L or reflects some artifacts in measurement protocols. In particular, the extreme values in the study of Simon (1987) have not been confirmed by other studies. Although these values are widely used to emphasize the high L values in mosses (Waite and Sack 2010), they reflect extreme density of the moss cushions studied by the author (Simon 1987). For example, in *Ceratodon purpureus*, the average number of stems per 1 cm^2 was 118, and corresponding value was 44 for *Tortula ruralis* (Simon 1987). To our knowledge this represents the upper end of moss stem densities ever reported. However, only 1 cm^2 patches of moss canopies were studied, but there is always some micro-heterogeneity in moss stem density in moss cover. Thus, more representative estimates might be obtained by using larger areas for estimating moss density.

A complication with some moss species can be the presence of unique morphological non-chlorophyll containing features including hyaline ("hairy") leaf tips of the awns, alar cells at the leaf base and costa, hyalocysts at the leaf base etc. (Crandall-Stotler and Bartholomew-Began 2007; Glime 2007).

Fig. 9.3. Representative images of stems of mosses *Rhytidiadelphus triquetrus* and *R. squarrosus* exhibiting characteristic gradients in growth and senescence. The border between "green" and "non-green" parts of the mosses is not clear-cut.

To our knowledge, the contribution of these almost transparent leaf parts to total *L* has been assessed only in the study of Vitt (1990). In *Drummondia prorepens*, it was estimated that non-green parts of foliage contribute to ca. 25 % of *L* (Vitt 1990), indicating that the presence of these structures can importantly affect *L* estimates. We suggest that in future studies, the contributions of hyaline and green leaf parts should be estimated for species possessing such unique foliar features.

Another potential caveat in estimating *L* in mosses is the distinction between dead and live biomass. From physiological perspective, only live, photosynthetically active tissue is relevant, and thus, *L* should only include live plant biomass. This is commonly achieved by separating green and non-green parts of the moss cushions, but the distinction on the basis of tissue greenness is not always clear-cut (Fig. 9.3), and there is a certain physiological activity deep in the canopy until almost all chlorophyll is depleted (section "Gradients of "leaf" traits in moss canopies: acclimation or senescence?"). Furthermore, greenness vs. "deadness" can vary among species (Davey et al. 2009), complicating separation between active and non-active moss parts. Such difficulties in separating between physiologically active and dead biomass can also partly contribute to the large range of *L* estimates across the studies (Table 9.1).

C. Moss Shoot Area Index

Shoot area index (*S*), i.e., the area of stem and attached branches has been determined in several studies (Table 9.1) (van der Hoeven et al. 1993; Bond-Lamberty and Gower 2007; Rice et al. 2011). Sometimes, *S* has been misleadingly called "leaf area index" (Bond-Lamberty and Gower 2007), but it is important to recognize that *L* is the product of *S* and shoot leaf area to shoot area ratio. Determination of shoot area index is less time-consuming, and can be achieved either by photo-electric planimeters (van der Hoeven et al. 1993; Rice et al. 2011) or by microscopy techniques (Bond-Lamberty and Gower 2007). In the case of planimetric methods, *S* has been always defined on the basis of projected area, while in the case of microscopy techniques, *S* for species with cylindrical shoots has been defined on the basis of half-of the total area by multiplying the shoot silhouette area by $\pi/2$ (Bond-Lamberty and Gower 2007).

Although determination of *S* is much faster, and it can be argued that moss branches and stems represent the fundamental units of light interception (Rice et al. 2011), there can be potential acclimation modifications in moss branch and stem architecture in

response to environment (see section "Acclimation of moss light harvesting across understory light environments"), altering light harvesting efficiency of shoots. Little research on such acclimation changes has been carried out in mosses, but in many vascular plants, in particular in conifers, shoot architecture – distribution of foliage inclination angles, spatial aggregation and leaf area density – strongly acclimate to local light environment within the canopy (Niinemets et al. 2006, Niinemets 2007). Also, shoot leaf area to shoot area ratio is expected to vary, for instance due to changes in moss water content (section "Controls of light interception in mosses by structure"). *L* will not be affected by changes in water content (although its efficiency for light interception changes, see section "Controls of light interception in mosses by structure"), but *S* will inevitably track such changes.

As with *L*, significant variation exists in the estimates of *S* among mosses (Table 9.1). Estimates of 1–5 $m^2\ m^{-2}$ were reported for *Pleurozium schreberi* (Rice et al. 2011), while values as high as 8.8–23.6 $m^2\ m^{-2}$ were observed in three moss species studied by van der Hoeven et al. (1993). Given that leaf area to shoot silhouette area ratio is ca. 3–5 $m^2\ m^{-2}$ for typical feather mosses (Tobias and Niinemets, unpublished data), *S* values for *Pleurozium schreberi* in Rice et al. (2011) are broadly comparable with *L* estimates for this species, and on average in mosses (Table 9.1). However, the values in van der Hoeven et al. (1993), if converted to *L*, represent the higher end of *L* estimates in mosses (Table 9.1).

Typically, *L* and *S* are defined for moss patches with full moss cover, and moreover, for areas with the cover of the same moss species. In practice, moss species may often grow in intermixed stands (Skré et al. 1983), and in vegetation there are patches with mosses and free of mosses. Thus, in Bond-Lamberty and Gower (2007), *S* values were scaled to whole stands including areas with and without mosses, overall resulting in low values of *S*. Such estimates certainly provide useful information for understanding the moss role in vegetation stands in general, for example in overall stand carbon balance. However, these estimates are not comparable with other studies on moss *S*. Another reason for low *S* values in Bond-Lamberty and Gower (2007) may also be the arbitrary distinction between dead and live biomass based on fixed depths of moss cushions. For example, in *Aulacomnium palustre*, *Tomentypnum nitens*, *Pleurozium schreberi*, *Ptilium crista-castrensis* only the upper 1.5 cm part, and in *Sphagnum fuscum* only the upper 0.5 cm part were considered alive (Bond-Lamberty and Gower 2007). These values are smaller than have been estimated for some of these species in previous studies (Tobias and Niinemets 2005, 2010; Rice et al. 2011), and again underscore the importance for accurate separation between dead and alive tissue in mosses.

D. Controls of Light Interception in Mosses by Structure

The large variability in *L* and *S* values among moss species has major implications for light harvesting. Apart from the large variability, the take home message from sections "Gradients in photosynthetic activity" and "Moss shoot area index" is that mosses in general tend to support very high leaf area indices. As discussed in section "Basics of light interception", such high *L* values would result in extreme reduction in light within the moss canopies unless foliage is clumped (Eq. 9.2, Fig. 9.1). In fact, canopies of many moss species tend to be clumped, and canopies of acrocarpous non-branching mosses tend to be especially strongly clumped (Fig. 9.4a–c). Nevertheless, even in branching mosses such as *Rhytidiadelphus* or *Calliergonella*, the apical extended parts of the moss shoots are strongly aggregated, whereas the foliage dispersion in deeper more strongly branched canopy becomes increasingly random (Fig. 9.4d, e). Finally, foliage dispersion in strongly branched canopies such as in typical feather mosses is essentially random (Fig. 9.4f), and in thalloid or planar mosses

Fig. 9.4. Representative photographs of moss canopies demonstrating various degrees of aggregation of foliage on stems and branches: (**a**) – *Rhodobryum roseum*; (**b**) – *Plagiomnium undulatum*; (**c**) – *Tortula ruralis*; (**d**), (**e**) – *Rhytidiadelphus triquetrus* side view (**d**) and top view (**e**); (**f**) – *Brachythecium rutabulum*. (**a**)–(**c**) represent strongly aggregated canopies formed of rosette-like moss stems. (**d**) and (**e**) demonstrate aggregation in upper canopy and increased randomness in lower canopy, and (**f**) represents a characteristic canopy with random dispersion of foliage elements.

and liverworts foliage dispersion becomes increasingly regular.

Comparisons of light gradients in moss species with varying structure are scarce (Fig. 9.5). In van der Hoeven et al. (1993), light gradient was steeper in the canopy of *Ctenidium molluscum* than in *Rhytidiadelphus squarrosus* and *Calliergonella cuspidata* (Fig. 9.5). In fact, the apparent extinction coefficient derived by fitting the simple Lambert-Beer relationship (Eq. 9.1) to R_Q vs. cumulative shoot area index (S_c) relationships was also larger in *C. molluscum* than in the other two species (van der Hoeven et al. 1993). Given that *C. molluscum* grows more strongly appressed to substrate and has more randomly dispersed foliage than either *R. squarrosus* and *C. cuspidata*, in which foliage is more strongly aggregated, this difference is in line with theoretical predictions (Eq. 9.2).

The importance of foliage aggregation is further emphasized by dynamic changes in moss light interception efficiency in response to alterations in the degree of moss hydration (Fig. 9.6). In many moss species, foliage becomes more strongly adhered to stems with decreasing moss water content (Bayfield 1973; Smith 1982; Seel et al. 1992; Barker et al. 2005). From the radiative transfer point of view, this implies stronger clumping, resulting in enhanced light transmission (Fig. 9.6, Davey and Ellis-Evans 1996; Zotz and Kahler 2007). Analogously, numerous studies demonstrate that reflectance of moss canopies in photosynthetically active spectral region increases with decreasing moss water status (Vogelmann and Moss 1993; Bryant and Baird 2003; Van Gaalen et al. 2007), compatible with enhanced clumping and reduced exposure of chlorophyll packed in appressed leaves. The capacity for

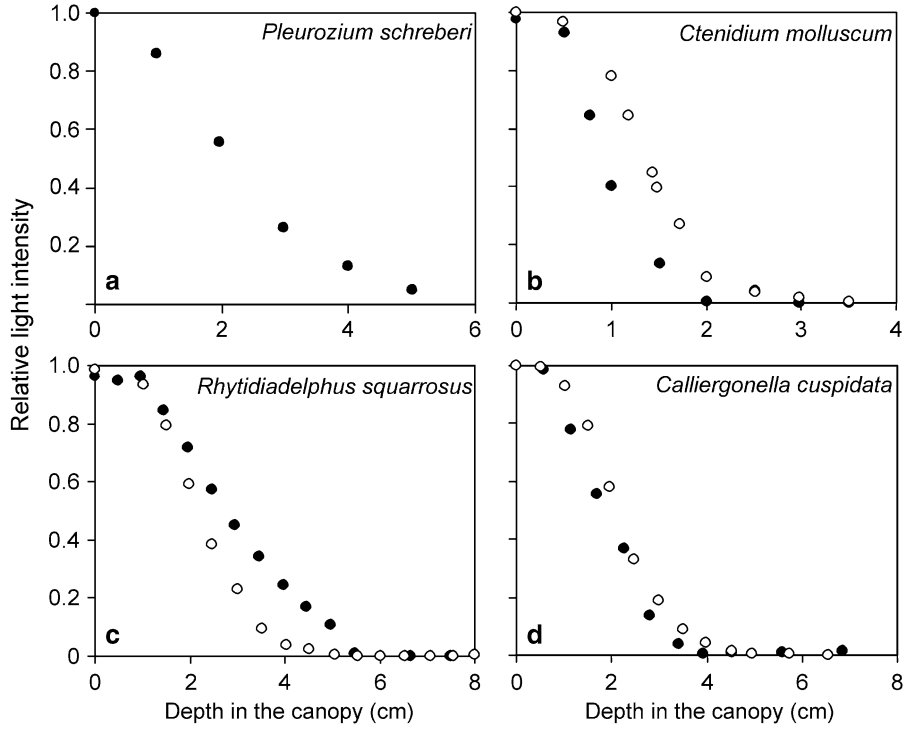


Fig. 9.5. Sample light gradients in canopies of four moss species. Data for (**a**) are from Tobias and Niinemets (2010), and data for the three other species are from van der Hoeven et al. (1993). In the latter study, the measurements were conducted in September (*filled symbols*) and in December (*open symbols*). In both studies, the measurements were conducted in the lab under diffuse light.

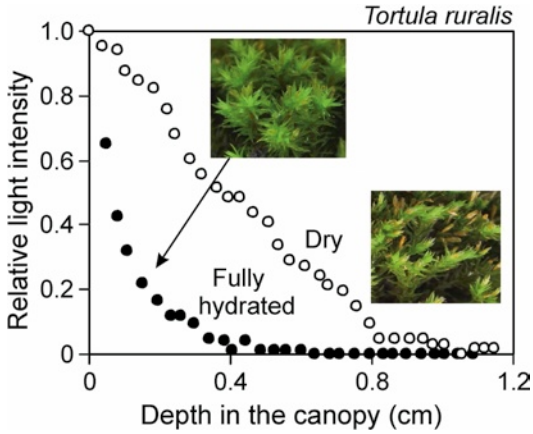


Fig. 9.6. Light gradients in the canopies of fully hydrated (water content 950 %) and dry (27 %) moss *Tortula ruralis* (Modified from Zotz and Kahler 2007).

leaf rolling and unrolling around the stem in response to changes in water availability appears to be larger in more xerophytic species (in particular in acrocarpous xerophytic species) than in less xerophytic species (Glime 2007). In fact, leaf rolling not only reduces light interception and probability for photoinhibition, but also reduces water evaporation, being thus, an important adaptive feature in mosses regularly undergoing cycles of desiccation and rehydration (Bayfield 1973; Glime 2007).

Changes in light gradients within moss canopies have been also observed during the season (Fig. 9.5). These changes were associated with changes in moss S_C values, and may also ultimately reflect changes in the degree of foliage aggregation, i.e., increased S_C being compatible with reduced degree of foliage aggregation (leaves less strongly appressed to stem resulting in greater stem surface area) (van der Hoeven et al. 1993). This suggestion is supported by reduced shoot area per shoot mass in this study in

September relative to December (van der Hoeven et al. 1993). We suggest that such seasonal changes in shoot area may be ultimately associated with seasonal changes in moss water content.

In light of the evidence of the importance of canopy architecture in determining light harvesting of mosses, clearly more quantitative information on moss canopy architecture is needed. New methods based on laser scanning (Rice et al. 2005) or stereo photogrammetry (Krumnikl et al. 2008) may provide a vehicle for rapid screening of moss canopies for relevant architectural traits such as the degree of foliage aggregation.

E. Moss Pigment Content and Light Harvesting

In addition to architecture, moss light interception characteristics are importantly driven by moss foliage optical properties. Unfortunately, data on moss leaf optics are scarce, and studies mainly report information on surface reflectance of moss cushions (Vogelmann and Moss 1993; Bubier et al. 1997; Hamerlynck et al. 2000; Lovelock and Robinson 2002; Arkimaa et al. 2009; Hallik et al. 2009b). However, surface reflectance is driven both by pigment contents and canopy structure and thus does not provide information on optical properties of single moss shoots or leaves. The problem with direct assessment of reflectance, transmittance and absorptance of moss foliage elements is their miniature size such that moss foliage elements, either single leaves, entire shoots or even stems, do not generally fill up the ports of integrated spheres routinely used in assessing vegetation optical characteristics. For small-sized objects such as conifer needles, methods have been developed to determine the optical properties considering the gap fraction of needles in the sample (e.g., Mesarch et al. 1999). However, certain non-uniformity of light field and difficulties in precisely estimating the gap fraction can introduce large errors in derivation of leaf optical characteristics by this method. Attempts have been made to study optical properties of single moss leaves by reducing the field of view with custom-made port covers, but these attempts were not successful due to low sensitivity of available instrumentation (a LI-1800 integrated sphere from Li-Cor, Inc., Lincoln, NE, USA with a Field Spec Pro Spectroradiometer from Analytical Spectral Devices, Inc. Boulder CO, USA; A. Cescatti and Ü. Niinemets, unpublished data 2004).

Given that leaf absorptance is primarily the function of leaf chlorophyll content (Evans 1993b; Vogelmann 1993; Carter and Knapp 2001), leaf absorptance in relation to leaf chlorophyll content per leaf area (χ_A, μmol m^{-2}) has been described by an empirical equation (Evans 1993b):

$$\zeta = \frac{\chi_A}{\chi_A + 76}. \tag{9.6}$$

This equation has been shown to provide excellent estimates of ζ for a wide range of species, except for hairy or waxy leaves (Evans 1993b; Evans and Poorter 2001).

The use of this equation requires information on chlorophyll content per area, but in mosses, chlorophyll content per dry (χ_M) or fresh mass is commonly estimated. Furthermore, the estimates generally constitute a weighted sample of moss parts with leaves that contain more chlorophyll and stems that contain less chlorophyll. Using the estimates of moss leaf area per shoot section mass (A_M) and chlorophyll content per shoot section mass (χ_M/A_M) (Tobias and Niinemets 2005, 2010), a range of moss leaf chlorophyll contents of 16–39 μmol m^{-2} is obtained for single-cell-layered leaves of feather moss *Pleurozium schreberi*. Calculating leaf chlorophyll content as χ_M/A_M does not consider presence of chlorophyll in stem, and thus, χ_A may be somewhat overestimated. Nevertheless, we suggest that these estimates can still serve as a first approximation of area-based pigment content. From equation (6), the range in chlorophyll contents obtained corresponds to a ζ range of 0.18–0.34. For comparison, typical values of leaf chlorophyll content for vascular

plants are 400–800 μmol m^{-2}, corresponding to leaf absorptance of 0.84–0.88 (Gabrielsen 1948; Masoni et al. 1994; Baldini et al. 1997; Carter and Knapp 2001). Thus, these estimates of leaf chlorophyll content and ζ are much lower than characteristic values in vascular plants, indicating that the impact of light scattering (Eq. 9.3) on light profiles within the canopy is of paramount significance in mosses (Fig. 9.1). Furthermore, chloroplast movements that have been shown to be especially significant in shade plants with thinner leaves (Williams et al. 2003) play an important role in mosses as well (Chap. 8 for a review). In particular, chloroplast movement is expected to reduce ζ at given foliage chlorophyll content at the top of the moss canopies, thereby reducing excess light interception (Chap. 8).

Not all mosses have single-cell-layered cells, and may have thickened central leaf parts (costa) as for example in species of *Campylopus*, *Dicranum*, *Holomitrium*, *Timmia*, *Weissia* etc., thickened multi-layered leaves such as in *Grimmia*, plications as in *Brachythecium* and *Drepanocladus*, or leaf lamellae as in *Polytrichaceae* spp. (Crandall-Stotler and Bartholomew-Began 2007; Glime 2007; Waite and Sack 2010). For *Polytrichum commune*, an average value of leaf area to shoot dry mass of 456 cm^2 g^{-1} was derived for the top 6 cm of the canopy (Tobias and Niinemets 2005 and Tobias and Niinemets unpublished). Given the average chlorophyll content of 6.4 μmol g^{-1} in this species (Martin and Churchill 1982; Masarovičová and Eliáshš 1987), chlorophyll content per area is 140 μmmol m^{-2}, corresponding to a ζ of 0.65. Compared with vascular plants, these values are still low and are compatible with estimates for young greening leaves, senescing leaves or for chlorotic leaves of chlorophyll-deficient mutants (Gabrielsen 1948; Adams et al. 1990; Masoni et al. 1994; Niinemets et al. 2004). Accordingly, even in relatively thick-leaved moss species, foliage pigment contents importantly alter the light gradients at given L_C and degree of foliage aggregation (Fig. 9.1b).

Leaves of many moss species have unique leaf characteristics such as papillae or mamillae on leaf surfaces, and concave or curled leaves (Crandall-Stotler and Bartholomew-Began 2007; Glime 2007). Calculation of leaf absorptance on the basis of leaf chlorophyll content does not consider effects of such anatomical characteristics. In vascular plants, it is known that epidermal thickenings may function as lenses improving light penetration into the leaf (Martin et al. 1991; Myers et al. 1994; Vogelmann et al. 1996a), and it is likely that epidermal modifications in bryophytes also improve light penetration into the chloroplasts.

On the other hand, presence of hyaline hairy leaf tips with high reflectance can dramatically reduce moss light interception, especially in a dry state when leaves are strongly adhered to the stem. Although being non-photosynthetic, hyaline leaf tips can be an important adaptive feature reducing radiation interception during dry periods in xerophytic mosses. Clearly more experimental work is needed to gain quantitative insight into optical characteristics of mosses and to understand how the leaf structural adaptations alter moss light harvesting efficiency.

F. Acclimation of Moss Light Harvesting Across Understory Light Environments

Despite that mosses are commonly considered shade plants, individuals of given moss species can colonize a range of light environments (Frego and Carleton 1995; Thomas et al. 2001; Tobias and Niinemets 2010). The key question is how moss light harvesting characteristics change upon acclimation to different understory light environments. Studies have demonstrated that in higher light, pleurocarpous mosses tend to branch more frequently and achieve higher canopy densities (Rincon and Grime 1989; Rincón 1993; Bergamini and Peintinger 2002). On the other hand, moss foliage chlorophyll contents tend to be larger in lower light (Rincón 1993; Tobias and Niinemets 2010). Thus, the question is how two contrasting trends, increasing foliage density and

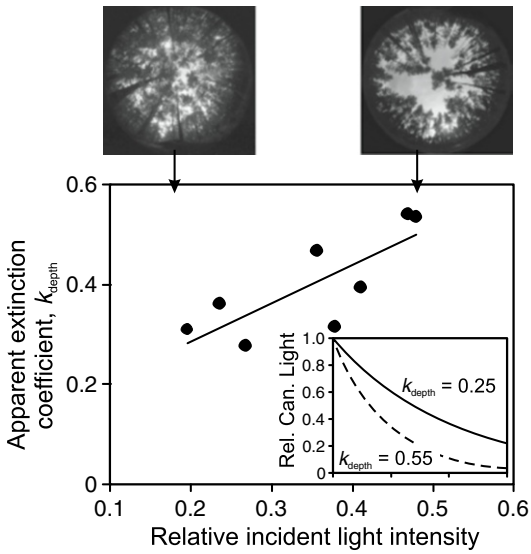


Fig. 9.7. Apparent extinction coefficient (k_{depth}) for within-canopy relative light (R_Q) vs. canopy depth (h) relationships in dependence on incident light availability above the moss layer (Modified from Tobias and Niinemets 2010). R_Q vs. h relationships were fitted by $R_Q = e^{-hk_{depth}}$, and the inset demonstrates variation in canopy light profiles for the range of k_{depth} values observed. The relative incident light availability in different forest habitats was characterized by hemispherical photography and representative hemispherical photographs in low and moderately high light are also demonstrated.

decreasing chlorophyll content with increasing light, sum up in affecting within-canopy light gradients. In *Pleurozium schreberi*, within-canopy gradient in light became steeper with increasing understory light availability (Fig. 9.7), suggesting that the variation in light gradients across the habitats was dominated by increases in foliage density. Clearly, further case studies are needed to gain more general insight into the potential variations in the moss light harvesting efficiency. Such studies are also pertinent given the huge range of variation in moss L_C values (section "Gradients in photosynthetic activity") that may not entirely be due to interspecific differences, but also reflect differences in light availability in sampled moss growth habitats, as for instance, has been observed in the study of Waite and Sack (2010).

III. Gradients of "Leaf" Traits in Moss Canopies: Acclimation or Senescence?

A. Gradients in Pigments

In simulations in Fig. 9.1, a constant leaf absorptance was assumed for all leaves in the canopy, but light gradients within the canopy are also importantly driven by variation in foliage pigment contents. In vascular plants, contrasting variation patterns in foliage pigment contents across the canopy have been reported (Niinemets 2007; Hallik et al. 2009a). In species that form all foliage throughout the canopy almost simultaneously such as temperate deciduous trees in spring, foliage chlorophyll content per dry mass increases with decreasing light availability from the top to the bottom of the canopy, and this is suggested to be an adaptive feature improving light absorptance per leaf dry mass (for reviews see Niinemets 2007; Niinemets and Anten 2009). However, in herbaceous species and in woody species with rapid growth, lower leaves formed first are continuously overtopped by foliage developing later and thus, light gradients are accompanied by gradients in leaf age and senescence. In herbaceous species, the increase of chlorophyll content per mass with decreased light is either less than in woody species (Evans 1993a, b; Aan et al. 2006; Hallik et al. 2009a, 2012), or chlorophyll content is not related to light at all (Aan et al. 2006; Hallik et al. 2009a) or chlorophyll content decreases with decreasing light availability (Boonman et al. 2007, 2009). This evidence collectively underscores the importance of interacting light and senescence gradients in affecting chlorophyll profiles within the canopy in herbaceous species with rapidly developing canopies.

The situation is similar with mosses where new leaves develop at the top and decompose at the bottom (Fig. 9.3). This pattern of foliage development is reflected in monotonous decreases of chlorophyll content from canopy top to bottom in acrocarpous moss *Tortula ruralis*, (Zotz and Kahler 2007).

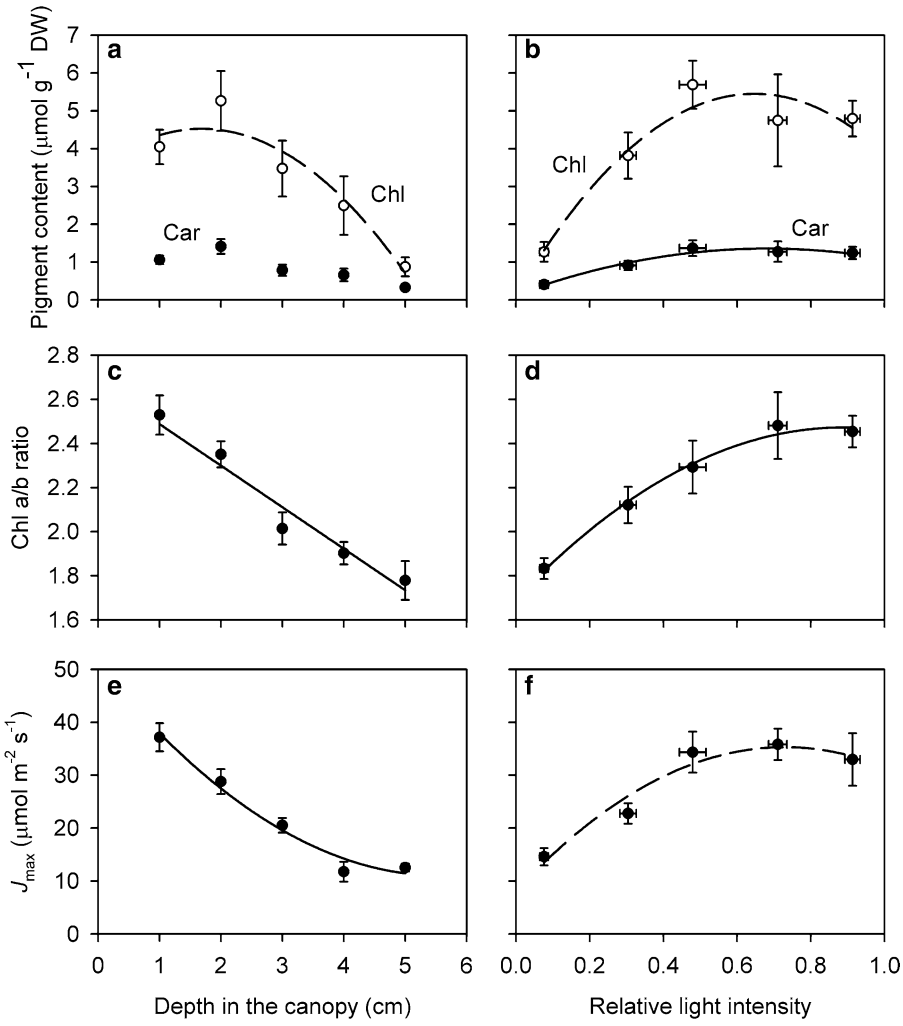


Fig. 9.8. Within-canopy gradients in chlorophyll and carotenoid contents (**a**, **b**), chlorophyll a/b ratio (**c**, **d**) and the capacity for photosynthetic electron transport (**e**, **f**) in the moss *Pleurozium schreberi* (Modified from Tobias and Niinemets 2010). Data were fitted by linear and second order polynomial regressions and regressions significant at least at $P<0.05$ ($r^2=0.91-0.96$) are shown by *solid lines* and those significant at $P<0.1$ ($r^2=0.83-089$) are shown by *dashed lines*.

However, in pleurocarpous moss *Pleurozium schreberi*, there is a curvilinear relationship of leaf chlorophyll content with canopy depth and light availability within the canopy, with uppermost foliage having moderately high chlorophyll contents, followed by a maximum and then by monotonous reduction of chlorophyll with further increases in canopy depth (Fig. 9.8a, b, Tobias and Niinemets 2010). This curvilinearity has been attributed to actively growing lateral branches (Fig. 9.8a, b, Tobias and Niinemets 2010), but lower chlorophyll contents at the top of the canopy can also constitute an high light avoidance response (Tobias and Niinemets 2010; Chap. 7).

After the maximum is reached, the reduction in chlorophyll contents with further increases of canopy depth can be suggested to demonstrate age-dependent modifications only. However, chlorophyll a/b ratio monotonically decreases with decreasing light

availability in the canopy (Fig. 9.8c, d, Tobias and Niinemets 2010). This reflects increases in light-harvesting pigment-binding complexes, LHC II, relative to the photosystems I and II (Bassi and Caffarri 2000). Such a modification in the stoichiometry of photosynthetic apparatus is a common shade-adaptation response in plants (Anderson et al. 2001; Niinemets 2007), indicating that the foliage in this species has a certain capacity to acclimate to low light. However, differently from *Pleurozium schreberi*, no changes in chlorophyll a/b ratio were observed in *Tortula ruralis*, suggesting that the within-canopy change in the stoichiometry of foliage photosynthetic apparatus is not a universal phenomenon in mosses.

The within-canopy variation in carotenoid content is analogous to chlorophyll (Fig. 9.8a, b, Tobias and Niinemets 2010). However, chlorophyll to carotenoid ratio at higher light was smaller than under intermediate light (Tobias and Niinemets 2010), likely indicating enhanced requirement for photoprotective carotenoids in the upper canopy. On the other hand, deeper in the canopy, chlorophyll to carotenoid ratio decreased again, and this may indicate accumulation of carotenoids in plastoglobuli of senescing leaves (Niinemets et al. 2012). Altogether, these data indicate that within-canopy variations in foliage pigment content reflect both overall reduction of pigment content due to senescence, and modifications in pigment stoichiometry being compatible with adaptation to variations in light availability.

B. Gradients in Photosynthetic Activity

Foliage photosynthesis is another important indicator of within-canopy variation in foliage physiological activity, and it is pertinent to ask whether gradients in photosynthesis accompany light gradients. In vascular plants, foliage photosynthetic capacity strongly increases with increasing light in the canopy, primarily due to enhanced investment of foliage nitrogen in Rubisco and rate-limiting components of photosynthetic electron transport (for reviews see Osmond et al. 1999; Pons and Anten 2004; Niinemets 2007; Niinemets and Anten 2009). As with other important functional traits, studies on moss within-canopy variation in photosynthesis are challenging due to miniature size of mosses, making the use of standard gas-exchange measurement practices difficult (Zotz and Kahler 2007). Nevertheless, gas-exchange measurements have demonstrated that photosynthetic capacity of current-year segments of *Hylocomium splendens* is much higher than in the canopy segments formed in the previous year (Sonesson et al. 1992). Analogously, subsequently removing 3 mm layers of *Tortula ruralis* canopy by razor blade, gas-exchange measurements combined with mathematical simulations were used to demonstrate that *T. ruralis* photosynthesis is strongly reduced from uppermost (layer 1) to lowest (layer 4) studied positions in the canopy (Zotz and Kahler 2007).

Higher spatial resolution data on moss photosynthesis can be obtained by labeling primary photosynthesis products by ^{14}C. According to this method, whole cryptogam cushions are exposed to $^{14}CO_2$, and after the exposure, the canopy is dissected into layers, and the amount of $^{14}CO_2$ fixed by each layer is determined. This method was used to gain insight into within-canopy variation of photosynthesis in *Sphagnum* (Johansson and Linder 1980), in the aquatic moss *Drepanocladus exannulatus* (Schwartz and Markager 1999) and in the lichen *Cladina stellaris* (Lechowicz 1983). As with standard gas-exchange measurements, ^{14}C studies have demonstrated large reduction of photosynthetic production with increasing canopy depth (Johansson and Linder 1980; Lechowicz 1983; Schwartz and Markager 1999). However, the disadvantage of the ^{14}C method with entire moss cushions is that there are inherent within-canopy light gradients during the exposure, such that plant parts at different depths in the canopy are exposed to different quantum flux densities. As the result, photosynthetic capacity in canopy interior may be underestimated.

The second key tool for gaining information on small-scale heterogeneity of photosynthetic

activity is chlorophyll fluorescence. Portable fluorescence instruments allow measurement of within-canopy photosynthetic profiles with high resolution and as actinic illumination is provided by the fluorescence instrument, light-saturated rates can be estimated at any position in the canopy (Tobias and Niinemets 2010). Chlorophyll fluorescence measurements demonstrate strong depth and light availability dependent variation within the canopy (Fig. 9.8e, f). In fact, significant photosynthetic activity is even observed in leaves with almost complete depletion of chlorophyll (Fig. 9.8). Due to low light, photosynthetic activity of leaves in the canopy interior may be below the observed photosynthetic capacity most of the time. However, during water stress when upper canopy leaves curl and become strongly appressed to the stem, leaves in more humid lower canopy can reach rates close to the theoretical maxima, partly compensating for the reduced activity of the upper canopy layers (Davey and Ellis-Evans 1996). This suggests that in determining moss canopy leaf area index, it is important to consider all foliage that is still physiologically active. Rather than using arbitrary methods, chlorophyll fluorescence may serve as an important tool for rapid and objective separation between alive and "dead" plant parts.

In addition to standard chlorophyll fluorescence techniques that require use of a sample holder (leaf clip), and multiple measurements

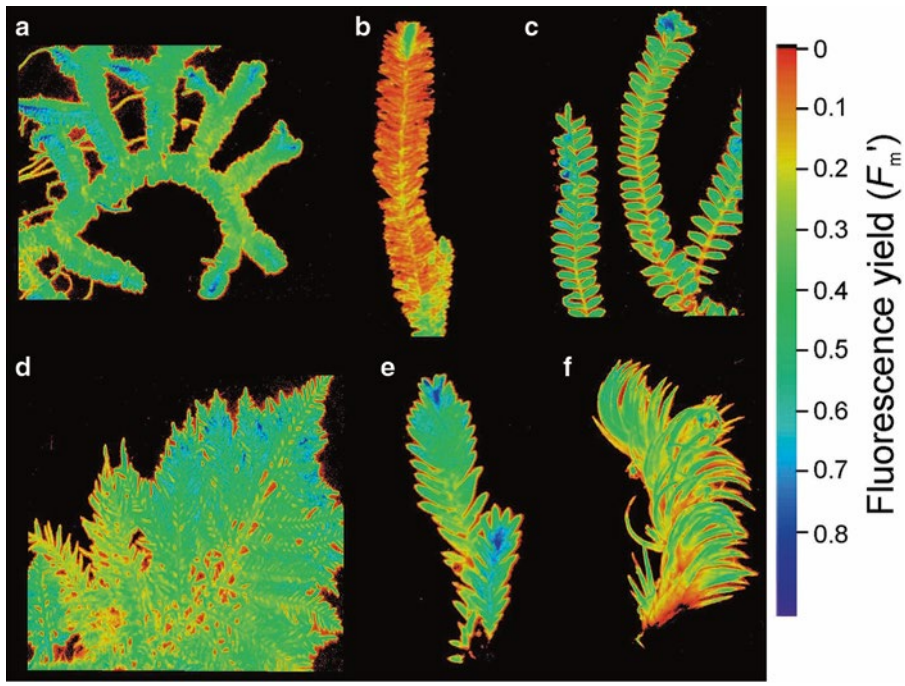


Fig. 9.9. Chlorophyll fluorescence images (light adapted maximum fluorescence yield, F_m') in six Hawaiian moss and liverwort species with plagiotropic growth form not forming a dense canopy: (**a**) – *Bazzania* sp., (**b**) – *Baldwiniella kealeensis* and (**c**) – *Plagiochila deflexa*; and with orthotropic growth form forming a canopy (**d**) – *Thuidium cymbifolium* (**e**) – *Fissidens pacificus*, and (**f**) – *Leucobryum seemannii*. The plant material was collected from the leeward side of Koolau mountains in Oahu (Tantalus trail, 21° 20′ N, 157° 48′ W, elevation ca. 400 m, (Peñuelas et al. 2010 for site description)). After sampling, the plants were kept in moist plastic bags, transported to the laboratory and chlorophyll fluorescence was measured with a Walz Imaging-PAM Mini fluorimeter (H. Walz, GmbH, Effeltrich, Germany). Moderately high actinic illumination of 400 μmol m^{-2} s^{-1} for 5 min. was used for light-adaptation of the samples. The taxonomy of mosses and liverworts follows (Staples et al. 2004; Staples and Imada 2006). Unpublished data of Ülo Niinemets and Mashuri Waite (2006).

to cover the whole within-canopy gradient, a new promising method for rapid assessment of spatial patterns in moss photosynthetic activity is imaging fluorescence (Siebke and Weis 1995; Baker et al. 2001; Chaerle et al. 2007). With imaging fluorescence, a whole moss stem can be sampled rapidly, and spatial variations in photosynthetic activity are immediately visualized. Pilot measurements demonstrate that foliage photosynthetic activity is essentially invariable within the shoots of plagiotropic mosses that do not form a canopy (Fig. 9.9a–c), but significant gradients from the top to bottom of the moss stem occur for mosses with orthotropic growth form that create a dense canopy (Fig. 9.9d–f). Overall, the photosynthetic data collectively demonstrate major variations in foliage photosynthetic characteristics from canopy top to bottom, but also that photosynthetic activity is preserved until almost entire depletion of chlorophyll.

IV. Conclusions

The available evidence demonstrates that most mosses do support high leaf area indices, on average much higher than leaf area indices supported by vascular plants. Such high leaf areas are accompanied by high degree of foliage aggregation reducing the light gradients within the moss stands. In addition, due to thin foliage elements, leaf chlorophyll contents are very low compared with vascular plants, expected to result in low leaf absorptances and much stronger impact of light scattering than in vascular plants. Within-canopy gradients in light are accompanied by gradients in foliage pigment contents and photosynthetic capacity that reflect both senescence in the bottom of the canopy as well as modifications in the stoichiometry of photosynthetic apparatus. Apart from the general patterns, there is high variability in light gradients across moss species driven by differences in the degree of foliage aggregation, canopy density and pigment content per area, but only limited number of species has been studied so far. We suggest that more case studies are needed to gain insight into the determinants of within-canopy light gradients. In particular, further studies are needed on moss leaf area indices and optical characteristics as well as on gradients in moss physiological characteristics.

Acknowledgements

Authors work on mosses has been supported by the Estonian Ministry of Science and Education (grant IUT 8-3), and the European Commission through European Regional Fund (the Center of Excellence in Environmental Adaptation).

References

Aan A, Hallik L, Kull O (2006) Photon flux partitioning among species along a productivity gradient of an herbaceous plant community. J Ecol 94:1143–1155

Adams WW III, Winter K, Schreiber U, Schramel P (1990) Photosynthesis and chlorophyll fluorescence characteristics in relationship to changes in pigment and element composition of leaves of *Platanus occidentalis* L. during autumnal leaf senescence. Plant Physiol 93:1184–1190

Anderson JM, Chow WS, Park Y-I, Franklin LA, Robinson SPA, van Hasselt PR (2001) Response of *Tradescantia albiflora* to growth irradiance: change versus changeability. Photosynth Res 67:103–112

Anten NPR, Hirose T (1999) Interspecific differences in above-ground growth patterns result in spatial and temporal partitioning of light among species in a tall-grass meadow. J Ecol 87:583–597

Anten NPR, Hirose T (2003) Shoot structure, leaf physiology, and daily carbon gain of plant species in a tallgrass meadow. Ecology 84:955–968

Arkimaa H, Laitinen J, Korhonen R, Moisanen M, Hirvasniemi T, Kuosmanen V (2009) Spectral reflectance properties of *Sphagnum* moss species in Finnish mires. In: 6th EARSeL SIG IS workshop, Imaging Spectroscopy: Innovative tool for scientific and commercial environmental applications, 16–19 March 2009, Tel-Aviv University, Tel-Aviv, Israel, 5 pp

Asner GP, Wessman CA (1997) Scaling PAR absorption from the leaf to landscape level in spatially heterogeneous ecosystems. Ecol Model 103:81–97

Baker NR, Oxborough K, Lawson T, Morison JIL (2001) High resolution imaging of photosynthetic

activities of tissues, cells and chloroplasts in leaves. J Exp Bot 52:615–621
Baldini E, Facini O, Nerozzi F, Rossi F, Rotondi A (1997) Leaf characteristics and optical properties of different woody species. Trees Struct Funct 12:73–81
Baldocchi DD, Bowling DR (2003) Modelling the discrimination of $^{13}CO_2$ above and within a temperate broad-leaved forest canopy on hourly to seasonal time scales. Plant Cell Environ 26:231–244
Barker DH, Stark LR, Zimpfer JF, Mcletchie ND, Smith SD (2005) Evidence of drought-induced stress on biotic crust moss in the Mojave Desert. Plant Cell Environ 28:939–947
Bassi R, Caffarri S (2000) Lhc proteins and the regulation of photosynthetic light harvesting function by xanthophylls. Photosynth Res 64:243–256
Bates JW (1998) Is 'life-form' a useful concept in bryophyte ecology? Oikos 82:223–237
Bayfield NG (1973) Notes on water relations of *Polytrichum commune* Hedw. J Bryol 7:607–617
Bergamini A, Peintinger M (2002) Effects of light and nitrogen on morphological plasticity of the moss *Calliergonella cuspidata*. Oikos 96:355–363
Bond-Lamberty B, Gower ST (2007) Estimation of stand-level leaf area for boreal bryophytes. Oecologia 151:584–592
Boonman A, Prinsen E, Gilmer F, Schurr U, Peeters AJM, Voesenek LACJ, Pons TL (2007) Cytokinin import rate as a signal for photosynthetic acclimation to canopy light gradients. Plant Physiol 143:1841–1852
Boonman A, Prinsen E, Voesenek LACJ, Pons TL (2009) Redundant roles of photoreceptors and cytokinins in regulating photosynthetic acclimation to canopy density. J Exp Bot 60:1179–1190
Brakke TW (1994) Specular and diffuse components of radiation scattered by leaves. Agric For Meteorol 71:283–295
Bryant RG, Baird AJ (2003) The spectral behaviour of *Sphagnum* canopies under varying hydrological conditions. Geophys Res Lett 30:1134
Bubier JL, Rock BN, Crill PM (1997) Spectral reflectance measurements of boreal wetland and forest mosses. J Geophys Res – Atm 102:29483–29494
Carter GA, Knapp AK (2001) Leaf optical properties in higher plants: linking spectral characteristics to stress and chlorophyll concentration. Am J Bot 88:677–684
Cescatti A, Niinemets Ü (2004) Sunlight capture. Leaf to landscape. In: Smith WK, Vogelmann TC, Chritchley C (eds) Photosynthetic adaptation: chloroplast to landscape, Ecological Studies, vol 178. Springer, Berlin, pp 42–85
Chaerle L, Leinonen I, Jones HG, Van Der Straeten D (2007) Monitoring and screening plant populations with combined thermal and chlorophyll fluorescence imaging. J Exp Bot 58:773–784
Crandall-Stotler BJ, Bartholomew-Began SE (2007) Morphology of mosses (phylum Bryophyta). In: Zander RH, Eckel PM (eds) Flora of North America north of Mexico (FNA), vol 27. www.efloras.org. Flora of North America Editorial Committee New York, Oxford, pp 3–13
Davey MC, Ellis-Evans JC (1996) The influence of water content on the light climate within Antarctic mosses characterized using an optical microprobe. J Bryol 19:235–242
Davey ML, Nybakken L, Kauserud H, Ohlson M (2009) Fungal biomass associated with the phyllosphere of bryophytes and vascular plants. Mycol Res 113:1254–1260
Dilks TJK, Proctor MCF (1979) Photosynthesis, respiration and water content in bryophytes. New Phytol 82:97–114
Duursma RA, Falster DS, Valladares F, Sterck FJ, Pearcy RW, Lusk CH, Sendall KM, Nordenstahl M, Houter NC, Atwell BJ, Kelly N, Kelly JWG, Liberloo M, Tissue DT, Medlyn BE, Ellsworth DS (2012) Light interception efficiency explained by two simple variables: a test using a diversity of small- to medium-sized woody plants. New Phytol 193:397–408
Evans JR (1993a) Photosynthetic acclimation and nitrogen partitioning within a lucerne canopy. I. Canopy characteristics. Aust J Plant Physiol 20:55–67
Evans JR (1993b) Photosynthetic acclimation and nitrogen partitioning within a lucerne canopy. II. Stability through time and comparison with a theoretical optimum. Aust J Plant Physiol 20:69–82
Evans JR, Poorter H (2001) Photosynthetic acclimation of plants to growth irradiance: the relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. Plant Cell Environ 24:755–767
Frego KA, Carleton TJ (1995) Microsite tolerance of four bryophytes in a mature black spruce stand: reciprocal transplants. Bryologist 98:452–458
Gabrielsen EK (1948) Effects of different chlorophyll concentrations on photosynthesis in foliage leaves. Physiol Plant 1:5–37
Glime J (2007) Bryophyte ecology. E-book sponsored by Michigan Technological University and the International Association of Bryologists
Goudriaan J (1977) Crop micrometeorology: a simulation study. Pudoc, Wageningen
Green TGA, Lange OL (1994) Photosynthesis in pokilohydric plants: a comparison of lichens and bryophytes. In: Schulze E-D, Caldwell MM (eds) Ecophysiology of photosynthesis, Ecological Studies, vol 100. Springer, Berlin, pp 319–341

Gutschick VP, Wiegel FW (1988) Optimizing the canopy photosynthetic rate by patterns of investment in specific leaf mass. Am Nat 132:67–86

Hallik L, Kull O, Niinemets Ü, Aan A (2009a) Contrasting correlation networks between leaf structure, nitrogen and chlorophyll in herbaceous and woody canopies. Basic Appl Ecol 10:309–318

Hallik L, Kull O, Nilson T, Peñuelas J (2009b) Spectral reflectance of multispecies herbaceous and moss canopies in the boreal forest understory and open field. Can J Remote Sens 35:474–485

Hallik L, Niinemets Ü, Kull O (2012) Photosynthetic acclimation to light in woody and herbaceous species: a comparison of leaf structure, pigment content and chlorophyll fluorescence characteristics measured in the field. Plant Biol 14:88–99

Hamerlynck EP, Tuba Z, Csintalan Z, Nagy Z, Henebry G, Goodin D (2000) Diurnal variation in photochemical dynamics and surface reflectance of the desiccation-tolerant moss, *Tortula ruralis*. Plant Ecol 151:55–63

Harris A (2008) Spectral reflectance and photosynthetic properties of *Sphagnum* mosses exposed to progressive drought. Ecohydrology 1:35–42

Hill MO, Preston CD, Bosanquet SDS, Roy DB (2007) BRYOATT: attributes of British and Irish mosses, liverworts and hornworts with information on native status, size, life form, life history, geography and habitat. NERC Centre for Ecology and Hydrology and Countryside Council for Wales, Cambridgeshire

Hirose T, Werger MJA (1987) Maximizing daily canopy photosynthesis with respect to the leaf nitrogen allocation pattern in the canopy. Oecologia 72:520–526

Johansson LG, Linder S (1980) Photosynthesis of *Sphagnum* in different microhabitats on a subarctic mire. In: Sonesson M (ed) Ecology of a subarctic mire, Ecological Bulletins, vol 30. Swedish Natural Science Research Council, Stockholm, pp 181–190

Krumnikl M, Sojka E, Gaura J, Motyka O (2008) A new method for bryophyte canopy analysis based on 3D surface reconstruction. In: 7th computer information systems and industrial management applications, IEEE, pp 210–211

Lechowicz MJ (1983) Age dependence of photosynthesis in the caribou lichen *Cladina stellaris*. Plant Physiol 71:893–895

Lovelock CE, Robinson SA (2002) Surface reflectance properties of Antarctic moss and their relationship to plant species, pigment composition and photosynthetic function. Plant Cell Environ 25:1239–1250

Martin CE, Churchill SP (1982) Chlorophyll concentrations and a/b ratios in mosses collected from exposed and shaded habitats in Kansas. J Bryol 12:297–304

Martin G, Myers DA, Vogelmann TC (1991) Characterization of plant epidermal lens effects by a surface replica technique. J Exp Bot 42:581–587

Masarovičová E, Eliáshš P (1987) Some ecophysiological features in woodland mosses in SW Slovakia. 2. Chlorophyll content and photosynthesis. In: Pócs T, Simon T, Tuba Z, Podani J (eds) Proceedings of the IAB conference of bryoecology. Akadémiai Kiadó, Budapest-Vacratot, Hungary, pp 113–123

Masoni A, Ercoli L, Mariotti M, Barberi P (1994) Changes in spectral properties of ageing and senescing maize and sunflower leaves. Physiol Plant 91:334–338

Mesarch MA, Walter-Shea EA, Asner GP, Middleton EM, Chan SS (1999) A revised measurement methodology for conifer needles spectral optical properties: evaluating the influence of gaps between elements. Remote Sens Environ 68:177–192

Monsi M, Saeki T (1953) Über den Lichtfaktor in den Pflanzengesellschaften und seine Bedeutung für die Stoffproduktion. Jap J Bot 14:22–52

Myers DA, Vogelmann TC, Bornman JF (1994) Epidermal focussing and effects on light utilization in *Oxalis acetosella*. Physiol Plant 91:651–656

Niinemets Ü (2007) Photosynthesis and resource distribution through plant canopies. Plant Cell Environ 30:1052–1071

Niinemets Ü (2010) A review of light interception in plant stands from leaf to canopy in different plant functional types and in species with varying shade tolerance. Ecol Res 25:693–714

Niinemets Ü, Anten NPR (2009) Packing the photosynthesis machinery: from leaf to canopy. In: Laisk A, Nedbal L, Govindjee (eds) Photosynthesis in silico: understanding complexity from molecules to ecosystems, Advances in photosynthesis and respiration, vol 29. Springer, Berlin, pp 363–399

Niinemets Ü, Kull O, Tenhunen JD (2004) Within canopy variation in the rate of development of photosynthetic capacity is proportional to integrated quantum flux density in temperate deciduous trees. Plant Cell Environ 27:293–313

Niinemets Ü, Tobias M, Cescatti A, Sparrow AD (2006) Size-dependent variation in shoot light-harvesting efficiency in shade-intolerant conifers. Int J Plant Sci 167:19–32

Niinemets Ü, García-Plazaola JI, Tosens T (2012) Photosynthesis during leaf development and ageing. In: Flexas J, Loreto F, Medrano H (eds) Terrestrial photosynthesis in a changing environment. The molecular, physiological and ecological bases of photosynthesis driving its response to the environmental changes. Cambridge University Press, Cambridge, pp 357–376

Nilson T (1971) A theoretical analysis of the frequency of gaps in plant stands. Agric Meteorol 8:25–38

Nilson T, Ross J (1997) Modeling radiative transfer through forest canopies: implications for canopy photosynthesis and remote sensing. In: Gholz HL, Nakane K, Shimoda H (eds) The use of remote sensing in the modeling of forest productivity, Forestry sciences, vol 50. Kluwer Academic, Dordrecht, pp 23–60

Osmond CB, Anderson JM, Ball MC, Egerton JG (1999) Compromising efficiency: the molecular ecology of light-resource utilization in plants. In: Press MC, Scholes JD, Barker MG (eds) Physiological plant ecology. The 39th Symposium of the British Ecological Society held at the University of York, 7–9 September 1998. Blackwell Science, Oxford, pp 1–24

Pedersen B, Hanslin HM, Bakken S (2001) Testing for positive density-dependent performance in four bryophyte species. Ecology 82:70–88

Peñuelas J, Sardans J, Llusià J, Owen SM, Carnicer J, Giambelluca TW, Rezende E, Waite M, Niinemets Ü (2010) Faster returns on 'leaf economics' and different biogeochemical niche in invasive compared with native plant species. Global Change Biol 16:2171–2185

Pons TL, Anten NPR (2004) Is plasticity in partitioning of photosynthetic resources between and within leaves important for whole-plant carbon gain in canopies? Funct Ecol 18:802–811

Proctor MC (1979) Structure and eco-physiological adaptations in bryophytes. In: Clarke G, Duckett J (eds) Bryophyte systematics. Academic, New York, pp 479–509

Proctor MCF (1984) Structure and ecological adaptation. In: Dyer AF, Duckett JG, Cronshaw J (eds) The experimental biology of bryophytes, Experimental botany: an international series of monographs. Academic, London, pp 9–37

Proctor MCF (1990) The physiological bases for bryophyte production. Bot J Linn Soc 104:61–77

Rice SK, Schneider N (2004) Cushion size, surface roughness, and the control of water balance and carbon flux in the cushion moss *Leucobryum glaucum* (Leucobryaceae). Am J Bot 91:1164–1172

Rice SK, Collins D, Anderson AM (2001) Functional significance of variation in bryophyte canopy structure. Am J Bot 88(9):1568–1576

Rice SK, Gutman C, Krouglicof N (2005) Laser scanning reveals bryophyte canopy structure. New Phytol 166:695–704

Rice SK, Neal N, Mango J, Black K (2011) Relationships among shoot tissue, canopy and photosynthetic characteristics in the feathermoss *Pleurozium schreberi*. Bryologist 114:367–378

Rincón E (1993) Growth responses of six bryophyte species to different light intensities. Can J Bot 71:661–665

Rincon E, Grime JP (1989) Plasticity and light interception by six bryophytes of contrasted ecology. J Ecol 77:439–446

Ross J (1981) The radiation regime and architecture of plant stands. Dr. W. Junk, The Hague

Sands PJ (1995) Modelling canopy production. I. Optimal distribution of photosynthetic resources. Aust J Plant Phys 22:593–601

Schwartz A-M, Markager S (1999) Light absorption and photosynthesis of a benthic moss community: importance of spectral quality of light and implications of changing light attenuation in the water column. Freshw Biol 42:609–623

Seel WE, Baker NR, Lee JA (1992) Analysis of the decrease in photosynthesis on desiccation of mosses from xeric and hydric environments. Physiol Plant 86:451–458

Siebke K, Weis E (1995) Imaging of chlorophyll-a-fluorescence in leaves: topography of photosynthetic oscillations in leaves of *Glechoma hederacea*. Photosynth Res 45:225–237

Simon T (1987) The leaf-area index of three moss species (*Tortula ruralis, Ceratodon purpureus*, and *Hypnum cupressiforme*). In: Pócs T, Simon T, Tuba Z, Podani J (eds) Proceedings of the IAB conference of bryoecology, Symposia Biologica Hungarica, 35. Akadémiai Kiadó, Budapest-Vacratot, Hungary, pp 699–706

Skré O, Oechel WC, Miller PM (1983) Moss leaf water content and solar radiation at the moss surface in a mature black spruce forest in central Alaska. Can J For Res 13:860–868

Smith AJE (1982) Bryophyte ecology. Chapman & Hall, London/New York

Sonesson M, Gehrke C, Tjus M (1992) CO_2 environment, microclimate and photosynthetic characteristics of the moss *Hylocomium splendens* in a subarctic habitat. Oecologia 92:23–29

Staples GW, Imada CT (2006) Checklist of Hawaiian Anthocerotes and Hepatics. Trop Bryol 28:15–47

Staples GW, Imada CT, Hoe WJ, Smith CW (2004) A revised checklist of Hawaiian mosses. Trop Bryol 25:35–69

Thomas SC, Liguori DA, Halpern CB (2001) Corticolous bryophytes in managed Douglas-fir forests: habitat differentiation and responses to thinning and fertilization. Can J Bot 79:886–896

Tobias M, Niinemets Ü (2010) Acclimation of moss *Pleurozium schreberi* photosynthetic characteristics

to among-habitat and within-canopy light gradients. Plant Biol 12:743–754

Tobias M, Niinemets Ü (2005) Acclimation to within-canopy light gradient in four moss species of contrasting architecture In: Abstracts. XVII International Botanical Congress, Vienna, Austria, 17–23 July 2005, pp 105–106

van der Hoeven EC, Huynen CIJ, During HJ (1993) Vertical profiles of biomass, light intercepting area and light intensity in chalk grassland mosses. J Hatt Bot Lab 74:261–270

Van Gaalen KE, Flanagan LB, Peddle DR (2007) Photosynthesis, chlorophyll fluorescence and spectral reflectance in *Sphagnum* moss at varying water content. Oecologia 153:19–28

Vitt DH (1990) Growth and production dynamics of boreal mosses over climatic, chemical and topographic gradients. Biol J Linn Soc 104:35–59

Vogelmann TC (1993) Plant tissue optics. Ann Rev Plant Physiol Plant Mol Biol 44:231–252

Vogelmann JE, Moss DM (1993) Spectral reflectance measurements in the genus *Sphagnum*. Remote Sens Environ 45:273–279

Vogelmann TC, Bornman JF, Yates DJ (1996a) Focusing of light by leaf epidermal cells. Physiol Plant 98:43–56

Vogelmann TC, Nishio JN, Smith WK (1996b) Leaves and light capture: light propagation and gradients of carbon fixation within leaves. Trends Plant Sci 1:65–70

Waite M, Sack L (2010) How does moss photosynthesis relate to leaf and canopy structure? Trait relationships for 10 Hawaiin species of contrasting light habitats. New Phytol 185:156–172

Williams WE, Gorton HL, Witiak SM (2003) Chloroplast movements in the field. Plant Cell Environ 26:2005–2014

Zotz G, Kahler H (2007) A moss "canopy" – small-scale differences in microclimate and physiological traits in *Tortula ruralis*. Flora 202:661–666

Chapter 10

Structural and Functional Analyses of Bryophyte Canopies

Steven K. Rice*
Department of Biological Sciences, Union College, Schenectady, NY 12308, USA

David T. Hanson
Department of Biology, University of New Mexico, Albuquerque, NM 87131, USA

Zach Portman
Department of Biology, Utah State University, Logan, UT 84322, USA

Summary

Although not often discrete, the canopy (i.e., the organization of branches, shoot systems and their extent) remains the most definable and useful unit of function in bryophytes. Chambers used for gas exchange provide an integrated summary of canopy photosynthetic function. However, other techniques can provide more information on spatial variation in physiological process in both the horizontal and vertical planes. Three examples of such studies are presented here. First, variation in photosystem II (PSII) function has been evaluated, along a canopy

*Author for correspondence, e-mail: rices@union.edu

D.T. Hanson and S.K. Rice (eds.), Photosynthesis in Bryophytes and Early Land Plants, Advances in Photosynthesis and Respiration 37, pp. 173–185, DOI 10.1007/978-94-007-6988-5_10,

surface, using an imaging chlorophyll fluorometer. We evaluated the quantum yield of PSII, ϕ_{PSII}, and calculated the relative rate of photosynthetic electron transport (RETR) on 7 cm diameter samples of ten *Sphagnum* species during drying. Spatial variation in RETR increased both during drying as well as in high light, which led to different relationships between mean RETR and its variation—across light gradients, the relationship was positive, but negative when RETR was reduced by tissue desiccation. Studies of photosynthetic function using chlorophyll fluorescence measurements need to match their sampling protocols to account for this difference. Further, combining a laser scanning approach that provides three-dimensional information on canopy structure with functional imaging allows assessment of function in three dimensions (3D) within the canopy. This is illustrated using a thermal imaging camera to measure temperature distribution within *Pleurozium schreberi* canopies under still conditions and with wind. This imaging system resolved 9 °C temperature differences within the canopy and localized shoot temperature relative to canopy height. Finally, computational canopy (i.e., virtual) models have been developed for bryophyte canopies, particularly ones with simple branching structure. A model of this type is shown here for the liverwort *Bazzania trilobata* and a light model implemented using a ray tracing algorithm. Output from this model followed the attenuation of light predicted by the Lambert-Beer Law and such a technique can be used to evaluate how branching architecture and density affect the dynamics of light capture in bryophytes. New approaches based on novel imaging technologies are in rapid development and present opportunities to further our understanding of function within bryophyte canopies.

I. Introduction

The canopy represents an important unit of matter and energy exchange in bryophytes; however, canopies do not display homogeneous function in either the horizontal or vertical planes. For example, plant water status, photosynthetic pigment concentration, photosynthetic capacity, photoinhibition, leaf or shoot area, light intensity, temperature and humidity can all vary along and within bryophyte canopies (Hayward and Clymo 1982; van der Hoeven et al. 1993; Gerdol et al. 1994; Davey and Ellis-Evans 1996; Zona et al. 2011), and affect rates of whole canopy photosynthesis (Zotz and Kahler 2007; Rice et al. 2008, 2011b; Tobias and Niinemets 2010). Unfortunately, this variation is normally neglected as methods of evaluating photosynthetic function often either provide measurements of average, coarse-scale properties (>4 cm scale or above the size of individual shoots; e.g., gas exchange methods) or they allow for fine-scale measurements (0.1–2 cm or at scale of large leaves or branches; e.g., fiber-optic evaluations of chlorophyll fluorescence parameters) that may obscure larger scale patterns. Although limitations of the latter may be overcome by dense sampling and exploring the variance structure of the data,

Abbreviations: Chl – Chlorophyll; ETR – Rate of photosynthetic electron transport as calculated from chlorophyll *a* fluorescence measurements; F_0 – Baseline fluorescence in dark-adapted tissue; F_0' – Baseline fluorescence in light-adapted tissue; F_m – Maximum chlorophyll *a* fluorescence in dark-adapted tissue at saturating light; F_v/F_m – Ratio of variable ($=F_m-F_o$) to maximal fluorescence in dark-adapted tissue; F_v'/F_m' – Ratio of variable to maximal fluorescence in light-adapted tissue; I_0 – Intensity of incident light at top of canopy; I_x – Intensity of incident light at depth x within canopy; K – Light extinction coefficient; K_{app} – Apparent light extinction coefficient, product of K and SAIx; PPFD – Photosynthetic photon flux density; PRI – Photochemical reflectance index; Q_{10} – Temperature coefficient; RETR – Relative rate of (calculated) photosynthetic electron transport; SAI_x – Shoot area index above depth x within canopy; ϕ_{PSII} – Quantum yield of photosynthesis as calculated from chlorophyll a fluorescence parameters

this is rarely done. Few methods have been developed that allow investigations across fine- and coarse-scales that would help provide an understanding of how physiological processes integrate across these scales within canopies and would allow for a more mechanistic understanding of physiological scaling of bryophytes.

Consequently, we have limited understanding of how variation in the horizontal and vertical dimensions affects bryophyte canopy function. Variation in the horizontal plane will be caused by extrinsic factors that affect microclimate, particularly those influencing energy and water budgets, as well as structural variation within the canopy that affect boundary layer processes and, hence water relations, or self-shading. When integration among adjacent shoots within the canopy is limited, portions of the canopy may experience different physiological conditions. This may be caused by uneven drying, or heating because of the resistance to capillary transport and heat movement within and among shoots. Depending on the scale of the underlying causes of spatial variation and the degree of spatial integration, physiological function may vary at fine- or coarse-scales, both, or neither. Fine-scale variation may occur when biotic or abiotic conditions vary among nearby leaves or shoots when there is limited physiological integration. For example when distal leaves or branches dry, there may be limited ability for plants to rehydrate from interior water stores causing high variability in the water status of distal branches.

Coarse-scale patterns may occur when local conditions within leaves and shoots are similar, but are patchy at larger spatial scales. This can be caused by development of different heights of shoot systems, existence of capillary networks that share water among nearby, but not distant shoots, proximity to local windshelters or regions of high humidity, or to the patchy distribution of ambient light. Recent studies of spatial variation in vascular plant leaf photosynthesis using 2D imaging chlorophyll fluorometers have localized regions of high photosynthetic activity or damage and related it to stomatal dynamics when combined with other imaging approaches (Oxborough 2004; Aldea et al. 2006; Chaerle et al. 2007; Morison and Lawson 2007; Baker 2008). In this chapter, we employ a similar approach to compare the structure of variation in photosynthetic electron transport, as inferred from chlorophyll a fluorescence measurements, during drying and across gradients of light availability in a multispecies comparison in *Sphagnum*. In addition, we introduce two methods that could prove promising for understanding physiological function and integration in studies of bryophyte canopies, namely 3D functional imaging combining laser scanning with thermal imaging and the use of simulated virtual canopies where processes can be explored using computer simulation modeling on 3D canopy representations.

II. Chlorophyll Fluorescence 2D Imaging in *Sphagnum*

Understanding the physiological mechanisms that underlie variation in whole plant or canopy function will require not only the ability to evaluate the performance of physiological units (e.g., leaves, branch systems or populations), but also variation in those units and their interaction. Using an imaging chlorophyll fluorometer (Fluorocam, PSI, Czech Republic) with a closed-chamber gas exchange system (LI-COR 6200, Lincoln, NB, USA), we have explored variation in photosynthetic characteristics obtained using chlorophyll fluorescence and CO_2 uptake simultaneously in a multi-species comparison in the genus *Sphagnum*.

A. Photosynthetic Drying and Light Response Curves

Samples of ten *Sphagnum* species that varied in their preferred habitat and morphology were collected in the field and reared in a greenhouse under 300 μmol quanta m^{-2} s^{-1} maximum photosynthetic photon flux density (PPFD) for over 90 days. Intact 7.5 cm

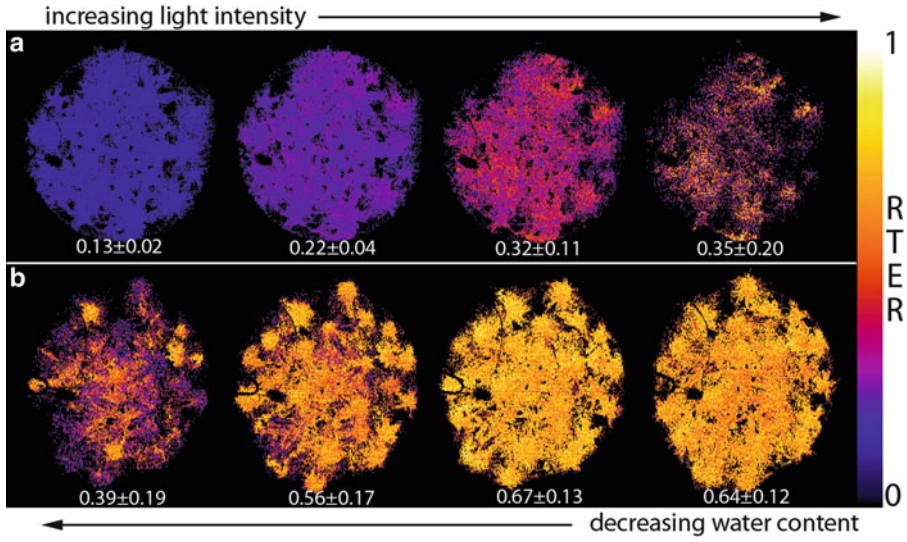


Fig. 10.1. Relative photosynthetic electron transport rates (*RETR*), as estimated from chlorophyll fluorescence measurements, at different water contents and light intensities in *Sphagnum recurvum* for 7.5 cm diameter samples. The quantum yield of Photosystem II, ϕ_{PSII}, as estimated from chlorophyll fluorescence parameters was measured at 0.3 mm resolution using 2D imaging. RETR was standardized relative to the maximum value in (**a**) during increasing light intensities (PPFD 60, 120, 250 and 500 µmol photons m^{-2} s^{-1}) and (**b**) during drying from optimal water content to zero. Mean RETR and standard deviations are shown. Higher RETR values and associated rates of photosynthesis at high light show elevated variation as evidenced by the increase in the standard deviation. The opposite relationship is shown when RTER was decreased during drying: where lower RETR is associated with high variation.

diameter canopies containing only new growth were used for analysis. Samples were placed in a 1.8 l closed chamber and CO_2 exchange measured in two 20 s intervals. Rates of net photosynthesis were assessed over a set of seven light intensities (30–730 µmol quanta m^{-2} s^{-1} PPFD) for each sample (n=3 per species). A photosynthetic drying curve (n = 1 per species) was also performed over a 2–3 days period. Details on sample collection, growth conditions and gas exchange are reported more fully in Rice et al. (2008). Following gas exchange measurements, a 2D imaging fluorometer (Fluorcam, PSI, The Czech Republic) was used to estimate both the photochemical yield (ϕ_{PSII}) and the relative rate of photochemical electron transport (RETR; rate of electron transport scaled between 0 and 1 for each sample) at 0.3 mm spatial scales (Fig. 10.1; for basics on chlorophyll *a* fluorescence, see Govindjee 2004; and other chapters in Papageorgiou and Govindjee (eds) 2004). LED panel lighting provided measuring light at 618 nm and a white saturating burst. The parameter ϕ_{PSII} was calculated as Fv'/Fm' (ratio of variable to maximum fluorescence in light adapted tissue) and ETR calculated as the product of ϕ_{PSII}, the light level and 0.5, the latter to account for energy going to photosystem II (Maxwell and Johnson 2000); absorption was assumed constant among species. This may deviate from 0.5 due to system state changes (see Papageorgiou and Govindjee 2011) and we report relative rates (RETR) to account for possible variation among species. The mean and standard deviation of RETR were derived and compared.

All samples showed characteristic light response curves with light compensation points varying between 37 and 72 µmol quanta m^{-2} s^{-1} PPFD and $PPFD_{95\ \%}$ (light level at 95 % of maximum net photosynthesis) between 491 and 648 µmol quanta m^{-2} s^{-1} PPFD (Rice et al. 2008). Within species, integrated values of ϕ_{PSII} and RETR showed typical

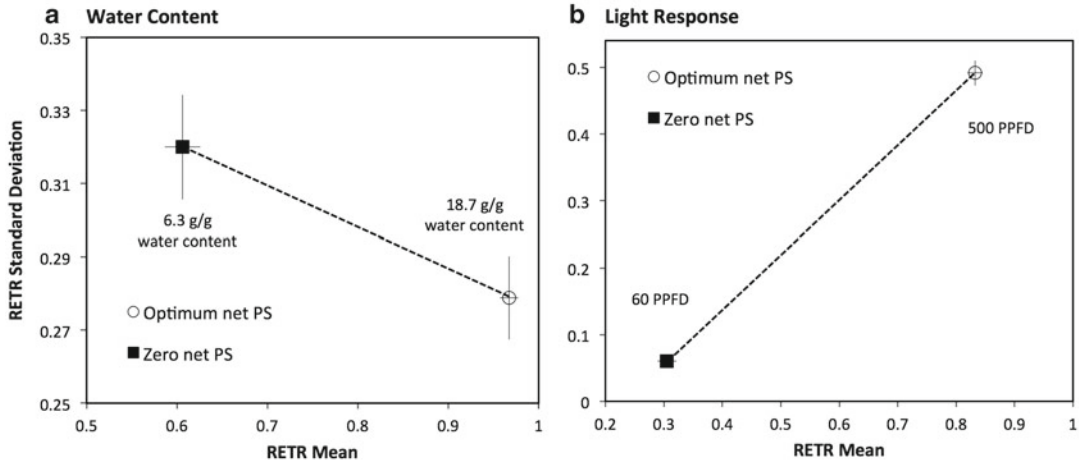


Fig. 10.2. Summary of spatial variation in relative rates of electron transport (*RTER*) estimated from imaging chlorophyll fluorescence associated with differences in plant water content (**a**) and light intensity (**b**). Means and standard errors are shown for n = 10 samples, each of a different *Sphagnum* species. Measurements of RETR assume constant fraction of excited electrons going to photosystem II within a sample (Note the difference in scales).

patterns with all displaying saturating responses through 730 μmol quanta m^{-2} s^{-1} PPFD. All species sampled showed characteristic responses of net photosynthesis and RETR to drying. At high water content, external water films reduce access to CO_2 by increasing diffusion resistance thereby lowering rates of photosynthesis. Photosynthesis is optimized at intermediate water content where externally held water is minimized, but cells remain in full turgor. This is achieved at water content between 10 and 22 g H_2O g^{-1} dry wt for the different species in this study. As plants dry, photosynthesis declines as cells desiccate. These responses were evident in the measures of net photosynthesis and RETR.

B. Fine- and Coarse-Scale Patterns of Electron Transport Rate

In the configuration used in this study, imaging fluorescence provides 0.3 mm spatial resolution of photosynthetic function for up to 50,000 points. This allows an evaluation of the variation as well as the spatial structure of the photosynthetic parameters. Mean and variation in RETR differed across both light and water content gradients (Fig. 10.1). During light response curves, both mean values and the variation, as represented by the standard deviation of RETR, increased at higher light intensities and correspondingly higher rates of net photosynthesis were obtained using gas exchange (Fig. 10.2). Increased dispersion of the measurements at high light is likely caused by enhanced sensitivity to noise in calculating the ratio F_v'/F_m', used to estimate RETR. The numerator, the difference between the maximum fluorescence (F_m') at the saturating burst and the minimum at ambient light (F_0'), is more sensitive to small changes in F_m' when F_0' is high under higher ambient light. Consequently, instead of indicating differences in the physiological function at fine spatial scales, higher spatial variation in RETR at high light is associated with greater measurement error.

In contrast, the relationship between variation and the mean RETR is reversed during drying (Fig. 10.1). In this case, as plants dehydrate and rates of photosynthesis and mean RETR decrease, the standard deviation of RETR increases (Fig. 10.2). During drying in *Sphagnum*, isolated distal branches, branch tips, and the edges of colonies dry first while areas of high branch and leaf density like the capitula retain water. This can

be observed in Fig. 10.1 and was common in other samples as well. This leads to spatial variation in physiological function and measures of RETR. Aggregation of regions of high photosynthetic activity increases at intermediate states of desiccation, as canopies continue to dry, the entire surface becomes desiccated and the regions of high activity become more dispersed. The cause of this pattern differs from that responsible for spatial variation in high light. During drying, high spatial variation in RETR is associated with uneven drying; it reflects underlying biophysical changes in the distribution of plant water. The large and non-uniform spatial variation observed in RETR derived from chlorophyll fluorescence studies should allow us to plan for sampling and measuring strategies in chlorophyll fluorescence studies of bryophytes. When fiber-optic probes are used, sufficient sampling should be performed to summarize variation as well as mean values.

III. 3D Thermal Mapping of Bryophyte Canopies

As the previous section illustrates, imaging systems are able to evaluate physiological function in two dimensions. Similarly, camera systems have been deployed in the field to monitor the physiological state of *Tortula princeps* (=*Syntrichia princeps*) during wet-dry cycles (Graham et al. 2006). With the development of new indices based on spectral reflectance data in the visible and near-infrared spectrum, there is now the potential to track both water status (Water Index) and physiological stress (PRI) remotely using imaging systems (Gamon et al. 1997; Lovelock and Robinson 2002; Harris 2008; Ollinger 2011). However, canopies are three-dimensional and even 2D representations will be limited in their ability to develop an understanding of canopy-level functional integration. Recent developments in 3D rendering of canopy structure using laser scanning may be combined with 2D imaging to explore physiological function in three dimensions. Below, we provide a proof-of-concept using thermal imaging to map the temperature distribution within *Polytrichum commune* canopies.

A. Combining Thermal Imaging and 3D Laser Scanning

A 3D thermal imaging system was developed by combining a thermal imaging camera (FLIR i7, Boston, MA, USA) with 0.1 °C temperature resolution with a laser scanning system using an optical camera. In brief, the laser scanning system positions a plane of laser light perpendicular to the surface and the intersection between the canopy surface and the light is captured from the camera at an angle of 45° from the normal. Following transformation, the horizontal and vertical locations of all points along the intersection are determined. Coarse-scale patterns in canopy height were removed by using residuals from a linear multiple regression using the axes of the horizontal plane as input. Following a scan, the apparatus was moved 0.5 cm and a new scan was obtained. Ten scans allowed for the scanning of a 5 cm × 5 cm area of each sample. Details of the laser scanning system are described more fully in Rice et al. (2005). The system was constructed so that the two cameras could be positioned sequentially to view the sample from the same viewpoint, allowing for registration between images of each type. Image registration used the Turboreg plugin (Thévenaz et al. 1998) implemented in ImageJ (Abramoff et al. 2004). This brought the resolution to the minimum of the two images (120 × 120 pixels for the thermal image). Final spatial resolution was 0.23 cm/pixel in the vertical plane and 0.12 cm/pixel in the horizontal plane. After registration, images from the laser scans were thresholded to identify the portion in the laser plane and those points were selected from the thermal image. Following transformation into Cartesian spatial dimensions, the data contain spatial coordinates and temperature distribution for over 500 points within the 5 cm × 5 cm canopy (Fig. 10.3).

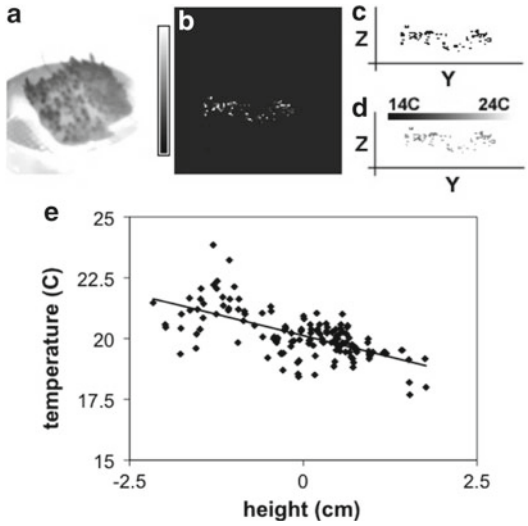


Fig. 10.3. Image acquisition and processing for 3D rendering of canopy temperatures in *Polytrichum commune*. Thermal images are taken (**a**) and visible light images are obtained from the same point of view. Images show intersection with the orthogonal plane of laser light (**b**). Images from the two sources are aligned and the laser—canopy intersection is highlighted using thresholding. The coordinates from the two images are generated following transformation (**c**). These points are identified in the thermal image, resulting in surface temperatures for the points within the canopy (**d**). Temperatures are graphed as a function of height relative to the mean canopy height (**e**).

B. Temperature Distribution in Polytrichum commune *Canopies*

We employed this system to explore differences in temperature distribution within canopies of *Polytrichum commune*, collected in Saratoga County, NY, USA. The stem density of the sample was 1.8 stems/cm^2. Samples were measured under laboratory conditions (25.5 °C, 10 % relative humidity) in still air as well as within an unconditioned flow generated by a fan (1.1 m/s average wind speed). Linear regressions were used to compare temperatures and heights within the canopy.

In still air, temperatures within the canopy ranged from 16.9 to 23.7 °C (19.9 °C mean, 1.3 °C standard deviation). With 1.1 m/s wind, temperatures were depressed and ranged from 15.2 to 23.8 °C with a 18.1 °C mean and 1.9 °C standard deviation. Under both conditions, there was a significant negative relationship with canopy height (Fig. 10.4a, b, $p < 0.0001$); however, they differed in their slopes and also in their explanatory power. In still air, the slope of the canopy height—temperature relationship was significantly more negative (-0.76 ± 0.04) than that in wind (-0.46 ± 0.10). Without wind, the linear model accounted for 24 % of variation in the data, whereas this was only 3 % with wind.

Clearly, temperature variation within bryophyte canopies is high. Although some may be due to measurement error as there is coarse resolution of the thermal imaging system used in this study, some is clearly related to evaporative cooling within bryophyte canopies, a phenomenon expected to be greater in the upper-canopy where boundary layers are thinner. The temperature ranges displayed are biologically significant, particularly for water loss, as evaporation is a temperature-dependent process. However, they are also significant for respiratory rates as the Q_{10} (temperature coefficient) of respiration is often >2 and even above 4 in bryophytes (Davey and Rothery 1997; Uchida et al. 2002), and although photochemistry does not normally display a similarly dramatic response to temperature, Q_{10} values can also be >2 for gross photosynthesis (Davey and Rothery 1997). Canopy photosynthesis models in bryophytes have only incorporated light and shoot-level acclimation responses (Zotz and Kahler 2007; Rice et al. 2011a, b), but not temperature differences that might impact shoot-level rates of net photosynthesis. Higher temperatures that allow for increased respiration together with low light in canopy interiors would further reduce the contribution of interior shoots to net carbon gain.

The approach described above provides sufficiently resolved 3D temperature data to develop thermal models for bryophyte canopies. When combined with the biomass or shoot-area distribution within the canopy, shoot temperatures would allow development of refined canopy-level evaporation and photosynthesis models. Indeed, the degree of integration experienced by shoots and branches within the canopy, especially of plant water status, affects whole-canopy

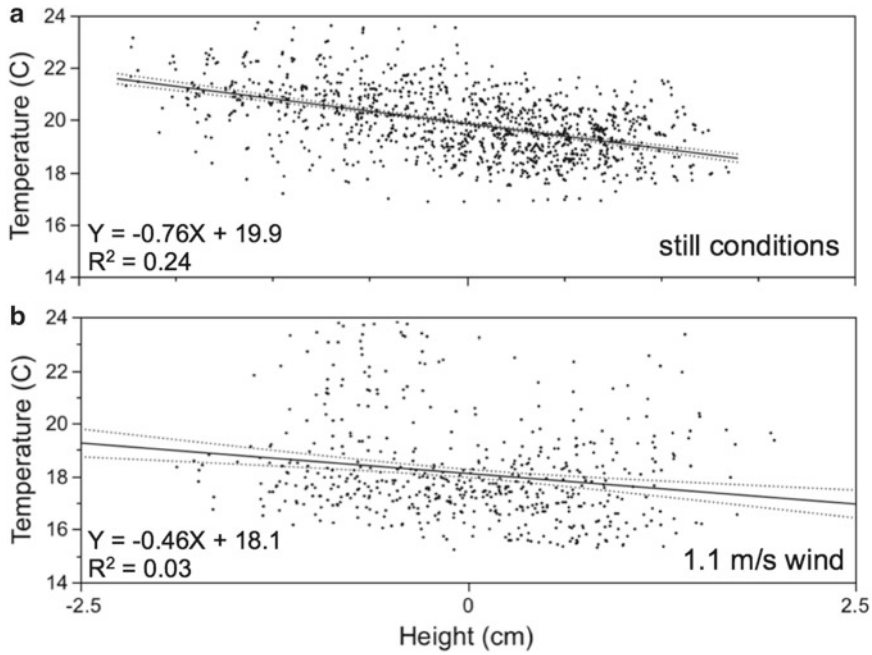


Fig. 10.4. Temperature distributions within *Polytrichum commune* canopies. Relationships are shown in still air (**a**) and under unconditioned 1.1 m/s wind (**b**) in laboratory conditions. Temperatures decline from the canopy interior to shoots that are above the mean canopy height with a more negative slope in still air (−0.76) than in wind (−0.46). Wind depressed overall temperatures and increased the variance.

function (Rice 2012) and 3D thermal imaging should allow a better understanding of this process.

In addition to thermal imaging, other imaging based physiological measurements are excellent candidates for use in 3D functional studies and include measurements of spectral reflectance to characterize indices that track water status or photosynthetic activity and 2D chlorophyll imaging systems as described in the first section of this chapter. Unfortunately, there are obstacles to developing these methods for 3D functional studies. Primarily, these approaches would be affected by changes in light intensity and/or quality within the canopy. In the case of spectral readings, reflectance from a shoot within the canopy interior is influenced by light absorption, transmission and reflection from the shoots above. Consequently, it will be difficult to determine the reflectance of the surface if the spectral quality of light falling on it is not well characterized. It might be useful to explore this problem using estimates of average spectral signatures within the canopy; however, we would have to investigate this further to determine if there is sufficient information available here to understand the physiology of the system. Similarly, measures of chlorophyll fluorescence parameters depend on saturating pulses and given the rapid attenuation of light within bryophyte canopies, the degree of saturation may affect interpretation of the measurements. In this case, trials can be run to determine whether or not saturation has been achieved (as were performed for the section described above) and measurements performed using empirically determined, saturating conditions.

IV. Light Dynamics in Virtual *Bazzania trilobata* Canopies

Physiological function within canopies also varies in the vertical dimension, which can be caused by temperature differences described above, but also by self-shading, gradients in

plant water status, and the age and acclimation of photosynthetic tissues. Although average light attenuation profiles within bryophyte canopies appear well modeled by the Lambert-Beer Law (van der Hoeven et al. 1993; Davey and Ellis-Evans 1996; Rice et al. 2011a, b), there is high variability due to the non-uniform distribution of shoot area within the canopy. For example, in wet samples of *Andreaea depressinervis*, the coefficient of variation of local light intensity was 0.66 and 0.95 at depths of 0.5 and 1.0 cm and was higher in dry shoots (Davey and Ellis-Evans 1996).

Computer simulations have been used to create virtual canopy models and explore the interaction between plant structure and light within canopies. These methods have performed well in studies of crops, landscaped systems and forest canopies (Soler et al. 2003). Due to the complexity of light in a plant canopy, which results from the interactions of reflected, scattered, and transmitted light with individual elements (i.e., leaves, branches, shoot systems), computer simulation is a useful way to explore light dynamics. Although never employed in bryophytes, this approach can enhance our understanding of the relationship between canopy structure and the absorption and extinction of light energy. This would help us improve canopy photosynthesis models that arise from an understanding of light distributions within the canopy.

Simulating the interaction of light within plant canopies involves the intersection of two different elements: plant models and light models. The approach used in this chapter draws on the L-systems language and the PlantGL package developed by Pradal et al. (2008) from the OpenAlea software suite to create the initial plant model; it models light within the plant model using the Caribu package, also from the OpenAlea software suite.

A. The Plant Model

Lindenmayer-systems, or L-systems, were initially developed as a mathematical theory to describe plant development, but they were soon extended into a flexible plant modeling tool and remain widely used (Prusinkiewicz and Lindenmayer 1990; Prusinkiewicz 2000). L-systems are a type of formal grammar similar to context free grammars, except all of the steps are performed in parallel. L-systems are well-suited for modeling plant development because they model the modularity and self similarity of plants in ways that mirror patterns of plant growth. The liverwort species *Bazzania trilobata* was chosen for canopy modeling due to its broad geographic range, ecological importance in boreal forests and relatively simple branching morphology, allowing it to be modeled more easily than other more structurally complex bryophytes.

Shoot structure and distributions were based on morphological measurements from 30 stems from two canopies collected in Rensselaer County, New York, USA, and measurements included: the height, width, depth, number of forks, angle of each fork, length to each fork, and length to shoot apices. The density of shoots on a ground area basis was also measured. Using morphological information, a structural model of *B. trilobata* shoots was constructed using L-systems and a canopy was simulated by populating a surface with shoots at a specified density. A custom set of L-system rules and an L-system generator were written for this project. The distribution of height, width, depth, and branch angle were incorporated into the individual shoot model using a normal distribution characterized by the means and standard deviations of the measured values. The plants were rendered using the PlantGL package of the OpenAlea software, a graphics package specializing in the visualization of plant structures.

The resulting individual morphology generated by the L-system is shown in Fig. 10.5. As the shoots grow, the lower parts senesce and these are not included in the model. Using multiple iterations of the model for individual *B. trilobata* shoots, a canopy was formed by generating plants and placing them at coordinates that matched the density of the naturally occurring specimens. Random variation was introduced along all three coordinate axes in order to produce a more realistic result. This canopy of plants was

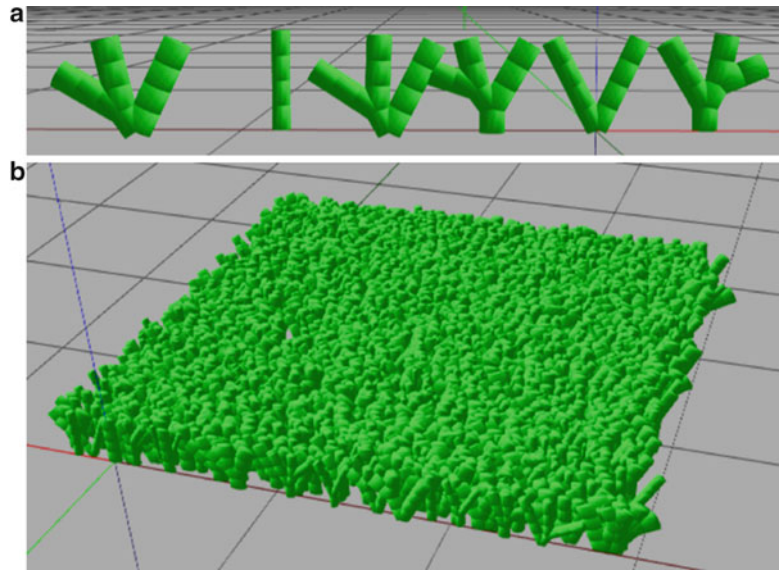


Fig. 10.5. Virtual canopy model of *Bazzania trilobata*. Some individual *B. trilobata* shoot morphologies constructed using L-systems to create individual shoots (**a**), which were used to populate a virtual *B. trilobata* canopy created by seeding individual shoots at natural densities (**b**).

then rendered using the PlantGL package. Due to the rules of the L-system used to generate the individuals, the proportion of each type of structure is the same in the computer-generated model as in the actual specimens.

B. Simulating Light Within the Canopy

Light was modeled within the canopy by the nested radiosity algorithm (Chelle and Andrieu 1998), implemented by the Caribu package of the OpenAlea software suite. The Caribu package works by modeling the light source as a diffuse mass, in this case located directly above the sample. Each individual plant that makes up the canopy is modeled as a number of discrete triangles, with potentially distinct properties of reflectiveness. The ground is also modeled and assigned its own reflectance properties. The simulation works by simulating rays of light from the source and, upon hitting each element, calculating how the light from that element affects all the other elements within a certain radius through reflectance. More sophisticated models can also incorporate transmittance, but that was not implemented in this work. For distant elements beyond that certain radius, the original element is grouped with its neighbors and their effects are averaged together.

The Caribu simulation was performed on different modeled canopies, but the results were similar and only one is shown. The visual results of the simulation are shown in Fig. 10.6. Light attenuation for the modeled canopies followed the same general pattern that is well described by the Lambert-Beer law adapted for bryophytes: $I_x = I_o \exp(-K \cdot SAI_x)$, where I_x and I_o are the light intensities at some depth in the canopy, x, and at the surface, K is the light extinction coefficient and SAI_x is the shoot area index above point x, the surface area of shoots per ground area (cm^2/cm^2). The apparent light extinction coefficient (K_{app}) was calculated following Zotz and Kahler (2007), which is the product of K and SAI_x.

The modeled results, in comparison with measurements of light attenuation within actual canopies, displayed a much more dramatic decline in light penetration. The K_{app} was calculated for five live canopies and for five modeled canopies of the same shoot density and compared using a t-test. Mean

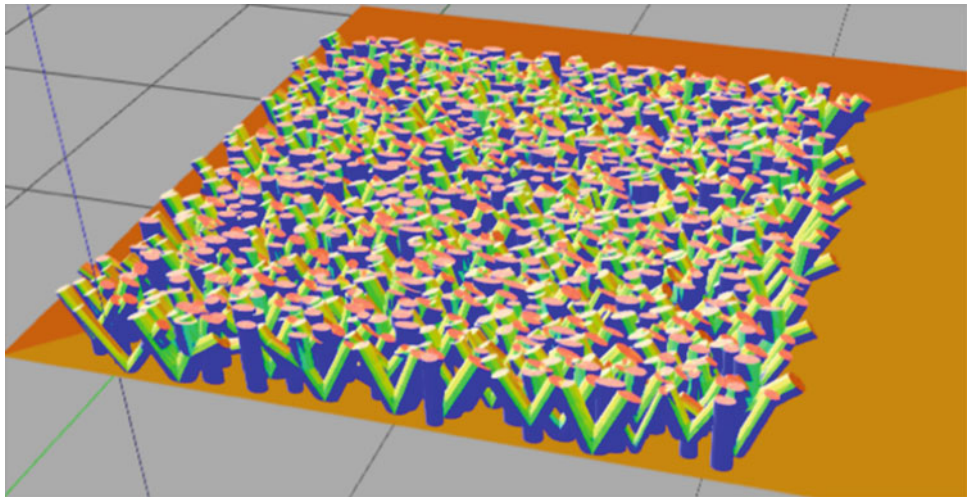


Fig. 10.6. Distribution of light within *Bazzania trilobata* canopy. A light model implementing reflected light shows the distribution of light within the canopy. Lighter colors (*red, yellow*) represent high light intensities and darker colors show lower intensity (*green, blue*).

K_{app}-value of the modeled canopies was significantly higher than the mean K_{app}-value of the actual measured canopies ($P < 0.0001$) indicating that light attenuated faster within the modeled canopies ($K_{app} = 2.7 +/- 0.1$ versus $1.3 +/- 0.2$, means with standard deviations). This is likely accounted for by two factors. First, as employed, the Caribu light model only allowed reflected, but not transmitted light within the canopy interior. *Bazzania trilobata* canopies are translucent and transmitted light might penetrate more deeply within the natural canopy. Second, the orientation of the shoots is vertical and the modeled branches that record light levels within the canopy are at acute angles to direct light from above. This contrasts with a light probe, which was oriented parallel to the ground surface. Light energy distributed on a vertical surface will be reduced in intensity by a function of the sine of the angle.

Bryophyte canopy structure emerges from the interaction of leaf, branch and stem traits and experimentally determining the functional significance of variation in these traits is often compounded by lack of suitable controls. As individual traits can be manipulated independently, virtual canopy modeling may be a useful approach to understand form-function relationships in bryophyte canopies. Further work should employ this approach to explore how variation in shoot structure affects light dynamics and incorporate the light distributions that result into a photosynthesis model to learn more about canopy-level photosynthetic processes within bryophytes.

V. Conclusions

Bryophytes present opportunities to further our understanding of canopy-level processes as whole canopies can be manipulated and evaluated by adapting techniques developed for vascular plant leaves. However, as described in this chapter, many of the traditional approaches have not allowed investigators to study functional variation in either the horizontal or vertical planes. In the simplest terms, understanding the distribution, not merely the mean values of functional traits, can help us in improving quantitative models of bryophyte canopy performance, especially when aggregation or interactions lead to non-normal distributions of functional traits. At a more complex level, these approaches will allow investigations into the physiological causes of functional variation within canopies.

For example, the capillary connection among shoots within a canopy varies among species and likely has important functional consequences. In many weft species, distal branches are often observed to desiccate first. Although this will reduce overall canopy photosynthesis, the dry shoots create additional boundary layer and prevent shoots in the interior from drying, allowing prolonged positive carbon uptake. This effect is only possible if capillary networks are not fully integrated. Species clearly differ in the degree of capillary integration among shoots, which is affected by the size, shape and spacing of leaves, by paraphyllia, and by shoot density and architecture. Just as our understanding of gas exchange in vascular plant leaves has improved by investigations of the spatial dynamics of stomatal control on leaf surfaces (Leinonen and Jones 2004; Chaerle et al. 2007; Morison and Lawson 2007; Rolfe and Scholes 2010), similar spatial dynamics are important features of bryophyte canopies in both principal planes, yet there has been little interest in exploring these processes. The novel approaches outlined in this chapter may help with such investigations.

Acknowledgements

The authors thank James Ross for conducting preliminary studies using 3D thermography, Mark Hooker, Kristina Streignitz for technical assistance. SKR thanks the Union College for sabbatical leave that facilitated writing of this chapter.

References

Abramoff MD, Magalhaes PJ, Ram SJ (2004) Image processing with image. J Biophotonics Int 11:36–42

Aldea M, Frank TD, DeLucia EH (2006) A method for quantitative analysis of spatially variable physiological processes across leaf surfaces. Photosynth Res 90:161–172

Baker NR (2008) Chlorophyll fluorescence: a probe of photosynthesis in vivo. Annu Rev Plant Biol 59:89–113

Chaerle L, Leinonen I, Jones HG, Van Der Straeten D (2007) Monitoring and screening plant populations with combined thermal and chlorophyll fluorescence imaging. J Exp Bot 58:773–784

Chelle M, Andrieu B (1998) The nested radiosity model for the distribution of light within plant canopies. Ecol Model 111:75–91

Davey MC, Ellis-Evans JC (1996) The influence of water content on the light climate within Antarctic mosses characterized using an optical microprobe. J Bryol 19:235–242

Davey MC, Rothery P (1997) Interspecific variation in respiratory and photosynthetic parameters in Antarctic bryophytes. New Phytol 137:231–240

Gamon JA, Serrano L, Surfus JS (1997) The photochemical reflectance index: an optical indicator of photosynthetic radiation use efficiency across species, functional types, and nutrient level. Oecologia 112:492–501

Gerdol R, Bonora A, Poli F (1994) The vertical pattern of pigment concentrations in chloroplasts of *Sphagnum capillifolium*. Bryologist 97:158–161

Govindjee (2004) Chlorophyll a fluorescence: a bit of basics and history. In: Papageorgiou GC, Govindjee (eds) Chlorophyll a fluorescence: a signature of photosynthesis, vol 19, Advances in photosynthesis and respiration. Springer, Dordrecht, pp 1–42

Graham EA, Hamilton MP, Mishler BD, Rundel PW, Hansen MH (2006) Use of a networked digital camera to estimate net CO_2 uptake of a desiccation-tolerant moss. Int J Plant Sci 167:751–758

Harris A (2008) Spectral reflectance and photosynthetic properties of *Sphagnum* mosses exposed to progressive drought. Ecohydrology 1:35–42

Hayward PM, Clymo RS (1982) Profiles of water content and pore size in Sphagnum and peat, and their relation to peat bog ecology. Proc Roy Soc Lond B Biol 215:299–325

Leinonen I, Jones HG (2004) Combining thermal and visible imagery for estimating canopy temperature and identifying plant stress. J Exp Bot 55:1423–1431

Lovelock CE, Robinson SA (2002) Surface reflectance properties of Antarctic moss and their relationship to plant species, pigment composition, and photosynthetic function. Plant Cell Environ 25:1239–1250

Maxwell K, Johnson GN (2000) Chlorophyll fluorescence—a practical guide. J Exp Bot 51:659–668

Morison JIL, Lawson T (2007) Does lateral gas diffusion in leaves matter? Plant Cell Environ 30:1072–1085

Ollinger SV (2011) Sources of variability in canopy reflectance and the convergent properties of plants. New Phytol 189:375–394

Oxborough K (2004) Using chlorophyll a fluorescence imaging to monitor photosynthetic performance.

In: Papageorgiou GC, Govindjee (eds) Chlorophyll a fluorescence: a signature of photosynthesis, vol 19, Advances in photosynthesis and respiration. Springer, Dordrecht, pp 409–428

Papageorgiou GC, Govindjee (eds) (2004) Chlorophyll a fluorescence: a signature of photosynthesis, vol 19, Advances in photosynthesis and respiration. Springer, Dordrecht, 818 pages

Papageorgiou GC, Govindjee (2011) Photosystem II fluorescence: slow changes – scaling from the past. J Photochem Photobiol B Biol 104:258–270

Pradal C, Dufour-Kowalski S, Boudon F, Fournier C, Godin C (2008) OpenAlea: a visual programming and component-based software platform for plant modeling. Funct Plant Biol 25:751–760

Prusinkiewicz P (2000) Simulation modeling of plants and plant ecosystems. Commun ACM 43:84–93

Prusinkiewicz P, Lindenmayer A (1990) The algorithmic beauty of plants. Springer, New York

Rice SK (2012) The cost of capillary integration for bryophyte canopy water and carbon dynamics. Lindbergia 35:53–62

Rice SK, Gutman C, Krouglicof N (2005) Laser scanning reveals bryophyte canopy structure. New Phytol 166:695–704

Rice SK, Aclander L, Hanson DT (2008) Do bryophyte shoot systems function like vascular plant leaves or canopies? Functional trait relationships in *Sphagnum* mosses (Sphagnaceae). Am J Bot 95:1366–1374

Rice SK, Neal N, Mango J, Black K (2011a) Modeling bryophyte productivity across gradients of water availability using canopy form-function relationships. In: Tuba Z, Slack NG, Stark LR (eds) Bryophyte ecology and global change. Cambridge University Press, Cambridge, pp 441–457

Rice SK, Neal N, Mango J, Black K (2011b) Relationships among shoot tissue, canopy and photosynthetic characteristics in the feathermoss *Pleurozium schreberi*. Bryologist 114:367–378

Rolfe SA, Scholes JD (2010) Chlorophyll fluorescence imaging of plant-pathogen interactions. Protoplasma 247:163–175

Soler C, Sillion F, Blaise F, Dereffye P (2003) An efficient instantiation algorithm for simulating radiant energy transfer in plant models. ACM T Graphic 22:204–233

Thévenaz P, Ruttimann UE, Unser M (1998) A pyramid approach to subpixel registration based on intensity. IEEE T Image Process 7:27–41

Tobias M, Niinemets Ü (2010) Acclimation of photosynthetic characteristics of the moss *Pleurozium schreberi* to among-habitat and within-canopy light gradients. Plant Biol 12:743–754

Uchida M, Muraoka H, Nakatsubo T, Bekku Y, Ueno T, Kanda H, Koizumi H (2002) Net photosynthesis, respiration, and production of the moss Sanionia uncinata on a glacier foreland in the high Arctic, Ny-Ålesund, Svalbard. Arct Antarct Alp Res 34: 287–292

Van der Hoeven EC, Huynen CIJ, During HJ (1993) Vertical profiles of biomass, light intercepting area and light intensity in chalk grassland mosses. J Hattori Bot Lab 74:261–270

Zona D, Oechel WC, Richards JH, Hastings S, Kopetz I, Ikawa H, Oberbauer S (2011) Light-stress avoidance mechanisms in a *Sphagnum*-dominated wet coastal Arctic tundra ecosystem in Alaska. Ecology 92:633–644

Zotz G, Kahler H (2007) A moss "canopy" – Small-scale differences in microclimate and physiological traits in *Tortula ruralis*. Flora 202:661–666

Chapter 11

Genetics and Genomics of Moss Models: Physiology Enters the Twenty-first Century

David J. Cove*
Centre for Plant Sciences, University of Leeds, Leeds, UK
Department of Biology, Washington University in St. Louis, St. Louis, MO, USA

Andrew C. Cuming
Centre for Plant Sciences, University of Leeds, Leeds, UK

Summary

Bryophytes, and in particular the mosses, *Physcomitrella patens* and *Ceratodon purpureus* have been developed for the genetic study of development and metabolism. Their ease of culture and the haploidy of their gametophyte stage, allow essentially microbiological techniques to be used. The discovery that gene targeting occurs at high frequency in both species, allows genes to be inactivated or modified, providing a very powerful tool for the analysis of gene function. The sequence of the *P. patens* genome is available and sequencing of the *C. purpureus* genome is in hand. The full potential of these experimental systems is now being realized for the study of photosynthesis.

*Author for correspondence, e-mail: d.j.cove@leeds.ac.uk

D.T. Hanson and S.K. Rice (eds.), Photosynthesis in Bryophytes and Early Land Plants, Advances in Photosynthesis and Respiration 37, pp. 187–199, DOI 10.1007/978-94-007-6988-5_11,

I. Introduction

Bryophytes were some of the earliest species to be used for genetic analysis in the post-Mendel era. Using natural variants and inter-specific crosses, von Wettstein analysed crosses and carried out some of the pioneering analyses to establish the basis of Mendelian genetics (reviewed in von Wettstein 1932). Bryophytes, both mosses and liverworts, were among the first species in which the utility of mutagenic treatments, both irradiation and chemicals, was established (Knapp 1935; Barthelmess 1953). Their utility for the genetic analysis of metabolism and development was also recognized early (Barthelmess 1941a). These studies had used a number of species, with *Marchantia polymorpha* and *Physcomitrium pyriforme* becoming preferred species. However, as a result of encouragement by Harold Whitehouse, an outstanding geneticist and enthusiastic "amateur" bryologist, *Physcomitrella patens* was used for mutant isolation and genetic analysis by Paulinus Engel (1968). Subsequently genetic methodology was extended and used for studying auxin and cytokinin action using chemically-induced mutants of *P. patens* (Ashton et al. 1979). *Funaria hygrometrica* continued to be used widely for physiological investigations, but the development of genetic technology for *P. patens,* including transformation, led to a steady expansion in the *P. patens* community. The discovery that in *P. patens,* transforming DNA containing genomic sequence was targeted to the corresponding genomic sequence at high frequencies, comparable to that in *Saccharomyces cerevisiae,* led to it becoming the preferred species for genetic studies and its choice as the first non-seed land plant to have its genome sequenced. Gene targeting has also been shown to occur in *Ceratodon purpureus* and genome sequencing of this species is now in progress. An application to sequence a *Sphagnum* genome is also in hand.

II. Propagation

A. Life Cycle

In common with all bryophytes, the dominant stage of the life cycle of *P. patens* is the haploid gametophyte. Haploid spores germinate to produce protonemata, cell filaments growing by the serial division of the apical cell. Protonemal sub-apical cells can also divide to allow branching of the filament, or to give rise to gametophores, the leafy shoot that produce gametes by mitosis. *P. patens* is monoicous, producing both sperm and eggs on the same gametophore. Self fertilisation is common. The sporophytes produced are therefore homozygous, allowing the production of large numbers of genetically-identical haploid spores (following meiosis). Cross fertilization does occur, and recently a technique has been devised that identifies hybrid sporophytes, using fluorescent markers (Perroud et al. 2011). The haploidy of the gametophyte has considerable technical advantages; for example, much research has been carried out on a single genotype, Gransden, the strain, originally isolated by Harold Whitehouse from a single spore, and it was this strain that was chosen for genome sequencing. Haploidy removes the need for inbred lines and allows the identification of mutant phenotypes directly, following mutagenesis of either spore or protonemally-derived protoplasts. *C. purpureus*, in contrast, has separate male and female gametophytes (*i.e.,* is dioicous), and currently a male and a female genome is being sequenced. Although, in this species, spores do not represent a uniform population of identical cells for mutagenesis, this is readily obtainable by way of protoplasting protonemal tissue.

B. Culture

P. patens, and many other moss species, are easily cultured on a simple medium containing only inorganic salts. Protonemal growth occurs on the surface of media solidified

with agar, but culture in liquid media is also possible. As culture employs essentially microbiological techniques, it is not surprising that vitamin auxotrophs were among the first artificially-induced mutants to be isolated (Engel 1968; Ashton and Cove 1977). By overlaying solid medium with sterile cellophane, and inoculating with blended protonemal tissue, it is possible to obtain within 7 days from 90 mm Petri dish, a uniform mat of protonemal tissue weighing about 30 mg dry weight (200 mg fresh weight). This tissue may be used for the extraction of DNA, RNA or protein, and for the enzymatic isolation of protoplasts for mutagenesis or transformation. Such protonemal mats may also be used for biolistic transformation.

If greater amounts of protonemal tissue are required, growth in liquid media may be employed. Batch culture was used originally (Wang et al. 1980), but stirred or airlift bioreactors have since been developed (Boyd et al. 1988a, b; Hohe et al. 2002; Decker and Reski 2007). Growth rates are enhanced by passing an air/CO_2 mix through the medium. In batch culture, development proceeds in a similar manner to growth on solid medium, but if the culture is diluted so that the tissue density remains more or less constant, development does not proceed beyond the protonemata. In the air lift bioreactor, the tissue is passed through a tissue homogeniser, restricting the size of tissue fragments to a maximum diameter of about 100 µm. Because of the strong regenerative capacity of protonemal tissue, growth is continuous and about 4 g dry weight (about 30 g fresh weight) can be harvested daily from a 6 l bioreactor (DJ Cove, unpublished data).

Protocols for most of the experimental manipulations that may be carried out with *P. patens* can be found in Cove et al. (2009).

C. Strain Storage

The storage of *P. patens* strains can be carried out in a number of ways. Where spores can be obtained, these can be kept for many years, but vegetative tissue can also be stored. Cryopreservation of protonemal tissue (Grimsley and Withers 1983) allows long term (at least 10 years) of some strains, but recovery from freezing is often unsuccessful. This leads to a need to freeze multiple samples of each strain. Alternatively, tissue can be maintained by storage at 10°–15° in a cycle of 1–2 h bright light per 24 h day. Either agar-grown cultures or harvested protonemal tissue, suspended in sterile distilled water, may be stored. Most strains survive well for at least 2 years when stored in this way *C. purpureus* can be stored by the same techniques, and is generally more robust than *P. patens*. We have successfully maintained fresh cultures of *C. purpureus* at low temperatures for 10 years.

III. Genetic Manipulation

A. Mutagenesis

The haploidy of bryophytes is a considerable advantage for the isolation of mutant strains. Providing the mutant phenotype is not lethal, mutant strains may be identified directly following mutagenesis. Although not yet employed extensively, the mutagenesis of diploid strains should allow the isolation of recessive-lethal mutants.

Successful mutagenesis of *P. patens* has been carried out using X-ray or UV-irradiation or chemicals to treat spores or protonemal tissue (Engel 1968; Ashton and Cove 1977; Boyd et al. 1988a, b). When protonemal tissue is treated, single-celled propagules can be obtained either by treating isolated protoplast directly or by protoplasting protonemal tissue after mutagenesis. Since *C. purpureus*, having separate sexes, is an obligate out-crosser, uniform samples of spores are not available, however somatic mutagenesis can readily be employed (Cove and Quatrano 2006). X-rays have been used to induce mutation in a number of other bryophytes including *Brachythecium rutabulum* (Moutschen 1954), *Marchantia*

polymorpha (Miller et al. 1962), *Physcomitrium pyriforme* (Barthelmess 1941b) and *Sphaerocarpus donnellii* (Knapp 1935; Schieder 1973).

B. Sexual Crossing

P. patens produces male and female gametangia on the same gametophore and so self-fertilization is possible and usual. Co-inoculation of two strains to be crossed, allows hybrid sporophytes to be produced, but these may be rare. However, early studies established that pure cultures of vitamin-requiring strains were unable to produce sporophytes unless vitamin supplementation was greatly increased. However, when two different and complementary vitamin-requiring mutants were co-inoculated and grown with the standard (low) level of vitamin supplementation, sporophytes were produced, all of which were hybrid (Courtice et al. 1978). The use of strains having vitamin auxotrophies is not always convenient, so recently an alternative method of identifying hybrid sporophytes has been devised. This uses transgenic strains expressing a fluorescent protein. Since the fluorescent phenotype is dominant, fluorescing sporophytes borne by a non fluorescently-tagged strain, co-inoculated with a tagged strain, must have been produced following fertilization by the tagged strain. Although hybrid sporophytes may be uncommon, they are nevertheless very easily identified (Perroud et al. 2011). *C. purpureus*, having separate sexes, needs no such technology, all sporophytes being hybrid.

The haploidy of the gametophyte, makes genetic analysis straightforward. "Gametophytic" segregation ratios are observed, i.e., 1:1 segregation for single gene differences (Engel 1968; Ashton and Cove 1977; Courtice et al. 1978), and 1:1:1:1 segregations are observed for two different unlinked genes (Ashton and Cove 1977). Early *P. patens* crosses detected no linkage but with the limited number of marked genes available and the high chromosome number (27) this was not surprising. With the availability of molecular markers, a detailed linkage map, anchored against the genome sequence, is now available (Kamisugi et al. 2008). A similar map of molecular markers is also available for *C. purpureus* (McDaniel et al. 2007) and it should be relatable to the genome sequence, once this is available. This study involved crosses between geographically widely separated strains and marked deviations from expected 1:1 segregation ratios were detected. Some formal genetic analysis has also been carried out in *Spaerocarpus donnellii* (Schieder 1973).

C. Somatic Hybridisation

Although the haploidy of the gametophyte is usually seen as an advantage for genetic studies, it does not allow allelic dominance to be studied directly. The sexual sterility of some developmental mutants is also a bar to their conventional genetic analysis. Both these problems can be overcome by the use of somatic hybridisation. Early studies obtained hybrid diploid gametophytes by aposporous regeneration of sporophyte tissue (von Wettstein 1932), but experimental selection of hybrid gametophytes is possible by the use of protoplast fusion. This was first demonstrated for *Sphaerocarpus donnellii* (Schieder 1974) and *P. patens* (Grimsley et al. 1977a). Somatic hybrids allow not only dominance relationships to be established but also allow complementation to be analysed (Grimsley et al. 1977b). Complementation analysis by way of somatic hybrid production is especially valuable in *C. purpureus*, where hybrids can be made between two mutant strains of the same sex (Cove and Quatrano 2006).

D. Transformation

Genetic transformation of *P. patens*, was first achieved by the treatment of isolated protoplasts with naked DNA in the presence of polyethylene-glycol (Schaefer et al. 1991), using a technique based on the transformation of angiosperm mesophyll protoplasts. This technique has remained essentially unchanged and is also effective for the transformation of *C. purpureus*. A number of

different selection cassettes are available, but geneticin- (G418) and hygromycin-resistance genes driven by a 35S promoter are most commonly used. Two classes of transformants are obtained. The most frequent only retains the transforming DNA sequence and the transformed phenotype, provided selection is maintained. Subculture onto, for example antibiotic-free medium, leads to loss of the transforming DNA. However, providing selection is maintained, the transforming DNA is replicated. The second class of transformant is stable and retains the transformed phenotype in the absence of selection. In this class, the transformed DNA is integrated into the genome and is inherited in a Mendelian manner. DNA containing no sequence homologous to genomic sequence is integrated randomly, but where the transforming DNA contains genomic sequence, integration is targeted to the corresponding genomic sequence (Schaefer and Zryd 1997).

E. Gene Targeting

The ability to deliver transforming DNA to a predetermined genetic locus provides a powerful tool for the analysis of gene function. Furthermore, this property is rare in eukaryotes, being straightforward only in the yeast, *Saccharomyces cerevisiae*, in which relatively short oligonucleotides can be delivered to cells and subsequently integrated at target loci by homologous recombination. Other eukaryotic systems in which gene targeting is routinely used include the chicken DT40 cell line (a terminally differentiated and cancerous B-cell-derived line in which the pathway for generation of antibody diversity by gene conversion is permanently switched on), and mouse embryonic stem cells, which can be transformed and regenerated, ultimately to generate "knockout mice" that can be used as models for the understanding of human inherited disorders. However, the distinguishing feature of targeted transgene integration in *P. patens* is its efficiency, which is several orders of magnitude greater than that in mouse ES cells: in some experiments, 100 % of transgenic moss plants have been found to have integrated the transgene at the designated locus (Kamisugi et al. 2005).

The parameters for efficient gene targeting in *P. patens* have been determined, and the principle is indicated in Fig. 11.1. Briefly, two targeting sequences, corresponding to the 5′- and 3′-ends of the gene to be targeted, are cloned on either side of a selectable marker cassette. The length of the targeting sequences (*i.e.* the extent of homology between the transforming DNA and the target gene) is the most crucial factor, with as little as 500 bp on either side of the selection cassette being sufficient to achieve a targeting frequency of 50 % (Kamisugi et al. 2005). For maximum efficiency, the construct is delivered as a linear fragment, either by recovery from the original cloning vector by restriction enzyme digestion, or, more conveniently, by PCR amplification of the targeting sequence. In a successful targeting experiment, the central sequence in the targeted gene is replaced by the selection cassette. The creation of multiply mutated strains by transformation with additional targeting cassettes can be achieved if the selection marker can be removed from the moss genome following targeted gene replacement. This is possible using an initial targeting vector in which the selection cassette is flanked by the bacteriophage lambda *loxP* sites. In this case, the selection cassette can be removed from the targeted locus by transient expression of the Cre recombinase gene, thereby allowing a further round of transformation and selection (Trouiller et al. 2006).

It is probable that other moss species share the ability to be mutagenised by gene targeting. Certainly, this has been achieved in *Ceratodon purpureus*: Brucker et al. (2005) generated specific point mutations in the *C. purpureus* heme oxygenase gene by delivery of linear fragments that had been specifically altered in a single base by site-directed mutagenesis. The targeted transformants were identified and selected by virtue of their acquisition of a mutant phenotype: the inability to respond phototropically to unilateral red light. Trouiller et al. (2007) compared targeting efficiencies in *P. patens*

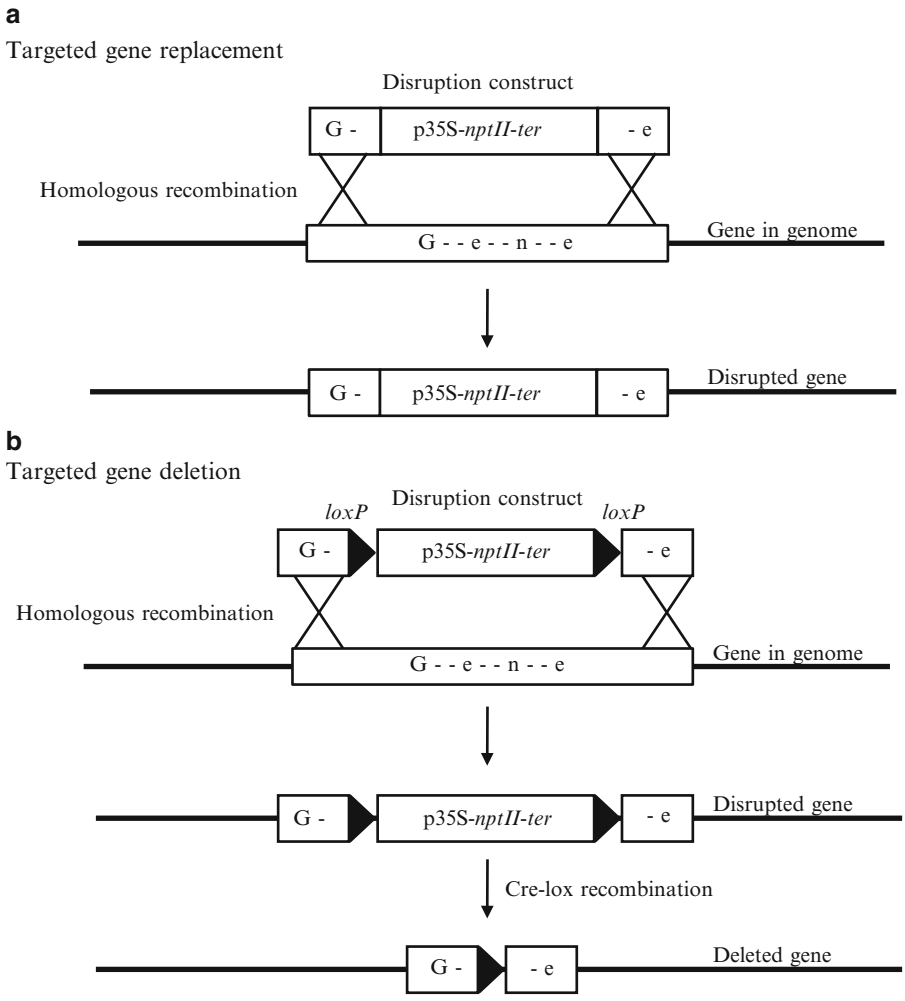


Fig. 11.1. Targeted transformation in *Physcomitrella* (**a**): Targeted gene replacement. A fragment of transforming DNA comprising a selection cassette flanked by *ca.*500–800 bp of sequence derived from the 5′- and 3′-ends of the gene sequence to be replaced is delivered to *Physcomitrella* protoplasts by polyethylene glycol-mediated transfection. Homologous recombination between each end of the construct and the corresponding sequence of the target gene results in replacement of the endogenous sequence. (**b**): Targeted gene deletion. In this instance, the transforming DNA contains two direct repeats of the *LoxP* recombination sequence from bacteriophage lambda on either side of the selection cassette. Following the selection and regeneration of transformants in which targeted gene replacement has occurred, delivery of an expression construct to protoplasts with subsequent transient expression of the Cre recombinase results in removal of the selection cassette, by recombination between the two *LoxP* sites. The mutant strain can now be re-transformed with another targeting cassette containing the original selection marker, to create multiply targeted strains.

and *C. purpureus* and found the targeting frequency in the latter to be *ca.* 10-fold less efficient than in *P. patens*, but still two orders greater than that achievable in flowering plants.

The realisation that efficient gene targeting was possible in *P. patens* led to a significant burgeoning of interest in the use of this organism as a vehicle for gene function analysis. In particular, the evolutionary distance between the mosses and flowering plants (*ca.* 200 million years between the respective divergences of the bryophyte and angiosperm lineages) suggested that

Physcomitrella might be most valuable as a model in which to study the evolution of gene function within the plant kingdom. However, in order to realize its potential, it was clearly important to develop resources that would enable any *Physcomitrella* gene to be cloned and manipulated without delay. The initiation of the *Physcomitrella* Genome Project met this requirement.

IV. Genomic Data and Applications

A. Genome Sequence

The first development of *Physcomitrella* genomic resources took the form of EST-based gene discovery projects in Freiburg, supported by substantial industrial funding from BASF, in Okazaki and in Leeds and St. Louis. Subsequently, at the annual moss conference, "Moss 2004" in Freiburg, a consortium was assembled to undertake the sequencing of the *Physcomitrella* genome, through the auspices of the Community Sequencing Program of the United States Department of the Energy's Joint Genome Institute. Co-ordinated by Brent Mishler and Ralph Quatrano, the proposal was successful, and the sequencing of the genome commenced in 2005.

Using a whole-genome shotgun sequencing approach, the first genome assembly was completed in 2006 and made available through the Joint Genome Institute website (http://genome.jgi-psf.org/Phypa1_1/Phypa1_1.home.html). The salient features of the genome were published early in 2008, following a community-wide gene curation and annotation effort (Rensing et al. 2008).

The "Version 1" genome assembly comprised nearly 2000 sequence scaffolds, corresponding to a sequence length of 480 Mbp. This was in good accord with previous genome size estimates based on flow cytometry of 511 Mbp (Schween et al. 2003), and is about 3× larger than the *Arabidopsis*. As is the case in most multicellular eukaryotes, the genome size is correlated with the proportion of repetitive DNA, rather than the number of genes, and although the initial annotation indicated a gene content of nearly 40,000 genes, subsequent annotations reported in the "Cosmoss" database (http://www.cosmoss.org) and replicated through the "Phytozome" resource (http://www.phytozome.net/physcomitrella) have reduced this number to *ca.* 32,000 protein coding loci.

Improvements to the genome assembly are continuing as additional genomic resources are developed. Importantly, the US Department of Energy has recognized the importance of the *Physcomitrella* genome in a commitment by the JGI to develop the genomic resources as one of its "flagship" plant genomes. Further enhancements to the genome assembly are expected to be released within the calendar year 2013, as a result of the integration of genetic linkage data in to the scaffold ordering process. Until recently, no genetic linkage map was available for *P. patens*, rendering "forward genetic" identification of the sequences underpinning mutant phenotypes problematic. However, a sequence-anchored linkage map based on molecular markers (Amplified Fragment Length Polymorphisms and Simple Sequence Repeats) was developed in 2008 (Kamisugi et al. 2008) and the development of a chromosome-scale genome assembly is being made possible through the high-density genotyping of SNP markers. These rapid improvements are being underpinned by next-generation sequencing technologies: data reported at the "Moss 2011" conference described the identification of nearly 600,000 SNPs between the standard "Gransden" laboratory strain and the genetically divergent "Villersexel" strain, collected by Michael Lüth, from the Haute Saône region of South-East France. This represents a SNP density of approximately 1/800 bp: a rich resource for linkage mapping (T Nishiyama, personal communication).

In addition to the nuclear genome, both organellar genomes have also been sequenced (Sugiura et al. 2003; Terasawa et al. 2007), as have those of a number of basal land plants, including the liverwort, *Marchantia polymorpha* (Ohyama et al. 1986; Oda et al.

1992) – the first plant chloroplast genome to be completely sequenced – and the hornwort, *Anthoceros formosa* (Kugita et al. 2003), together with organelle genomes from the charophyte algae (Turmel et al. 2002, 2006): a group believed to represent the immediate aquatic ancestors of the land plants.

With the rapid advance in genome sequencing technologies, *Physcomitrella* is no longer the only bryophyte with a genome sequencing programme. The genome sequence of the liverwort, *Marchantia polymorpha*, is currently being annotated prior to release by a JGI consortium led by John Bowman (Monash University). This will substantially enhance the potential for investigating the roots of the land plant lineage, since *M. polymorpha* resources include an efficient Agrobacterium-mediated transformation system (although without the ability to undertake gene targeting, as in mosses) and the potential to identify mutants through genetic map-based cloning methodologies. Additionally programmes to sequence the genomes of *Ceratodon purpureus* and *Sphagnum* are in progress, whilst EST collections are available for a number of aquatic algae (including *Chara, Coleochaete* and *Spirogyra* – Timme and Delwiche 2010).

B. Genomics

What questions have the availability of a moss genome sequence allowed us to address? Principally, the interesting questions are evolutionary. How has the form and function of land plants evolved, and what processes led to the domination of terrestrial ecosystems by green plants? – an event of fundamental importance to the natural history of our planet.

One of the defining features of plant evolution, in general, is the propensity for polyploidization to act as an engine for evolutionary change, by enabling immediate reproductive isolation. Evidence of polyploidy is widespread throughout the Plant Kingdom, and *Physcomitrella* is not excepted from this. Analysis of the genome sequence enabled the identification of a large-scale (probably whole-genome) duplication having occurred relatively recently in its evolutionary history (between 30 and 60 MYA). Originally predicted from the similarity of paralagous gene pairs identified in the EST collection (Rensing et al. 2007), the remnants of this event can be found in the genome assembly where syntenic regions of conserved genes are found interspersed with additions or deletions (Fig. 11.2).

Since modern bryophytes are the direct descendants of the first plants to colonize terrestrial habitats, it is likely that a number of *Physcomitrella* genes encode "primitive" traits that were advantageous for this step-change in plant evolution. Comparative functional analysis of genes between *Physcomitrella* and angiosperms can highlight some of these traits.

A particularly elegant example of this derives from the identification of two *Physcomitrella* orthologs of the *Arabidopsis* gene, *RDH6*, encoding a transcription factor regulating root hair development. Targeted knockout of the *Physcomitrella* genes demonstrated that their function was to regulate rhizoid formation, and the conservation of a biological function necessary for interaction with the substratum was further demonstrated by the ability of one of the moss genes to cross-complement a mutant *rdh6* allele and restore root hair development in *A. thaliana* (Menand et al. 2007).

The first plants to colonise the land must necessarily have developed adaptations to resist a wide range of abiotic stresses associated with the terrestrial environment. Chief among these are resistance to drought and enhanced radiation, and genes known to be required for resistance to both stresses in flowering plants play similar functions in *Physcomitrella*. Thus, the genes encoding the ABA-mediated drought stress response are present in the moss genome, and regulate a similar suite of responses as are seen in angiosperms. This includes the ABA-activated closure of stomata (present on the moss sporophyte) (Chater et al. 2011), ABA signalling using the ABI1-like protein phosphatases (Komatsu et al. 2009) and the transcriptional

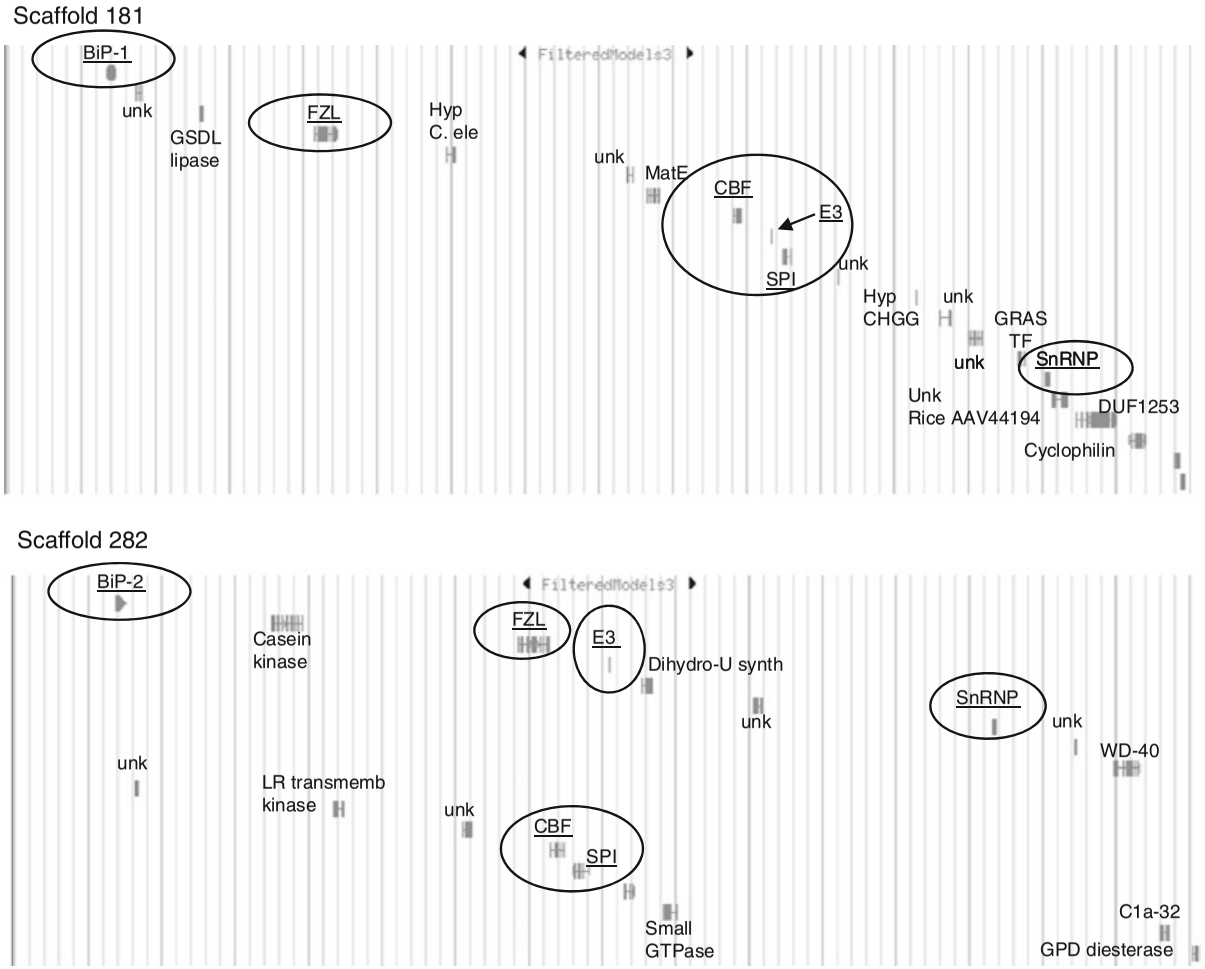


Fig. 11.2. Example of internal synteny within the *Physcomitrella* genome. This shows two screenshots from the JGI *Physcomitrella* genome browser, corresponding to *ca.* 300-kilobase regions in sequence scaffolds 181 *(top)* and 282 *(bottom),* respectively. Genes duplicated and in a syntenic relationship to one another are circled and identified by underlined annotations: "BiP" (ER-chaperone) "FZL" (Mitochondrial GTPase) CBF (Histone fold transcription factor CBF), "SPI" (serine proteinase inhibitor), "E3" (E3 ubiquitin ligase), "SnRNP" (Splicing factor). The intervening genes differ between the two scaffolds.

activation of *LEA* gene expression by ABA (Cuming et al. 2007) through the activity of ABI3 transcription factors (Khandelwal et al. 2010) – a function restricted to seed development in angiosperms. Vascular plants possess only a single *ABI3* gene, whereas in *Physcomitrella* there are three *ABI3* orthologs with redundant function in the initiation of the drought response. It is suggested that evolutionary loss of the additional copies in the vascular plants led to the sequestration of ABA-mediated desiccation tolerance in the seed development pathway (Rensing et al. 2008).

By contrast with the conservation of the ABA signalling pathway, the status of the gibberellin signalling pathway is less clearly defined. The *Physcomitrella* genome contains genes encoding enzymes of the gibberellin biosynthetic pathway (*ent*-kaurene synthetase, and GA20- and GA3-oxidases). It also contains genes encoding the vascular plant GA receptor (GID) protein and its interacting DELLA growth-repressive factor, but whilst knockout mutants of the *ent-kaurene* synthetase gene produce an abnormal protonemal phenotype (Hayashi et al. 2010), it is unclear whether this can be ascribed to a gibberellin-like activity, at least so far as this is understood in vascular plants. Analyses of the *Physcomitrella* GID-DELLA interaction and its participation in GA signalling indicate that the PpGID protein does not bind gibberellins, and that while the GID-DELLA interaction is fully functional in the lycophyte *Selaginella*, this is not the case in *Physcomitrella*, indicating that this hormone signalling pathway evolved subsequent to the divergence of the bryophytes, and is characteristic of the vascular plant lineages (Hirano et al. 2007; Yasumura et al. 2007).

V. Potential for Photosynthetic Studies

The availability of genomic tools and resources for mosses, together with the ease with which the model species can be grown and manipulated in culture provides new opportunities for photosynthetic research. Thus, whilst the parameters of photosynthesis established in both *Physcomitrella* and *Ceratodon* appear essentially similar to those in vascular plants, the ability to undertake a genetic analysis of these processes – either through "forward genetic" mutagenesis or "reverse genetic" approaches – offers considerable promise. Because *Ceratodon* is more amenable to heterotrophic growth than *P. patens* , this species has been used to generate a large number of mutants (via UV-irradiation) that are deficient in photosynthetic processes, exhibiting characteristic alterations in chlorophyll fluorescence, and in chlorophyll synthesis and accumulation (Thornton et al. 2005). The development of genomic resources for this species over the next 2 years should enable a map-based cloning pipeline to be established by which such mutants can be characterised.

The availability of genomic resources in *Physcomitrella* is already being exploited for photosynthetic studies. A bioinformatic interrogation of the photosynthetic gene set has been used to to determine the evolution of the photosynthetic process during the colonisation of land by green plants (Alboresi et al. 2008), and the capacity for gene targeting has enabled the interrogation of components of the photosynthetic light harvesting and electron transport chain under varying environmental conditions (Alboresi et al. 2010, 2011; Gerotto et al. 2011).

Other aspects of chloroplast function addressed in *Physcomitrella patens* include the mechanisms of chloroplast division and of protein import into plastids. Indeed, the very first mutant phenotype to be obtained by gene targeting in *P.* patens was the formation of giant chloroplasts, that resulted from the disruption of the *FtsZ* gene (Strepp et al. 1998) – an ancient member of the tubulin family, subsequently shown to form cytoskeletal networks in chloroplasts (KIessling et al. 2000). Both plastid and cytosolic isoforms of FtsZ exist, and both isoforms are required for the correct specification of plastid division (Gremillon et al. 2007).

Import of proteins by plastids – an essential process in the establishment of the original

endosymbiotic event that gave rise to the green plants – is (unsurprisingly) conserved between moss and angiosperms (Hoffman and Theg 2003), suggesting that the targeted mutagenesis of moss import proteins could provide a powerful means of identifying key residues in the protein uptake polypeptides.

In summary, the existing and future genomic resources available for the molecular analysis of life processes in bryophytes will aid us in the unravelling of the complexity of the evolution of gene function throughout the Plant Kingdom.

References

Albanese A, Caffarri S, Nogué F, Bassi R, Morosinotto T (2008) In silico and biochemical analysis of *Physcomitrella patens* photosynthetic antenna: identification of subunits which evolved upon land adaptation. PLoS ONE 3:e2033

Alboresi A, Gerotto C, Giacometti GM, Bassi R, Morosinotto T (2010) *Physcomitrella patens* mutants affected on heat dissipation clarify the evolution of photoprotection mechanisms upon land colonization. Proc Natl Acad Sci U S A 107:11128–11133

Alboresi A, Gerotto C, Cazzaniga S, Bassi R, Morosinotto T (2011) A red shifted antenna protein associated to photosystem II in *Physcomitrella patens*. J Biol Chem 286:28978–28987

Ashton NW, Cove DJ (1977) The isolation and preliminary characterisation of auxotrophic mutants of the moss, *Physcomitrella patens*. Mol Gen Genet 154:87–95

Ashton NW, Grimsley NH, Cove DJ (1979) Analysis of gametophytic development in the moss, *Physcomitrella patens*, using auxin and cytokinin resistant mutants. Planta 144:427–435

Barthelmess A (1941a) Über Beziehungen zwischen Genetik, Systematik, Morphologie und Entwicklungsphysiologie und die Möglichkeit einer Zusammenarbeit. Zeitschrift für die gesamte Naturwissenschaften H3(4):84–92

Barthelmess A (1941b) Mutationsversuche mit einem Laubmoos *Physcomitrium piriforme* II. Morphologische und physiologische analyse der univalenten und bivalenten protonemen einiger mutanten. Zeitschrift für induktive Abstammungs- und Vererbunglehre 79:153–170

Barthelmess A (1953) Mutationsauslösung mit chemikalien. Naturwissenschaften 22:583–584

Boyd PJ, Grimsley NH, Cove DJ (1988a) Somatic mutagenesis of the moss, *Physcomitrella patens*. Mol Gen Genet 211:545–546

Boyd PJ, Hall J, Cove DJ (1988b) An airlift fermenter for the culture of the moss *Physcomitrella patens*. In: Glime JM (ed) Methods in bryology. Proceedings of Bryological Methods Workshop, Mainz. Hattori Botanical Laboratory, Nichinan, pp 41–45

Brucker G, Mittmann F, Hartmann E, Lamparter T (2005) Targeted site-directed mutagenesis of a heme oxygenase locus by gene replacement in the moss *Ceratodon purpureus*. Planta 220:864–874

Chater C, Kamisugi Y, Movahedi M, Fleming A, Cuming AC, Gray JE, Beerling JD (2011) Regulatory mechanism controlling stomatal behavior conserved across 400 million years of land plant evolution. Curr Biol 21:1025–1029

Courtice GRM, Ashton NW, Cove DJ (1978) Evidence for the restricted passage of metabolites into the sporophyte of the moss, *Physcomitrella patens*. J Bryol 10:191–198

Cove DJ, Quatrano RS (2006) Agravitropic mutants of the moss, *Ceratodon purpureus*, do not complement mutants having a reversed gravitropic response. Plant Cell Environ 29:1379–1387

Cove DJ, Perroud P-F, Charron J, McDaniel SF, Khandelwal A, Quatrano RS (2009) The Moss *Physcomitrella patens*: a novel model system for plant development and genomic studies. In: Behringer RR, Johnson AD, Krumlauf RE (eds) Emerging model organisms: a laboratory manual, vol 1. Cold Spring Harbor Laboratory Press, New York, pp 69–104

Cuming AC, Cho SH, Kamisugi Y, Graham H, Quatrano RS (2007) Microarray analysis of transcriptional responses to abscisic acid and osmotic, salt and drought stress in the moss, *Physcomitrella patens*. New Phytol 176:275–287

Decker EL, Reski R (2007) Moss bioreactors producing improved biopharmaceuticals. Curr Opin Biotechnol 18:393–398

Engel PP (1968) The induction of biochemical and morphological mutants in the moss *Physcomitrella patens*. Am J Bot 55:438–446

Gerotto C, Alborese A, Giacometti GM, Bassi R, Morosinotto T (2011) Role of PSBS and LHCSR in *Physcomitrella patens* acclimation to high light and low temperature. Plant Cell Environ 34:922–932

Gremillon L, Kiessling J, Hause B, Decker EL, Reski R, Sarnighausen E (2007) FtsZ isoforms specifically interact in the chloroplasts and in the cytosol of *Physcomitrella patens*. New Phytol 176:299–310

Grimsley NH, Withers LA (1983) Cryopreservation in the moss, *Physcomitrella patens*. Cryo-Lett 4:251–258

Grimsley NH, Ashton NW, Cove DJ (1977a) The production of somatic hybrids by protoplast fusion in the moss, *Physcomitrella patens*. Mol Gen Genet 154:97–100

Grimsley NH, Ashton NW, Cove DJ (1977b) Complementation analysis of auxotrophic mutants of the moss, *Physcomitrella patens*, using protoplast fusion. Mol Gen Genet 155:103–107

Hayashi K, Horie K, Hiwatashi Y, Kawaide H, Yamaguchi S, Hanada A, Nakashima T, Nakajima M, Mander LM, Yamane H, Hasebe M, Nozaki H (2010) Endogenous diterpenes derived from ent–kaurene, a common gibberellin precursor, regulate protonemal differentiation of the moss *Physcomitrella patens*. Plant Physiol 153:1085–1097

Hirano K, Nakajima M, Asano K, Nishiyama T, Sakakibara H, Kojima M, Katoh E, Xiang H, Tanahashi T, Hasebe M, Banks JA, Ashikari M, Kitano H, Ueguchi-Tanaka M, Matusoka M (2007) The GID1-mediated gibberellin perception mechanism is conserved in the lycophyte *Selaginella moellendorffii* but not in the bryophyte *Physcomitrella patens*. Plant Cell 19:3058–3079

Hoffman NR, Theg SM (2003) Chloroplast protein transport in the moss, *Physcomitrella patens*: conserved machineries between vascular and non-vascular plants. Plant Mol Biol 53:643–654

Hohe A, Decker EL, Gorr G, Schween G, Reski R (2002) Tight control of growth and cell differentiation in photoautotrophic growing moss (*Physcomitrella patens*) bioreactor cultures. Plant Cell Res 20:1135–1140

Kamisugi Y, Cuming AC, Cove DJ (2005) Parameters determining the efficiency of gene targeting in the moss *Physcomitrella patens*. Nucleic Acids Res 33:e173

Kamisugi Y, von Stackelberg M, Lang D, Care M, Reski R, Rensing SA, Cuming AC (2008) A sequence-anchored genetic linkage map for the moss, *Physcomitrella patens*. Plant J 56:855–866

Khandelwal A, Cho SH, Marella H, Sakata Y, Perroud P-F, Pan A, Quatrano RS (2010) Role of ABA and ABI3 in desiccation tolerance. Science 327:546

Kiessling J, Kruse S, Rensing SA, Harter K, Decker EL, Reski R (2000) Visualisation of a cytoskeleton-like FtsZ network in chloroplasts. J Cell Biol 151:945–950

Knapp E (1935) Untersuchungen über die Wirkung von Röntgenstrahlen an dem Lebermoos, Sphaerocarpus, mit Hilfe der Tetraden-Analyse. Zeitschrift für induktive Abstammungs- und Vererbungslehre 70:309–349

Komatsu K, Nishikawa Y, Ohtsuka T, Taji T, Quatrano RS, Tanaka S, Sakata Y (2009) Functional analyses of the ABI1-related protein phosphatase type 2C reveal evolutionarily conserved regulation of abscisic acid signaling between *Arabidopsis* and the moss *Physcomitrella patens*. Plant Mol Biol 70:327–340

Kugita M, Kaneko A, Yamamoto Y, Takeya Y, Matsumoto T, Yoshinaga K (2003) The complete nucleotide sequence of the hornwort (*Anthoceros formosae*) chloroplast genome: insight into the earliest land plants. Nucleic Acids Res 31:716–721

McDaniel SF, Willis JH, Shaw J (2007) A linkage map reveals a complex basis for segregation distortion in an interpopulation cross in the moss *Ceratodon purpureus*. Genetics 176:2489–2500

Menand B, Yi K, Jouannic S, Hoffmann L, Ryan E, Linstead P, Schaefer DG, Dolan L (2007) An ancient mechanism controls the development of cells with a rooting function in land plants. Science 316:1477–1480

Miller MW, Gauber ED, Voth PD (1962) Nutritionally deficient mutants of *Marchantia polymorpha* induced by X-rays. Bot Gaz 124:94–102

Moutschen J (1954) Contribution a l'étude de la génétique des mousses. Action des rayons X et des rayons gamma. La Cellule 46:181–210

Oda K, Yamato K, Ohta E, Nakamura Y, Takemura M, Nozato N, Akashi K, Kanegae T, Ogura Y, Kohchi T, Ohyama K (1992) Gene organization deduced from the complete sequence of liverwort *Marchantia polymorpha* mitochondrial DNA: a primitive form of plant mitochondrial genome. J Mol Biol 223:1–7

Ohyama K, Fukuzawa H, Kohchi T, Shirai H, Sano T, Sano S, Umezono K, Shiki Y, Takeuchi M, Chang Z, Aota S-I, Inokuchi H, Ozeki H (1986) Chloroplast gene organization deduced from complete sequence of liverwort *Marchantia polymorpha* chloroplast DNA. Nature 322:572–574

Perroud P-F, Cove DJ, Quatrano RS, McDaniel SF (2011) An experimental method to facilitate the identification of hybrid sporophytes in the moss *Physcomitrella patens* using fluorescent tagged lines. New Phytol 191:301–306

Rensing SA, Ick J, Fawcett JA, Lang D, Zimmer A, Van de Peer Y, Reski R (2007) An ancient genome duplication contributed to the abundance of metabolic genes in the moss *Physcomitrella patens*. BMC Evol Biol 7:130

Rensing SA, Lang D, Zimmer AD, Terry A, Salamov A, Shapiro H, Nishiyama T, Perroud P-F, Lindquist E, Kamisugi Y, Tanahashi T, Sakakibara K, Fujita T, Oishi K, Shin-I T, Kuroki Y, Toyoda A, Suzuki Y, Hashimoto S, Yamaguchi K, Sugano S, Kohara Y, Fujiyama A, Anterola A, Aoki S, Ashton N, Barbazuk WB, Barker E, Bennetzen JL, Blankenship R, Cho SH, Dutcher SK, Estelle M, Fawcett JA, Gundlach H, Hanada K, Heyl A, Hicks KA, Hughes J,

Lohr M, Mayer K, Melkozernov A, Murata T, Nelson D, Pils B, Prigge M, Reiss B, Renner T, Rombauts S, Rushton PJ, Sanderfoot A, Schween G, Shiu S, Stueber K, Theodoulou FL, Tu H, Van de Peer Y, Verrier PJ, Waters E, Wood A, Yang L, Cove D, Cuming AC, Hasebe M, Lucas S, Mishler BD, Reski R, Grigoriev IV, Quatrano RS, Boore JL (2008) The genome of the moss *Physcomitrella patens* reveals evolutionary insights into the conquest of land by plants. Science 319:64–69

Schaefer DG, Zryd J-P (1997) Efficient gene targeting in the moss *Physcomitrella patens*. Plant J 11:1195–1206

Schaefer DG, Zryd J-P, Knight CD, Cove DJ (1991) Stable transformation of the moss *Physcomitrella patens*. Mol Gen Genet 226:418–424

Schieder O (1973) Untersuchungen an Nicotinsaüre-auxotrophen Stämmen von *Sphaerocarpus donnellii* Aust. Zeitschrift für Planzenphysiologie 70:185–189

Schieder O (1974) Selektion einer somatischen Hybride nach Fusion von Protoplasten auxotropher Mutanten von *Sphaerocarpus donnellii*. Aust Zeitschrift für Planzenphysiologie 74:357–365

Schween G, Gorr G, Hohe A, Reski R (2003) Unique tissue-specific cell cycle in *Physcomitrella*. Plant Biol 5:50–58

Strepp R, Scholz S, Kruse S, Speth V, Reski R (1998) Plant nuclear gene knockout reveals a role in plastid division for the homolog of the bacterial cell division protein FtsZ, and ancestral tubulin. Proc Natl Acad Sci U S A 95:4368–4373

Sugiura C, Kobayashi Y, Aoki S, Sugita C, Sugita M (2003) Complete chloroplast DNA sequence of the moss *Physcomitrella patens*: evidence for the loss and relocation of rpoA from the chloroplast to the nucleus. Nucleic Acids Res 31:5324–5331

Terasawa K, Odahara M, Kabeya Y, Kikugawa T, Sekine Y, Fujhiwara M, Sato N (2007) The mitochondrial genome of the moss *Physcomitrella patens* sheds new light on mitochondrial evolution in land plants. Mol Biol Evol 24:699–709

Thornton LE, Keren N, Ohad I, Pakrasi HB (2005) *Physcomitrella patens* and *Ceratodon purpureus*, mosses as model organisms in photosynthesis studies. Photosynth Res 83:87–96

Timme RE, Delwiche CF (2010) Uncovering the evolutionary origin of plant molecular processes: comparison of *Coleochaete* (Coleochaetales) and *Spirogyra* (Zygnematales) transcriptomes. BMB Plant Biol 10:96

Trouiller B, Schaefer DG, Charlot F, Nogué F (2006) MSH2 is essential for the preservation of genome integrity and prevents homeologous recombination in the moss *Physcomitrella patens*. Nucleic Acids Res 34:232–242

Trouiller B, Charlot F, Choinard S, Schaefer DG, Nogué F (2007) Comparison of gene targeting efficiencies in two mosses suggests that it is a conserved feature of Bryophyte transformation. Biotechnol Lett 29:1591–1598

Turmel M, Otis C, Lemieux C (2002) The chloroplast and mitochondrial genome sequences of the charophyte *Chaetosphaeridium globosum*: insights into the timing of the events that restructured organelle DNAs within the green algal lineage that led to land plants. Proc Natl Acad Sci U S A 99:11275–11280

Turmel M, Otis C, Lemieux C (2006) The chloroplast genome sequence of *Chara vulgaris* sheds new light into the closest green algae relatives of land plants. Mol Biol Evol 23:1324–1338

von Wettstein F (1932) Genetik. In: Verdoorn F (ed) Manual of bryology. Martinus Nijhoff, The Hague, pp 232–272

Wang TL, Cove DJ, Beutelmann P, Hartmann E (1980) Isopentenyladenine from mutants of the moss, *Physcomitrella patens*. Phytochemistry 19:1103–1105

Yasumura Y, Crumpton-Taylor M, Fuentes S, Harberd NP (2007) Step-by-step acquisition of the gibberellin-DELLA growth-regulatory mechanism during land-plant evolution. Curr Biol 17:1225–1230

Chapter 12

Photosynthesis in Aquatic Bryophytes

Janice M. Glime*
Professor Emerita, Department of Biological Sciences,
Michigan Technological University, Houghton, MI 49931, USA

Summary

Aquatic bryophytes occupy streams, lakes, and wetlands where they face limited CO_2 in solution, limited CO_2 diffusion, high boundary layer resistance, and loss of light with depth, especially red light. Limitations to photosynthesis in the water are therefore somewhat different from those on land. Of primary importance is the availability of CO_2 and hence, *p*H is important in determining the availability of this gas. There is also limited evidence that some mosses might be able to convert bicarbonates to CO_2 at the moss surface or within the cell to increase access to carbon. The often one-cell-thick leaves permit light and CO_2 to reach photosynthetic cells directly, but boundary-layer resistance can reduce CO_2 uptake.

*Author for correspondence, e-mail: jmglime@mtu.edu

D.T. Hanson and S.K. Rice (eds.), Photosynthesis in Bryophytes and Early Land Plants,
Advances in Photosynthesis and Respiration 37, pp. 201–231, DOI 10.1007/978-94-007-6988-5_12,

Other nutrients can be somewhat limiting, especially phosphorus and nitrogen. Sedimentation, and overgrowth by diatoms, other algae, and detrital complex, can block light, and water decreases the light with depth. This is further complicated by the rapid attenuation of red light. The aquatic environment protects chlorophyll from UV radiation, and in areas with high light intensity, at least some bryophytes produce enhanced pigmentation to serve as a filter. In dry seasons, lack of water can limit or halt photosynthesis. Temperature also can be a problem at this time, with exposed but still hydrated mosses in some cases reaching temperatures unknown in submersed conditions, and causing elevated respiration that can exceed photosynthetic fixation. High temperatures may greatly limit the presence of many cosmopolitan species of aquatic bryophytes in tropical regions. Contrarily, many aquatic mosses have temperature optima in the 0–20 °C range, with optima depending on their usual habitats.

I. Introduction: History of Photosynthesis in Aquatic Bryophytes

In many of the fundamental studies of photosynthesis, bryophytes served as models, particularly the aquatic bryophyte *Fontinalis antipyretica* (Plaetzer 1917; Harder 1921, 1923). Blackman and Smith (1910) included this species in their study on effects of CO_2 levels on photosynthesis and respiration and demonstrated that the rate limitation was due to the factor that had reached limiting availability, in this case CO_2. This revelation contradicted the paradigm that it was loss of an optimum that caused productivity to drop off at higher CO_2 levels.

It appears that discovery of photorespiration involved *Fontinalis*, wherein Bode (1940) puzzled over a kind of respiration that occurred in light and that was different from that in dark, although this German study seems to have been overlooked. Dilks (1976) further elucidated this observation of photorespiration by comparing many bryophyte species, including aquatic taxa, and demonstrated a lower rate of $^{14}CO_2$ loss in light compared to dark. Dilks considered three possible explanations: partial reassimilation of $^{14}CO_2$ produced by photosynthesis, partial inhibition of dark respiration by light, or low rate of glycolate synthesis and oxidation. Understanding that dark and light respiration were different led to rethinking the determination of gross productivity. In the same study on *Fontinalis* Bode (1940) discovered, apparently for the first time (see Emerson and Lewis 1943) that the greatest respiration occurred in blue light and greatest photosynthesis in red light. In 1970 Bolhár-Nordenkampf verified this in *F. antipyretica*.

II. The Role of Plant and Habitat Structure in Photosynthesis

The habitat for aquatic bryophytes is largely dictated by their photosynthetic needs. In lakes and streams, bryophytes occupy locations where other plants are unable to survive. On the other hand, aquatic (and all) bryophytes are absent in marine waters, only a few are able to invade the brackish waters at river mouths (e.g. *Fontinalis* spp.; Carroll 2003), and a few survive in pools or splash zones where they can become inundated by coastal water [e.g. *Muelleriella crassifolia* (formerly *Orthotrichum crassifolium*) & *Ulota phyllantha* (Orthotrichaceae); Rod Seppelt, 23 July 2011, personal communication] or survive in its spray (e.g. *Schistidium maritimum*; Bates and Phoon 2008). In calcareous waters, bryophytes often become encrusted with carbonates that

Abbreviations: CAM – Crassulacean Acid Metabolism; DW – Dry Weight; LSA – Leaf Specific Area; RuBP – Ribulose-1,5-bisphosphate; W m^{-2} – Watts per m^2

interfere with light needed for photosynthesis, as seen in species of *Didymodon* that create rock formations called didymodontoliths (Boros 1925; Wilkinson and Ormerod 1994).

Aquatic habitats present a variety of inorganic stressors, including high flow velocity, ice cover, scouring, too much or too little irradiance, nutrient limitation, low CO_2 concentrations, pollutants, salinity, siltation, and high temperatures while exposed out of water but hydrated (Lacoul and Freedman 2006). Biological stressors include competition, disease, and herbivory.

The requirement for water seems to be of paramount importance to aquatic bryophytes, with both physiological and morphological plasticity responding to differences among their many habitats. Some bryophytes occupy a wide range of habitats, whereas others are restricted to more narrow water conditions, making bryophyte species assemblages indicative of year-round conditions (Lacoul and Freedman 2006; Fritz et al. 2009).

Aquatic bryophytes lack the complex structures known for tracheophytes, and this changes the way they regulate light, nutrients, and CO_2 capture for photosynthesis. Aquatic bryophytes have little or no cuticle, no epidermis to interfere with gas exchange, and no stomata to control CO_2 uptake. In fact, it would appear that structural control of CO_2 uptake is impossible, and that the only control is due to *p*H, enzyme limitation, and habitat conditions. The lack of aerenchyma (tissue with air spaces) in all aquatic bryophytes but Polytrichaceae and a few aquatic liverworts prevents the bryophytes from bathing their photosynthetic cells in gases and provides them with no place to store this gas for even a brief period of time. Tremp (2003) attributes their limited growth to this lack of aerenchyma tissue. On the other hand, all leaf cells of aquatic bryophytes are typically photosynthetic, with *Sphagnum* being a notable exception. Even in aquatic *Sphagnum* species, as well as many other aquatic bryophytes (many stream species are exceptions), the two primary cell faces are in constant contact with their surrounding medium.

A. Quiet Water – Lakes

In lakes, bryophytes are able to occupy deep waters that are uninhabitable for the greater light-requiring tracheophytes (Fogg 1977; Chambers and Kalff 1985; Riis and Sand-Jensen 1997). In deep, cold lakes, bryophytes thrive in the upper hypolimnion (zone of water cut off from the upper lake by steep temperature cline) where they benefit from higher CO_2 levels and nutrients arising from deep lake decomposition, and their slow growth rates allow them to tolerate the low light levels and cold year-round temperatures (Riis and Sand-Jensen 1997; Osmond et al. 1981; Maberly 1985b). Such deep-water communities are known from the Canadian High Arctic (Hawes et al. 2002), Crater Lake, Oregon, USA, and Lake Tahoe on the California-Nevada border, USA (McIntire et al. 1994). In clear, cold-bottomed lakes, they can occupy positions as deep as 140 m (McIntire et al. 1994).

Lake bryophytes are structurally different from those living in fast water (rheophytes). Westlake (1971) contended that light was a limiting factor, and that, given enough space, aquatic plants would develop the optimal arrangement of leaves to take the greatest advantage of available light. One such adaptation is that bryophyte leaves are often spaced further apart in deeper water (Lodge 1960; Beever 1995), and also in submerged compared to aerial stems (Priddle 1979; Rice and Schuepp 1995), a trait shared with aquatic tracheophytes (Spence 1976; Spence and Dale 1978). By cultivating the aquatic bryophytes *Warnstorfia fluitans* (formerly *Drepanocladus fluitans*) and *W. exannulata* (formerly *D. exannulatus*), Lodge (1960) demonstrated plasticity that resulted in greater internode length with decreasing light intensity. Furthermore, aquatic bryophytes [e.g. *Warnstorfia sarmentosa* (formerly *Calliergon sarmentosum*), *Drepanocladus* cf. *aduncus*] often have larger leaves than terrestrial species (Priddle 1979).

The simple leaf structure provides little structural interference with light reception, and aquatic bryophytes must rely on pigments

and light absorbance properties of water to regulate light reaching the chlorophyll. On the other hand, attenuation of red light as it passes through water can be a severe limitation to growth at great depths, yet bryophytes are able to grow at greater depths than can tracheophytes and most algae (Fogg 1977; Chambers and Kalff 1985; Riis and Sand-Jensen 1997), in part because of lack of interference with light by their own tissues. Leaf Specific Area (LSA) (or alternatively Shoot Specific Area) provides a means of comparing light-capturing surface area to biomass. For example, *Scapania undulata* at 5 cm depth had an LSA of 317 $cm^2\ g^{-1}$ DW, but at 45 cm the LSA increased to 399 $cm^2\ g^{-1}$ DW (Mártínez-Abaigar et al. 1993). This shift in biomass allocation resulted in a Leaf Specific Weight that was reduced from 3.16 to 2.50 mg cm^{-2}.

Zastrow (1934) and Loeske (1926) demonstrated such changes as loss of the central strand from the stem, loss of leaf cell papillae, loss of leaf borders, reduction of costa (moss version of a midrib), and loss of differentiated alar cells (cells at base of leaf) in response to submersion (in non-flowing water), factors that could influence their photosynthetic efficiency by providing more cells dedicated to photosynthesis. Whereas curved leaves, an adaptation that helps protect terrestrial mosses from UV radiation by causing leaves to overlap, can provide strength in flowing water (Watson 1919), in quieter water loss of curvature presents greater surface area to gather the limited light available. For example, when grown submerged in an aquarium rather than as an emergent plant, *Drepanocladus aduncus* (formerly var. *kneiffii*) had straight leaves instead of curved ones (Loeske 1922).

Many submerged species in quiet water have leaves that are only one cell thick, with most exceptions being floating thallose liverworts. This lack of layering, along with flat, widely spaced leaves, provides maximum surface area for CO_2 exchange and light interception. This typical one-cell thickness differs even from multiple layers of cells of some terrestrial mosses.

B. Mires

In wetlands such as fens and bogs, nutrient depravity and abundance are major factors in determining the species composition, with *Sphagnum* species occupying habitats that generally have very low nutrient concentrations. In these habitats, the atmosphere and decomposition contribute to the CO_2 supply, but changing water levels can present hydration challenges to their photosynthesis and survival.

In mires (bogs and fens), squarrose or recurved leaves and falcation are significantly more common compared to river and stream mosses (Hedenäs 2001). On the other hand, whereas stream mosses have longer costae, the frequency of these does not differ between mire species and bryophytes in general. Mire mosses are more likely to have inflated alar cells, a condition that can facilitate water uptake and storage, but probably more importantly for these taxa, they spread their leaves when they are hydrated, facilitating greater light and CO_2 capture. Hedenäs only examined pleurocarpous mosses; it would be interesting to see if these same differences are present among acrocarpous taxa – a group found only infrequently in moving water but more common in mires. Although Hedenäs found no significant difference in the multistratose leaf character between stream/river species (88 % unistratose) or mire species (97 % unistratose) and pleurocarpous mosses in general, it can vary greatly within species. Spitale and Petraglia (2010) found this to be the most variable character measured in *Palustriella falcata*, varying not only between springs, but within individual plants! It is perhaps responsive to environmental conditions at the time of development of those cells (e.g. stagnant water, emergence), but does not seem to be widely adaptive. See Chap. 10 for further discussion of these adaptations.

Submerged *Sphagnum* species have greater relative growth rates and greater allocation of biomass to photosynthetic tissue (higher plant chlorophyll content) than hummock/terrestrial taxa (Rice 1995).

This is accomplished by having greater allocation to photosynthetic cells than to hyaline cells, but can also result from a biochemical difference that partitions photosynthetic cells to favor light-reaction proteins. The submerged taxa, like lake bryophytes, have longer, thinner branch leaves with greater internode distances, and photosynthetic cells are more exposed at the leaf surface, i.e., hyaline cells cover less of the photosynthetic cells between them (Rice and Schuepp 1995). Rice and Schuepp suggest that the leaf arrangement of aquatic *Sphagnum* taxa may have other adaptive consequences, and hypothesize they would have adaptations to reduce resistance to CO_2 diffusion. They demonstrated that the aquatic *S. trinitense* had thinner boundary layers compared to the non-aquatic *S. recurvum*, hence having lower overall resistance to CO_2 uptake than its non-aquatic counterpart.

But *Sphagnum* species are typically sun plants, and many of them can avoid desiccation by transporting water through capillary spaces along stems, then storing it in hyaline cells. This bathes their photosynthetic cells in water when other mosses are drying out and permits them to continue photosynthesis.

C. Flowing Water – Streams and Rivers

In streams, bryophytes are most common in cool headwaters and mountain streams where flowing water replenishes nutrients and CO_2 constantly and helps in removal of silt and the periphyton-detrital complex that blocks light and gas exchange. Furthermore, their ability to adhere to rocks and survive rapidly flowing water permits certain bryophytes to live in locations where tracheophytes are torn to shreds. Such shredding seems to be less detrimental to bryophytes, and many seem able to survive the abrasion with little harm and are even dispersed by it (Conboy and Glime 1971; Glime et al. 1979). Rather, it seems to affect primarily the older, often senescent leaves (Conboy and Glime 1971). Fortunately for bryophytes, even if part of a leaf is torn and tattered, the remaining portion can continue photosynthesis. Nevertheless, preservation of tissues requires different adaptive strategies.

In streams and rivers, abrasion makes multistratose leaves, arrangement of leaves all around the stem, shorter internodes, borders, and costae more practical solutions, while the movement of the water lessens the need for the flat growth habit and wide spacing. *Fissidens grandifrons* survives waterfalls and steep glacial melt streams with its multistratose leaves, stiff stems, and tight overlap between leaves (personal observations; Richard Zander, 23 July 2011, personal communication). By contrast, *F. fontanus*, a lake species, has widely spaced leaves and lax stems. A similar comparison can be made between *F. strictus*, a rheophyte (fast-water plant) that has stiff, compact shoots, and *F. berterii*, a lake species that has soft, flexible stems with widely spaced leaves (Beever 1995). Stiff stem structure is important in maintaining a plant in strong flow, but in quiet water it can be helpful to have a flexible stem that can move in response to light and limited water movement (Biehle et al. 1998; Miler et al. 2010). Longer plants increase drag. Nevertheless, the longest bryophyte known is the brook moss *Fontinalis*. This genus, especially in fast water, has a stem structure with thick-walled cells in the outer part and thin-walled parenchyma cells in the center. Those species that typically grow in fast water, with greater drag, have more strengthening tissue and greater elasticity in their stems (Biehle et al. 1998). At the same time, they are able to maintain more biomass that can take advantage of sunflecks, and turbulence can alternately expose various leaves to the most light while those no longer exposed can continue to process carbon fixed during that more illuminated period.

Multistratose leaves permit *Vittia pachyloma* to live on rocks in rapid South Georgian streams and the splash of waterfalls (Ochyra and Lightowlers 1988; Bernard Goffinet, 23 July 2011, personal communication). Among multistratose-leafed species are *Muelleriella crassifolia* (formerly *Orthotrichum crassifolium*), a moss that occurs in the marine splash zone of sub-Antarctic McQuarrie Island,

and the bistratose genus *Pachyglossa*, a moss often found in lakes of the island (Rod Seppelt, 23 July 2011, personal communication).

Growth form plays a role not only in water movement and retention, but also in the ability to capture both light and CO_2 needed for photosynthesis. Mat-forming liverworts like *Nardia compressa* and *Scapania undulata* in streams access maximum light per biomass while reducing boundary-layer resistance that interferes with CO_2 uptake by living in areas of high flow (Jenkins and Proctor 1985). On the other hand, the streamer form of *Fontinalis* spp. maximizes surface area, whereas its boundary-layer resistance is high, especially during times of low flow.

III. Resource Availability and Utilization in Aquatic Bryophytes

A. CO_2

CO_2 is typically a limiting factor in the aquatic environment. Its availability is diminished by high *p*H, stagnant water, use by algae and plants, and high temperatures. Higher productivity can be expected when more CO_2 is available. For example, the aquatic moss *Hygrohypnum ochraceum* exhibited increased growth as CO_2 concentrations were increased up to the level of its natural environment (6 mg L^{-1}) (Sanford et al. 1974). But mosses in general seem to have difficulty obtaining CO_2, with their lack of aerenchyma limiting photosynthesis (Marschall and Proctor 2004). For those plants living in water, this gas is even more difficult to get, with the diffusion coefficient of CO_2 in water roughly four orders of magnitude less than that in air (Proctor 1984).

Aquatic plants, including bryophytes, can obtain considerable CO_2 from sediment decomposition (Maberly 1985b) where carbon has been stored for a long time, thus altering the typical ^{13}C:^{12}C ratios (Keeley et al. 1986; Aravena et al. 1992). In a 1-m deep lake, Maberly demonstrated a lower *p*H and higher alkalinity at 0.1 m above the sediment than elsewhere in the water column. Nutrient levels were not limiting for *Fontinalis antipyretica* growing in this CO_2-enriched water. In fact, during 4 months of study, no factor seemed to be the primary cause of limitation to photosynthesis, but rather, that limitations changed seasonally. Photon irradiance seemed to limit productivity in November, whereas in March, temperature was limiting. CO_2 only became limiting in August due to increased photosynthetic activity of phytoplankton.

Low temperatures (4 °C in deep lakes) hold CO_2 in solution longer. For example, in a low-nutrient lake in Denmark, Riis and Sand-Jensen (1997) found that *Warnstorfia exannulata* and *Sphagnum subsecundum* grew faster in deep than in shallow water. *Sphagnum subsecundum* represents this deepwater response with lower dark respiration (1.3-fold; suppressed by high CO_2) and higher photosynthesis (3.3-fold) at 9.5 m than at 0.7 m. This CO_2 saturation, coupled with a low light compensation point, permits aquatic bryophytes to occupy greater depths than that possible for tracheophytes.

Maberly (1985a) found a CO_2 compensation point in UK *Fontinalis antipyretica* consistent with that of a C_3 plant, and no evidence of photoinhibition. However, the light compensation point was somewhat low. Temperature increases affected the slope of the photosynthesis vs CO_2 concentration curve in a way that suggested effects of boundary-layer resistance.

1. Flow Rate

Fast-moving water can trap and carry gaseous CO_2 from the atmosphere, rapidly replacing CO_2 lost by photosynthesis and diffusion, hence providing a renewed source for bryophytes. But boundary-layer resistance at the bryophyte surface can complicate obtaining CO_2 from flowing water. Maberly (1985a) suggested that boundary layer resistance decreased photosynthetic activity, citing a linear increase in the slope of photosynthesis vs [CO_2] as evidence. In *Fontinalis*, a flow rate of less than 0.01 mm s^{-1} limits photosynthetic ability (Jenkins and Proctor 1985),

whereas increasing flow rates to as little as 1 cm s^{-1} seems to result in no limiting effect (Proctor 1984). On the other hand, Madsen et al. (1993) found a significant net decrease in photosynthesis as flow rate increased from 1 to 8.6 cm s^{-1}, but suggested this may have been partly due to the concomitant decrease in dark respiration.

Compensating for lack of aerenchyma and problems of boundary layer resistance, some bryophytes such as *Fontinalis* streamers have high boundary layer resistance in stream velocities of 0.01–0.2 m s^{-1}, resulting in CO_2 boundary-layer resistance between ~180 and 15 s mm^{-1} (Jenkins and Proctor 1985). Other taxa, such as the leafy liverworts *Nardia compressa* and *Scapania undulata* have a high leaf area index that results from mat formation. But even these latter two species face steep decline in photosynthetic rate when flow rate decreases below ~10 cm s^{-1}. *Nardia compressa* and *Scapania undulata* had resistances of ~35–5 and 70–9 s mm^{-1} at water velocities of 0.02–0.2 m s^{-1}, respectively. Proctor (1984) suggests that turbulence is important for moving fresh CO_2-containing water into small spaces between leaves, disrupting the boundary layer resistance. Overlapping arrangement of leaves often reduces effective surface area so that shoot area may be a more appropriate way to express CO_2 uptake and photosynthesis, at least in some cases (see Chap. 9). In marine algae, Wheeler (1988) stressed the importance of microturbulence and found that low-velocity laminar flow restricts nutrient transfer, but that microturbulence can improve the transfer. Similarly to results of Jenkins and Proctor (1985) for the leafy mat-forming liverworts, Wheeler found the optimum range of flow rate to be 0.01–0.2 m s^{-1} for diffusion of nutrients into the algae.

In transplant experiments, Aronsuu et al. (1999) determined that in controlled flow, *Fontinalis dalecarlica* did not grow well compared to sites with short-term regulated flow, a difference they attributed to loss of substrates during winter in the controlled flow site. In a Swedish study, Englund et al. (1997) likewise found that low flow velocity reduced abundances of *Fontinalis antipyretica* and *F. dalecarlica*. Furthermore, both reduced flow and regulated flow reduced taxonomic richness of bryophyte species. On the other hand, abundance of both *Blindia acuta* and *Schistidium agassizii* was higher than predicted where flow was regulated but not reduced. The bryophytes must live where there is a suitable balance among CO_2 renewal, boundary layer resistance, and damage due to drag and movement of substrate.

The importance of moving water for replenishment of the CO_2 supply and that of other nutrients is suggested by experiments with *Fontinalis duriaei* (Glime 1987b). At its optimum temperature of 15 °C, this species grew only 4 mm per week in standing water, whereas at the same light intensity in turbulent flow it grew 15 mm per week.

2. Temperature

Although playing a somewhat minor role, temperature can influence where aquatic bryophytes live. For example, *Fontinalis* species are common and abundant at high latitudes and altitudes, but in the lowland tropics this genus is absent (Ruttner 1955). At higher temperatures, CO_2 rapidly leaves the water and respiration increases more rapidly than photosynthesis. The Q_{10} for *Fontinalis antipyretica* was 2.0 for maximum gross photosynthesis and 2.1 for respiration in the range of 5–15 °C, but in the range of 10–20 °C it changed to 1.4 and 2.0 respectively, demonstrating the shift to net loss in photosynthate due to relatively depressed photosynthesis (Maberly 1985a), explaining the relatively low temperature optima of most aquatic bryophytes. For example, some species of *Fontinalis* have a temperature optimum near 5 °C (Glime 1987a) and some bryophytes continue photosynthesis at temperatures below freezing, although that would only be possible in aquatic systems in rapidly moving water that experiences supercooling. In a lake that occasionally has year-round ice cover, *Calliergon* sp. managed to survive a *p*H of 3.7 and grow 10–30 mm per year at a depth of 22 m (Hawes et al. 2002),

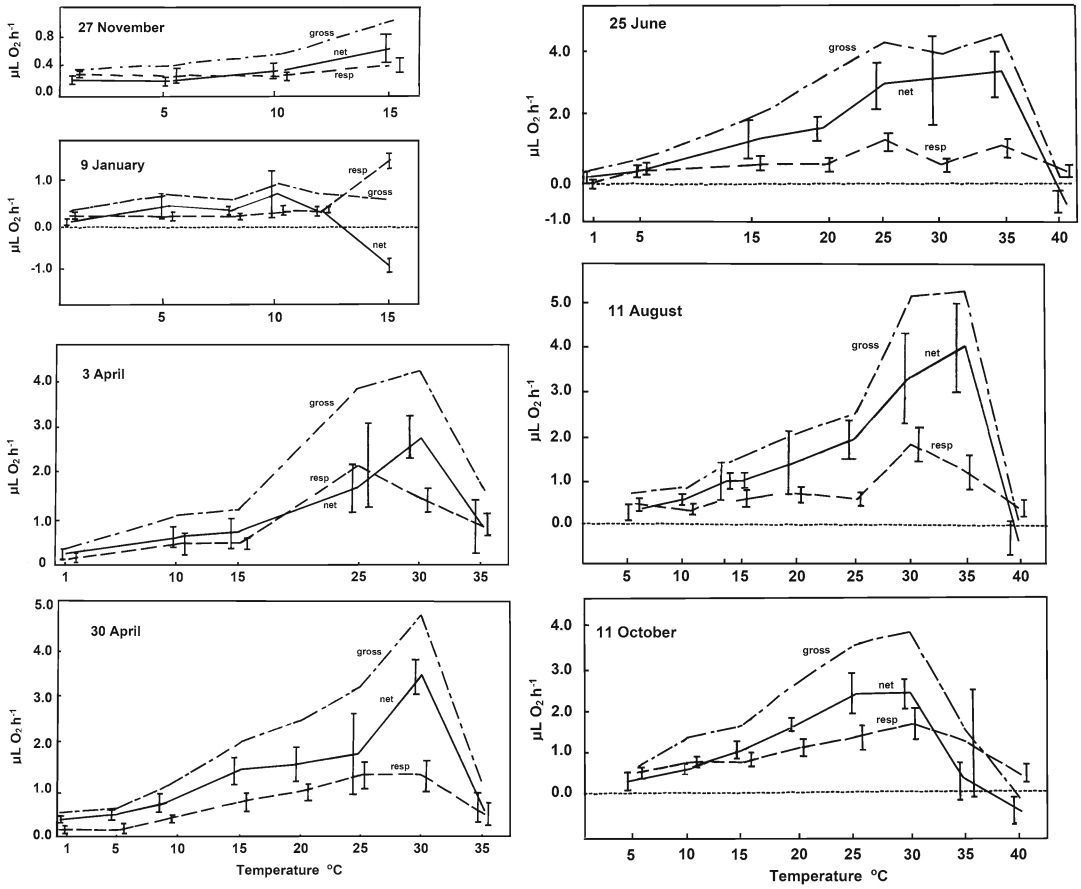


Fig. 12.1. Left: Changes in temperature optima (measured in lab) in field-acclimated *Fontinalis duriaei* from late autumn (27 November) through winter to early spring (30 April) (stream temperatures remained at 0–1 °C, but light intensity was higher than in summer due to snow reflectance and leaf loss from canopy). *Right*: Changes from early summer (25 June) to mid autumn (11 October) (stream temperatures, measured on sampling dates, were mostly 10–20 °C) (Redrawn from Fornwall (1978)).

where the temperature would most likely remain at 4 °C year-round. For some typically terrestrial species (*Leptobryum* sp. and *Bryum pseudotriquetrum*) in the Antarctic, aquatic habitats provide refuges from severe temperatures (Kudoh et al. 2003; see also Chap. 17).

Carballeira et al. (1998) found no difference in temperature response (pigment ratio, photosynthetic rate, respiratory rate) between *Fontinalis antipyretica* from a normal river and those collected from one with abnormally high temperatures resulting from a hot spring. Even plants maintained at 30 °C for 10 days recovered completely. On the other hand, Fornwall and Glime (1982) found that field-acclimated *Fontinalis duriaei* exhibited an Arrhenius response for its net assimilation, with a cold photosynthetic response in plants grown at 0–1 °C and a warm response at 9–16 °C. This suggests a shift in enzymes. The right enzymes could alter the ability to trap and use CO_2 (Fig. 12.1).

In some cases, it is the variation in the temperature that becomes limiting (Vanderpoorten et al. 1999). The leafy liverwort *Chiloscyphus pallescens*, thallose liverwort *Pellia endiviifolia*, and moss *Hygroamblystegium tenax* occupied streams where the annual standard deviation in

temperature was less than 4 °C and in that geographic range, their frequency decreased as the variation increased. On the other hand, the moss *Cinclidotus danubicus* is absent in streams where the annual standard deviation is less than 4 °C. Some bryophytes can take advantage of short exposures to higher temperatures, but are unable to sustain in longer exposures. In short-term experiments, *Fontinalis duriaei* was able to have positive photosynthesis up to ~30 °C, but when it was maintained at light intensities close to those in the field at 20 °C for 3 weeks, its growth rate was reduced, chlorosis occurred, and eventually many tissues died (Glime and Acton 1979; Fornwall and Glime 1982; Glime 1987a, b).

Other factors (CO_2, nutrients, light) determine response to temperature elevation. Lovalvo et al. (2010) reported *Fontinalis* with morphology of *F. novae-angliae* but genetic markers of *F. antipyretica* at 28 m in Yellowstone Lake in association with geothermal vents at 32–34 °C. The light intensity showed an 8,000-fold decrease from that at the surface, in part due to suspended particulates. Temperatures exceeded most temperature maxima known for the genus, but water was supersaturated with CO_2. This high CO_2 could provide two compensations for the high temperature: suppression of respiration and removal of photosynthetic CO_2 limitation. Temperature affects CO_2 compensation point, with greater CO_2 concentrations required at higher temperatures, and both of these compensation points are affected by light intensity (Rastorfer 1971; Dilks and Proctor 1975).

3. p*H, Bicarbonates, and Carbonates*

Poor diffusion and rapid loss of CO_2 are not the only problems for aquatic bryophytes needing CO_2. The solubility of CO_2 depends on the *p*H, with low *p*H waters holding CO_2, medium *p*H having mostly bicarbonate, and high *p*H water having predominantly carbonates. Like tracheophytes, bryophytes use the enzyme carbonic anhydrase to convert bicarbonates to CO_2 within the cells (Steeman Nielsen and Kristiansen 1949; Arancibia and Graham 2003). Some vascular plants use extracellular carbonic anhydrase to convert bicarbonates to free CO_2 (Allen and Spence 1981), but evidence suggests that this capability does not exist for aquatic mosses (James 1928; Ruttner 1947; Steeman Nielsen 1947; Bain and Proctor 1980; Allen and Spence 1981; Osmond et al. 1981; Glime and Vitt 1984; Prins and Elzenga 1989; Madsen et al. 1993; Ballesteros et al. 1998; Raven et al. 1998). Members of Chlorophyta (green algae) and Anthocerotophyta (hornworts) have pyrenoids that serve as CO_2-concentrating mechanisms (Raven 1991; Smith and Griffiths 1996; Hanson et al. 2002; Chap. 6), but such structures are unknown among other bryophytes.

The problem of explaining species that occur in habitats with a *p*H too high for free CO_2 to exist has continued to plague bryophyte physiologists. Steeman Nielsen (1947) tried to determine if *Fontinalis* had a means to use bicarbonates (HCO_3^-) as an inorganic carbon source, but was unable to demonstrate it. Many of the attempts to determine bicarbonate use have relied on *p*H measurements to model the CO_2-bicarbonate equilibrium, demonstrating that no free CO_2 should be available at higher *p*H levels. Peñuelas (1985) provided evidence that *Fontinalis antipyretica* could use $NaHCO_3$ as a carbon source. During photosynthesis, this moss increased the *p*H to 9.6, indicating a CO_2 compensation point of 1.1 mM m^{-3} CO_2. The photosynthetic rates were higher than could be explained by CO_2 alone and increased at higher levels of HCO_3^- when CO_2 was held constant. In fact, photosynthesis was not depressed to zero until the *p*H reached 11.8–12.0 in *F. antipyretica* and 10.10 in *Fissidens grandifrons* – a species known to thrive in alkaline streams. But in a different stream, Peñuelas found that the *F. antipyretica* could not use HCO_3^- to photosynthesize, suggesting either different physiological races or different acclimation to conditions. Shaw and Allen (2000) have recently discovered genetic differences among members of this highly diverse species that might explain differences

in capability. But we still do not know what physiological mechanisms support those differences.

Of course, as CO_2 is used, we should expect some bicarbonate to be converted to CO_2, possibly accounting for the added advantage of water with higher levels of bicarbonate. But an equilibrium is dynamic and time delays exist. New sources constantly arise as new CO_2 enters water from decomposition on the bottom or from bacteria and fungi on adhering sediments or the leaves themselves; closely associated bryophytes can absorb this newly released CO_2 before all of it converts to bicarbonates or carbonates. CO_2 is also renewed by dissolution from the atmosphere as water tumbles over rocks or falls through the atmosphere in a waterfall. The ability of the moss to lower the *p*H at its surface (through cation exchange) could permit a local change in the balance of CO_2 and bicarbonate around leaves (see Chap. 13). Could CO_2 be stored at night by some as yet unknown concentrating mechanism when everything else is respiring?

In any case, bryophytes exist in streams with *p*H levels above 7, indicating that they have some means of obtaining CO_2 even in these alkaline waters (Glime and Vitt 1984). Catteneo and Fortin (2000) demonstrated that *p*H played at least a minor role in the distribution of aquatic species. Furthermore, bryophytes seem to be more sensitive to changes in *p*H than their tracheophyte associates (Stephenson et al. 1995).

To further complicate the story, Farmer et al. (1986) determined that *Fontinalis antipyretica* has no PEP carboxylase and uses only Rubisco for its fixation of CO_2 in photosynthesis, supporting the earlier statement of Steeman Nielsen (1947) that *F. antipyretica* could not use bicarbonates from the water for its photosynthesis. But Harder (1921) had already shown that *F. antipyretica* increased its net assimilation from 0.66 to 3.14 as HCO_3^- was increased from 0.01 to 0.64 %, and Burr (1941) had likewise demonstrated greater productivity in this species in water with more bicarbonate than that with CO_2. Steeman Nielsen and Kristiansen (1949) suggested that CO_2 might enter photosynthetic reactions in its hydrated form, i.e. as bicarbonate. Bain and Proctor (1980) tested 20 aquatic species from a range of habitats. In all cases (except the hornwort *Anthoceros husnotii* with pyrenoids), they found that *p*H compensation points were in the range expected for CO_2-dependent C_3 plants. Extensive evidence supports the concept that all aquatic mosses are C_3 plants (Ruttner 1947; Allen and Spence 1981; Osmond et al. 1981; Salvucci and Bowes 1981; Raven 1991; Raven et al. 1987, 1994, 1998), even though some are able to exist in conditions that have CO_2 concentrations below the expected CO_2 compensation point.

We still need to explain the fact that bicarbonates seem able to provide the CO_2 needed for bryophyte photosynthesis (James 1928; Burr 1941; Peñuelas 1985; Vitt et al. 1986). *Fissidens* cf. *manateensis* demonstrated an ability to concentrate CO_2, a phenomenon we only understand in C_4 or CAM metabolism (Salvucci and Bowes 1981). And this appears to be facultative, based on temperature. In winter conditions (12 °C, 10 h day length), *Fissidens cf. manateensis* had a typical C_3 compensation point (Salvucci and Bowes 1981). But in summer (30 °C, 14 h day length), when CO_2 gas has short residency in water, this species evidenced the ability to concentrate CO_2. Raven et al. (1998) suggested that *Fissidens cf. manateensis* and *Fontinalis antipyretica* have some sort of CO_2-concentrating mechanism, but its exact nature is still a mystery. Or could there be some sort of active transport of inorganic carbon, perhaps in a non-gaseous state, across membranes?

In one study *Fontinalis antipyretica* had a higher carbon uptake rate than did its counterpart *Isoetes bolanderi*, a CAM plant (Sandquist and Keeley 1990). Another curious phenomenon for *F. antipyretica* is that it seems to have a delay in carbon fixation (Søndergaard and Sand-Jensen 1979), consistent with performing some sort of transformation first. In *F. antipyretica*, this delay is low, resulting in an underestimation of only 0.2 % of the first hourly rate of photosynthesis, compared to underestimations in

the aquatic tracheophytes *Elodea* (8 %) and *Littorella* (14 %). Søndergaard and Sand-Jensen suggest that this difference reflects a difference in reserved CO_2 in lacunae in *Elodea*, more lacunae in *Litorella*, and none in *Fontinalis*. Furthermore, *Litorella* was able to move CO_2 from the roots for initial use in the light. Such reserves would cause a delay in usage of external $^{14}CO_2$. But when the plants were given $^{14}CO_2$ leading up to the experiment, there was some shortening of the delay in the two tracheophytes, but not in *Fontinalis*. The initial delay in all these plants may reflect the direction of diffusion, with dark-treated plants having a CO_2 flow outward from respiration, delaying the movement direction inward to the chloroplast when light is available.

4. Carboxylase Activity

In a comparison between the aquatic moss *Leptodictyum riparium* and tracheophyte *Elodea canadensis*, the moss had nearly double the Rubisco (Ribulose-1,5-bisphosphate carboxylase oxygenase) and PEP carboxylase activity (11.8 vs 6.0 μM mg^{-1} chl h^{-1} and 0.7 vs 0.3 μM mg^{-1} chl h^{-1}, respectively) compared to *E. canadensis* (Keeley et al. 1986). Using *Fissidens rigidulus*, Yeoh et al. (1981) demonstrated the highest K_m(RuBP) of RuBP carboxylase activity in this moss, compared to that of four algae, five aquatic monocots, and two aquatic dicots. The PEP carboxylase activity in the moss is unusual because PEP carboxylase appears to be absent in *Fontinalis antipyretica* and unexpected in a C_3 plant (Farmer et al. 1986). *Elodea canadensis* is among the many aquatic plants that can use bicarbonates (Farmer et al. 1986), but in this case the superior activity of *L. riparium* may represent an amplification of enzymes rather than any concentrating mechanisms. Yet proof of this seems to be lacking.

5. Alternative CO_2 Sources or Mechanisms

One avenue that has been difficult to explore is the role of the detrital complex in providing CO_2 at the cell surface. Bacterial activity in breaking down organic sediments that collect on bryophyte surfaces (Johnson 1978; Hoppe et al. 1988) could provide a source of CO_2 that is grabbed and used by bryophyte cells before it escapes into the water column and converts to a less soluble form. One indication of its importance is that Raven (1991) found CO_2-concentrating mechanisms in aquatic plants to be negatively correlated with areas of CO_2 enrichment such as microbial respiration of organic carbon. Other factors had less effect, including low temperatures during the growing season, low *p*H external to the plant, and rapid flow of CO_2-containing water over the plants. But for bryophytes, these may all be important; furthermore, lack of an epidermis may make microbial-generated CO_2 absorption greater than that experienced by tracheophytes.

Sphagnum cells are very acid due to the presence of numerous cation exchange sites on the cell walls. These exchange sites trade H^+ ions for other cations dissolved in the water, making the *p*H of these cells, and of water immediately surrounding the plants, acid. By this activity, *Sphagnum* ensures that its environment is suitable for the solution of CO_2 in nearby water. But other mosses may likewise use this mechanism to obtain CO_2 from water where free CO_2 is otherwise not available. Little research has been done on cation exchange capacity of non-*Sphagnum* bryophytes, but we know that at least some other bryophytes are capable of cation exchange (Clymo 1963, 1964; Glime et al. 1982; Soudzilovskaia et al. 2010). Glime et al. (1982) found that other fen bryophyte species had varying degrees of cation exchange capacity. Hence, we might expect some aquatic bryophytes that live in highly alkaline habitats to convert bicarbonates or even carbonates at the bryophyte surface, freeing CO_2 that is then taken into the moss before it all escapes from that location.

Vitt et al. (1986) found species such as *Fissidens grandifrons* in streams with a *p*H of 7.3 or higher. In this case, rapidly flowing meltwater (~1 °C) created aeration that helped to maintain CO_2 in solution, but if these mosses also had good cation exchange capacity, it could explain their abundance in the stream bed away from the major turbulence.

Sphagnum has other tricks. There is a biochemical shift that favors light-reactive proteins (Rice 1995), permitting it to respond more efficiently to limited available light. And Graham et al. (2010) found that *Sphagnum compactum* under carbon or light limits, can take up exogenous sugars, thus maintaining a positive carbon balance (see Chap. 2).

Hence, CO_2 from decomposition, dynamic equilibrium of CO_2 – bicarbonate, and cation exchange could all contribute to the ability of bryophytes to live in water where *p*H would otherwise be too high to provide sufficient free CO_2 for net photosynthetic gain. It is clear that we need more investigation on microbial contributions and cation exchange mechanisms.

B. Nutrients

Aquatic bryophytes require the same nutrients as tracheophytes in order to grow, but require them in much smaller quantities. Among these, nitrogen and phosphorus are the most likely to limit productivity if factors of light, temperature, and CO_2 are adequate. In lakes, competition with algae and tracheophytes can severely limit nutrients, but in streams bryophytes benefit from constant renewal by flowing water.

It appears that bryophytes might be less affected by nutrients than algae. Ylla et al. (2007) examined the effects of enhanced nutrients and light on both algae and mosses. Algae (*Cladophora glomerata*) were much more productive under all conditions (control, enhanced nutrients, enhanced nutrients + enhanced light, enhanced light), having net primary productivity of 3–18 mg oxygen per mg chlorophyll per hour, whereas the range for mosses was only 0.4–1.8 mg oxygen per mg chlorophyll per hour. Both algae and mosses required high light intensity to take advantage of the higher nutrient levels. These results suggest that during spring and fall in temperate systems, when leaves are off the trees, higher levels of bryophyte productivity are possible.

Aquatic bryophytes can use many forms of **nitrogen**: nitrate (NO_3^-), ammonium (NH_4^+) (Schwoerbel and Tillmanns 1972; Rudolph and Voigt 1986), nitrite (NO_2^-) (Schwoerbel and Tillmanns 1964, 1977), and amino acids (Sharma et al. 1960; Basile 1965; Simola 1975; Kielland 1997; Alghamdi 2003). Nitrogen is typically provided by the bacterial breakdown of organic matter in the water and by nitrogen fixation by Cyanobacteria or other micro-organisms. Many bryophytes absorb NH_4^+ more easily than they absorb NO_3^- (Schwoerbel and Tillmanns 1974; Simola 1975; Miyazaki and Satake 1985; Schuurkes et al. 1986). Differences in NH_4^+-N can explain differences in aquatic *Amblystegium* (sensu lato) distributions in river systems (Vanderpoorten 2000). Schwoerbel and Tillmanns (1974, 1977) found that *Fontinalis antipyretica* uses NO_3^- and NH_4^+, with NH_4^+ being taken up first if provided together with NO_3^-, but Frahm (1975) found that the brook moss *Fontinalis gigantea* had low tolerance for NH_4^+. These apparent differences may be associated with different physiological races, or even different species (see Shaw and Allen 2000).

In lakes, often the highest concentration of nutrients is in the hypolimnion, where it is safe from use by most plants that are unable to live in deep waters where light and temperatures are low. Here some bryophytes can benefit from the cool C_3-friendly temperatures, supersaturated CO_2, and constant nutrient renewal. In a Denmark lake, Riis and Sand-Jensen (1997) found that *Sphagnum subsecundum* and *Warnstorfia exannulata* (formerly *Drepanocladus exannulatus*) were more abundant in deep water than near the surface, attributing their success to low temperatures, CO_2 supersaturation, and high levels of nutrients.

Nutrient conditions select for different communities. *Chiloscyphus pallescens*, *Pellia endiviifolia*, and *Hygroamblystegium tenax* (formerly *Amblystegium tenax*) live in oligotrophic conditions in the Alsatian Rhine floodplain, in contrast to the more nutrient-rich

conditions that favor *Hygroamblystegium fluviatile* (formerly *Amblystegium fluviatile*), *Cinclidotus danubicus*, *C. riparius*, and *Fissidens crassipes* (Vanderpoorten et al. 1999). *Leptodictyum riparium* (formerly *Amblystegium riparium*), *Fontinalis antipyretica*, and *Platyhypnidium riparioides* (formerly *Rhynchostegium riparioides*) occupy a wide range of nutrient conditions, but their frequencies increase in eutrophic streams in Europe. *Leptodictyum riparium* and *Platyhypnidium riparioides* increase in frequency as ammonia N increases.

Many *Sphagnum* species require most of their nitrogen as NH_4^+ (Schuurkes et al. 1986), the predominant form in bogs and poor fens (Rosswall and Granhall 1980). But other *Sphagnum* species (*S. nemoreum*, *S. fimbriatum*) grow well on the amino acids alanine and arginine (Simola 1975, 1979). In fact, McKane et al. (1993) found that glycine was actually preferred over NH_4^+ and NO_3^- in *Sphagnum* sp. and *Aulacomnium palustre*. Kielland (1997) likewise found glycine to be the preferred form of N in *Sphagnum rubellum* in the Arctic and supported the contention of others that amino acids were used more extensively in habitats low in inorganic N forms. But Schuler et al. (1955) found that the floodplain liverwort *Sphaerocarpos texanus* had a more typical growth form when grown on a mix of amino acids than when grown with NH_4NO_3 alone, suggesting that the importance of amino acids is not restricted to nutrient-poor habitats. Furthermore, bryophytes may be able to move organic molecules such as amino acids and dipeptides from older, senescing tissues to the growing apex (Brown 1982).

Organic sources of nitrogen, such as amino acids, are available in the sediments, but they are also likely to be available among moss leaves. Sediments that accumulate among leaf axils will contain not only inorganic silt, but also decaying organisms, and the latter will not only provide CO_2 release, but will also contain protein break-down products – amino acids.

Not all amino acids are equally useful for bryophytes. Burkholder (1959) tested 20 amino acids on *Atrichum undulatum* and found that only glycine, L-cystine, L-cysteine, and L-tyrosine were sufficient for this moss to retain its green color. Alghamdi (2003) examined five common soil water-soluble amino acids (glycine, methionine, serine, arginine, and alanine) and found that four of them induced branching in the aquarium moss *Taxiphyllum barbieri* relative to controls (no N source), but that there were no branches in mosses grown with methionine. Methionine caused an increase in biomass and decrease in growth in length with concentration increases (1, 10, 30 mg L^{-1}). Alanine, on the other hand, caused both an increase in growth rate and in biomass accumulation with concentration, creating a more robust plant at higher concentrations, with a higher biomass to length ratio than that of controls. For glycine, a greater concentration was required (20 and 30 mg L^{-1}) to exceed growth in the controls, but at those higher concentrations both their length and biomass increased considerably over that of controls.

Absence of the right form of N can cause photosynthetic problems due to its effect on chlorophyll. In his experiments with *Taxiphyllum barbieri*, Alghamdi (2003) found that using NO_2^- caused little improvement in biomass or length compared to N-free controls, but that there was considerable increase in chlorophyll *a*. On the other hand, the amount of chlorophyll *b* per biomass was even less than that of controls. Even inorganic sources of N such as potassium nitrate can enhance the green color of *Fontinalis novae-angliae* and *F. dalecarlica* (J. M. Glime, unpublished). One might ponder what effect concentrations and forms have in nature where chlorophyll *b* is important as a light-gathering accessory pigment in low light. Does this suggest that amino acids from decomposition are important in deep water for chlorophyll *b* production, further adapting the mosses to a deep-water habitat?

Effects of N form and concentration on branching could also be adaptive. Presumably, the highest organic N will be in sediments, hence in deepest water. It is interesting that

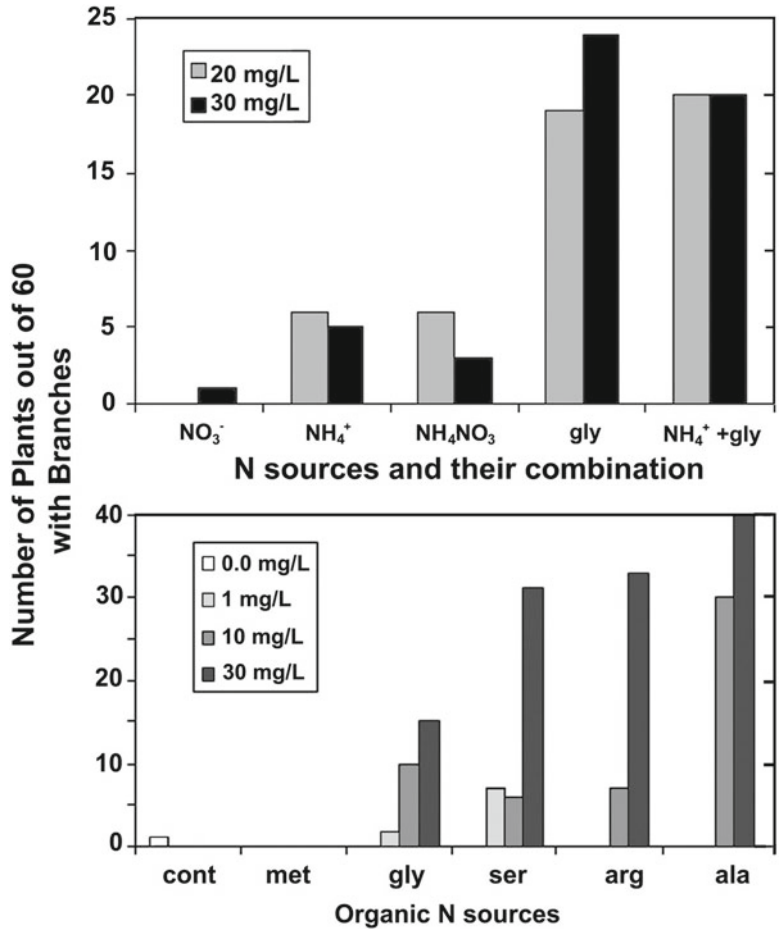


Fig. 12.2. Comparison of effects of inorganic *(upper)* and organic *(lower)* N sources with those of glycine (gly) on branching in *Taxiphyllum barbieri cont* no N, *met* methionine, *gly* glycine, *ser* serine, *arg* arginine, *ala* alanine (Redrawn from Alghamdi (2003)).

higher concentrations of some amino acids increase branching (as well as increasing stem length) in *Taxiphyllum barbieri* over that in inorganic sources or other water-soluble amino acids, giving the moss greater surface area for absorbing light (Alghamdi 2003). A straight vertical stem extended upward would not be very efficient at trapping light, whereas spreading branches would increase that ability. Might the availability of these branch-promoting amino acids explain the presence or absence of aquatic bryophytes in some lakes? And could these same amino acids cause these branched bryophytes to be absent in fast-flowing but organically rich water where the additional drag caused by branches could dislodge them? (Fig. 12.2)

Phosphorus is initially provided by bedrock, but for most organisms this has been further processed by recycling from plants. For bryophytes, the supply is likely to come from sediments, where it is released by decomposition. Could it be that nucleic acids or other organic decay compounds supply aquatic bryophytes with significant phosphorus as well (Whitton et al. 2005)? These bound nutrients would not show up in measurements of dissolved nutrients in the water and may mislead us as to nutrient availability and needs of bryophytes. Ellwood and Whitton (2007) found that activity of

phosphomonoesterase and phosphodiesterase did correlate with uptake of phosphorus by bryophytes (*Warnstorfia fluitans*) in the field in a system with low *p*H. In the lab, these axenic bryophytes had greater activity of these enzymes with organic phosphorus than with inorganic phosphorus. However, Ellwood et al. (2008) found no relationship between phosphatase activity and organic phosphorus concentration in several bryophytes. The use of organic phosphorus needs further study to determine which sources are useful and how other environmental factors affect that use.

It appears that P limitation may exist in a number of aquatic habitats. In an Alaskan stream, *Hygrohypnum alpestre* and *H. ochraceum* responded with an increase in cover to an addition of inorganic P (Bowden et al. 1994; Benstead et al. 2007). Li et al. (1993) demonstrated an increase in growth of *Sphagnum magellanicum* and *S. papillosum* with added inorganic P over field levels in laboratory experiments. Both species exhibited their maximum growth in both length and dry mass in 0.100 mM H_2PO_4. *Sphagnum magellanicum* produces red pigments in response to deficiency of phosphorus, a response that adapts it to high light levels when nutrients are limiting at the end of the growing season. But it also produces red pigments in response to high concentrations of phosphorus, a response that may lack adaptive value.

Christmas and Whitton (1998) found that the N:P ratio for *Fontinalis antipyretica* and *Platyhypnidium riparioides* varied with availability of these nutrients in the stream. The highest phosphomonoesterase activity in these mosses occurred in late summer. *Fontinalis antipyretica* typically showed twice the enzyme activity of *P. riparioides*. Enzyme activity increased when P content was less than 0.3 % or tissue N:P content was above 9:1. It appears that these mosses are able to modulate their N and P to maintain a less variable condition in the apical 2 cm than in other parts (Ellwood et al. 2008). In *Warnstorfia fluitans* organic phosphorus is more important than inorganic phosphorus, eliciting greater phosphomonoesterase and phosphodiesterase activity (Ellwood and Whitton 2007).

One might expect current velocity to be important in nutrient availability, as it is in CO_2 replenishment. Meyer (1979) found that phosphorus uptake by the leafy liverwort *Scapania undulata* in a New Hampshire, USA, stream depended on both flow rate and concentration. There was evidence of boundary layer resistance; high flow rates resulted in less change in incorporated P concentration, but total P taken up was greater. This observation was fortified by realization that efficiency of removal of added P from the water stream was lowered at increased flow rates. Surprisingly, initial concentration did not seem to affect rate of uptake. Croisetière et al. (2001), on the other hand, demonstrated that current velocity had no effect on accumulation of Cd in *Fontinalis dalecarlica* in lab or field, suggesting the uptake for at least some ions may be more complicated and that current velocity may be of only minor importance for at least some of them. We need to answer the question whether differences in accumulation relative to flow rate relate to availability vs need, with micronutrients that behave like Cd being unaffected because of low need.

Wood ash, currently being applied to forests as an additional source of nutrients and to restore acidified soils, can also provide nutrients for *Fontinalis antipyretica* (Aronsson and Ekelund 2006). Runoff brings this ash into streams, and one might expect the added nutrients to be beneficial to tracheophytes to the detriment of low-nutrient bryophytes. However, after 9 weeks of treatment, stem growth of *F. antipyretica* was enhanced significantly at higher concentrations (1–10 g L^{-1}), and more secondary branches formed. Nevertheless, photosynthesis was significantly lower in untreated (alkaline) wood ash compared to wood ash adjusted to *p*H 7.5 or the control (no wood ash), perhaps due to CO_2 limitations or reduced light. Optical density increased with wood ash. The short duration of the experiment does not permit us to understand

long-term effects. Tracheophytes could respond more slowly to greater nutrient levels and eventually outcompete bryophytes.

C. Light

Bryophytes are basically shade plants. Their light saturation occurs at moderate levels of light, as indicated by fluorescence saturation at levels typical of shade species (Proctor and Smirnoff 2011). Nevertheless, sun species such as *Sphagnum* do not have saturation of their relative electron transport rate at high light levels, indicating adaptation to high light. But even at low (limited) CO_2 levels, their O_2 reduction remains high. This may, in part, be explained by the high capacity of bryophytes for photosynthetic electron transport to oxygen, providing protection from high excitation energy. Proctor and Smirnoff further supported this position by demonstrating that bryophytes have a high resistance to Paraquat, a herbicide that generates superoxide in photosystem I.

But Sand-Jensen and Madsen (1991) were unable to find any correlation between depth of growth and growth rate or respiration rate in low light. Primarily, these plants had high efficiency in low light and high growth rate in high light. With little disturbance or herbivory, bryophytes survive by growing slowly with little destruction.

The simple leaf structure of bryophytes provides little structural interference with light reception, and aquatic bryophytes must rely on pigments and light absorbance properties of water to regulate light reaching the chlorophyll. On the other hand, attenuation of red light as it passes through water can be a severe limitation to growth at great depths, yet bryophytes are able to grow at greater depths than can tracheophytes and most algae (Fogg 1977; Chambers and Kalff 1985; Riis and Sand-Jensen 1997), in part because of lack of interference with light by their own tissues.

Although bryophytes have long been considered shade plants, their restriction to shade may not be due to their pigments. Marschall and Proctor (2004) concluded that bryophytes include both shade and sun plants, but that their light-saturation levels in sun are lower than those for vascular plants. This, they suggest, is due to their lack of "ventilated photosynthetic tissues" in which to provide photosynthetic cells with CO_2.

Aquatic habitats present two problems in getting sufficient light. Turbidity causes a decrease in productivity in macrophytes, presumably due to its blockage of light (Robel 1961; Jones et al. 1983), and for bryophytes on a lake bottom, sedimentation may exceed growth, burying the bryophytes. But light attenuation through the water column can present an increasing problem with depth. For bryophytes (*Drepanocladus aduncus*) living at depths up to 140 m (McIntire et al. 1994), one must wonder how they attain sufficient light, when only 1 % of surface light reached 100 m. Yet, in Waldo Lake, Oregon, 13 bryophyte species grow at depths of 40–128 m, growing 1.5–3 cm per year (Wagner et al. 2000).

Light attenuation is not the only light-gathering problem for aquatic bryophytes. Spectral quality changes with depth. Red light has long wavelengths with low energy, causing it to be absorbed quickly with increasing depth in water. Blue light, with short, high-energy waves, also decreases, but more slowly. These are the two most important wavelengths for photosynthesis. In Lake Waldo, liverworts are the most common, comprising ~98 % of the bryomass, with the remainder being almost entirely pleurocarpous mosses (Wagner et al. 2000). Wagner et al. suggested that the liverworts might be more viable than mosses under attenuated blue light. They found that some of the liverworts possessed red pigments down to about 70 m, but that these were lost in most species at 40–50 m. *Sphagnum*, on the other hand, retained its red color down to 100 m. Red algae are likewise common at greater depths, and this success has been attributed to the ability of the red pigments to capture the green and blue light that penetrates to greater depths, then to transfer the light energy to the reaction center of chlorophyll *a*. But liverworts and pleurocarpous mosses are also

flatter and therefore able to capture more light per unit of biomass.

Water clarity can degrade due to dissolved organic carbon (DOC), causing even greater attenuation of blue light with depth, as seen in Grane Langsoe (Schwarz and Markager 1999). Under conditions of light attenuation due to both water and DOC, *Warnstorfia exannulata* (=*Drepanocladus exannulatus*) accounted for 70 % of lake biomass in Grane Langsoe. The moss had the greatest pigment concentration in the youngest parts, where it also had maximum light absorption.

1. Chlorophyll and Accessory Pigments

The chlorophyll *a*:*b* ratios of aquatic bryophytes (2–3) differ little from those of terrestrial bryophytes (Peñuelas 1984; Peñuelas et al. 1988; López and Carballeira 1989; Martínez-Abaigar et al. 1994; Martínez-Abaigar and Núñez-Olivera 1998), but are lower than those for tracheophytes (2.4–3.7) (Martin and Churchill 1982). For example, in Yuan-Yang Lake in China, aquatic bryophytes seem to have lower chlorophyll *a*:*b* ratios (mean 2.41) than the two species of aquatic tracheophytes (mean 3.08), being typical shade plants (Yang et al. 1994). Nevertheless, these bryophyte values were higher than most values reported for bryophytes in the literature and may indicate their ability to acclimate to the higher light intensities of this lake (250 μmol m^{-2} s^{-1}). Tuba (1987) suggested that low *a*:*b* ratios in bryophytes are advantageous because bryophytes are poikilohydric and must depend on atmospheric moisture to regulate internal water content. This adaptation in an atmosphere that is typically dry during the day and has the most available moisture during periods of low light, stormy days or early morning and late evening selects for chlorophyll adjusted to low light levels, but a slightly higher light compensation point than that of shade-adapted tracheophytes. This same higher compensation point (that level of light intensity at which photosynthetic gain and respiratory loss are equal) permits bryophytes to take advantage of sunflecks, but the advantage of sunflecks to aquatic bryophytes remains to be studied.

In the thallose liverwort genus *Riccia*, terrestrial *R. discolor* displayed the highest chlorophyll concentrations in shade conditions, whereas the floating *R. fluitans* had the lowest (Patidar et al. 1986). But the chlorophyll *a*:*b* ratios did not differ between these two species. Similarly, in *Sphagnum fimbriatum* both chlorophyll *a* and *b* increased in dim light (Koskimies-Soininen and Nyberg 1991). But temperature played a role in the response. At 25 °C in dim light, the ratio increased only slightly, whereas at 15 °C, there was no increase. Rincón (1993) found a similar response in six bryophyte species; the highest level of total chlorophyll occurred at the lowest light level, but the chlorophyll *a*:*b* ratio differed little among treatments.

In some cases, greater water depth results in reduction of chlorophyll (Zastrow 1934), a characteristic that seems to be the opposite of tracheophyte response (Bowes et al. 1977; McMillan and Phillips 1979; Wiginton and McMillan 1979; Westlake 1981; Barko and Filbin 1983). Mártinez-Abaigar et al. (1993) failed to demonstrate any relationship between either light or water availability and chlorophyll concentration among the aquatic species in their study. Submersion, on the other hand, does seem to result in higher chlorophyll concentrations. In 32 rivers in Galicia, Spain, bryophytes (*Brachythecium rivulare*, *Fontinalis antipyretica*, *Platyhypnidium riparioides*, and *Scapania undulata*) had higher chlorophyll concentrations than those of some terrestrial bryophytes (López and Carballeira 1989). Similarly, the greatest chlorophyll concentration is often in the basal (deepest) portion of the plant (Ikusima 1965, 1966).

Schistidium rivulare, an emergent moss with dark-colored cell walls and high concentration of chlorophyll, seems to be an exception (Mártinez-Abaigar et al. 1993). One might explain this seeming anomaly by its lack of water for long periods of time (it lives on emergent rocks) during which chlorophyll is easily damaged, and the need to take advantage of the most light possible

Table 12.1. Chlorophyll concentrations as mg m^{-2} for bryophyte species occurring in full sun, sun, shade, and deep shade and five water availabilities.

	Chl mg m^{-2}	Light availability	Water availability	LSA cm^2 g^{-1}	LSW mg cm^{-2}
Schistidium rivulare	351 ± 17	Full sun	I-E-D	133 ± 7	7.51 ± 0.4
Fontinalis squamosa	341 ± 14	Sun	I	271 ± 13	3.7 ± 0.18
Fontinalis antipyretica	290 ± 14	Full sun	I	226 ± 16	4.42 ± 0.31
Fissidens grandifrons	289 ± 13	Full sun	I	222 ± 4	4.5 ± 0.08
Platyhypnidium riparioides	257 ± 4	Deep shade	I-E	224 ± 9	4.47 ± 0.18
Cinclidotus fontinaloides	250 ± 13	Full sun	I-E-D	164 ± 15	6.11 ± 0.56
Cratoneuron filicinum	246 ± 4	Full sun	I-E-D	274 ± 15	3.65 ± 0.2
Fissidens grandifrons	244 ± 11	Deep shade	I	211 ± 8	4.73 ± 0.18
Jungermannia eucordifolia	173 ± 6	Full sun	I	351 ± 15	2.85 ± 0.12
Hygrohypnum duriusculum	157 ± 8	Full sun	I-E-D	313 ± 25	3.2 ± 0.26
Scapania undulata	150 ± 7	Shade	I-E-D	262 ± 10	3.81 ± 0.15
Palustriella commutata	121 ± 10	Full sun	E	187 ± 25	5.36 ± 0.72
Brachythecium rivulare	116 ± 5	Full sun	I	456 ± 41	2.19 ± 0.2
Pellia endiviifolia	97 ± 7	Shade	E	446 ± 15	2.24 ± 0.08

From Mártínez-Abaigar et al. (1993)
Species are arranged from highest to lowest chlorophyll concentrations
I immersed, *E* emerged, *D* dry, *LSA* Leaf Specific Area, *LSW* Leaf Specific Weight

during its brief periods of hydration (Table 12.1).

We know from terrestrial studies that bryophytes living in shaded habitats have larger grana in their chloroplasts (Karunen and Aro 1979). Other studies demonstrate increase in chlorophyll *b*. And it appears that polyunsaturated fatty acids are more abundant in mosses in extreme conditions (Gellerman et al. 1972; Valanne 1984), including those of low light (Karunen and Aro 1979). There is no comprehensive study to determine if these are important factors for deep-water bryophytes.

Bryophytes, like tracheophytes, use antenna pigments to transfer light energy to reaction centers of chlorophyll *a*. Using aquatic bryophytes, Martínez-Abaigar and Núñez-Olivera (1998) demonstrated that the two dimers of chlorophyll *a* absorb best at 680 and 700 nm (red light) and very poorly in the range between 450 and 650 nm. Chlorophyll *b* is able to absorb in that poor absorption range of chlorophyll *a* and to transfer light energy to chlorophyll *a*. Other frequent antenna pigments, including those in aquatic bryophytes (Martínez-Abaigar and Núñez-Olivera 1998), include α- and β-carotene, lutein, neoxanthin, violaxanthin, and zeaxanthin (Taylor et al. 1972; Schmidt-Stohn 1977; Czeczuga 1980, 1985; Czeczuga et al. 1982; Huneck 1983; Farmer et al. 1988; Boston et al. 1991). These pigments broaden the spectrum of light that can be absorbed, but can also facilitate the dissipation of excess absorbed light. *Fontinalis antipyretica* stands out by having the additional pigment auroxanthin (Bendz et al. 1968).

Schwarz and Markager (1999) found that *Warnstorfia exannulata* increased chlorophyll *a* per gram dry weight at 10 m depth compared to 2 m, but the ratio of accessory pigments did not change. Most of the photosynthesis occurred in the youngest parts of plants where the highest concentrations of pigments occurred. These mosses showed no ability to shift pigment ratios when added carbon in the lake caused a decrease in blue light available at greater depths.

2. Photoinhibition

Whereas too little light prevents photosynthesis from reaching its maximum, too much

destroys DNA and chlorophyll. But bryophytes, as do many tracheophytes, have a physiological response that helps to protect them from damage by high light intensity. The gaseous hormone ethylene (C_2H_4) is produced in response to stress and inhibits synthesis of carotenoids and chlorophyll (Kang and Burg 1972). This step reduces the danger of over-excitation of chlorophyll by reducing both antenna pigments and chlorophyll itself.

Low temperatures, in particular, can cause over-excitation of chlorophyll in high light intensity. Ethylene is inhibited by CO_2 – a molecule that is in low concentration in water. And it requires the presence of oxygen for its formation. Hence, when photosynthesis is geared up too high from high light intensities, conditions are suitable for ethylene production. *Fontinalis antipyretica* responds to a combination of low temperatures and high light intensity by producing red pigments in nature (Glime 1984). When this species was cultured with various concentrations of ACC, an ethylene precursor, a water-soluble red pigment was produced as a wall pigment (Glime and Rohwer 1983).

Chlorophyll content generally does not correlate linearly with leaf area in tracheophytes (Martínez-Abaigar and Núñez-Olivera 1998), several *Sphagnum* species, or terrestrial mosses (Hoddinot and Bain 1979; Austin and Wieder 1987; Gaberscik and Martincic 1987), resulting in no linear relationship with growth (Martínez-Abaigar and Núñez-Olivera 1998). On the other hand, under conditions of limiting light, both bryophytes (Hearnshaw and Proctor 1982; Kershaw and Webber 1986; Gaberscik and Martincic 1987; McCall and Martin 1991) and tracheophytes (Frost-Christensen and Sand-Jensen 1992) exhibit a correlation between photosynthesis and chlorophyll concentration. For aquatic bryophytes, chlorophyll concentrations reported range from 2 to 15 mg g^{-1} dry weight and from 100 to 350 mg m^{-2} (Glime 1984; Peñuelas 1984; Peñuelas et al. 1988; López and Carballeira 1989; Martínez-Abaigar et al. 1994). Compared to chlorophyll contents of obligate aquatic bryophytes, concentrations are lower in terrestrial bryophytes, with emergent species behaving more like terrestrial bryophytes (Mártínez-Abaigar et al. 1994; Rice 1995).

In mountain streams, UV-B levels can be high, and the temperature is typically cold. In these environs, UV-absorbing compounds can help to protect bryophytes from the damage of high UV activity (Arróniz-Crespo et al. 2004; see Chap. 7). Mosses and liverworts may respond differently. In these streams, liverworts had high levels of UV-absorbing pigments, whereas mosses had low levels. Structural differences such as sclerophylly seemed to have little effect. Only *Polytrichum commune* among the mosses was able to protect itself against the UV radiation by any of the means examined (Arróniz-Crespo et al. 2004). When irradiated with UV-B in laboratory experiments, responses were somewhat different. The leafy liverwort *Jungermannia exsertifolia* subsp. *cordifolia* and moss *Fontinalis antipyretica* exhibited decreases in chlorophyll and carotenoid concentrations, chlorophyll *a*:*b* ratios, chlorophyll:phaeopigment ratios, net photosynthetic rates, light saturation point, maximum quantum yield of photosystem II (F_v/F_m), and apparent electron transport rate (ETR) (Martínez-Abaigar et al. 2003; Núñez-Olivera et al. 2004). (F_v is total variable fluorescence; F_m is maximum fluorescence. Hence, F_v/F_m = maximum quantum efficiency of photosystem II.) Their sclerophylly index increased, as did the dark respiration rate. Furthermore, *J. exsertifolia* subsp. *cordifolia* irradiated with UV-B only showed a decrease in F_v/F_m, perhaps the most sensitive physiological variable to UV-B. This subtle response was accompanied by an increase in UV-absorbing compounds in this species. Young shoots, the site of most active growth and photosynthesis, exhibited higher values for F_v/F_m and higher carotenoid and UV-absorbing compound concentrations than in older shoots (Arróniz-Crespo et al. 2008b). But at cold temperatures (2 and 10 °C) the physiological damage to *Jungermannia exsertifolia* subsp. *cordifolia*, except for damage to growth, hardly was noticeable compared to that exhibited by *F. antipyretica*

(Núñez-Olivera et al. 2004). This damage was evident not only after extended treatments of 32–82 days, but also after only 78 h at 2 °C (Núñez-Olivera et al. 2005). Both sun- and shade-acclimated *Fontinalis antipyretica* exhibited high sensitivity, but shade samples were more sensitive to UV-B treatment. The F_v/F_m ratio decreased 42 % in shade samples, but only 27 % in those from sun.

In some plants, UV-B can cause irreversible photosynthetic damage due to chlorophyll damage. However, in the aquatic thallose liverwort *Riella helicophylla*, photosynthesis was least at noon during the period of highest light intensity in both UV-screened and non-screened plants, while electron transport reached its highest levels (Conde-Al'varez et al. 2002). Recovery of the photosynthetic process occurred the same afternoon, indicating that any damage caused by ambient UV radiation was not irreversible.

One response to higher UV-B intensity is production of higher levels of *p-coumaroylmalic* acid, as seen in *Jungermannia exsertifolia* subsp. *cordifolia* (Otero et al. 2006). In mountain streams of mid-latitude Spain, new shoots of *Jungermannia exsertifolia* subsp. *cordifolia* accumulated higher levels of *p-coumaroylmalic* acid in response to UV radiation than did those from other latitudes (Arróniz-Crespo et al. 2008a; Núñez-Olivera et al. 2009). Fabón et al. (2010) demonstrated that increased levels of UV-B absorbing compounds were induced by higher levels of UV-B radiation in this taxon, but of five hydroxycinnamic acid derivatives identified, only p-coumaroylmalic acid (soluble fraction) and p-coumaric acid (cell wall-bound fraction) increased under enhanced UV-B.

UV-B intensity varies with season, and its danger to aquatic bryophytes depends in part on water temperature. Concentrations of UV-absorbing compounds varied seasonally in both *Bryum pseudotriquetrum* and *Fontinalis antipyretica*, with *B. pseudotriquetrum* having three to four times the concentration of that in *F. antipyretica* (Núñez-Olivera et al. 2010). The researchers suggested that both species had efficient DNA repair mechanisms, resulting in the absence of any evidence of DNA damage. *Jungermannia exsertifolia* subsp. *cordifolia* experienced greater DNA damage when UV-B radiation was accompanied by exposure to cadmium, possibly due to inhibition of the DNA enzymatic repair mechanism (Otero et al. 2006).

3. Light Compensation Point

More typically, aquatic bryophytes from deep water are likely to have the lowest light compensation points in that habitat where light penetration is greatly reduced. *Sphagnum inundatum* from deep water had extremely low light compensation points, as did the tracheophyte *Juncus bulbosus* (Wetzel et al. 1985). The moss *Drepanocladus* (*sensu lato*) in Antarctic lakes has a light compensation point of 0.11 W m^{-2+}, ≃0.5 μmol m^{-2} s^{-1} (0.03 % ambient light), a value similar to that of the algal communities, but *Calliergon* sp. from shallow water had a light compensation point of 0.64 W m^{-2}, ≃2.9 μmol m^{-2} s^{-1} (0.16 % ambient light) (Priddle 1980). *Fontinalis* sp. at 5 °C had a light compensation point of 15 lux (~0.3 μmol m^{-2} s^{-1}); at 20 °C it was 40 lux (~0.8 μmol m^{-2} s^{-1}) (Burr 1941). The flattened leafy liverwort *Chiloscyphus rivularis*, by contrast, had a light compensation point of 1,750 lux (~3.5 μmol m^{-2} s^{-1}) (Farmer et al. 1988). Larcher (1983) compares compensation points of bryophytes to those of other photosynthetic groups (Table 12.2).

IV. Desiccation

Hydrated bryophytes exposed to air in warm temperatures can soon surpass their light or CO_2 photosynthetic compensation points, with respiration exceeding photosynthesis. For many bryophytes, this is their most vulnerable state.

Ueno and Kanda (2006) suggest that the required water content for maximum photosynthesis is related to the degree of physiological adaptation to an aquatic environment. In their study of the emergent

Table 12.2. Comparison of light compensation and saturation points for photosynthetic aquatic organisms from various habitats.

Photosynthetic group	Compensation light intensity I_k in Klux (μmol m^{-2} s^{-1})	Light saturation I_S in Klux (μmol m^{-2} s^{-1})
Mosses (aquatic and terrestrial)	**Up to 3 (~24)**	**0.6–3.5 (~10.8–63)**
Planktonic algae		(7) 15–20
Tidal-zone seaweeds	1–2 (~18–36)	10–20 (~180–360)
Deep-water algae		1–2 (~18–36)
Seed plants	<1–2 (<~18–32)	(5) 10–30 (~180–540)

From Larcher (1983), compiled from various authors

species *Calliergon giganteum*, they found that maximum photosynthesis (1.2–1.6 mg CO_2 g^{-1} h^{-1}) occurred at a water content of 1,500–1,700 %; at half maximum it was 980 %, the highest reported among wetland mosses. Titus et al. (1983) found that the species of *Sphagnum* in their study experienced a decline in photosynthetic rate at less than 500 % water content. Davey (1997) found that aquatic Antarctic species had a more rapid decline in photosynthesis with water loss than did terrestrial species.

Although *Fontinalis* has been considered desiccation-intolerant, it can survive out of water, but its recovery following desiccation can be slow (Glime 1971; Cruz de Carvalho et al. 2011). Under desiccating conditions in *Fontinalis antipyretica*, respiration increased not only in light, but also in the dark (Cruz de Carvalho et al. 2008; Cruz de Carvalho 2009). Gross photosynthesis decreased significantly, and ultimately the moss was not able to recover until 5 or more days later. Cruz de Carvalho (2009) attributed this delay in recovery to membrane damage and significant loss of electrolytes during desiccation. The percentage of water loss played a greater role in recovery than did rate of water loss, suggesting this moss may have limited capacity to prepare for desiccation. Nevertheless, Cruz de Carvalho et al. (2011) determined that it should be considered desiccation tolerant. To achieve full pre-desiccation state, drying must occur slowly. Following rehydration, the photochemical quenching coefficient decreases, potentially lessening effects of excess energy on photosystem I. At the same time, non-photochemical quenching permits an energy shift that can further protect the cell from excitation damage.

One response to dehydration is the loss of chlorophyll *a* (Peñuelas 1984). Aquatic bryophytes lost 50 % or their chlorophyll *a* within a few weeks. Likewise, phaeo-pigment proportion – indicative of chlorophyll breakdown – was sensitive to rainfall and humidity changes. Peñuelas found that among the species in the River Muga, NE Spain, *Cinclidotus fontinaloides* was most tolerant and *F. antipyretica* the least. Within 2 months of dehydration, all species lost their green leaves and exhibited considerable morphological decay.

V. Storage Compounds

Once CO_2 has been fixed in the photosynthetic pathway, it can be converted into multiple kinds of compounds. In a study that included *Fontinalis*, Norris et al. (1954) demonstrated that much less sucrose was stored in plants containing only photosynthetic tissue (bryophytes and algae) compared to those with non-photosynthetic tissue, suggesting that other compounds ultimately resulted from photosynthesis. For example, within 2 h the aquatic leafy liverworts *Plagiochila asplenioides* and *Scapania undulata* exhibited the presence of C from $^{14}CO_2$ uptake in the amino acids asparagine, glutamine, and glutamic acid and in citric and malic acids (Gupta 1976). *Plagiochila* also exhibited labelled fumaric, glycolic, and

succinic acids. Typical, and expected (see Valanne 1984), soluble carbohydrates stored included a series of fructans plus glucose, mannitol, and sucrose. The two species differed in concentrations of carbohydrates stored. *Plagiochila asplenioides* stored the greatest soluble carbohydrate as volemitol, whereas *Scapania undulata* stored mostly sucrose. Gupta (1976) showed that malic acid exhibited the most labelling among organic acids in both liverwort species, causing one to wonder if there is some sort of CO_2-concentrating mechanism that used this compound, as in CAM plants.

There are trade-offs among the storage compounds. For example, herbivores can have multiple effects on net productivity when measured as biomass increase. Not only do they reduce biomass directly by eating the tissues, but Graham et al. (2010) suggested that production of polyphenolic compounds, an adaptation to extensive herbivory, may preclude production of large quantities of glucose (hence reducing growth), a carbon and energy trade-off that keeps bryophytes small while protecting them from annihilation by herbivores. LaCroix (1996) found polyphenolic compounds in *Fontinalis antipyretica*; it is likely that other aquatic species produce them as well, thus altering the storage of sugars.

VI. Productivity

Aquatic bryophytes may have slow growth rates compared to tracheophytes, but the constant presence of water and ability to photosynthesize in cold water of winter permits them to have photosynthesis throughout most, if not all, of the year. Hence, many perennial aquatic bryophytes can accumulate more biomass than accompanying tracheophytes that must restart leafy shoots each year.

Productivity is dependent upon available nutrients, light intensity and quality, CO_2 availability, temperature, and moisture content. As the limit of any of these factors is approached, the photosynthetic rate attenuates. Figure 12.3 compares the light intensity response of the bryophyte *Fontinalis antipyretica* with two charophyte algae and three aquatic tracheophytes, indicating that the moss response is similar to that of these other aquatic organisms.

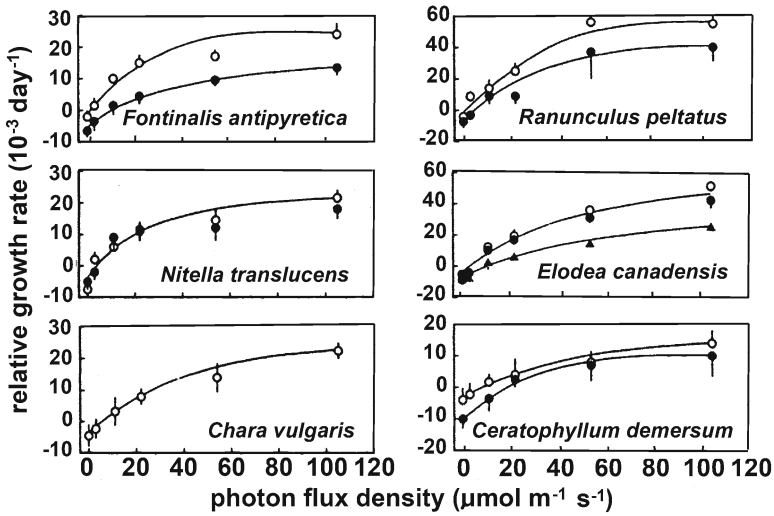


Fig. 12.3. Comparison of mean relative growth rates (±95 % CI) among the aquatic bryophyte *Fontinalis antipyretica*, two charophyte algae (*Nitella translucens*, *Chara vulgaris*), and three aquatic tracheophytes (*Ranunculus peltatus*, *Elodea canadensis*, *Ceratophyllum demersum*) over 25–30 days at 7 °C. [10^{-3} day^{-1} = mmol C (mol cell C)$^{-1}$ day^{-1}] [o = July, ● = August, ▲ = October, n = 4 or 5] (Redrawn from Sand-Jensen and Madsen (1991)).

Fontinalis can exhibit negative productivity during the warm part of the year, with a positive net photosynthesis only in winter (Naiman and Sedell 1980). But among 4th order and higher order boreal streams this moss was the most productive species, with 3.9×10^{10} g year^{-1} productivity, compared to that of periphyton (2.1×10^{10} g year^{-1}) (Naiman 1983). In a north temperate stream, field measurements demonstrated that *Fontinalis duriaei* grew an average of 12 mm per week from November to July (Glime 1987b). In the lab it achieved a mean of 15 mm per week for 15 weeks in turbulent flow at 15 °C. Its peak assimilation occurred at 10 °C (testing range of 1–20 °C following 3 weeks of acclimation at that temperature) and 5,400 lux, explaining its absence in hotter portions of the world (Glime and Acton 1979).

In an arctic stream fertilized with phosphorus, bryophyte productivity increased from 2.3 to 6.3 g C h^{-1} (Arscott et al. 1998). The two species of *Hygrohypnum* had the higher productivity (1,676–6,342 μg O_2 g^{-1} dry mass h^{-1}) compared to *Schistidium agassizii* (428–1,163 μg O_2 g^{-1} dry mass h^{-1}). The amphibious thallose liverwort *Riccia fluitans* had a relative growth rate of 0.011 day^{-1} at low light and CO_2 availability, and at 0.138 day^{-1} at high light and CO_2 (Andersen and Pedersen 2002).

Productivity can be measured in terms of gas exchange or in terms of the ultimate product of growth. Measurement of growth in biomass requires destructive sampling and either laboratory culture or massive sampling of marked specimens. Growth in length is more easily measured, but measuring branches that may be major expressions of that growth is quite time consuming. When using oxygen production per unit of chlorophyll *a*, Arscott et al. (1998) demonstrated that epilithic algae had a greater productivity rate in an arctic tundra stream than did bryophytes (*Schistidium agassizii*, *Hygrohypnum* spp.). However, when measured on the basis of substrate area, bryophyte productivity exceeded that of algae. But Martin and Adamson (2001) found the opposite; they demonstrated that when productivity of bryophytes is calculated on the basis of chlorophyll, differences in rate compared to that of tracheophytes disappear.

VII. Seasons

The ability of aquatic bryophytes to acclimate to the range of temperatures they experience with changing seasons is a major determinant in their distributions and contributions to ecosystems. As seasons change, so does quality and quantity of light reaching aquatic bryophytes. Tree canopies create a green filter that absorbs red light during the growing season; winter brings slanted rays that are highly reflected by snow. And ice cover filters the light and provides a substrate where light-blocking snow can accumulate. We might expect bryophytes to respond with seasonal changes in chlorophyll content. When Martínez-Abaigar et al. (1994) examined pigments, it appeared that summer degradation of chlorophyll and decrease in chlorophyll *a*:*b* ratio, accompanied by increase in carotenoid:chlorophyll ratio, was most evident in those species suffering from desiccation. Those bryophytes that remained wet exhibited more subtle changes that correlated with changes in light conditions, particularly canopy state. In sun-exposed habitats where species were submerged year-round, both chlorophyll content and *a*:*b* ratio remained high throughout the year with little seasonal pigment variation.

Glime (1987b) developed an annual growth model for *Fontinalis duriaei* based on a combination of laboratory cultures at five temperatures and ca 8-week field measurement intervals. The laboratory data predicted the seasonal growth patterns observed in the field. Fornwall and Glime (1982) demonstrated that *Fontinalis duriaei* had its greatest productivity during spring and early summer when temperatures were optimal and canopy cover was not developed, providing high light intensity. Much of winter seemed to be spent recovering from loss of chlorophyll during the hot summer

(Fornwall and Glime 1982; Glime 1987b). Johnson (1978) found that *Fontinalis* sp. in a north Swedish river had its greatest shoot growth starting in ice-free rapids at 0 °C, with its highest rate just before the stream reached its highest temperature.

VIII. Future Research

Both physiological and ecological questions remain for the aquatic bryophytes. We have not addressed the role of sunflecks in forested aquatic systems and the degree to which they contribute to photosynthetic pulses. Attendant to this is a lack of understanding of induction time for net photosynthesis in bryophytes and how long they might continue to store carbon after a flash of light from a sunfleck. Since streams themselves create partial openings in the forest canopy in temperate ecosystems, sunflecks may be more common than on the forest floor and could play a significant role in photosynthesis there. Are these sunflecks sufficient to cause DNA and chlorophyll damage in shallow water, and if so, do the bryophytes produce protective pigments or have other mechanisms to avoid that damage?

Our understanding of the ability of bryophytes to obtain CO_2 in high *p*H water is muddled, with conflicting results among studies. It would be interesting to investigate the role of cation exchange and enzyme concentration (and diversification?) as they relate to the conversion of carbonates and bicarbonates at the bryophyte surface or utilization within the cell. And we need an accurate method to assess the role of the periphyton-detrital complex in providing CO_2 at the surface of the leaves.

Progress has been made in understanding the uptake of nutrients, but further clarification is needed on the mechanisms that control observed differences in uptake of macronutrients and micronutrients, particularly as influenced by flow rate, temperature, and other ecological variables. We are just beginning to understand the ability of aquatic bryophytes to use amino acids (and probably other organic sources) as sources of nitrogen and phosphorus, and we do not understand the resulting biochemical effects that result in morphological changes and differences in chlorophyll concentrations.

Understanding all of these physiological mechanisms will not only help us to assess ecosystem processes more completely, but they will guide us in our understanding of the evolution of bryophytes in general.

References

Alghamdi AA (2003) The effect of inorganic and organic nitrogen sources and their combination on growth and metabolism of *Vesicularia dubyana*. PhD dissertation, Michigan Technological University, Houghton

Allen ED, Spence DHN (1981) The differential ability of aquatic plants to utilize the inorganic C supply in freshwater. New Phytol 87:269–283

Andersen T, Pedersen O (2002) Interactions between light and CO_2 enhance the growth of *Riccia fluitans*. Hydrobiologia 477:163–170

Arancibia P, Graham L (2003) Carbonic anhydrase. In: Charophyceae and bryophytes. Abstract, Botany 2003, Aquatic and wetland plants: wet and wild. Botanical Society of America, Mobile, p 65

Aravena R, Warner BG, MacDonald GM, Hanf KL (1992) Carbon isotope composition of lake sediments in relation to lake productivity and radiocarbon dating. Quat Res 37:333–345

Aronsson KA, Ekelund NG (2006) Effects on growth, photosynthesis and pigments of the freshwater moss *Fontinalis antipyretica* Hedw. after exposure to wood ash solution. Sci Total Environ 372:236–246

Aronsuu I, Vuori K-M, Nieminen M (1999) Survival and growth of transplanted *Fontinalis dalecarlica* (Bryophyta) in controlled flow and short-term regulated flow sites in the Perhonjoki River, Western Finland. River Res Appl 15:87–97

Arróniz-Crespo M, Núñez-Olivera E, Martínez-Abaigar J, Tomás R (2004) A survey of the distribution of UV-absorbing compounds in aquatic bryophytes from a mountain stream. Bryologist 107:202–208

Arróniz-Crespo M, Núñez-Olivera E, Martínez-Abaigar J (2008a) Hydroxycinnamic acid derivatives in an aquatic liverwort as possible bioindicators of enhanced UV radiation. Environ Pollut 151:8–16

Arróniz-Crespo M, Phoenix G, Núñez-Olivera E, Martínez-Abaigar J (2008b) Age-specific physiological

responses to UV radiation in the aquatic liverwort *Jungermannia exsertifolia* subspecies *cordifolia* (Dumort.). In: Mohamed H, Bakar BB, Boyce AN, Yuen PLK (eds) Bryology in the new millennium: proceedings of the world bryology conference 2007, Kuala Lumpur, Malaysia. Institute of Biological Sciences, University of Malaya, Kuala Lumpur, pp 339–345

Arscott DB, Bowden WB, Finlay JC (1998) Comparison of epilithic algal and bryophyte metabolism in an arctic tundra stream, Alaska. J N Am Benthol Soc 17:210–227

Austin KA, Wieder RK (1987) Effects of elevated H+, SO4-, NO3-, and NH4+ in simulated acid precipitation on the growth and chlorophyll content of 3 North American *Sphagnum* species. Bryologist 90:221–229

Bain JT, Proctor MCF (1980) The requirement of aquatic bryophytes for free CO_2 as an inorganic carbon source: some experimental evidence. New Phytol 86:393–400

Ballesteros D, García-Sánchez MJ, Heredia MA, Felle H, Fernández JA (1998) Inorganic carbon acquisition in *Riccia fluitans* L. J Exp Bot 49:1741–1747

Barko JW, Filbin GJ (1983) Influences of light and temperature on chlorophyll composition in submersed freshwater macrophytes. Aquat Bot 15:249–255

Basile DV (1965) Growth, development and gemma formation in the liverwort *Scapania nemorosa* as influenced by L-arginine, L-histidine and L-glutamic acid. Am J Bot 52:443–454

Bates J, Phoon X (2008) Salinity tolerance and survival of the rocky-seashore bryophyte Schistidium maritimum. In: Mohamed H, Bakar BB, Boyce AN, Yuen PLK (eds) Bryology in the new millennium: proceedings of the world bryology conference 2007, Kuala Lumpur, Malaysia. Institute of Biological Sciences, University of Malaya, Kuala Lumpur, pp 327–338

Beever JE (1995) Studies of *Fissidens* (Bryophyta: Musci) in New Zealand: *F. strictus* Hook. f. and Wils. and *F. berteroi* (Mont.) C. Muell., with a discussion of aquatic adaptations. N Z J Bot 33:291–299

Bendz G, Lööf LG, Martensson O (1968) Moss pigments. 8. The carotenoids of *Fontinalis antipyretica* L. ex Hedw. Acta Chem Scand 22:2215–2218

Benstead JP, Green AC, Deegan LA, Peterson BJ, Slavik K, Bowden WB, Huryn AD, Hershey AE (2007) Recovery of three Arctic stream reaches from experimental low-level nutrient enrichment. Freshw Biol 52:1077–1089

Biehle G, Speck T, Spatz HC (1998) Hydrodynamics and biomechanics of the submerged water moss *Fontinalis antipyretica* – a comparison of specimens from habitats with different flow velocities. Bot Acta 111:42–50

Blackman FF, Smith AM (1910) Experimental researches on vegetable assimilation and respiration. IX.- On assimilation in submerged water-plants, and its relation to the concentration of carbon dioxide and other factors. Proc R Soc Biol Sci Ser B 83:389–412

Bode O (1940) Assimilation, Atmung und Plastidenfarbstoffe in verschiedenfarbigen Licht aufgezogenen *Fontinalis*-Pflanzen. Jahrb Wiss Bot 89:208–244

Bolhár-Nordenkampf H (1970) Die Wirkung von Atrizin auf den pflanzenlich Gasstoffwechsel. Biochem Physiol Pflanzen 161:110–158

Boros A (1925) Two fossil species of mosses from the diluvial lime tufa of Hungary. Bryologist 28:29–32

Boston HL, Farmer AM, Madsen JD, Adams MS, Hurley JP (1991) Light-harvesting carotenoids in two deep-water bryophytes. Photosynthetica 25:61–66

Bowden WB, Finlay JC, Maloney PE (1994) Long-term effects of PO_4 fertilization on the distribution of bryophytes in an Arctic river. Freshw Biol 32:445–454

Bowes G, Van TK, Garrard LA, Haller WT (1977) Adaptation to low light levels by *Hydrilla*. J Aquat Plant Manag 15:32–35

Brown DH (1982) Mineral nutrition. In: Smith AJE (ed) Bryophyte ecology. Chapman & Hall, London, pp 383–444

Burkholder PR (1959) Organic nutrition of some mosses growing in pure culture. Bryologist 62:6–15

Burr GO (1941) Photosynthesis of algae and other aquatic plants. In: Needham JG (ed) Symposium on hydrobiology. University of Wisconsin Press, Madison, pp 163–181

Carballeira A, Díaz S, Vázquez MD, López J (1998) Inertia and resilience in the responses of the aquatic bryophyte *Fontinalis antipyretica* Hedw. to thermal stress. Arch Environ Contam Toxicol 34:343–349

Carroll DM (2003) Bryophytes as indicators of water level and salinity change along the Northeast Cape Fear River. MS thesis, University of North Carolina at Wilmington

Cattaneo A, Fortin L (2000) Moss distribution in streams of the Quebec Laurentian Mountains. Can J Bot 78:748–752

Chambers PA, Kalff J (1985) Depth distribution and biomass of submersed aquatic macrophyte communities in relation to Secchi depth. Can J Fish Aquat Sci 42:701–709

Christmas M, Whitton BA (1998) Phosphorus and aquatic bryophytes in the Swale–Ouse river system, north-east England. 1. Relationship between ambient phosphate, internal N:P ratio and surface phosphatase activity. Sci Total Environ 110–111:389–399

Clymo RS (1963) Ion exchange in *Sphagnum* and its relation to bog ecology. Ann Bot N S 27:309–324

Clymo RS (1964) The origin of acidity in *Sphagnum* bogs. Bryologist 67:427–431

Conboy DA, Glime JM (1971) Effects of drift abrasives on *Fontinalis novae-angliae* Sull. Castanea 36:111–114

Conde-A'lvarez RM, Pérez-Rodríguez E, Altamirano M, Nieto JM, Abdala R, Figueroa FL, Flores-Moya A (2002) Photosynthetic performance and pigment content in the aquatic liverwort *Riella helicophylla* under natural solar irradiance and solar irradiance without ultraviolet light. Aquat Bot 73:47–61

Croisetière L, Hare L, Tessier A (2001) Influence of current velocity on cadmium accumulation by an aquatic moss and the consequences for its use as a biomonitor. Environ Sci Technol 35:923–927

Cruz de Carvalho R (2009) Desiccation in the aquatic bryophyte *Fontinalis antipyretica*. In: XVII Simpósio Botânica Criptogâmica, 24 Sept 2009. Abstract http://lisboa.academia.edu/RicardoCruzdeCarvalho/Talks/36958/Desiccation_in_the_aquatic_bryophyte_Fontinalis_antipyretica. 17 Aug 2011

Cruz de Carvalho R, Branquinho C, da Marques Silva J (2008) Oxygen evolution and chlorophyll fluorescence under extreme desiccation in the aquatic bryophyte *Fontinalis antipyretica*. Photosynth Energy Sun 24:1425–1430

Cruz de Carvalho R, Branquinho C, da Marques Silva J (2011) Physiological consequences of desiccation in the aquatic bryophyte *Fontinalis antipyretica*. Planta 234:195–205

Czeczuga B (1980) Investigation on carotenoids in Embryophyta. I. Bryophyta. Bryologist 83:21–28

Czeczuga B (1985) Investigations on carotenoids in Embryophyta. III. Representatives of the Hepaticae. Phyton 25:113–121

Czeczuga B, Gutkowski R, Czerpak R (1982) Investigations of carotenoids in Embryophyta. II. Musci from the Antarctic. Nova Hedw 36:695–701

Davey MC (1997) Effects of short-term dehydration and rehydration on photosynthesis and respiration by Antarctic bryophytes. Environ Exp Bot 37:187–198

Dilks TJK (1976) Measurement of the carbon dioxide compensation point and rate of loss of $^{14}CO_2$ in the light and dark in some bryophytes. J Exp Bot 27:98–104

Dilks TJK, Proctor MCF (1975) Comparative experiments on temperature responses of bryophytes: assimilation, respiration and freezing damage. J Bryol 8:317–336

Ellwood NTW, Whitton BA (2007) Phosphatase activities of the aquatic moss *Warnstorfia fluitans* (Hedw.) Loeske from an acidic stream in North-East England. Hydrobiologia 575:95–107

Ellwood NTW, Haile SM, Whitton BA (2008) Aquatic plant nutrients, moss phosphatase activities and tissue composition in four upland streams in northern England. J Hydrol 350:246–260

Emerson R, Lewis CM (1943) The dependence of the quantum yield of *Chlorella* photosynthesis on wave length of light. Am J Bot 30:165–178

Englund G, Jonsson B-G, Malmqvist B (1997) Effects of flow regulation on bryophytes in north Swedish rivers. Biol Conserv 79:79–86

Fabón G, Martínez-Abaigar J, Tomás R, Núñez-Olivera E (2010) Effects of enhanced UV-B radiation on hydroxycinnamic acid derivatives extracted from different cell compartments in the aquatic liverwort *Jungermannia exsertifolia* subsp. *cordifolia*. Physiol Plant 140:269–279

Farmer AM, Maberly SC, Bowes G (1986) Activities of carboxylation enzymes in freshwater macrophytes. J Exp Bot 37:1568–1573

Farmer AM, Boston HL, Adams MS (1988) Photosynthetic characters of a deepwater bryophyte from a clear, oligotrophic lake in Wisconsin, USA. Int Ver Theor Angew Limnol Verh 23:1912–1915

Fogg GE (1977) Aquatic primary production in the Antarctic. Proc R Soc Biol Sci Ser B 279:27–38

Fornwall MD (1978) Primary productivity of Fontinalis duriaei Schimp. and its relation to seasonal changes in stream temperature. MS thesis, Michigan Technological University, Houghton, 121 pp

Fornwall MD, Glime JM (1982) Cold and warm-adapted phases in *Fontinalis duriaei* Schimp. as evidenced by net assimilatory and respiratory responses to temperature. Aquat Bot 13:165–177

Frahm J-P (1975) Toxitoleranzversuche an Wassermoosen. Gewass Abwass 57(58):59–66

Fritz KM, Glime JM, Hribljan J, Greenwood JL (2009) Can bryophytes be used to characterize hydrologic permanence in forested headwater streams. Ecol Indic 9:681–692

Frost-Christensen H, Sand-Jensen K (1992) The quantum efficiency of photosynthesis in macroalgae and submerged angiosperms. Oecologia 91:377–384

Gaberscik A, Martincic A (1987) Seasonal dynamics of net photosynthesis and productivity of *Sphagnum papillosum*. Lindbergia 13:105–110

Gellerman JL, Anderson WH, Schlenk H (1972) Highly unsaturated lipids of *Mnium*, *Polytrichum*, *Marchantia* and *Matteuccia*. Bryologist 75:550–557

Glime JM (1971) Response of two species of *Fontinalis* to field isolation from stream water. Bryologist 74:383–386

Glime JM (1984) Theories on adaptations to high light intensity in the aquatic moss *Fontinalis*. J Bryol 13:257–262

Glime JM (1987a) Phytogeographic implications of a *Fontinalis* (Bryopsida) growth model based on temperature and flow conditions for six species. Mem N Y Bot Gard 45:154–170

Glime JM (1987b) Growth model for *Fontinalis duriaei* based on temperature and flow conditions. J Hattori Bot Lab 62:101–109

Glime JM, Acton DW (1979) Temperature effects on assimilation and respiration in the *Fontinalis duriaei-periphyton* association. Bryologist 82:382–392

Glime JM, Rohwer F (1983) The comparative effects of ethylene and 1-amino-cyclopropane-1-carboxylic acid on two species of *Fontinalis*. J Bryol 12:611–616

Glime JM, Vitt DH (1984) The physiological adaptations of aquatic Musci. Lindbergia 10:41–52

Glime JM, Nissila PD, Trynoski SE, Fornwall MD (1979) A model for attachment of aquatic mosses. J Bryol 10:313–320

Glime JM, Wetzel RG, Kennedy BJ (1982) The effects of bryophytes on succession from alkaline marsh to *Sphagnum* bog. Am Midl Nat 108:209–223

Graham LE, Kim E, Arancibia-Avila P, Graham JM, Wilcox LW (2010) Evolutionary and ecophysiological significance of sugar utilization by the peat moss *Sphagnum compactum* (Sphagnaceae) and the common charophycean associates *Cylindrocystis brebissonii* and *Mougeotia* sp. (Zygnemataceae). Am J Bot 97:1485–1491

Gupta RK (1976) Soluble constituents and photosynthetic products biosynthesized from $^{14}CO_2$ in leafy liverworts, *Plagiochila asplenioides* (L.) Dum. and *Scapania undulata* (L.) Dum. Indian J Exp Biol 14:595–598

Hanson DT, Andrews TJ, Badger MR (2002) Variability of the pyrenoid-based CO_2-concentrating mechanism in hornworts (Anthocerotophyta). Funct Plant Biol 29:407–416

Harder R (1921) Kritische Versuche zu Blackmans Theorie der begrenzenden Faktoren bei der Kohlensaureassimilation. Jahrb Wiss Bot 60:531–571

Harder R (1923) Bemerkungen über die variationsbreite des Kompensationspunktes beim Gaswechsel der Pflanzen. Ber Deutsch Bot Gesell 41:194–198

Hawes I, Anderson DT, Pollard WH (2002) Submerged aquatic bryophytes in Colour Lake, a naturally acidic polar lake with occasional year-round ice-cover. Arctic 55:380–388

Hearnshaw GF, Proctor MCF (1982) The effect of temperature on the survival of dry bryophytes. New Phytol 90:221–228

Hedenäs L (2001) Environmental factors potentially affecting character states in pleurocarpous mosses. Bryologist 104:72–91

Hoddinott J, Bain J (1979) The influence of simulated canopy light on the growth of six acrocarpous moss species. Can J Bot 57:1236–1242

Hoppe H-G, Kim S-J, Gocke K (1988) Microbial decomposition in aquatic environments: combined process of extracellular enzyme activity and substrate uptake. Appl Environ Microbiol 54:784–790

Huneck S (1983) Chemistry and biochemistry of bryophytes. In: Schuster RM (ed) New manual of bryology. Hattori Botanical Laboratory, Nichinan, pp 1–116

Ikusima I (1965) Ecological studies on the productivity of aquatic plant communities 1. Measurement of photosynthetic activity. Bot Mag Tokyo 78:202–211

Ikusima I (1966) Ecological studies on the productivity of aquatic plant communities 2. Seasonal changes in standing crop and productivity of a natural submerged community of *Vallisneria denseserrulata*. Bot Mag Tokyo 79:7–19

James WO (1928) Experimental researches on vegetable assimilation and respiration. XIX. The effect of variations of carbon dioxide supply upon the rate of assimilation of submerged water plants. Proc R Soc Biol Sci Ser B 103:1–42

Jenkins JT, Proctor MCF (1985) Water velocity, growth-form and diffusion resistances to photosynthetic CO_2 uptake in aquatic bryophytes. Plant Cell Environ 8:317–323

Johnson T (1978) Aquatic mosses and stream metabolism in a North Swedish River. Proceedings: congress in Denmark 1977 Part 3. Ver Int Ver Theor Angew Limnol 20:1471–1477

Jones RC, Walti K, Adams MS (1983) Phytoplankton as a factor in decline of the submersed macrophyte *Myriophyllum spicatum* L. in Lake Wingra, Wisconsin. Hydrobiologia 107:213–219

Kang BG, Burg SP (1972) Involvement of ethylene in phytochrome-mediated carotenoid synthesis. Plant Physiol 49:631–633

Karunen P, Aro EM (1979) Fatty acid composition of polar lipids in *Ceratodon purpureus* and *Pleurozium schreberi*. Physiol Plant 45:265–269

Keeley JE, DeNiro MJ, Sternberg LO (1986) The use of stable isotopes in the study of photosynthesis in freshwater plants. Aquat Bot 26:213–223

Kershaw KA, Webber MR (1986) Seasonal changes in the chlorophyll content and quantum efficiency of the moss Brachythecium rutabulum. J Bryol 14:151–158

Kielland K (1997) Role of free amino acids in the nitrogen economy of Arctic cryptogams. Ecoscience 4:75–79

Koskimies-Soininen K, Nyberg H (1991) Effects of temperature and light on the glycolipids of *Sphagnum fimbriatum*. Phytochemistry 30:2529–2536

Kudoh S, Kashino Y, Imura S (2003) Ecological studies of aquatic moss pillars in Antarctic lakes. 3. Light response and chilling and heat sensitivity of photosynthesis. Polar Biol 16:33–42

Lacoul P, Freedman B (2006) Environmental influences on aquatic plants in freshwater ecosystems. Environ Rev 14:89–136

LaCroix JJ (1996) Phenolics from *Fontinalis antipyretica* Hedw. and light as causes of differential distribution of *Asellus militaris* Hay in Gooseneck Creek. Unpublished MS thesis, Michigan Technological University, Houghton

Larcher W (1983) Physiological plant ecology, corrected printing of the second printing. Springer, New York

Li Y, Glime JM, Drummer TD (1993) Effects of phosphorus on the growth of *Sphagnum magellanicum* Brid. and *S. papillosum* Lindb. Lindbergia 18:25–30

Lodge E (1960) Studies of variation in British material of *Drepanocladus fluitans* and *Drepanocladus exannulatus*. II. An experimental study of the variation. Svensk Bot Tidskr 54:387–393

Loeske L (1922) Bryologische Notizen. Herbarium 61:121–123; 62:129–132

Loeske L (1926) Der Einfluss des Wassers auf Papillen und Mamillen. Folia Cryptogam Z Erforsch ungarischen Kryptogamenflora 1:215–219

López J, Carballiera J (1989) A comparative study of pigment contents and response to stress in five species of aquatic bryophytes. Lindbergia 15:188–194

Lovalvo D, Clingenpeel SR, McGinnis S, Macur RE, Varley JD, Inskeep WP, Glime J, Nealson K, McDermott TR (2010) A geothermal-linked biological oasis in Yellowstone Lake, Yellowstone National Park, Wyoming. Geobiology 8:327–336

Maberly SC (1985a) Photosynthesis by *Fontinalis antipyretica*. I. Interactions between photon irradiance, concentration of carbon dioxide and temperature. New Phytol 100:127–140

Maberly SC (1985b) Photosynthesis by *Fontinalis antipyretica*. II. Assessment of environmental factors limiting photosynthesis and production. New Phytol 100:141–155

Madsen TV, Enevoldsen HO, Jorgensen TB (1993) Effects of water velocity on photosynthesis and dark respiration in submerged stream macrophytes. Plant Cell Environ 16:317–322

Marschall M, Proctor MCF (2004) Are bryophytes shade plants? Photosynthetic light responses and proportions of chlorophyll *a*, chlorophyll *b* and total carotenoids. Ann Bot 94:593–603

Martin CE, Adamson VJ (2001) Photosynthetic capacity of mosses relative to vascular plants. J Bryol 23:319–323

Martin CE, Churchill SP (1982) Chlorophyll concentrations and *a*/*b* ratios in mosses collected from exposed and shaded habitats in Kansas. J Bryol 12:297–304

Martinez-Abaigar J, Núñez-Olivera E (1998) Ecophysiology of photosynthetic pigments in aquatic bryophytes. In: Bates JW, Ashton NW, Duckett JG (eds) Bryology for the twenty-first century. Maney Publishing and the British Bryological Society, Leeds, pp 277–292

Mártínez-Abaigar J, Núñez-Olivera E, Sánchez-Díaz M (1993) Effects of organic pollution on transplanted aquatic bryophytes. J Bryol 17:553–566

Mártínez-Abaigar J, Núñez-Olivera E, Sánchez-Díaz M (1994) Seasonal changes in photosynthetic pigment composition of aquatic bryophytes. J Bryol 18:97–113

Martínez-Abaigar J, Nuñez-Olivera E, Beaucourt N, Garcia-Alvaro MA, Tomas R, Arróniz M (2003) Different physiological responses of two aquatic bryophytes to enhanced ultraviolet-B radiation. J Bryol 25:17–30

McCall KK, Martin CE (1991) Chlorophyll concentrations and photosynthesis in three forest understory mosses in northeastern Kansas. Bryologist 94:25–29

McIntire CD, Phinney HK, Larson GL, Buktenica M (1994) Vertical distribution of a deep-water moss and associated epiphytes in Crater Lake, Oregon. Northwest Sci 68(1):11–21

McKane R, Johnson L, Shaver G, Nadelhoffer K, Fry B, Rastetter E, Giblin A, Laundre J (1993) Differentiation in uptake of ^{15}N by depth, season, and chemical form in an arctic tussock tundra plant community. In: Proceedings of the 78th annual ESA meeting, 31 July–4 Aug 1993. Bull Ecol Soc Amer Program and Abstracts, vol 74(Suppl 2), p 354

McMillan C, Phillips RC (1979) Differentiation in habitat response among populations of new world seagrasses. Aquat Bot 7:185–196

Meyer JL (1979) The role of sediments and bryophytes in phosphorus dynamics in a headwater stream ecosystem. Limnol Oceanogr 24:365–375

Miler O, Albayrak I, Nikora V, Crane T, O'Hare M (2010) Biomechanics of aquatic plants and its role in flow-vegetation interactions. In: Dittrich A, Kill K, Aberle J, Geisenhainer P (eds) River flow 2010. Bundesanstalt fur Wasserbau, Karlsruhe, pp 245–251

Miyazaki T, Satake K (1985) In situ measurements of uptake of inorganic carbon and nitrogen by the aquatic liverworts *Jungermannia vulcanicola* Steph. and *Scapania undulata* (L.) Dum. in an acid stream, Kashiranashigawa, Japan. Hydrobiologia 124:29–34

Naiman RJ (1983) The annual pattern and spatial distribution of aquatic oxygen metabolism in boreal forest watersheds. Ecol Monogr 53:73–94

Naiman RJ, Sedell JR (1980) Relationships between metabolic parameters and stream order in Oregon. Can J Fish Aquat Sci 37:834–847

Norris L, Norris RE, Calvin M (1954) A survey of the rates and products of short-term photosynthesis in plants of 9 phyla. Lawrence Berkeley National Laboratory, 22 pp. http://escholarship.org/uc/item/3dp3827f. 17 Aug 2011

Núñez-Olivera E, Martínez-Abaigar J, Tomás R, Beaucourt N, Arróniz-Crespo M (2004) Influence of temperature on the effects of artificially enhanced UV-B radiation on aquatic bryophytes under laboratory conditions. Photosynthetica 42:201–212

Núñez-Olivera E, Arróniz-Crespo M, Martínez-Abaigar J, Tomás R, Beaucourt N (2005) Assessing the UV-B tolerance of sun and shade samples of two aquatic bryophytes using short-term tests. Bryologist 108:435–448

Núñez-Olivera E, Otero S, Tomás R, Martínez-Abaigar J (2009) Seasonal variations in UV-absorbing compounds and physiological characteristics in the aquatic liverwort *Jungermannia exsertifolia* subsp. *cordifolia* over a 3-year period. Physiol Plant 136:73–85

Núñez-Olivera E, Otero S, Tomás R, Fabón G, Martínez-Abaigar J (2010) Cyclic environmental factors only partially explain the seasonal variability of photoprotection and physiology in two mosses from an unforested headwater stream. Bryologist 113:277–291

Ochyra R, Lightowlers PJ (1988) The south Georgian moss flora: *Vittia*. Br Antarct Surv Bull 80:121–127

Osmond CB, Valanne N, Haslam SM, Votila P, Roksandic Z (1981) Comparisons of $\delta^{13}C$ values in leaves of aquatic macrophytes from different habitats in Britain and Finland: some implications for photosynthetic processes in aquatic plants. Oecologia 50:117–124

Otero S, Núñez-Olivera E, Martínez-Abaigar J, Tomás R, Arróniz-Crespo M, Beaucourt N (2006) Effects of cadmium and enhanced UV radiation on the physiology and the concentration of UV-absorbing compounds of the aquatic liverwort *Jungermannia exsertifolia* subsp. *cordifolia*. Photochem Photobiol Sci 5:760–769

Patidar KC, Solanki CM, Kaul A (1986) Chlorophyll concentration and a/b ratios in response to habitats in three species of *Riccia*. Yushania 3(4):1–4

Peñuelas J (1984) Pigments of aquatic mosses of the river Muga, NE Spain, and their response to water pollution. Lindbergia 10:127–132

Peñuelas J (1985) HCO_3^- as an exogenous carbon source for aquatic bryophytes *Fontinalis antipyretica* and *Fissidens grandifrons*. J Exp Bot 36:441–448

Peñuelas J, Murillo J, Azcón-Bieto J (1988) Actual and potential dark respiration rates and different electron transport pathways in freshwater aquatic plants. Aquat Bot 30:115–128

Plaetzer H (1917) Untersuchungen über die Assimilation und Atmung von Wasserpflanzen. Phys Med Gesel (Wurzburg) 45:31–101

Priddle J (1979) Morphology and adaptation of aquatic mosses in an Antarctic lake. J Bryol 10:517–531

Priddle J (1980) The production ecology of benthic plants in some Antarctic lakes. 1. *In situ* production studies. J Ecol 68:141–153

Prins HBA, Elzenga JTM (1989) Bicarbonate utilization: function and mechanism. Aquat Bot 34:59–83

Proctor MCF (1984) Structure and ecological adaptation. In: Dyer AF, Duckett JG (eds) The experimental biology of bryophytes. Academic, New York, pp 9–37

Proctor MCF, Smirnoff N (2011) Ecophysiology of photosynthesis in bryophytes: major roles for oxygen photoreduction and non-photochemical quenching? Physiol Plant 141:130–140

Rastorfer JR (1971) Effects of temperature on carbon dioxide compensation points of the moss *Drepanocladus uncinatus*. Antarct J U S 6(5):162–163

Raven JA (1991) Implications of inorganic carbon utilization: ecology, evolution, and geochemistry. Can J Bot 69:908–924

Raven JA, MacFarlane JJ, Griffiths H (1987) The application of carbon isotope techniques. In: Crawford RMM (ed) Plant life in aquatic and amphibious habitats. British Ecological Society Special Symposium, Blackwell, Oxford, pp 129–144

Raven JA, Johnson AM, Newman JR, Scrimgeour CM (1994) Inorganic carbon acquisition by aquatic photolithotrophs of the Dighty Burn, Angus, UK: uses and limitations of natural abundance measurements of carbon isotopes. New Phytol 127:271–286

Raven JA, Griffiths H, Smith EC, Vaughn KC (1998) New perspectives in the biophysics and physiology of bryophytes. In: Bates JW, Ashton NW, Duckett JG (eds) Bryology in the twenty-first century. Maney Publishing and the British Bryological Society, Leeds, pp 261–275

Rice SK (1995) Patterns of allocation and growth in aquatic *Sphagnum* species. Can J Bot 73:349–359

Rice SK, Schuepp PH (1995) On the ecological and evolutionary significance of branch and leaf morphology in aquatic *Sphagnum* (Sphagnaceae). Am J Bot 82:833–846

Riis T, Sand-Jensen K (1997) Growth reconstruction and photosynthesis of aquatic mosses: influence of light, temperature and carbon dioxide at depth. J Ecol 85:359–372

Rincòn E (1993) Growth responses of six bryophyte species to different light intensities. Can J Bot 71:661–665

Robel RJ (1961) Water depth and turbidity in relation to growth of sago pond weed. J Wildl Manag 25:436–438

Rosswall T, Granhall U (1980) Nitrogen cycling in a subarctic ombrotrophic mire. In: Sonesson M (ed) Ecology of a subarctic mire. Ecol Bull (Stockholm) 30:209–234

Rudolph HJ, Voigt JU (1986) Effects of NH4+-N and NO3-N on growth and metabolism of Sphagnum magellanicum. Physiol Plant 66:339–343

Ruttner F (1947) Zur Frage der Karbonatassimilation der Wasserpflanzen, I. Österr Bot Zeits 94:265–294

Ruttner F (1955) Zur Okologie tropischer Wassermoose. Arch Hydrobiol 21(Suppl):343–381

Salvucci ME, Bowes G (1981) Induction of reduced photorespiration activity in submersed and amphibious aquatic macrophytes. Plant Physiol 67:335–340

Sand-Jensen K, Madsen TV (1991) Minimum light requirements of submerged freshwater macrophytes in laboratory growth experiments. J Ecol 79:749–764

Sandquist DR, Keeley JE (1990) Carbon uptake characteristics in two high elevation populations of the aquatic CAM plant *Isoetes bolanderi* (Isoetacae) [sic]. Am J Bot 77:682–688

Sanford GR, Bayer DE, Knight AW (1974) An evaluation of environmental factors affecting the distribution of two aquatic mosses in the Sacramento River near Anderson, California University Departments of Botany, Water Science Engineering

Schmidt-Stohn G (1977) Aenderungen der plastidenpigmente bei *Sphagnum magellanicum* Brid. in Abhaengigkeit von Standort, Verfaerbungsgrad und Alter. Z Pflanzenphysiol 81:289–303

Schuler JF, Diller VM, Fulford M, Kerstein HJ (1955) Plant Physiol 30:478–482

Schuurkes JAAR, Kok CJ, Den Hartog C (1986) Ammonium and nitrate uptake by aquatic plants from poorly buffered and acidified waters. Aquat Bot 24:131–146

Schwarz A-M, Markager S (1999) Light absorption and photosynthesis of a benthic moss community: importance of spectral quality of light and implications of changing light attenuation in the water column. Freshw Biol 42:609–623

Schwoerbel J, Tillmanns GC (1964) Untersuchungen über die Stoffwechseldynamik in Fliebgewassern. Arch Hydrobiol 28(Suppl):259–267

Schwoerbel J, Tillmanns GC (1972) Adaptation to ammonia in situ by submerged macrophytes. Arch Hydrobiol 42(Suppl):139–141

Schwoerbel J, Tillmanns GC (1974) Assimilation of nitrogen from the medium and nitrate reductase activity in submerged macrophytes: *Fontinalis antipyretica* L. Arch Hydrobiol 47(Suppl):282–294

Schwoerbel J, Tillmanns GC (1977) Uptake of nitrate from the water and activity of nitrate reductase by *Fontinalis antipyretica* L. under light and dark conditions. Arch Hydrobiol 48(Suppl):412–423

Sharma KK, Diller VM, Fulford M (1960) Studies on the growth of *Haplomitrium*. II. Media containing amino acids. Bryologist 63:203–212

Shaw AJ, Allen B (2000) Phylogenetic relationships, morphological incongruence, and geographic speciation in the Fontinalaceae (Bryophyta). Mol Phylogenet Evol 16:225–237

Simola LK (1975) The effect of several protein amino acids and some inorganic nitrogen sources on the growth of *Sphagnum nemoreum*. Physiol Plant 35:194–199

Simola LK (1979) Dipeptide utilization by *Sphagnum fimbriatum*. J Hattori Bot Lab 46:49–54

Smith EC, Griffiths H (1996) The occurrence of the chloroplast pyrenoid is correlated with the activity of a CO_2-concentrating mechanism and carbon isotope discrimination in lichens and bryophytes. Planta 198:6–16

Søndergaard M, Sand-Jensen K (1979) The delay in ^{14}C fixation rates by three submerged macrophytes, a source of error in the ^{14}C technique. Aquat Bot 6:111–119

Soudzilovskaia NA, Cornelissen JHC, During HJ, van Logtestijn RSP, Lang SI, Aerts R (2010) Similar cation exchange capacities among bryophyte species refute a presumed mechanism of peatland acidification. Ecology 91:2716–2726

Spence DHN (1976) Light and plant response in freshwater. In: Evans RB, Rockham O (eds) Light as an ecological factor: II. 16th symposium of the British Ecological Society, March 1974. Blackwell Scientific Publications, Oxford, pp 93–133

Spence DHN, Dale HM (1978) Variations in the shallow water form of *Potamogeton richardsonii* induced by some environmental factors. Freshw Biol 8:251–268

Spitale D, Petraglia A (2010) *Palustriella falcata* (Brid.) Hedenäs (Amblystegiaceae, Bryopsida) with pluristratose lamina: morphological variability of specimens in springs of the Italian Alps. Plant Syst Evol 286:59–68

Steeman Nielsen E (1947) Photosynthesis of aquatic plants with special reference to the carbon source. Dansk Bot Ark 12(8):1–71

Steeman Nielsen E, Kristiansen J (1949) Carbonic anhydrase in submersed autotrophic plants. Physiol Plant 2:325–331

Stephenson SL, Studlar SM, McQuattie CJ, Edwards PJ (1995) Plant and environment interactions. Effects of acidification on bryophyte communities in West Virginia mountain streams. J Environ Qual 24:116–125

Taylor AO, Jesper NM, Christeller JT (1972) Plants under climatic stress. III. Low temperature, high light effects on photosynthetic products. Plant Physiol 47:798–802

Titus JE, Wagner DJ, Stephens MD (1983) Contrasting water relations of photosynthesis for two *Sphagnum* mosses. Ecology 64:1109–1115

Tremp H (2003) Ecological traits of aquatic bryophytes and bioindication. http://www.uni-hohenheim.de/www320/german/homepages/horst/image/pdfs/aquatic_bryophytes.pdf. 31 Oct 2003

Tuba Z (1987) Light, temperature and desiccation responses of CO_2-exchange in the desiccation-tolerant moss, *Tortula ruralis*. Symp Biol Hung 35:137–149

Ueno T, Kanda H (2006) Photosynthetic response of the arctic semi-aquatic moss *Calliergon giganteum* to water content. Aquat Bot 85:241–243

Valanne N (1984) Photosynthesis and photosynthetic products in mosses. In: Dyer AF, Duckett JG (eds) The experimental biology of bryophytes. Academic, New York, pp 257–274

Vanderpoorten A (2000) Hydrochemical determinism and molecular systematics in the genus *Amblystegium* (Musci). Application to the biomonitoring of surface waters. Dissertation de docteur en sciences agronomiques et ingénierie biologique, Faculté Universitaire des Sciences Agronomiques de Gembloux, Gembloux, Belgium

Vanderpoorten A, Klein J-P, Stieperaere H, Trémolières M (1999) Variations of aquatic bryophyte assemblages in the Rhine Rift related to water quality. I. The Alsatian Rhine floodplain. J Bryol 21:17–23

Vitt DH, Glime JM, LaFarge-England C (1986) Bryophyte vegetation and habitat gradients of montane streams in western Canada. Hikobia 9:367–385

Wagner DH, Christy JA, Larson DW (2000) Deep-water bryophytes from Waldo Lake, Oregon. Lake Reserv Manag 16:91–99

Watson W (1919) The bryophytes and lichens of fresh-water. J Ecol 7:71–83

Westlake DF (1971) Water plants and the aqueous environment. Biol Hum Aff 36:10

Westlake DF (1981) Temporal changes in aquatic macrophytes and their environment. In: Hoestlandt H (ed) Dynamique de populations et qualité de l'eau, Actes Symp Inst Ecol Bassin Somme, Chantilly, 1979. Gauthier-Villars, Paris, pp 110–138

Wetzel RG, Brammer ES, Lindström K, Forsberg C (1985) Photosynthesis of submersed macrophytes in acidified lakes: II. Carbon limitation and utilization of benthic CO_2 sources. Aquat Bot 22:107–112

Wheeler WN (1988) Algal productivity and hydrodynamics – a synthesis. Prog Phycol Res 6:23–58

Whitton BA, Al-Shehri AM, Ellwood NTW, Turner BL (2005) Chapter 10. Ecological aspects of phosphatase activity in Cyanobacteria, eukaryotic algae and bryophytes. In: Turner BL, Frossard E, Baldwin DS (eds) Organic phosphorus in the environment. CAB International, Wallingford, pp 205–242

Wiginton JR, McMillan C (1979) Chlorophyll composition under controlled light conditions as related to the distribution of seagrasses in Texas and the U.S. Virgin Islands. Aquat Bot 6:171–184

Wilkinson SM, Ormerod SJ (1994) The effect of catchment liming on bryophytes in upland Welsh streams, with an assessment of the communities at risk. Aquat Conserv Mar Freshw Ecosyst 4(4):297–306

Yang C-M, Hsu J-C, Shih C-F (1994) Response of chlorophyll a/b ratios in Yuan-Yang Lake bryophytes to the alteration of light intensity. Proc Natl Sci Counc Repub China Part B, Life Sci 18(3):134–137

Yeoh H-H, Badger MR, Watson L (1981) Variations in kinetic properties of ribulose-1,5-bisphosphate carboxylases among plants. Plant Physiol 67:1151–1155

Ylla I, Romaní AM, Sabater S (2007) Differential effects of nutrients and light on the primary production of stream algae and mosses. Fundam Appl Limnol/Arch Hydrobiol 170:1–10

Zastrow E (1934) Experimentelle Studien aber die Anpassung von Wasser-und Sumpfmoosen. Pflanzenforschung 17:1–70

Chapter 13

Physiological Ecology of Peatland Bryophytes

Tomáš Hájek*
Institute of Botany, Academy of Sciences of the Czech Republic, Dukelská 135, 379 82 Třeboň, Czech Republic
Faculty of Science, University of South Bohemia, Branišovská 31, 370 05 České Budějovice, Czech Republic

Summary

Bryophytes, notably mosses of the genus *Sphagnum*, are significant and essential primary producers in peatlands. Peatland bryophytes face specific physical conditions; they are exposed to direct sunlight, but due to their permanent hydration they do not escape by drying as typical xeric bryophytes of open habitats. Being desiccation avoiders they are actually sensitive to drought. During photosynthesis, hydration increases the diffusion resistance to CO_2, which can be supplied also from respiration in the underlying peat. The distance to the water table affects the degree of hydration, but also influences nutrient availability as mineral nutrients can be carried in capillary water. Consequently, gradients of nutrient and water availability are related in peatlands and their variation in addition to light maintains bryophyte species diversity in peatlands. Habitats with low stress intensity, typically forested peatlands and wet microhabitats of open bogs and fens, host mosses with competitive life strategies, characterized by high rates of photosynthesis, growth and production. In contrast,

*Author for correspondence, e-mail: hajek@botany.cas.cz

D.T. Hanson and S.K. Rice (eds.), Photosynthesis in Bryophytes and Early Land Plants, Advances in Photosynthesis and Respiration 37, pp. 233–252, DOI 10.1007/978-94-007-6988-5_13,

mosses inhabiting sun-exposed, nutrient poor microhabitats, typically hummocks, must cope with low water availability and photodamage. Their stress-tolerance/avoidance strategy is reflected by slow photosynthetic and growth rates, and allocation to water holding tissues.

In this chapter, I review the effects of ecologically relevant (stress) factors affecting photosynthesis and growth, especially in *Sphagnum*. Potential consequences of global climate change are also discussed. I mention how the non-uniform experimental conditions used in photosynthetic gas exchange measurements may affect the diffusion resistance to CO_2 and consequent estimates of photosynthesis and evaporation. Suggestions for further research are proposed.

I. Introduction

This chapter deals with bryophyte ecophysiology in temperate to sub-arctic peatlands. These habitats are characterized by the abundance of mosses, which are often the key primary producers. Their biomass undergoes very slow decomposition and thus accumulates as peat. The dominance of mosses and peat accumulation are conditioned by high water level near the surface. If groundwater feeds the peatland surface, minerotrophic fen vegetation develops. The concentration of dissolved ions, particularly calcium and bicarbonate greatly determines the vegetation composition, classifying fens from poor up to extremely rich. If rainwater is the exclusive source of nutrients, namely where the accumulated peat isolates the surface from groundwater, ombrotrophic bog vegetation prevails. The high soil water level in fens and bogs prevents development of dense tree cover so living (peat accumulating) peatlands are often sunny habitats. For bryophytes that dominate the peatland surface, the excess or shortage of water, nutrients and/or light are the main environmental factors limiting photosynthetic light harvesting, CO_2 fixation, growth and production. These limiting factors drive differentiation of ecological niches and consequently bryophyte species diversity in peatlands (Rydin and Jeglum 2006; Vitt and Wieder 2009).

Mosses of the genus *Sphagnum* (peat mosses, sphagna) are the most characteristic representatives of peatlands. Due to their ecological success in these ecosystems, which is based on their unique morphology, physiology and chemistry, they are likely the most successful plant genus in the world, at least in terms of biomass (Clymo and Hayward 1982). It is then not surprising that sphagna have been the subject of intensive research, making them one of the most investigated bryophyte genera. Therefore, the majority of this chapter is devoted to *Sphagnum* ecophysiology.

II. Specific Adaptations of Peatland Bryophytes

A. Microtopography Gradients

Differences in water availability and its chemical composition lead to differentiation not only among bogs and various types of fens, but they also represent the key ecological factors characterizing within-mire differentiation of surface microforms, such as bog pools, wet hollows and carpets, mesophytic lawns, and elevated ombrotrophic hummocks. Species' ecological niches have differentiated along this so-called hummock–hollow gradient. Hummock-forming sphagna usually belong to the section Acutifolia while typical hollow species belong to the section

Abbreviations: A – photosynthetic CO_2 assimilation (rate); E – evaporation rate; F_v/F_m – maximum quantum yield of PSII photochemistry; NPQ – non-photochemical quenching of chlorophyll fluorescence; QY PSII – quantum yield of PSII photochemistry; PPFD – photosynthetic photon flux density; PSII – photosystem II; RETR – relative electron transport rate; RH – relative air humidity; WC – water content

Cuspidata. There are general morphological, ecological and physiological differences between sections (Rydin 1993; Rydin et al. 2006). *Sphagnum* species dominating in hollows have a capacity for higher rates of photosynthesis, growth and production. Hollow species, if not limited by drought, may utilize their greater photosynthetic capacity for growth and competition, taking advantage of the relatively rich nutrients and low acidity. Moreover, Cuspidata species also have greater potential to assimilate experimental additions of nitrogen (N) and CO_2 for biomass production (Jauhiainen et al. 1998a, b). In hummocks, however, individual shoots must cooperate in water retention by forming tight cushions and by reducing surface roughness to limit evaporation (Clymo 1973). Individuals that are capable of quicker growth cannot overgrow their neighbors since their growth is regulated by desiccation. This phenomenon is best seen where individual shoots of species from the section Cuspidata penetrate between hummock sphagna, supported by their high capacity of water retention and conduction. They may protrude above the hummock surface only during wet periods but they desiccate quickly during drier periods (Rydin 1985; Rydin and McDonald 1985; Robroek et al. 2007a).

Similarly, Rice et al. (2008) described a principal trade-off between sphagna with higher and lower metabolic cost. High metabolic cost, represented particularly by high investment in water holding capacity, such as in hummock sphagna, is characteristic of species tolerating environmental stress. On the contrary, species with metabolically inexpensive tissue experiencing low stress, such as those in hollows, are capable of efficient photosynthetic and growth rates, having also greater vertical distribution of photosynthesis by allowing deeper light penetration. In addition, Laine et al. (2011) observed typical ruderal life strategy (*sensu* Grime 1977) in *S. fimbriatum* (section Acutifolia). This pioneer species with low competitive but great dispersion potential formed low hummocks on shallow peat in successionally young mires, which are characterized by high water level and nutrient availability. Photosynthetic, growth and production rates of *S. fimbriatum* even exceeded those in hollow species in successionally older mires.

B. Water and CO_2 Economy

Sphagna have a unique competitive strategy among bryophytes. Due to their large capacity to store external capillary water, *Sphagnum* shoots remain photosynthetically active for longer than shoots of other bryophyte groups, particularly in sun-exposed habitats. As a typical trade-off, this competitive advantage brings costs based in great resistance for CO_2 diffusion due to thick water films of external water. Since the C_3 type of moss photosynthesis is not equipped with a CO_2 concentrating mechanism (Bain and Proctor 1980; Raven 1991), the high CO_2 diffusion resistance limits CO_2 fixation by the Calvin–Benson cycle, increasing the need to utilize excess excitation energy.

Sphagnum leaves are unistratose, consisting of a single cell layer. The cells are organized into a mesh of living chlorophyllous cells interposed by larger empty hyaline cells. The hyaline cells form from 60 % (in aquatic habitats) up to 95 % of the leaf volume (Rice 1995; Rice et al. 2008) and are normally filled by capillary water keeping the adjacent chlorophyllous cells turgid. The leaves overlap along the central branch so the spaces between the leaves and branches form another fraction of capillary water. The developing branches in the shoot apex compose the capitulum, which is the growing shoot segment and also the main shoot part exposed to sun and free atmosphere. Below capitula, branches elongate and the leaves senesce if they become too shaded by capitula above; this usually happens at the depth of 1–10 cm below the capitulum surface, depending on the capitulum and shoot bulk density (leaf area index) but it is common that 99.9 % of light is absorbed within top 3 cm (Rice et al. 2008; Robroek et al. 2009; see also Chap. 9).

It is evident that the organization of the *Sphagnum* photosynthetic tissue differs from leaves of tracheophytes, which are arranged above the ground often exposed to turbulent air that reduces the laminar boundary layer and thus the diffusion resistance for CO_2; moreover air-filled leaf mesophyll allows quick CO_2 diffusion to photosynthesizing cells. In contrast, the ground moss cover or its individual leaves are sunk within a thick laminar boundary layer and the water films further reduce CO_2 diffusion by factor of 10^4. As a result, CO_2 availability limits the rate of photosynthetic assimilation of CO_2 (*A*) in hydrated mosses, particularly sphagna. The CO_2 limitation by diffusive resistance through water films in *Sphagnum* is evidenced by the existence of distinct optimum of water content (WC) for *A* (see below), by lack of this optimum after sufficient increase of CO_2 availability (Silvola 1990; Jauhiainen and Silvola 1999), and by ^{13}C-enrichment of biomass in water-saturated sphagna caused by low discrimination against $^{13}CO_2$ by diffusion towards Rubisco (Williams and Flanagan 1996; Loisel et al. 2009).

The rate of *A* is therefore constrained by shoot WC from both directions: too low WC is accompanied by water loss from hyaline and chlorophyllous cells, turgor loss and inhibition of cellular biochemistry, while excessive water reduces carboxylation in the Calvin–Benson cycle by CO_2 shortage. This indicates that the optimum shoot WC is relatively narrow, representing a state between low and high WC. Fully turgid *Sphagnum* capitula free of capillary water contain about 1.7–2.4 g H_2O g^{-1} of shoot dry mass, depending on the species (Hájek and Beckett 2008), which is the theoretical but unreachable optimum WC for CO_2 diffusion and thus photosynthesis. Published values of optimum WC measured either in the field or laboratory conditions lie in a wide range between 5 and 30 g g^{-1} (Table 13.1).

There are few data on the optimum WC for photosynthesis of non-*Sphagnum* peatland mosses, but the general relationship described above is valid for all poikilohydric plants. Among bryophytes, Polytrichaceae have low water holding capacity that corresponds with their low optimum WC for photosynthesis (below 5 g g^{-1}; Skre and Oechel 1981; Silvola 1991). Their leaves possess lamellae protected by surface waxes from filling the interlamellar spaces with water; lamellae enhance CO_2 exchange by a factor of 6 or more (Proctor 2005). Polytrichaceae may afford maintaining low WC since they have efficient internal conducting tissue (Skre et al. 1983a) and tolerance to desiccation (Proctor et al. 2007a). Therefore they may replace sphagna on high hummocks (*Polytrichum strictum*) or they may form the uppermost moss layer above carpet of *Sphagnum* capitula in forested mires (*P. commune*). Optimum WC of feather mosses in forested mires is clearly lower than that for sphagna, ranging between 3–4 and 6–7 g g^{-1} for *Hylocomium splendens* and *Pleurozium schreberi* respectively (Skre and Oechel 1981; Silvola 1991). On the other hand, variable optimum WC was reported in semiaquatic brown mosses in fens: 16 g g^{-1} in *Calliergon giganteum* and about 5 g g^{-1} in *C. sarmentosum* (*Warnstorfia sarmentosa*) (Ueno and Kanda 2006; Oechel and Collins 1976, respectively).

Most of the variability in the optimum WC for photosynthesis between (and partly also within) the published works originates from incomparable measurement conditions. In *Sphagnum*, capitula usually represent the majority of sample biomass used in CO_2 assimilation measurements. As in the field, the water evaporates predominantly from the capitulum surface so the uppermost leaves lose the capillary water first, being replaced by air. Capitula become relatively well supplied by CO_2 but the chlorophyllous cells have already lost some water. In the field, the moss carpet evaporates water relatively slowly and the lost water is continuously replenished by capillary transport from the deeper water-saturated peat. In a ventilated gas exchange chamber, isolated apical stem segments may dry relatively rapidly, missing the continuous water replenishment. The uppermost layer may dry and become photosynthetically

Table 13.1. Optimum shoot water content (WC) for photosynthetic CO_2 assimilation measured for various Sphagnum species under close-to-ambient CO_2 concentration using various gas-exchange apparatuses.

Water content (g_{H_2O} g_{dm}^{-1})	*Sphagnum* species	References
6–10	*S. angustifolium*	Murray et al. (1989)
10–12	*S. angustifolium*	Silvola and Aaltonen (1984)
12–16	*S. angustifolium*	T. Hájek, unpublished (in part Hájek et al. 2009)
7	*S. balticum*	Rydin and McDonald (1985)
9	*S. balticum*	Schipperges and Rydin (1998)
7	*S. capillifolium*	Titus et al. (1983)
9	*S. capillifolium*	Titus and Wagner (1984)
7–10	*S. capillifolium*	Silvola (1991)
≤15	*S. capillifolium*	Gerdol et al. (1996)
11–13	*S. centrale*	Silvola (1991)
14–25	*S. cristatum*	Maseyk et al. (1999)
≥30	*S. cuspidatum*	Robroek et al. (2009)
7–8	*S. cuspidatum*	Schipperges and Rydin (1998)
5	*S. fallax*	Titus et al. (1983)
7	*S. fallax*	Titus and Wagner (1984)
10–17	*S. fallax*	T. Hájek, unpublished (in part Hájek et al. 2009)
≤15	*S. fallax*	Gerdol et al. (1996)
14–22	*S. flexuosum*	T. Hájek, unpublished (in part Hájek et al. 2009)
6	*S. fuscum*	Silvola (1990)
≥7	*S. fuscum*	Rydin and McDonald (1985)
7–9	*S. fuscum*	Silvola and Aaltonen (1984)
8	*S. fuscum*	Schipperges and Rydin (1998)
8–13	*S. fuscum*	Jauhiainen and Silvola (1999)
5–7	*S. magellanicum*	T. Hájek, unpublished (in part Fig. 13.1)
9	*S. magellanicum*	Schipperges and Rydin (1998)
11–13	*S. magellanicum*	Silvola (1991)
12–16	*S. magellanicum*	T. Hájek, unpublished (in part Hájek et al. 2009)
13–25	*S. magellanicum*	T. Hájek, unpublished (in part Hájek et al. 2009)
≤15	*S. magellanicum*	Gerdol et al. (1996)
24–30	*S. magellanicum*	Robroek et al. (2009)
27	*S. magellanicum*	Rudolph (1968)
6–12	*S. papillosum*	T. Hájek, unpublished (in part Fig. 13.2)
8–13	*S. papillosum*	Schipperges and Rydin (1998)
10–20	*S. papillosum*	T. Hájek, unpublished (in part Hájek et al. 2009)
12–21	*S. rubellum*	Robroek et al. (2009)
10–13	*S. russowii*	T. Hájek, unpublished (in part Hájek et al. 2009)
6–8	*S. squarrosum*	Murray et al. (1989)
6–9	*S. subsecundum*	Skre and Oechel (1981)
8	*S. tenellum*	Rydin and McDonald (1985)
7–8	*S. teres*	Van Gaalen et al. (2007)
6–8	*S.* sec. Acutifolia	Williams and Flanagan (1996)

The within-reference variability can be usually attributed to species differences while the contrasting WC optima among references represent various gas exchange set-ups. As a result, measured optimum WC, for example, in *S. magellanicum* may vary as much as between 5 and 30 g g^{-1}

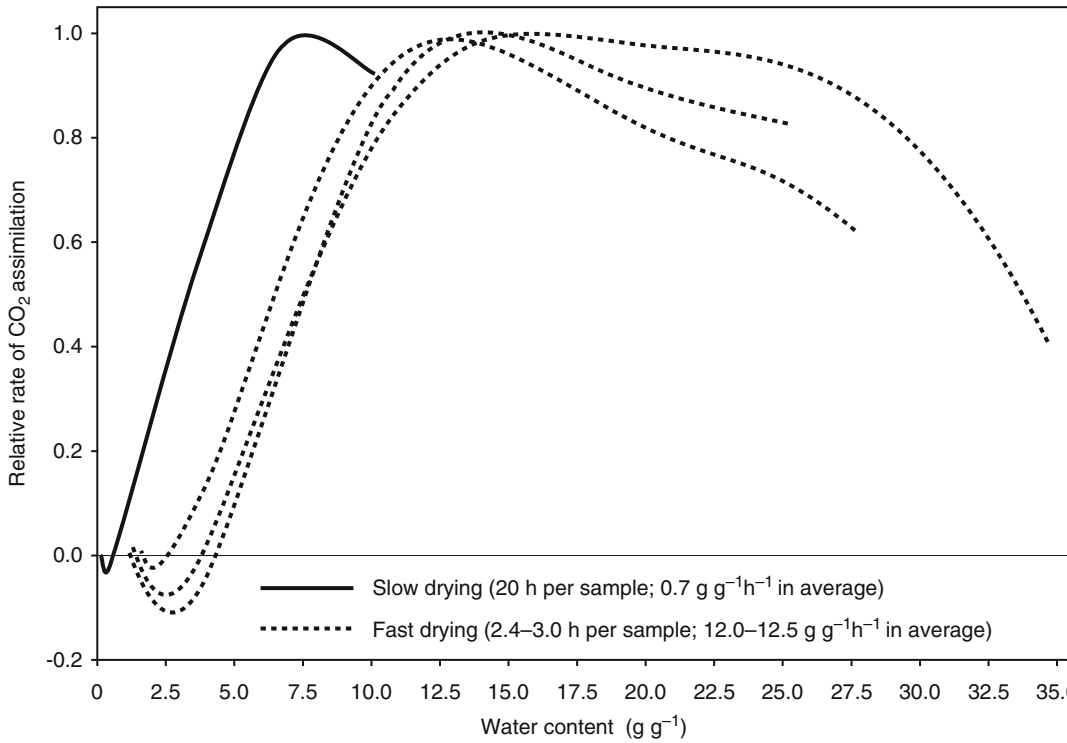


Fig. 13.1. Photosynthetic CO_2 assimilation rate of *Sphagnum magellanicum* capitula (10 mm apical shoot segments) in relation to capitulum water content. Photosynthetic and evaporation rates were continuously measured under saturation irradiance by infra-red gas analyzers (GFS-3000, Heinz Walz GmbH, Germany for low drying and LI-6400, Li-Cor Inc., USA for fast drying) while the samples were desiccated inside the gas-exchange chamber. The water content was then reconstructed using the evaporation data. The example demonstrates that the instrumental set-up may greatly affect the desiccation rate and therefore also the optimum and compensation water content for photosynthesis.

inefficient while the leaves deeper inside the capitula are still water saturated. Experimental settings used for measurements of *A*, such as airflow, speed of the internal chamber fan, or incoming relative air humidity (RH) always affect the heterogeneity in capitulum WC via interlinked factors of evaporation rate (*E*) and the thickness of boundary layer. Anatomical and morphological differences between species/populations from leaf up to the carpet organization level may be obscured by the variation in diffusion resistance due to capillary water (Rice and Giles 1996) or experimental conditions in gas exchange chambers, as also indicated by comparison of species or ecological groups across the literature (Table 13.1). For instance, relatively higher and wide optimum WC of 10–20 g g^{-1} was found when photosynthesis was measured under low RH of incoming air and efficient air mixing inside the chamber (Fig. 13.1). This setup greatly reduced the boundary layer but also greatly increased *E* up to 25 mmol m^{-2} s^{-1} (*i.e.* 1.6 kg m^{-2} h^{-1}!). These conditions led to heterogeneity in capitulum WC. On the other hand, slow air mixing and higher RH of incoming air reduced *E* (3–4 mmol m^{-2} s^{-1}) and shifted the optimum WC of the same species down to 7 g g^{-1} (Fig. 13.1); these values are comparable to those of Williams and Flanagan (1996). Thicker boundary layer is indicated by narrow range of the optimum WC, which is achieved after relatively long times. This complicates routine measurements of *A* under defined conditions such as

optimum WC because experimental designs (*e.g.*, number of replicates or treatment levels) are usually limited by the time-consuming photosynthetic measurements. To accelerate the measurements, it is desirable to widen the optimum WC of the sample by tuning the parameters controlling *E* and boundary layer thickness inside the gas-exchange chamber, together with the initial WC of the bryophyte sample.

However, even the lowest *E* occurring inside gas exchange chambers—standardly equipped with air-mixing fan—probably still overestimate the field reality where we can expect slower net water loss due to thicker boundary layers. Data on E from moss cover, unaffected by artificial air mixing are however rare. Bond-Lamberty et al. (2011) measured and modeled *E* of boreal forest; the maximum values (annual means), which were found in sphagna from bogs, did not exceed 0.9 mmol m^{-2} s^{-1}. Evaporation rates of about 0.5 mmol m^{-2} s^{-1} were measured in water-saturated *Sphagnum* cores under RH of 93 %, no air mixing and photosynthetic photon flux density (PPFD) of 500–1,000 μmol m^{-2} s^{-1} (Skre et al. 1983a). Recalculated cumulative evaporation data from greenhouse *Sphagnum* cultivation (Robroek et al. 2007b) showed that the mean half-year *E* for four sphagna are close to 1 mmol m^{-2} s^{-1} at RH ~75 %. In another greenhouse experiment with lower RH (45 %), *E* of hydrated sphagna slightly exceeded 2 mmol m^{-2} s^{-1} (Robroek et al. 2009). Although the last three examples represent artificial conditions, the boundary layer and *E* more likely resemble field conditions more so than those used in gas-exchange experimental chambers where the boundary layer is purposefully minimized.

Nevertheless, the WC of well hydrated apical green shoot segment ranges between 15 and 30 g g^{-1} (*e.g.*, Schipperges and Rydin 1998; Rice et al. 2008) suggesting that field-grown sphagna rather rarely utilize the optimum WC, which is closely connected to desiccation stress (however, *cf*. Williams and Flanagan 1996). In open mires, desiccation sensitive sphagna occupy either wet hollows or form compact hummocks and remain hydrated in the sun where most of the excitation energy from PSII cannot be utilized for CO_2 fixation. Excess excitation generates reactive oxygen species (ROS) and causes uncontrolled photodamage to photosynthetic apparatus. Photorespiration is a significant alternative sink for excessive electrons preventing the over reduction of the photosynthetic electron transport pathway (Wilhelm and Selmar 2011). Photorespiration may reduce net photosynthesis to one half in *Sphagnum* as well as in feather mosses (Skre and Oechel 1981). Beside this, sphagna and other bryophytes of unshaded habitats possess non-saturating electron flow through PSII, where the excess excitation energy is continuously taken from PSII, although the carboxylation/oxidation capacity of Rubisco is already light-saturated (Fig. 13.2). Proctor and Smirnoff (2011) identified molecular oxygen as the electron acceptor at high PPFD (Mehler reaction; see also Chap. 7). Because O_2 is reduced to O_2^- (superoxide), these mosses are also highly tolerant to ROS. The results of Proctor and Smirnoff (2011) stress the importance of Mehler reaction in bryophytes, being probably more of a significant alternative electron sink than in C_3 trachophytes where it consumes only <10 % of transported electrons (Badger et al. 2000). In *Sphagnum*, the non-saturation electron flow was first reported in sun-grown, brown colored *S. cristatum* while the shade-grown green plants of the same species showed light saturation at PPFD ~200 μmol m^{-2} s^{-1} (Maseyk et al. 1999). Similarly, Laine et al. (2011) revealed the saturation character of *A* relative to RETR, indicating efficient electron flow to alternative sinks in all studied sun-grown species at high light intensities.

Examples of two light response curves measured under contrasting levels of diffusion resistance to CO_2 are demonstrated (Fig. 13.2). Similar course of quantum yield of PSII photochemistry (QY PSII; estimated by chlorophyll fluorescence) and of relative electron transport rate (RETR = QY PSII × PPFD) in the two samples indicates that electron flow is almost unaffected by diffu-

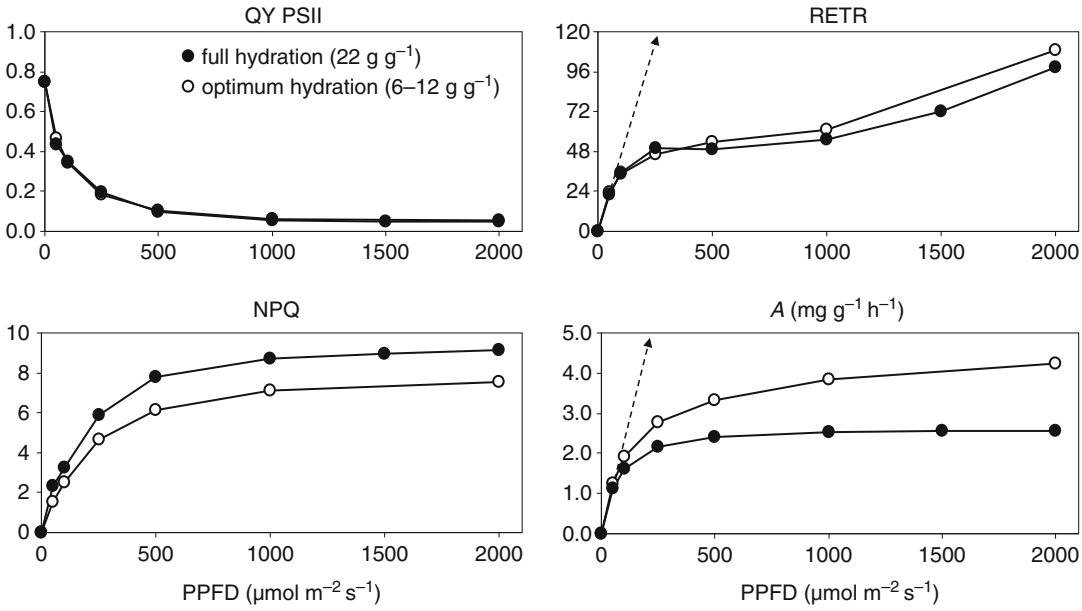


Fig. 13.2. Photosynthetic response of *Sphagnum papillosum* capitula (6 mm) to photosynthetic photon flux density (PPFD) measured under two contrasting levels of diffusion resistance to CO_2. The light curves were measured under full hydration (22 g g^{-1}) and limited sample aeration (high diffusion resistance to CO_2; *closed symbols*), and under optimum hydration (6–12 g g^{-1}) and thorough sample aeration (low diffusion resistance to CO_2; *open symbols*) using GFS-3000 Portable Gas Exchange Fluorescence System (Heinz Walz GmbH, Germany). CO_2 concentration was set to 400 ppm and temperature to 24 °C. *QY PSII* quantum yield of photosystem II photochemistry, *RETR* relative electron transport rate (RETR = QY_PSII × PPFD), *NPQ* non-photochemical quenching of chlorophyll fluorescence, *A* photosynthetic CO_2 assimilation rate. Measurements started in light-acclimated state and finished by dark measurement. Value of QY in the *dark* represents maximum QY PSII (F_v/F_m) after 12-h dark acclimation and the associated NPQ is zero by definition. Means of two replicates are shown. *Dashed arrows* show the rate of photon absorption, illustrating the huge fraction of excess light that the photosynthetic apparatus must cope with (the excess light may be visualized as area included between the light response curve and the *arrow*).

sion resistance to CO_2 but the low diffusion resistance clearly limits *A*. It should be noted that majority of the chlorophyll fluorescence signal comes from the uppermost leaves in capitula and thus the parameters of chlorophyll fluorescence are not as sensitive to CO_2 diffusion as *A*. The RETR become PPFD-saturated at about 500 μmol m^{-2} s^{-1} but then continues to rise with higher light levels, contrary to *A*. The high saturation level of *A*–PPFD curve in the sample with low diffusion resistance to CO_2 results from efficient air-mixing through the canopies of moss capitula. The curve thus integrates CO_2 exchange across the entire 6-mm gradient of light penetration through the sample, resulting in its gradual inflexion and higher estimate of saturation PPFD. The slightly greater NPQ in the sample with high diffusion resistance reflects the greater proportion of heat-dissipated excess excitation energy that could not be taken by photochemistry.

C. Desiccation

Bryophytes as poikilohydric plants generally tolerate some level of cytoplasmic dehydration. Species of xeric habitats are equipped with constitutive desiccation tolerance to survive rapid, irregular and repeated drought, but species of mesic and hydric habitats may afford inducible tolerance by hardening (acclimation; Proctor et al. 2007b).

Since *Sphagnum* mosses are typical desiccation avoiders, they usually posses only limited or no ability to recover their physiological functions and growth after natural or experimental drying (Abel 1956; Clymo 1973; Wagner and Titus 1984; Schipperges and Rydin 1998; Bragazza 2008). Desiccated *Sphagnum* cells are able to maintain constant QY PSII until they lose turgor (Hájek and Beckett 2008), but exact determination of shoot compensation WC for photosynthesis is similarly problematic as the exact determination of optimum WC (Fig. 13.1) due reasons described above. Within the genus *Sphagnum*, species forming hummocks have greater water holding capacity than those occupying hollows. As a consequence, hollow species became more quickly desiccated in the field in comparison to hummock formers (Wagner and Titus 1984; Rydin 1985). Better recovery of the hollow species after experimental drought was interpreted as a trade-off, *i.e*. that the hollow species lack the avoidance strategy of hummock sphagna (anatomical adaptation), but possess better physiological tolerance to desiccation (Wagner and Titus 1984). This hypothesis has been supported only by single published study (Sagot and Rochefort 1996). By contrast, hummock sphagna showed better recovery of photosynthetic parameters after slow drying in the dark (Hájek and Beckett 2008); however, the desiccation did not proceed by same rates for all species. Subsequent experiments on controlled very slow desiccation of *Sphagnum* capitula supported the hypothesis of Wagner and Titus (1984)—hollow sphagna were able to recover their photosynthesis better after severe desiccation treatment because they have better biochemical hardening to drought during initial phases of protoplast desiccation (T. Hájek and E. Vicherová, unpublished). Application of abscisic acid had comparable effects as acclimation by slow desiccation. Accordingly, only slow desiccation to *Sphagnum* capitula in the field, ensured by capillary contact with hydrated basal stem parts (Schipperges and Rydin 1998), seem to provide sufficient time for the biochemical induction of desiccation tolerance at least down to WC of 0.2 g g^{-1} (Wagner and Titus 1984) or even 0.07 g g^{-1} (Rydin and McDonald 1985), corresponding to WC of *Sphagnum* capitula dried under 80 and 40 % relative air humidity (read from the water sorption curves in Clymo and Hayward 1982).

During desiccation of bryophyte shoots, air replaces water from capillary spaces between leaves and in case of *Sphagnum* also from the interior of hyaline cells. In desiccated *Sphagnum* capitula, reflectance of photosynthetic active radiation increases roughly twofold (Vogelmann and Moss 1993; Harris et al. 2005; Van Gaalen et al. 2007), *i.e*. the excess PSII excitation pressure will decrease. Reflectance in the infrared (water absorption bands) increases even more, notably in the mid-infrared (Vogelmann and Moss 1993; Harris et al. 2005), preventing overheating when evaporative cooling is absent.

III. Specific Properties of Peatlands

A. Peat as Carbon Source for Photosynthesis

Peatland bryophytes are mostly terrestrial plants utilizing atmospheric CO_2 for photosynthesis. Most of the photosynthetically fixed carbon entering the acrotelm, the upper aerobic peat layer in mires, is mineralized back to CO_2 before it reaches catotelm, the deeper water-logged anoxic peat layer. Reducing conditions in the catotelm facilitate methane production. Mire bryophytes form an interface between the soil or water and the atmosphere. The peat-derived gases diffuse upwards through the peat pore water or in air pockets, but they move also internally within central parenchyma of *Sphagnum* stems (Rydin and Clymo 1989). Both pathways were found to represent significant C source for photosynthesis and growth in *Sphagnum* mosses. In the case of hummock forming sphagna in a boreal bog, long-term C refixation could represent about 15–25 % of the total C assimilated (Tolonen et al. 1992) based on analysis of peat profiles for ^{14}C originating from nuclear weapons

testing in 1960s. Turetsky and Wieder (1999) estimated that about 5 % of ^{14}C incorporated to living *Sphagnum fuscum* had been refixed within 90 days. Additional, substrate-derived C was found to be indispensable for normal development of *S. magellanicum* shoots in wet conditions supporting C allocation to structural tissues (Smolders et al. 2001).

Hyaline cells in *Sphagnum* leaves provide suitable microhabitat for methanotrophic bacteria. They may oxidize up to 100 % of methane produced in the catotelm (Whalen 2005), leaving CO_2 that can be readily assimilated by neighbouring chlorophyllous cells. Methane-derived CO_2 represents 5–35 % of photosynthetically assimilated CO_2 in many *Sphagnum* species, particularly in aquatic conditions (Raghoebarsing et al. 2005; Kip et al. 2010; Larmola et al. 2010). Liebner et al. (2011) also found the moss–methanotroph associations in "brown" mosses; they estimated that *Scorpidium scorpioides*, a dominant moss in pools of polygonal tundra, obtained as much as 70 % of assimilated CO_2 from methane oxidation.

In summary, peat-derived CO_2 serves as a significant source of C for *A* in peatland mosses, particularly in aquatic habitats. This locally elevated CO_2 may be understood as a kind of compensation for high leaf diffusion resistance to atmospheric CO_2 caused by large content of extracellular capillary water which is typical notably for *Sphagnum* shoots (see above).

B. CO_2 Availability in Rich Fens

In terms of their total area, rich and extremely rich fens represent only a small fraction of the world's peatlands. The plant cover here is strongly influenced by chemistry of groundwater, often running over the surface. Such water is rich in calcium bicarbonate that originates from dissolving of calcium carbonate underground. When the groundwater reaches the surface, the free and bicarbonate-bound CO_2 releases from the water into the atmosphere. This equilibration is accompanied by a pH increase in the fen water (Shotyk 1988). In extremely-rich fens this process may lead to precipitation of calcium carbonate as tufa resulting in the lack of free CO_2 for photosynthesis. Such water chemistry is tolerated by only a few species, typically brown mosses from the family Amblystegiaceae such as *Cratoneuron* or *Scorpidium* species. Although direct uptake of bicarbonate could be a competitive advantage in bryophytes submerged in rich fen water, this adaptation has been only indicated in the aquatic moss *Fontinalis antipyretica* (Peñuelas 1985), and has not been observed in rich fen mosses (Ruttner 1947; Bain and Proctor 1980; see Chap. 12 for further details). There is also no evidence that moss–methanotroph associations facilitate moss growth in such hard waters. Therefore the photosynthesizing moss shoots growing at the groundwater–atmosphere interface assimilate aerial CO_2. Alternatively, CO_2-enriched groundwater provides CO_2 until its concentration becomes equilibrated with the air. After that, CO_2 consumption down to the photosynthetic CO_2 compensation point leads to CO_2 release from bicarbonate followed by carbonate precipitation. Such re-equilibration of CO_2/bicarbonate/carbonate may explain bryophyte growth in nearly stagnant alkaline waters (Bain and Proctor 1980). Active proton extrusion from protoplasts and also cation exchange in the cell walls may acidify the bicarbonate and increase CO_2 concentration near the photosynthesizing shoots. The capacity of these two mechanisms is, however, low when compared to the buffering capacity of the excess hard groundwater.

C. Life in Water-Saturated and Sunny Environment

Bryophyte-dominated peatlands are often treeless due high water levels and associated anoxic conditions in the catotelm. Especially in bogs and partly in fens, *Sphagnum* biomass is the key contributor to the peat formation and thus the lack of trees may be understood as a result of competition between rootless bryophytes and rooting tracheophytes (vascular plants). *Sphagnum*

mosses are typical desiccation avoiders, storing large amount of capillary water that prevents drying. In most terrestrial habitats, bryophytes get dry and physiologically inactive in full sunshine, activating their metabolism during wet, *i.e*. mostly cloudy periods. On the other hand, permanent shoot hydration is common in shady habitats. Therefore, the combination of full sunlight irradiance and permanent hydration in *Sphagnum* places demands on efficient photoprotection because the rate of chlorophyll excitation greatly exceeds the capacity for CO_2 fixation.

Synthesis of red or brown cell-wall pigments is the most conspicuous photoprotective mechanism in sun-grown sphagna, while the same species are deep green in the shade (*e.g*., sun grown *S. capillifolium* and *S. magellanicum* are often red to crimson while *S. teres* and *S. fuscum* are brown). These pigments simply shield those involved in light harvesting (chlorophylls and carotenoids) by reflecting and absorbing part of the photosynthetically active radiation. If the readily-reversible protective mechanisms (notably non-photochemical quenching provided largely by xanthophyll cycle; Bukhov et al. 2001), as well as photochemical quenching by alternative electron sinks such as photorespiration, are insufficient, the photosynthetic apparatus faces photodamage (Raven 2011; Wilhelm and Selmar 2011) resulting in long term depression of chlorophyll excitation and reduction of growth and production. These symptoms were evident in experimentally shaded and unshaded *Sphagnum* in a subarctic mire, as well as under controlled laboratory cultivation of subarctic and temperate *Sphagnum* samples (Murray et al. 1993). Specifically, mosses treated by sun or moderately high PPFD showed impairment of PSII (lowered F_v/F_m) resulting in decreased A and growth; this impairment persisted for the 2-week experimental period. Similar conclusions were provided by a screening of gas exchange and chlorophyll fluorescence parameters in dominant species from pristine (open) and forested (shaded) halves of single boreal poor fen (Hájek et al. 2009); similar patterns were found in *S. cristatum* from New Zealand (Maseyk et al. 1999). Analogously, the uppermost capitulum level of arctic sphagna experiencing 24-h daylight showed substantially reduced F_v/F_m, notably in the late season, in contrast to the subcapitulum shoot level that experienced lower irradiance (Zona et al. 2011).

These results indicate that F_v/F_m is a sensitive indicator of light stress in peatland mosses, which is also true for other bryophytes. Bukhov et al. (2001) concluded that the F_v/F_m of moss gametophytes does not exceed 0.80 and suggested that this was due to photoinhibition, *i.e*. heat dissipation of excess excitation energy in damaged PSII. This indicates that mosses are generally incapable of the rapid and/or full photoacclimation of PSII known from tracheophytes whose sun leaves reach $F_v/F_m > 0.80$ within minutes after darkening. Instead, F_v/F_m in sun sphagna may remain below 0.60 after 12–20 min of dark acclimation (Hájek et al. 2009; Laine et al. 2011), but shade sphagna may regularly exceed the value of 0.80 after overnight dark acclimation as observed in many species from boreal spruce mires in Southern Finland (T. Hájek, unpublished). This result suggests that mosses do not possess a kind of constitutive photoinhibition since their PSII is capable of full shade acclimation. Permanent F_v/F_m reduction in the sun may be also considered as an acclimation mechanism of avoiding excess excitation when the fast-reversible dissipation mechanisms are insufficient (Štroch et al. 2004). Since the photoinhibitory components of non-photochemical quenching are slowly reversible and very heterogenous, the length of the dark acclimation period for F_v/F_m should be indicated when the parameter values are compared and interpreted, especially in bryophytes.

Thus, the shady forest floor seems to be a favorable habitat in terms of low light and also low evaporation rate. Moreover, the understory rainwater is substantially enriched by nutrients deposited in and leached out of the forest canopy. These nutrients can then be

readily taken up by nutrient-limited mosses (Tamm 1964). Low nutrients, particularly nitrogen in *Sphagnum* tissue are hypothesized to be responsible for the inability of photoacclimation to high light conditions (Murray et al. 1993). Close correlation between mass-based shoot N content and maximum quantum efficiency of *A*, a good indicator of light stress, has been reported for bryophytes (Waite and Sack 2010). Direct experimental evidence supporting the hypothesis of Murray et al. (1993) is however missing.

In summary, forested peatlands are relatively unstressful habitats for bryophytes in terms of light, water, and nutrients. This allows them to maximize their photosynthesis, photosynthate allocation to growth, and production, which is necessary for efficient competition. Sphagnum mats in nutrient-rich sparsely forested peatlands are thus one of the most productive peatland habitats (Brock and Bregman 1989). On the other hand, sphagna in open peatlands do not seem to maximize their photosynthetic and biomass production. They have already suppressed their vascular competitors (van Breemen 1995). Therefore they can afford the reduction of light acquisition efficiency in PSII protecting their photosynthetic apparatus against more severe damage that may result from over excitation.

D. Mineral Nutrition

Peatlands have typically slow biomass mineralization rates resulting in low nutrient availability in the soil; these nutrients are however utilized preferentially by rooting tracheophytes (Malmer et al. 1994). Especially in bogs, bryophytes must rely rather on efficient nutrient acquisition from atmospheric deposition and nutrient recycling from senescent shoots. Numerous recent studies question the effect of anthropogenically raised atmospheric concentrations and deposition of nutrients, particularly nitrogen, on peatland ecosystems (Limpens et al. 2011). Despite nutrient-poor characteristics of peatlands, production of most *Sphagnum* populations is currently not N-limited, except some remote sites with very low (but probably still not pre-industrial) atmospheric deposition of biologically active N (reviewed by Limpens et al. 2006, 2011; Wieder 2006). Consequently, (co-)limitation with other nutrients results, notably phosphorus (P) and potassium (K) (Bragazza et al. 2004; Limpens et al. 2011). Only few studies investigated the effect of N availability on *Sphagnum* photosynthesis. Foliar N content correlates well with net photosynthesis in tracheophytes (Wright et al. 2004), but perhaps less in bryophytes (Waite and Sack 2010) including *Sphagnum* capitula (but not whole canopies; Rice et al. 2008). Maximum *A* and in part also F_v/F_m increased with tissue N and chlorophyll contents along the North–South gradient of increasing background wet N deposition across N–W Europe (Granath et al. 2009a). Specifically, *A* increased 3–6 times from low deposition rates of 0.3 up to 1.1 or 1.4 g N m^{-2} $year^{-1}$ depending on species, but production showed only weak, if any, trend across Europe. In another study, *A* in *Sphagnum* decreased with artificial N oversaturation of 6–23 g N m^{-2} $year^{-1}$ (van der Heijden et al. 2000). Correspondingly, moderate experimental N deposition of 1.5 g N m^{-2} $year^{-1}$ resulted in greater *A* (but not F_v/F_m) than under deposition of 0.2 and 3.0 g N m^{-2} $year^{-1}$ in *S. balticum* (Granath et al. 2009b, 2012). However, *A* (but neither F_v/F_m nor production) of *S. fallax* and *S. fuscum* benefited from experimental N deposition up to 5.6 g N m^{-2} $year^{-1}$ (Granath et al. 2012), resulting in the capitulum N content about 1.3 %. Above this optimum N content, *Sphagnum* production generally decreases, perhaps due to shifted nutrient stoichiometry (Limpens et al. 2011); the optimum capitulum N content was about 1.3 % which is in a good agreement with the general limit for sphagna above which the production decreases (Limpens et al. 2011; see also Granath et al. 2012). *Sphagnum magellanicum* in Patagonian bogs receives only about 0.1 g N m^{-2} $year^{-1}$ in precipitation. When the mosses were experimentally overloaded by 4 g N m^{-2} $year^{-1}$, their capitulum responded

by doubled N (1.5 %) and chlorophyll contents but *A* and production of entire moss carpets did not change at all due to strong P limitation (Fritz et al. 2012). Even though the combined N and P treatment stimulated *A* and especially production, P failed to alleviate the stress imposed by excess N.

As mentioned above, Murray et al. (1993) hypothesized that generally low N availability to mire sphagna may limit PSII recovery from photoinhibition. Nitrogen may significantly limit the PSII recovery in areas of very low N deposition, which has been indicated by low F_v/F_m in such conditions (Granath et al. 2009a; Hájek et al. 2009). On the other hand, experimental shading (60 % reduction of PPFD) of four *Sphagnum* species in an open Central-European mountain bog had only a negligible effect on the relatively high $F_v/F_{m;}$ during the whole year (increased from 0.71 to 0.72), although the total wet N deposition did not exceed 0.5 g N m^{-2} $year^{-1}$ and capitulum N content did not differ from that in Murray et al. (1993) (T. Hájek and E. Vicherová unpublished data). Interspecific differences in the ability to recover the PSII efficiency may be also important; in a boreal mire with low N deposition, *S. angustifolium* and *S. magellanicum* dominated in both open and shaded habitats, but only *S. magellanicum* exhibited strong and long-term reduction of F_v/F_m and *A* in the open, while these parameters did not differ between habitats in *S. angustifolium* (Hájek et al. 2009).

In sites with non-limiting N deposition, ammonium was found to accumulate in *Sphagnum* cells where it is considered to directly inhibit the moss physiology (Limpens and Berendse 2003). Although cytosolic ammonium *per se* was, however, not found to be toxic to plants, its inhibitory action to photosynthesis is diverse and complex (Britto and Kronzucker 2002). Among other mechanisms, ammonium was found to reduce non-photochemical dissipation of excitation energy by xanthophyll cycle pigments, the key photoprotective mechanism in plants. This reduction may be compensated by alternative photochemical processes of energy dissipation such as photorespiration and O_2 reduction via Mehler reaction (Britto and Kronzucker 2002). The latter mechanism is particularly efficient in sun growing bryophytes including sphagna (Proctor and Smirnoff 2011). On the other hand, high ammonium availability may be accompanied by an increase in chlorophyll content (Tomassen et al. 2003; Bonnett et al. 2010; Fritz et al. 2012; but cf. Rudolph and Voigt 1986), which may further increase chlorophyll excitation and risk of photodamage.

IV. Seasonal Variability of Photosynthesis and Respiration

Optimum conditions for growth and production often occur in spring (Gaberščik and Martinčič 1987; Brock and Bregman 1989) when the supply of water exceeds evaporation, where summer production can be limited by drought. A second growth optimum may come in late summer/autumn (Johansson and Linder 1980; Lindholm 1990; Asada et al. 2003). In general, moisture distribution is a key factor explaining seasonal variability in *Sphagnum* growth (Backéus 1988), particularly in hollows, which host species with low water retention capacity (Asada et al. 2003). Hulme and Blyth (1982) found that wetter bog microhabitats have a longer growth season for *Sphagnum* species. Thus, aquatic conditions allowed whole-year growth in *S. cuspidatum*, while sphagna on flat hummocks or sphagna transplanted from these hummocks to pools grew only for about 6 months in the temperate climate of South Scotland. The last finding emphasizes species control on the length of growth season. Corresponding with the contrasting ecological strategies of hummock and hollow sphagna described above, hollow species maximize their growth and competitive ability also by maximizing the length of their growth season.

Seasonal patterns of *Sphagnum* photosynthesis and production rates are, however, not always correlated. Gaberščik and

Martinčič (1987) measured the greatest photosynthetic and dark respiration rates as well as highest chlorophyll contents of *S. papillosum* in summer, peaking in early August, after the period of maximum growth and production. Similarly, Skre and Oechel (1981) reported a gradual increase in net photosynthetic rates of *Sphagnum* and other spruce mire mosses to a maximum in August, but dark respiration did not follow this seasonal pattern. The model of Williams and Flanagan (1998) revealed that maximum carboxylation rate by Rubisco in *Sphagnum* peaks in summer while the dark respiration was again stable. This seasonal pattern of *A*, respiration, and growth could be explained by seasonality in resource allocation. There is specific allocation to photosynthetic capacity in summer. In the late season, photosynthates may be stored in capitula as lipids (Karunen and Salin 1982) or carbohydrates (monosaccharides and reserve polysaccharides; Skre et al. 1983b); the carbohydrates also provide osmotic protection to survive forthcoming freeze. In the spring, the photosynthetic apparatus is still acclimated to winter dormancy. The low efficiency in light harvesting and CO_2 fixation may be compensated by utilization of the capitulum storage accumulated in previous autumn. This corresponds with the finding of Backéus (1988) who revealed that the water availability in August is a good predictor of *Sphagnum* growth for the next year. Moreover, the reduced *A* may be compensated by efficient C translocation from senescent shoot segments to the capitula (Rydin and Clymo 1989).

Induction of winter dormancy in sphagna may be independently triggered by both short photoperiod (Li and Glime 1991; Gerdol 1995) and chilling, *i.e.* low night temperature of about 5 °C and lower (Gerdol 1995; Gerdol et al. 1998). Chilling seems to induce rapid degradation of chlorophylls (Gerdol et al. 1994) accompanied by synthesis of sphagnorubins, red cell-wall anthocyanin-derived flavonoid pigments (Rudolph et al. 1977; Rudolph and Jöhnk 1982), produced perhaps by all species. Gerdol et al. (1998) observed no reduction in chlorophylls, but great reduction in photosynthetic rate and growth after low night temperatures of 5 °C. This indicates that the induction of dormancy in the light-harvesting apparatus probably requires lower temperatures than the reactions involved in *A*. Seasonal changes in chlorophylls and sphagnorubins are important in reduction of excess excitation that cannot be utilized for C fixation since biochemical processes are more temperature dependent than the light harvesting. Sphagnorubins, which are also synthesized in sun conditions without chilling (Rudolph et al. 1977), acts partly as a shield reflecting red (photosynthetically active) radiation and partly as a heater absorbing photosynthetically inactive radiation. The quantitative importance of both effects is however unknown. Moreover, sphagnorubins seem to be synthesized also under other stress conditions, such as nutrient imbalance (Li et al. 1993).

V. Photosynthesis and Production in a Warmer and Richer World

The most discussed global climate-related environmental changes are increases in temperature, atmospheric CO_2 concentration, nutrient deposition, UV irradiance, and irregularity of water supply. Much interest is currently being focused on peatlands and their C balance since these habitats are likely to be greatly impacted due to their distribution in colder and (thus) wetter parts of the world, to their primary production being nutrient limited, and to their production showing CO_2 limitation.

Short-term increases of air CO_2 concentration lead to temporarily proportional increases of photosynthetic rates in *Sphagnum* mosses (Silvola 1985), but over a few days (van der Heijden et al. 2000) or months (Jauhiainen and Silvola 1999) photosynthesis is down-regulated so final *A* only slightly exceed the original ones. Relatively high N fertilization and elevated CO_2 stimulated half-year production of *Sphagnum fallax* taken from

minerotrophic fen (van der Heijden et al. 2000); bog sphagna, however, did not respond to elevated CO_2 for 3 years, although the experimental sites were distributed along the gradient of aerial N deposition across N–W Europe (Hoosbeek et al. 2001). Their analysis of nutrient stoichiometry revealed that K and partly also P limited the production under elevated CO_2.

Depending on species and initial temperatures, increased temperature may stimulate or reduce production of peatland bryophytes through several pathways such as direct physiological response or indirect effects like drought stress (due to increased evaporation or reduced water holding capacity of the new biomass), increased nutrient availability (due to enhanced mineralization of organic matter), expansion of vascular or bryophyte competitors or pathogen infection. Although the optimum temperature for net *A* varies during the growth season (Gaberščik and Martinčič 1987), the summer optimum temperature seems to be relatively high (20 °C), even in tundra and taiga ecosystems (Harley et al. 1989; Skre and Oechel 1981, respectively). Studies linking direct photosynthetic responses to elevated temperature with indirect growth and production responses are, however, absent. For example, high temperature stress alone (36 and 43 °C) temporarily reduced F_v/F_m and net *A* in two fully hydrated hummock sphagna under moderate PPFD (Gerdol and Vicentini 2011); however such heat stress is usually accompanied by drought and high PPFD in the field, conditions that may be already lethal (Bragazza 2008). Thus, the indirect impacts of increased temperature such as desiccation or competitive pressure can be considered as the threat to peatland bryophytes, whereas the (slightly) increased temperature *per se* may have rather positive effect on *A*, growth and production (Gerdol 1995; Dorrepaal et al. 2003), notably in colder zones. Increased temperature in warmer zones, accompanied by elevated N deposition rates is, however, detrimental to *Sphagnum* production due to imbalanced nutrient stoichiometry (Limpens et al. 2011) or risk of desiccation due to reduced water retention capacity (Manninen et al. 2011).

The effects of UV radiation on bryophytes, including sphagna have been recently reviewed elsewhere (see also Chap. 7). Briefly, neither current nor expected elevated fluxes of UV radiation seem to have substantial effects on *A*, pigments content (chlorophylls, carotenoids, UV-absorbing compounds), and biomass production in *Sphagnum*. Sphagna seem to be equipped with inherent UV protection rather than with prompt repair mechanisms. The protection may be provided by UV-B absorbing phenolic compounds fixed within cell-walls, namely lignin-like polymers and flavonoid pigments. Although this hypothesis has not been tested in *Sphagnum* yet, the cell-wall pigments were found to provide efficient UV-screen in Antarctic bryophytes (Chap. 7). Nonetheless, UV radiation may become harmful in combination with other stress factors associated with climate change such as severe desiccation.

Desiccation stress is currently becoming more frequent under ongoing climatic change, which is characterized by irregularity in seasonal distribution of precipitation. Robroek et al. (2009) emphasized that lack of precipitation, rather than a low water table *per se*, inhibits *A* in *Sphagnum*, particularly in hollow more than in hummock species. Bragazza (2008) documented extensive and permanent die off in hummock sphagna following a spell of hot and dry weather in Italian Alps.

In summary, most of the individual factors representing climate change such as realistically elevated temperature, CO_2, UV radiation or N supply do not have direct and substantial negative effects on peatland bryophytes, represented by several *Sphagnum* species, at least in terms of photosynthesis and biomass production. Current levels of biologically active N and CO_2 considerably exceed pre-industrial levels. Therefore species' response to our current 'control' levels may obscure the effect of the experimentally increased levels. Water availability is perhaps the most variable and most significant

environmental factor limiting photosynthesis and production in the field conditions. It can be expected that drought, when metabolic activity including repair mechanisms is retarded, will be intensified by stress induced by climate change and exacerbated by excess PPFD.

VI. Suggestions for Further Research

This review pointed out several questions that deserve further interest. For example, there are only indications that the recovery of PSII from photoinhibition is limited by low N availability in traditionally nutrient deficient peatlands. The general question remains, what is the nutrient control on light harvesting and CO_2 assimilation in peatland bryophytes in field conditions?

We have quite good overview of how moss shoot WC retards diffusion of atmospheric CO_2 (and its assimilation) in individual experimental measuring set-ups. But how does it work in the field? We need a model for field CO_2 exchange that includes conductance to water vapor and CO_2 within the natural moss cover, taking into account also laminar boundary layer thickness (wind speed, surface roughness), CO_2 source (air and peat-derived) or water pressure deficit.

We have almost no data on the combined effects of desiccation stress and other stress factors (high PPFD, nutrient availability, heat or elevated UV radiation and ozone concentration) on photosynthesis and growth in peatland bryophytes. Generally high sensitivity of *Sphagnum* to desiccation combined with exclusively inducible desiccation tolerance has been a barrier to deeper experimental work on this topic. The high sensitivity, however, has a potential to affect these bryophytes differently than in the more desiccation tolerant species of mesic and xeric habitats. The induction of *Sphagnum* desiccation tolerance, when technically mastered, may open new experimental possibilities.

Sphagnorubins, the cell-wall pigments, are synthesized under several stress conditions, but their expected ecophysiological significance (reflection of photosynthetically active radiation, absorption of other wavelengths including UV) has not been subjected to specific experimental testing. Moreover, the synthesis of these flavonoid pigments—secondary metabolites—may interact with the current increase in N availability mediated through phenylalanine pools, an amino acid that is the primary resource for sphagnorubin synthesis (Tutschek 1982; Chap. 7).

This review also revealed how the ecophysiological research of brown mosses—in comparison with sphagna—is neglected although they are key species in nutrient rich peatlands. They are tightly connected with the relatively alkaline groundwater rich in calcium and bicarbonate, which may lead up to precipitation of calcium carbonate. Such water chemistry represents a stress factor that may interact with other abovementioned abiotic stress factors typical for sun-exposed peatland habitats.

Acknowledgements

I am grateful to the editors for helpful comments and language correction. This work was supported by long-term research development project no. RVO 67985939 (Academy of Sciences of the Czech Republic) and grant GAP505/10/0638 (The Czech Science Foundation).

References

Abel WO (1956) Die Austrocknungsresistenz der Laubmoose. Sitzungsberichte. Österreichische Akademie der Wissenschaften. Mathematisch-naturwissenschaftliche Klasse, Abt. I 165:619–707

Asada T, Warner BG, Banner A (2003) Growth of mosses in relation to climate factors in a hypermaritime coastal peatland in British Columbia, Canada. Bryologist 106:516–527

Backéus I (1988) Weather variables as predictors of *Sphagnum* growth on a bog. Holarct Ecol 11:146–150

Badger MR, von Caemmerer S, Ruuska S, Nakano H (2000) Electron flow to oxygen in higher plants and algae: rates and control of direct photoreduction

(Mehler reaction) and Rubisco oxygenase. Philos Trans R Soc Lond B Biol Sci 355:1433–1445

Bain JT, Proctor MCF (1980) The requirement of aquatic bryophytes for free CO_2 as an inorganic carbon source: some experimental evidence. New Phytol 86:393–400

Bond-Lamberty B, Gower ST, Amiro B, Ewers BE (2011) Measurement and modeling of bryophyte evaporation in a boreal forest chronosequence. Ecohydrology 4:26–35

Bonnett SAF, Ostle N, Freeman C (2010) Short-term effect of deep shade and enhanced nitrogen supply on *Sphagnum capillifolium* morphophysiology. Plant Ecol 207:347–358

Bragazza L (2008) A climatic threshold triggers the die-off of peat mosses during an extreme heat wave. Glob Change Biol 14:2688–2695

Bragazza L, Tahvanainen T, Kutnar L, Rydin H, Limpens J, Hájek M, Grosvernier P, Hájek T, Hájková P, Hansen I, Iacumin P, Gerdol R (2004) Nutritional constraints in ombrotrophic *Sphagnum* plants under increasing atmospheric nitrogen depositions in Europe. New Phytol 163:609–616

Britto DT, Kronzucker HJ (2002) NH_4^+ toxicity in higher plants: a critical review. J Plant Physiol 159:567–584

Brock TCM, Bregman R (1989) Periodicity in growth, productivity, nutrient content and decomposition of *Sphagnum recurvum* var. mucronatum in a fen woodland. Oecologia 80:44–52

Bukhov NG, Heber U, Wiese C, Shuvalov VA (2001) Energy dissipation in photosynthesis: does the quenching of chlorophyll fluorescence originate from antenna complexes of photosystem II or from the reaction center? Planta 212:749–758

Clymo RS (1973) The growth of *Sphagnum*: some effects of environment. J Ecol 61:849–869

Clymo RS, Hayward PM (1982) The ecology of *Sphagnum*. In: Smith AJE (ed) Bryophyte ecology. Chapman and Hall, New York, pp 229–289

Dorrepaal E, Aerts R, Cornelissen JHC, Callaghan TV, van Logtestijn RSP (2003) Summer warming and increased winter snow cover affect *Sphagnum fuscum* growth, structure and production in a sub-arctic bog. Glob Change Biol 10:93–104

Fritz C, van Dijk G, Smolders AJ, Pancotto VA, Elzenga TJ, Roelofs JG, Grootjans AP (2012) Nutrient additions in pristine Patagonian *Sphagnum* bog vegetation: can phosphorus addition alleviate (the effects of) increased nitrogen loads. Plant Biol 14:491–499

Gaberščik A, Martinčič A (1987) Seasonal dynamics of net photosynthesis and productivity of *Sphagnum papillosum*. Lindbergia 13:105–110

Gerdol R (1995) The growth dynamics of *Sphagnum* based on field measurements in a temperate bog and on laboratory cultures. J Ecol 83:431–437

Gerdol R, Vicentini R (2011) Response to heat stress of populations of two *Sphagnum* species from alpine bogs at different altitudes. Environ Exp Bot 74:22–30

Gerdol R, Bonora A, Poli F (1994) The vertical pattern of pigment concentrations in chloroplasts of *Sphagnum capillifolium*. Bryologist 97:158–161

Gerdol R, Bonora A, Gualandri R, Pancaldi S (1996) CO_2 exchange, photosynthetic pigment composition, and cell ultrastructure of *Sphagnum* mosses during dehydration and subsequent rehydration. Can J Bot 74:726–734

Gerdol R, Bonora A, Marchesini R, Gualandri R, Pancaldi S (1998) Growth response of *Sphagnum capillifolium* to nighttime temperature and nutrient level: mechanisms and implications for global change. Arct Alp Res 30:388–395

Granath G, Strengbom J, Breeuwer A, Heijmans MMPD, Berendse F, Rydin H (2009a) Photosynthetic performance in *Sphagnum* transplanted along a latitudinal nitrogen deposition gradient. Oecologia 159:705–715

Granath G, Weidermann MM, Strengbom J (2009b) Physiological responses to nitrogen and sulphur addition and raised temperature in *Sphagnum balticum*. Oecologia 161:481–490

Granath G, Strengbom J, Rydin H (2012) Direct physiological effects of nitrogen on *Sphagnum*: a greenhouse experiment. Funct Ecol 26:353–364

Grime JP (1977) Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. Am Nat 111:1169–1194

Hájek T, Beckett RP (2008) Effect of water content components on desiccation and recovery in *Sphagnum* mosses. Ann Bot 101:165–173

Hájek T, Tuittila E-S, Ilomets M, Laiho R (2009) Light responses of mire mosses – a key to survival after water-level drawdown? Oikos 118:240–250

Harley PC, Tenhunen JD, Murray KJ, Beyers J (1989) Irradiance and temperature effects on photosynthesis of tussock tundra *Sphagnum* mosses from the foothills of the Philip Smith Mountains, Alaska. Oecologia 79:251–259

Harris A, Bryant RG, Baird AJ (2005) Detecting near-surface moisture stress in *Sphagnum* spp. Remote Sens Environ 97:371–381

Hoosbeek MR, van Breemen N, Berendse F, Grosvernier P, Vasander H, Wallén B (2001) Limited effect of increased atmospheric CO_2 concentration on ombrotrophic bog vegetation. New Phytol 150:459–463

Hulme PD, Blyth AW (1982) The annual growth period of some *Sphagnum* species on the Silver Flowe National Nature Reserve, south-west Scotland. J Bryol 12:287–291

Jauhiainen J, Silvola J (1999) Photosynthesis of *Sphagnum fuscum* at long-term raised CO_2 concentrations. Ann Bot Fennici 36:11–19

Jauhiainen J, Silvola J, Vasander H (1998a) Effects of increased carbon dioxide and nitrogen supply on mosses. In: Bates JW, Ashton NW, Duckett JG (eds) Bryology for the twenty-first century. Maney Publishing and the British Bryological Society, Leeds, pp 343–360

Jauhiainen J, Wallén B, Malmer N (1998b) Potential $^{15}NH_4{}^{15}NO_3$ uptake in seven *Sphagnum* species. New Phytol 138:287–293

Johansson LG, Linder S (1980) Photosynthesis of *Sphagnum* in different microhabitats on a subarctic mire. In: Sonesson M (ed) Ecology of a subarctic mire, Ecological bulletins, 30. Swedish Natural Science Research Council, Stockholm, pp 181–190

Karunen P, Salin M (1982) Seasonal changes in lipids of photosynthetically active and senescent parts of *Sphagnum fuscum*. Lindbergia 8:35–44

Kip N, van Winden JF, Pan Y, Bodrossy L, Reichart G-J, Smolders AJP, Jetten MSM, Damste JSS, Op den Camp HJM (2010) Global prevalence of methane oxidation by symbiotic bacteria in peat-moss ecosystems. Nat Geosci 3:617–621

Laine A, Juurola E, Hájek T, Tuittila E-S (2011) *Sphagnum* growth and ecophysiology during mire succession. Oecologia 167:1115–1125

Larmola T, Tuittila E-S, Tiirola M, Nykäne H, Martikainen JP, Yrjälä K, Tuomivirta T, Fritze H (2010) The role of *Sphagnum* mosses in the methane cycling of a boreal mire. Ecology 91:2356–2365

Li Y, Glime JM (1991) Growth response of two *Sphagnum* species to photoperiod. Can J Bot 69: 2643–2646

Li Y, Glime JM, Drummer TD (1993) Effects of phosphorous on the growth of *Sphagnum magellanicum* Brid. and *S. papillosum* Lindb. Lindbergia 18:25–30

Liebner S, Zeyer J, Wagner D, Schubert C, Pfeiffer E-M, Knoblauch C (2011) Methane oxidation associated with submerged brown mosses reduces methane emissions from Siberian polygonal tundra. J Ecol 99:914–922

Limpens J, Berendse F (2003) Growth reduction of *Sphagnum magellanicum* subjected to high nitrogen deposition: the role of amino acid nitrogen concentration. Oecologia 135:339–345

Limpens J, Heijmans MMPD, Berendse F (2006) The nitrogen cycle in boreal peatlands. In: Wieder RK, Vitt DH (eds) Boreal peatland ecosystems, vol 188, Ecological studies. Springer, Berlin/Heidelberg, pp 195–230

Limpens J, Granath G, Gunnarsson U, Aerts R, Bayley S, Bragazza L, Bubier J, Buttler A, van den Berg LJL, Francez A-J, Gerdol R, Grosvernier P, Heijmans MMPD, Hoosbeek MR, Hotes S, Ilomets M, Leith I, Mitchell EAD, Moore T, Nilsson MB, Nordbakken JF, Rochefort L, Rydin H, Sheppard LJ, Thormann M, Wiedermann MM, Williams BL, Xu B (2011) Climatic modifiers of the response to N deposition in peat-forming *Sphagnum* mosses: a meta-analysis. New Phytol 191:496–507

Lindholm T (1990) Growth dynamics of the peat moss *Sphagnum fuscum* on hummocks on a raised bog in southern Finland. Ann Bot Fennici 27:67–78

Loisel J, Garneau M, Hélie J-F (2009) Modern *Sphagnum* $\delta^{13}C$ signatures follow a surface moisture gradient in two boreal peat bogs, James Bay lowlands, Québec. J Quart Sci 24:209–214

Malmer N, Svensson BM, Wallén B (1994) Interactions between Sphagnum mosses and field layer vascular plants in the development of peat forming systems. Folia Geobot Phytotaxon 29:483–496

Manninen S, Woods C, Leith ID, Sheppard LJ (2011) Physiological and morphological effects of long-term ammonium or nitrate deposition on green and red (shade and open grown) *Sphagnum capillifolium*. Environ Exp Bot 72:140–148

Maseyk KS, Green TGA, Klinac D (1999) Photosynthetic responses of New Zealand *Sphagnum* species. N Z J Bot 37:155–165

Murray KJ, Harley PC, Beyers J, Walz H, Tenhunen JT (1989) Water content effects on the photosynthetic response of Sphagnum mosses from the foothills of the Philip Smith Mountains, Alaska. Oecologia 79:244–250

Murray KJ, Tenhunen JD, Nowak RS (1993) Photoinhibition as a control on photosynthesis and production of *Sphagnum* mosses. Oecologia 96:200–207

Oechel WC, Collins NJ (1976) Comparative CO_2 exchange patterns in mosses from two tundra habitats at Barrow, Alaska. Can J Bot 54:1355–1369

Peñuelas J (1985) HCO_3^- as an exogenous carbon source for aquatic bryophytes *Fontinalis antipyretica* and *Fissidens grandifrons*. J Exp Bot 36:441–448

Proctor MCF (2005) Why do Polytrichaceae have lamellae? J Bryol 27:219–227

Proctor MCF, Smirnoff N (2011) Ecophysiology of photosynthesis in bryophytes: major roles for oxygen photoreduction and non-photochemical quenching? Physiol Plant 141:130–140

Proctor MCF, Ligrone R, Duckett JG (2007a) Desiccation tolerance in the moss *Polytrichum formosum*: physiological and fine-structural changes during desiccation and recovery. Ann Bot 99:75–93

Proctor MCF, Oliver MJ, Wood AJ, Alpert P, Stark LR, Cleavitt NL, Mishler BD (2007b) Desiccation-tolerance in bryophytes: a review. Bryologist 110:595–621

Raghoebarsing AA, Smolders AJP, Schmid MC, Rijpstra WI, Wolters-Arts M, Derkens J, Jetten MSM, Schouten S, Sinninghe Damste JS, Lamers LPM, Roelofs JGM, Op den Camp HJM, Strous M (2005) Methanotrophic symbionts provide carbon for photosynthesis in peat bogs. Nature 436:1153–1156

Raven JA (1991) Implications of inorganic carbon utilization: ecology, evolution, and geochemistry. Can J Bot 69:908–924

Raven JA (2011) The cost of photoinhibition. Physiol Plant 142:87–104

Rice SK (1995) Patterns of allocation and growth in aquatic *Sphagnum* species. Can J Bot 73:349–359

Rice SK, Giles L (1996) The influence of water content and leaf anatomy on carbon isotope discrimination and photosynthesis in *Sphagnum*. Plant Cell Environ 19:118–124

Rice SK, Aclander L, Hanson DT (2008) Do bryophyte shoot systems function like vascular plant leaves or canopies? Functional trait relationships in *Sphagnum* mosses (Sphagnaceae). Am J Bot 95:1366–1374

Robroek BJM, Limpens J, Breeuwer A, van Ruijven J, Schouten MGC (2007a) Precipitation determines the persistence of hollow *Sphagnum* species on hummocks. Wetlands 27:979–986

Robroek BJM, Limpens J, Breeuwer A, Schouten MGC (2007b) Effects of water level and temperature on performance of four *Sphagnum* mosses. Plant Ecol 190:97–107

Robroek BJM, Schouten MGC, Limpens J, Berendse F, Poorter H (2009) Interactive effects of water table and precipitation on net CO_2 assimilation of three co-occurring *Sphagnum* mosses differing in distribution above the water table. Glob Change Biol 15:680–691

Rudolph H (1968) Gaswechselmessungen an *Sphagnum magellanicum*. Ein Beitrag zur Membranochromie der Sphagnen (III). Planta 79:35–43

Rudolph H, Jöhnk A (1982) Physiological aspects of phenolic compounds in the cell walls of Sphagna. J Hattori Bot Lab 53:195–203

Rudolph H, Voigt JU (1986) Effects of NH_4^+-N and NO_3^--N on growth and metabolism of *Sphagnum magellanicum*. Physiol Plant 66:339–343

Rudolph H, Kabsch U, Schmidt-Stohn G (1977) Änderungen des Chloroplastenpigment-Spiegels bei *Sphagnum magellanicum* im Verlauf der Synthese von Sphagnorubin und anderer membranochromer Pigmente. Z Pflanzenphysiol 82:107–116

Ruttner F (1947) Zur Frage der Karbonatassimilation der Wasserpflanzen. I. Teil: Die beiden Haupttypen der Kohlenstoffaufnahme. Ost Bot Z 94:265–294

Rydin H (1985) Effect of water level on desiccation of *Sphagnum* in relation to surrounding Sphagna. Oikos 45:374–379

Rydin H (1993) Mechanisms of interactions among *Sphagnum* species along water level gradients. Adv Bryol 5:153–185

Rydin H, Clymo RS (1989) Transport of carbon and phosphorus compounds about *Sphagnum*. Proc R Soc Lond B 237:63–84

Rydin H, Jeglum JK (2006) The biology of peatlands. Oxford University Press, New York

Rydin H, McDonald AJS (1985) Photosynthesis in *Sphagnum* at different water contents. J Bryol 13:579–584

Rydin H, Gunnarsson U, Sundberg S (2006) The role of *Sphagnum* in peatland development and persistence. In: Wieder RK, Vitt DH (eds) Boreal peatland ecosystems, vol 188, Ecological studies. Springer, Berlin/Heidelberg, pp 49–65

Sagot C, Rochefort L (1996) Tolérance des sphaignes à la dessiccation. Cryptogam Bryol Lichénol 17:171–183

Schipperges B, Rydin H (1998) Response of photosynthesis of *Sphagnum* species from contrasting microhabitats to tissue water content and repeated desiccation. New Phytol 148:677–684

Shotyk W (1988) Review of the inorganic geochemistry of peats and peatland waters. Earth Sci Rev 25:95–176

Silvola J (1985) CO_2 dependence of photosynthesis in certain forest and peat mosses and simulated photosynthesis at various actual and hypothetical CO_2 concentrations. Lindbergia 11:86–93

Silvola J (1990) Combined effects of varying water content and CO_2 concentration on photosynthesis in *Sphagnum fuscum*. Holarctic Ecol 13:224–228

Silvola J (1991) Moisture dependence of CO_2 exchange and its recovery after drying in certain boreal forest and peat mosses. Lindbergia 17:5–10

Silvola J, Aaltonen H (1984) Water content and photosynthesis in the peat mosses *Sphagnum fuscum* and *S. angustifolium*. Ann Bot Fennici 21:1–6

Skre O, Oechel WC (1981) Moss functioning in different taiga ecosystems in interior Alaska. I. Seasonal, phenotypic, and drought effects on photosynthesis and response patterns. Oecologia 48:50–59

Skre O, Oechel WC, Miller PM (1983a) Moss leaf water content and solar radiation at the moss surface in a mature black spruce forest in central Alaska. Can J For Resour 13:860–868

Skre O, Oechel WC, Miller PM (1983b) Patterns of translocation of carbon in four common moss

species in a black spruce *Picea mariana* dominated forest in interior Alaska. Can J For Resour 16:869–878

Smolders AJP, Tomassen HBM, Pijnappel H, Lamers LPM, Roelofs JGM (2001) Substrate-derived CO_2 is important in the development of *Sphagnum* spp. New Phytol 152:325–332

Štroch M, Špunda V, Kurasová I (2004) Non-radiative dissipation of absorbed excitation energy within photosynthetic apparatus of higher plants. Photosynthetica 42:323–337

Tamm CO (1964) Growth of *Hylocomium splendens* in relation to tree canopy. Bryologist 67:423–426

Titus JE, Wagner DJ (1984) Carbon balance for two *Sphagnum* mosses: water balance resolves a physiological paradox. Ecology 65:1765–1774

Titus JE, Wagner DJ, Stephens MD (1983) Contrasting water relations of photosynthesis for two *Sphagnum* mosses. Ecology 64:1109–1115

Tolonen K, Possnert G, Jungner H, Sonninen E, Alm J (1992) High resolution ^{14}C dating of surface peat using the AMS technique. Suo 43:271–276

Tomassen HBM, Smolders AJP, Lamers LPM, Roelofs JGM (2003) Stimulated growth of *Betula pubescens* and *Molinia caerulea* on ombrotrophic bogs: role of high levels of atmospheric nitrogen deposition. J Ecol 91:357–370

Turetsky MR, Wieder RK (1999) Boreal bog *Sphagnum* refixes soil-produced and respired $^{14}CO_2$. Ecoscience 6:587–591

Tutschek R (1982) An evaluation of phenylpropanoid metabolism during cold-induced sphagnorubin synthesis in *Sphagnum magellanicum* BRID. Planta 155:301–306

Ueno T, Kanda H (2006) Photosynthetic response of the arctic semi-aquatic moss *Calliergon giganteum* to water content. Aquat Bot 85:241–243

van Breemen N (1995) How *Sphagnum* bogs down other plants. Trends Ecol Evol 10:270–275

van der Heijden E, Verbeek SK, Kuiper PJC (2000) Elevated atmospheric CO_2 and increased nitrogen deposition: effects on C and N metabolism and growth of peat moss *Sphagnum recurvum* P. Beauv. var. mucronatum (Russ.) Warnst. Glob Change Biol 6:201–212

van Gaalen KE, Flanagan LB, Peddle DR (2007) Photosynthesis, chlorophyll fluorescence and spectral reflectance in *Sphagnum* moss at varying water contents. Oecologia 153:19–28

Vitt DH, Wieder RK (2009) The structure and function of bryophyte-dominated peatlands. In: Goffinet B, Shaw AJ (eds) Bryophyte biology, 2nd edn. Cambridge University Press, Cambridge, pp 357–392

Vogelmann JE, Moss DM (1993) Spectral reflectance measurements in the genus *Sphagnum*. Remote Sens Environ 45:273–279

Wagner DJ, Titus JE (1984) Comparative desiccation tolerance of two *Sphagnum* mosses. Oecologia 62: 182–187

Waite M, Sack L (2010) How does moss photosynthesis relate to leaf and canopy structure? Trait relationships for 10 Hawaiian species of contrasting light habitats. New Phytol 185:156–172

Whalen SC (2005) Biogeochemistry of methane exchange between natural wetlands and the atmosphere. Environ Eng Sci 22:73–94

Wieder RK (2006) Primary production in boreal peatlands. In: Wieder RK, Vitt DH (eds) Boreal peatland ecosystems, vol 188, Ecological studies. Springer, Berlin/Heidelberg, pp 145–164

Wilhelm C, Selmar D (2011) Energy dissipation is an essential mechanism to sustain the viability of plants: the physiological limits of improved photosynthesis. J Plant Physiol 168:79–87

Williams TG, Flanagan LB (1996) Effect of changes in water content on photosynthesis, transpiration and discrimination against $^{13}CO_2$ and $C^{18}O^{16}O$ in *Pleurozium* and *Sphagnum*. Oecologia 108: 38–46

Williams TG, Flanagan LB (1998) Measuring and modelling environmental influences on photosynthetic gas exchange in *Sphagnum* and *Pleurozium*. Plant Cell Environ 21:555–564

Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin FS, Cornelissen JHC, Diemer M, Flexas J, Garnier E, Groom PK, Gulias J, Hikosaka K, Lamont BB, Lee T, Lee W, Lusk C, Midgley JJ, Navas M-L, Niinemets Ü, Oleksyn J, Osada N, Poorter H, Poot P, Prior L, Pyankov VI, Roumet C, Thomas SC, Tjoelker MG, Veneklaas E, Villar R (2004) The world-wide leaf economics spectrum. Nature 428:821–827

Zona D, Oechel WC, Richards JH, Hastings S, Kopetz I, Ikawa H, Oberbauer S (2011) Light stress avoidance mechanisms in a *Sphagnum*-dominated wet coastal Arctic tundra ecosystem in Alaska. Ecology 92:633–644

Chapter 14

Interacting Controls on Ecosystem Photosynthesis and Respiration in Contrasting Peatland Ecosystems

Lawrence B. Flanagan*
Department of Biological Sciences, Water and Environmental Science Building, University of Lethbridge, 4401 University Drive, Lethbridge, AB T1K 3M4, Canada

Summary

Photosynthesis in moss contributes significantly to carbon gain in northern peatland ecosystems. In turn, these northern peatland ecosystems contain a large fraction of the global soil carbon stock, which has been suggested to be vulnerable to warming and drying associated with climate change. The fate of this vast peatland carbon stock depends on the relative responses of ecosystem photosynthesis and respiration to climate change-induced shifts in environmental conditions. This chapter reviews some recent studies of the controls on ecosystem photosynthesis and respiration in contrasting peatland ecosystems in northern Alberta, Canada, a region where peatlands occupy a significant fraction of the landscape. In particular, it is highlighted how (i) differences in dominant plant functional type, (ii) interactions between variation in water table depth and temperature, and (iii) ecosystem succession, can all strongly control the rate of net carbon sequestration in peatland ecosystems and influence the response of these ecosystems to variation in environmental conditions associated with anticipated climate change. Prediction of future climate change effects on peatland ecosystems would be improved if global-scale models could include more details of the biological variability among peatlands (both spatial and temporal), with realistic parameterizations of the responses of photosynthesis and respiration to variation in temperature, water table depth and soil moisture.

*Author for correspondence, e-mail: larry.flanagan@uleth.ca

D.T. Hanson and S.K. Rice (eds.), Photosynthesis in Bryophytes and Early Land Plants, Advances in Photosynthesis and Respiration 37, pp. 253–267, DOI 10.1007/978-94-007-6988-5_14,

I. Introduction

Photosynthesis in *Sphagnum* (peat moss) and several other moss species contribute significantly to carbon gain in northern peatland ecosystems (Glenn et al. 2006; Flanagan and Syed 2011). In turn, these northern peatland ecosystems contain between one-quarter to one-third of the global soil carbon pool (Gorham 1991; Turunen et al. 2002). The carbon stock in peatlands has accumulated over thousands of years because of moderate rates of ecosystem photosynthesis that exceed decomposition and autotrophic respiration, the latter two processes being limited by the cool temperatures and waterlogged conditions that typically occur in peatlands (Gorham 1991; Davidson and Janssens 2006). The fate of this vast peatland carbon stock, under anticipated warmer and drier conditions associated with climate change, depends on the relative responses of ecosystem photosynthesis and respiration to shifts in environmental conditions. Concern has been expressed that exposure of peatlands to warmer and drier conditions could alter the balance between ecosystem photosynthesis and respiration/decomposition thus increasing atmospheric carbon dioxide concentration and providing a positive feedback to further climate change (Moore et al. 1998; Davidson and Janssens 2006; Tarnocai 2006). Simple ecosystem models usually predict that respiration and decomposition are more sensitive than photosynthesis to increases in temperature and reduction in water availability. However, these simple model predictions ignore other important ecological controls that can influence ecosystem CO_2 exchange processes and affect the rate of net carbon sequestration in peatland ecosystems. This chapter reviews some recent studies of the controls on ecosystem photosynthesis and respiration in contrasting peatland ecosystems in northern Alberta, Canada, a region where peatlands occupy a significant fraction of the landscape. In particular, I highlight how (i) plant functional type, (ii) interactions between variation in water table depth and temperature, and (iii) ecosystem succession, can all strongly control net carbon sequestration in peatland ecosystems and influence the response of these ecosystems to variation in environmental conditions associated with anticipated climate change.

Abbreviations: A_{max} – maximum photosynthetic capacity; GEP – gross ecosystem photosynthesis; NEP – net ecosystem productivity; R_{10} – respiratory capacity at 10 °C; TER – total ecosystem respiration

II. Characteristics of Study Sites and Ecosystem CO_2 Flux Measurements

Peatlands are defined in Canada as wetland ecosystems that have a minimum depth of 40 cm of peat soil (National Wetlands Working Group 1988; Johnson et al. 1995; Vitt et al. 1998). Peat refers to the partially decomposed remains of plants, containing over 65 % organic matter by dry weight with less than 20–35 % inorganic material. The criterion of 40 cm depth was chosen because, at this peat thickness, most wetland vascular plants present at a site would be completely rooted within the peat soil (Johnson et al. 1995). Peatlands can be further classified into fens and bogs, with fens having water input from precipitation and groundwater that has come into contact with mineral soils, while bogs only access precipitation. In continental western Canada, peatlands are distributed along a bog-rich fen gradient based on water characteristics and plant species composition (Vitt 1994; Vitt et al. 1998). The water of bogs has low pH, low conductivity, and low base cation concentrations and these three components increase along the gradient axis between bogs and rich fens. Water that is more alkaline, high in conductivity and base cation concentration is characteristic of rich fen ecosystems. The dominant vegetation in rich fens are sedges (*Carex spp.*) and "brown moss" species including *Drepanocladus aduncus* and *Aulacomnium palustre*, while *Sphagnum* moss typically dominates in bogs and poor fens. (Vitt 1994; Johnson et al. 1995; Vitt et al. 1998).

This chapter reviews case studies conducted at three sites, all located in northern Alberta,

Canada. The first site, Tony's Fen, is a rich fen dominated by the sedge, *Carex lasiocarpa* with a discontinuous mat of *Drepanocladus aduncus* and *Aulacomnium palastre* (Glenn et al. 2006). Comparative studies were conducted between Tony's fen and May Tower, a poor fen site with open, wet pools or "flarks" alternating with slightly elevated and drier "strings" that were orientated perpendicular to the direction of water flow through the site (Glenn et al. 2006). The poor fen is dominated by *Sphagnum* moss that forms a continuous ground cover with the following species: *S. angustifolium*, *S. magellanicum*, *S. fuscum*. Other species present at the poor fen included *Andromedia polifolia*, *Smilacina trifolia*, *Carex limosa*, along with dwarf trees (*Picea mariana* and *Larix laricina*, less than 0.75 m tall) that were present along the strings of the fen (Glenn et al. 2006). The third site, La Biche River, is a moderately-rich treed fen and is an example of the most common type of peatland found in western Canada (Vitt et al. 1998). The dominant plant species of the La Biche River site are stunted trees (2–3 m tall) of *Picea mariana* and *Larix laricina* which contribute approximately two-thirds of the aboveground biomass (Syed et al. 2006). The site also has a relatively high abundance of a broad-leaf deciduous shrub, *Betula pumila*, and a range of moss species including *Sphagnum angustifolium*, *S. fuscum* and other *Sphagnum* species, *Drepanocladus aduncus*, *Aulocomium palaustre*, and the feather moss, *Pleurozium schreberi*. Several other herb and dwarf shrub species are also present (Syed et al. 2006).

In order to study CO_2 exchange processes at the ecosystem-scale, we used the eddy covariance technique to make measurements of net ecosystem CO_2 flux. Eddy covariance is a micro-meteorological technique involving fast-response (10–20 Hz) measurements of vertical wind speed and associated changes in atmospheric CO_2 concentration (Moncrieff et al. 2000; Baldocchi 2003). The fast-response measurements are used to calculate the net flux of CO_2 across a plane between the ecosystem and the atmosphere at the height that the wind speed and CO_2 concentration measurements are made. The net CO_2 flux is sampled over an area upwind of the sensors and its size depends on the height of the instruments and turbulence characteristics of the local atmosphere. During unstable atmospheric conditions, rough guidelines suggest a ratio of 100:1 between the distance sampled upwind from the instruments and their height (Moncrieff et al. 2000). So instruments mounted on a tower 3 m above the surface would sample CO_2 flux across a distance of 300 m upwind of the sensors, although the peak contribution to the measured flux would come from an area much closer (25–50 m) to the tower. The flux footprint rotates on a radius around the instrument tower as the direction of the predominant wind changes. The CO_2 flux measured directly by eddy covariance represents the net difference between the uptake of CO_2 in photosynthesis and the release of CO_2 in respiration by all organisms located above- and below-ground within the flux footprint (Moncrieff et al. 2000; Baldocchi 2003). A number of approaches are available to partition the net CO_2 flux into its major components, ecosystem photosynthesis and total ecosystem respiration (Barr et al. 2004; Moffat et al. 2007; Desai et al. 2008). In general, these approaches make use of a series of night-time measurements of net ecosystem CO_2 flux (which represent ecosystem respiration only) over a range of different temperatures, in order to develop a temperature-response function for ecosystem respiration. The temperature-response function for respiration is used during the daytime along with temperature measurements to calculate respiration rates while simultaneous net CO_2 flux measurements are made. Ecosystem photosynthesis rates during the day can then be determined from the measured net CO_2 flux rate and the calculated daytime ecosystem respiration measurements. In this paper I use a positive sign convention for both photosynthesis and respiration CO_2 fluxes, and so net ecosystem productivity (NEP) is positive when the photosynthesis rate exceeds the respiration rate and the ecosystem is a net sink for carbon dioxide.

III. Comparison of a *Sphagnum*-Dominated Poor Fen and a *Carex*-Dominated Rich Fen

Recent comparative studies conducted by my lab group have shown distinct differences between a poor fen (May Tower) and an extreme-rich fen (Tony's Fen) for their responses of NEP measured by eddy covariance to inter-annual variation in temperature and water table conditions during three study years (Fig. 14.1; Adkinson et al. 2011). The rates of growing season (May-October) cumulative NEP at the poor fen were very similar among years with an average (± SD) of 110.1 ± 0.5 g C m^{-2} $period^{-1}$. By contrast, the growing season cumulative NEP at the extreme-rich fen varied substantially among years and the extreme-rich fen was, on average (76.6 g C m^{-2} $period^{-1}$), a lower net sink for CO_2 than the poor fen. Consistent with the eddy covariance net CO_2 uptake measurements, analysis of ^{210}Pb-dated peat cores also showed higher recent net rates of carbon accumulation in the poor fen than in the extreme-rich fen (36.3 versus 20.6 g C m^{-2} $year^{-1}$; Adkinson et al. 2011). The differences between sites resulted from several factors including: (i) contrasting plant functional types; (ii) environmental differences, particularly the importance of water table fluctuations at the extreme-rich fen; and (iii) interactions between temperature and water table depth for effects on ecosystem CO_2 exchange. Each of these three factors will be discussed further in the paragraphs below.

Because of differences in the functional type of the dominant plant species, the poor fen had a longer period of net CO_2 uptake, with net uptake in at least 5 months (May through September), while the rich fen was normally only a net sink during 3 months of the year (June through August). While significant seasonal variation in biochemical capacity for photosynthesis and respiration does occur in *Sphagnum* (Williams and Flanagan 1998), these mosses do not need to build entirely new photosynthetic tissue at the start of the growing season and complete senescence of tissue does not occur at the end of the growing season. In addition, *Sphagnum* can also begin to photosynthesize almost immediately following snowmelt when

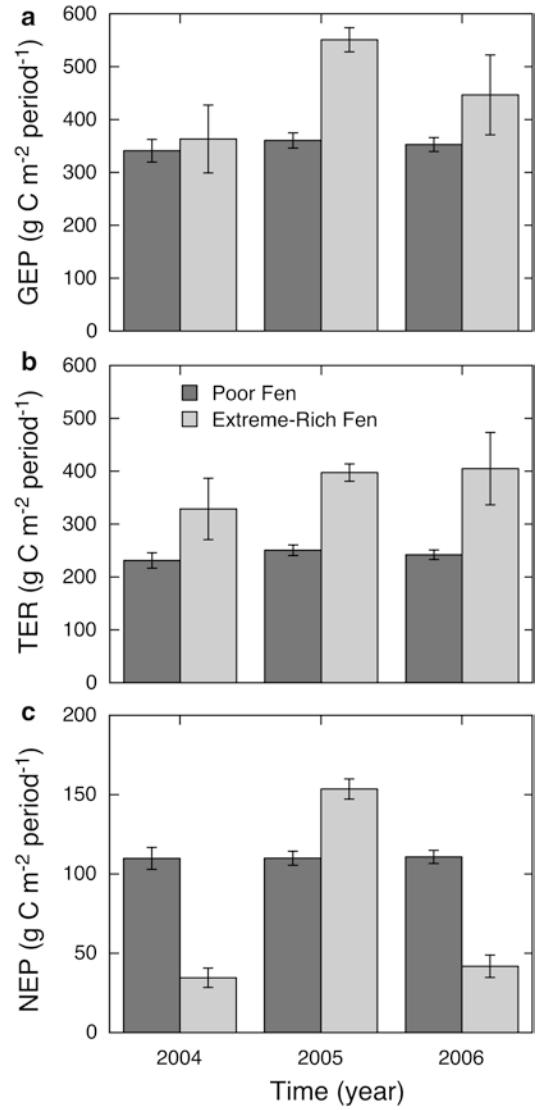


Fig. 14.1. Comparisons of the integrated growing season (1 May to 31 October) values of: (**a**) gross ecosystem photosynthesis (*GEP*); (**b**) total ecosystem respiration (*TER*); and (**c**) net ecosystem productivity (NEP; NEP = GEP – TER) in 2004, 2005 and 2006 at the poor fen and extreme-rich fen study sites. Cumulative growing season values were determined using a combination of measured and modeled half-hourly flux data as described by Adkinson et al. (2011). *Error bars* represent total uncertainty estimates (This figure is based on data in Adkinson et al. (2011)).

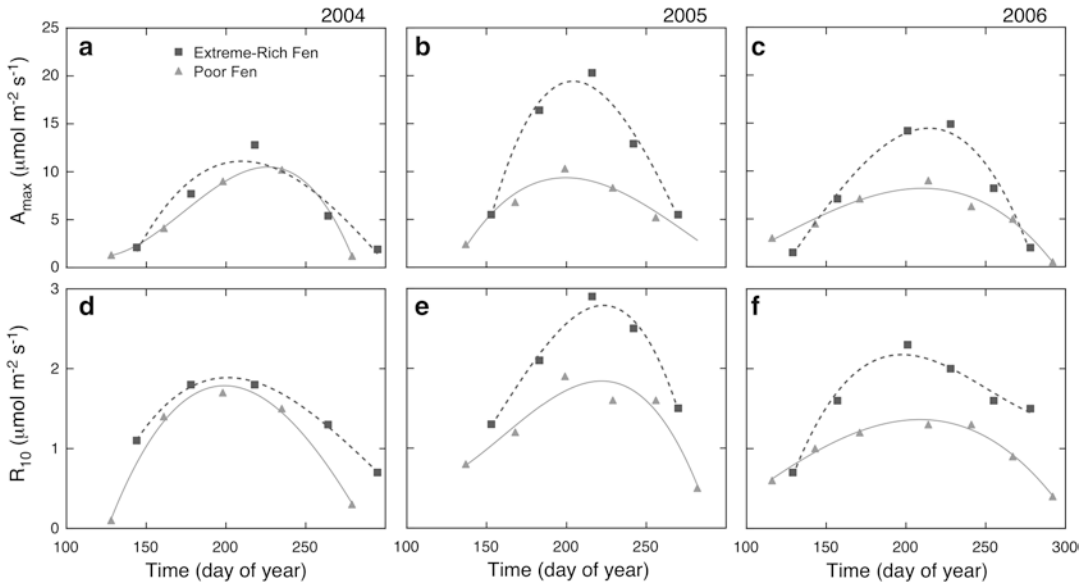


Fig. 14.2. Seasonal trends in fitted parameters describing ecosystem CO_2 exchange in three different study years (2004–2006) at the poor fen and extreme-rich fen sites: (**a**–**c**) the maximum photosynthetic capacity (A_{max}); and (**d**–**f**) the respiratory capacity at 10 °C (R_{10}). *Points* represent parameter estimates derived by fitting the following equation to measured net ecosystem productivity (NEP) data as described by Adkinson et al. (2011):

$$NEP = \frac{A_{max}\alpha\, PPFD}{A_{max} + \alpha\, PPFD} - R_{10}Q_{10}^{\left(\frac{T-10}{10}\right)}$$

Lines represent third order polynomial regressions fitted to the parameter estimates as a function of time (day of year) (This figure is based on data in Adkinson et al. (2011)).

only the surface of the moss has thawed (Bubier et al. 1998; Lafleur et al. 2003; Moore et al. 2006). Therefore, *Sphagnum* mosses and other evergreen vascular plants at the poor fen can remain photosynthetically active for a longer period of time within the growing season than the deciduous vegetation can at the extreme-rich fen. Also, photosynthetic and respiratory capacities at the poor fen varied less among years, even though both sites were exposed to similar inter-annual variation in environmental conditions (Fig. 14.2, Adkinson et al. 2011). We believe this to result from the fact that *Sphagnum* does not need to develop completely new photosynthetic tissue at the beginning of each growing season. By contrast, the growth of new leaf tissue at the extreme-rich fen, and the capacity for photosynthesis and respiration, may be influenced more strongly by environmental variation among years (Fig. 14.2). The development of new leaf tissue, particularly in *Carex lasiocarpa*, at the extreme-rich fen appeared to respond very quickly to variation in spring and early summer temperatures (Adkinson et al. 2011; and see additional text below). The differences in dominant plant functional types between the sites, therefore, appeared to control the magnitude of possible response to environmental change.

The two study sites also differed significantly in water table fluctuations and peat density. The extreme-rich fen had relatively large fluctuation in water table depth (Fig. 14.3; Adkinson et al. 2011) and high peat density (83.1 ± 18.3 kg m^{-3}, mean ± SD, n = 14). In contrast, the poor fen had low peat density (46.5 ± 7.5 kg m^{-3}, mean ± SD, n = 16) and an unstable surface that was likely not anchored to the bottom mineral soil sediments. Variation in the water table at the poor fen, therefore, may have been associated with similar changes in peat surface height, so that there was little or no movement of the water table relative to the peat surface (Adkinson et al. 2011). In this case, moisture availability in the peat at the poor fen would not vary significantly within the growing season or on an inter-annual

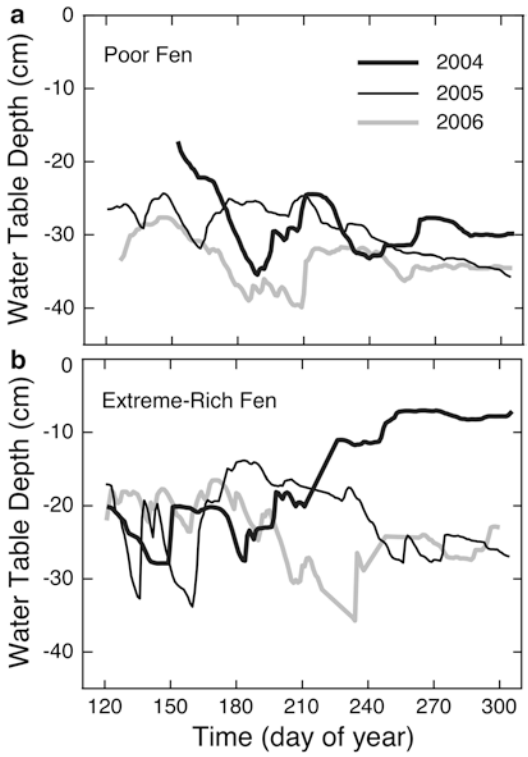


Fig. 14.3. Comparison of growing season daily average water table depths among study years at: (**a**) the poor fen site; and (**b**) the extreme-rich fen site. Water table depth was measured relative to a fixed height on the water well and expressed relative to the average hummock height measured in 2004 (This figure is reproduced from Adkinson et al. (2011)).

basis. However, variation in water table depth likely caused significant changes in "soil" moisture availability at the extreme-rich fen. Previous studies have shown that change in water table depth has little influence on peatland ecosystem CO_2 exchange if the water table variation is not accompanied by significant change in soil moisture content (Parmentier et al. 2009).

Important interactions can occur between temperature and water availability in peatland ecosystems with significant effects on ecosystem CO_2 exchange processes. For example, some previous studies have shown that warm and dry summer conditions can reduce net CO_2 uptake in peatlands by limiting photosynthesis and/or increasing respiration, and by promoting earlier leaf senescence in deciduous plants (Bellisario et al. 1998; Alm et al. 1999; Arneth et al. 2002; Bubier et al. 2003; Aurela et al. 2007; Cai et al. 2010). The effects of moisture stress on photosynthesis and respiration have been observed in both sedge-dominated and *Sphagnum*-dominated peatlands (Shurpali et al. 1995; Silvola et al. 1996; Alm et al. 1999; Griffis et al. 2000; Aurela et al. 2007; Sonnentag et al. 2010). In our comparative studies, ecosystem photosynthesis and respiration at the poor fen did not respond to significant inter-annual variation in temperature and water table depth, while the extreme-rich fen showed strong inter-annual variation in ecosystem CO_2 exchange (Figs. 14.1 and 14.2). In addition, the interacting effects of combined variation in temperature and water table depth at the extreme-rich fen contributed to the fluctuations in CO_2 exchange observed at that site (Adkinson et al. 2011). For example, during 2005 warm spring temperatures were accompanied by sufficient water availability throughout the period of leaf growth and this resulted in very high ecosystem photosynthetic capacity (A_{max}) at the extreme-rich fen (Fig. 14.2). This was likely primarily caused by high leaf area index, but possibly also by enhanced photosynthetic rates per unit leaf area. In contrast, the cool spring temperatures in 2004 appeared to inhibit A_{max} and leaf area development at the extreme-rich fen. The reduced water availability at the extreme-rich fen from the beginning of July 2006 onward, however, constrained development of high ecosystem photosynthetic capacity relative to 2005, despite the warm spring and summer temperatures in 2006 (Fig. 14.2). Prior to the decline in water table, conditions for growth were favorable in 2006, with warmer air and soil temperatures and a higher water table than in 2005, suggesting that reduced water availability was the primary factor contributing to the difference in peak photosynthetic capacity between 2005 and 2006 (Adkinson et al. 2011). In addition, warm temperatures in 2006, and the associated increase in evaporative demand, may have exacerbated the effect of declining water table on maximal leaf development in July and August. This

demonstrates the important interactions that occur between temperature and water availability in peatland ecosystems.

Our study of ecosystem response to inter-annual environmental variation has relevance to consideration of the potential responses of these two ecosystems to future climate change. Because of its floating nature, the poor fen will probably be quite resilient to anticipated warmer and drier conditions, as long as the current hydrological features of the site are not disrupted. By contrast, our data indicate that the extreme-rich fen should be much more susceptible to predicted warmer and drier conditions. The response of the extreme-rich fen to the very warm conditions in 2006 was constrained by reduced moisture availability in mid-growing season, and this occurred in a year when the cumulative precipitation during May-October was higher than normal (439 mm in 2006 versus the long-term average (± SD) of 381 ± 81 mm; Adkinson et al. 2011). This suggests that in the future, combined warmer- and drier-than-normal conditions could substantially affect ecosystem CO_2 exchange and net carbon sequestration at the extreme-rich fen site.

IV. Sensitivity of CO_2 Exchange in a Moderately-Rich Fen to Warmer and Drier Conditions

In this second case study, the sensitivities of gross ecosystem photosynthesis (GEP), total ecosystem respiration (TER) and NEP to variations in temperature and water table depth were examined in a moderately-rich treed fen (La Biche River), the most abundant peatland type in western Canada, in a region where peatland ecosystems are a significant landscape component (approximately 20 %; National Wetlands Working Group 1988; Vitt et al. 1998; Tarnocai 2006). During the 6-year study period, the average growing season (May-October) water depth declined approximately 38 cm (Fig. 14.4b), with the reduction in water table depth primarily caused by progressively lower precipitation in May-August throughout the study period

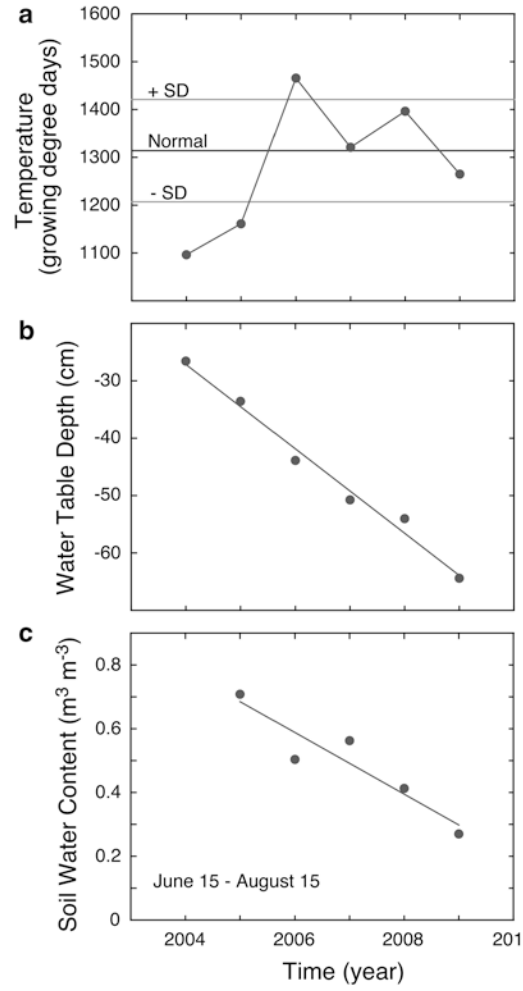


Fig. 14.4. Comparison of inter-annual variation in: (**a**) temperature (expressed as cumulative growing degree days, March-October); (**b**) water table depth (average value during May-October); and (**c**) average soil (peat) water content during mid-summer (June 15–August 15) at the La Biche River flux station. The line fitted to the data in panel (**b**) was: $y = -7.3531\ x + 14{,}709$, $r^2 = 0.98$. The line fitted to the data in panel (**c**) was: $y = -0.0967\ x + 194.61$, $r^2 = 0.87$. Soil water content measurements represent the average of three probes located at depths of 7.5, 10 and 12.5 cm below the surface in *Sphagnum* moss (This figure is based on data published in Flanagan and Syed (2011)).

(Fig. 14.5). For example, the cumulative precipitation during May-August in the final year of the study (134 mm in 2009) was less than 50 % of the long-term average ± SD (306 ± 68 mm) during this summer time period. In addition, during the study there

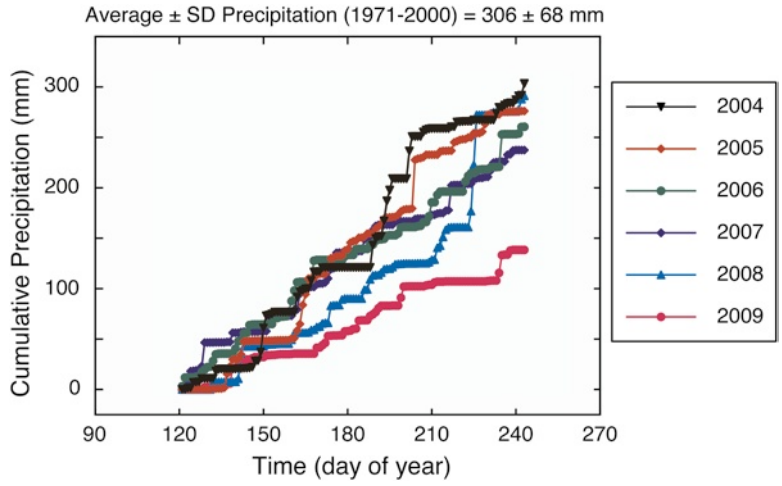


Fig. 14.5. Comparison of inter-annual variation in daily precipitation recorded during May-August at the La Biche River flux station in northern Alberta, Canada. The indicated average (± SD) is based on the long-term (1971–2000) Environment Canada weather records measured at nearby Athabasca, Alberta (This figure is reproduced from Flanagan and Syed (2011)).

was an associated significant linear decline in the average soil water content in the middle of the growing season (June 15–August 15; Fig. 14.4c). Temperature, expressed as cumulative growing degree days (GDD) during March-October, varied from a minimum of approximately 1,096 in 2004 to a high of 1,466 in 2006 (Fig. 14.4a). Based on the long-term (1971–2000) Environment Canada weather records at nearby Athabasca, Alberta, the average (± SD) GDD for the region was 1,310 ± 105 (Flanagan and Syed 2011). The difference of 370 GDD apparent among years of our study is equivalent to a change in elevation of approximately 600 m. We suggest, therefore, that observation of ecosystem response to the relatively large inter-annual environmental variation in temperature and moisture conditions apparent during our study period has relevance to understanding the potential response of this important ecosystem type to future climate change (Flanagan and Syed 2011).

Both GEP and TER showed similar increases in response to the warmer and drier conditions experienced during the 6-year study period (Fig. 14.6a–d). Photosynthesis and respiration were positively correlated with cumulative GDD (Pearson product–moment correlation coefficient (r); GEP, $r=0.762$; TER, $r=0.743$), and they were both negatively correlated with average water table depth (GEP, $r=-0.781$; TER, $r=-0.839$). In an observational study such as this, it is difficult to separate the effects temperature and water table changes because the two environmental changes were coincident and negatively correlated ($r=-0.569$). We conducted a variety of statistical analyses, however, to evaluate the relative importance of temperature and moisture effects on ecosystem photosynthesis and respiration, as described below.

The two-dimensional graphs shown in Fig. 14.6a–d assume independent responses to variation in temperature and changes in water table depth, when the environmental conditions were actually negatively correlated. Partial correlation analysis suggested that the response to changes in water table depth were more important than changes in temperature (Flanagan and Syed 2011). In addition, as shown in Fig. 14.6a–d we fitted the observed ecosystem photosynthesis and respiration data to optimum (bell-shaped) response functions for simultaneous change in both GDD and water table depth (dotted lines) and compared this with the optimum response function considering only one

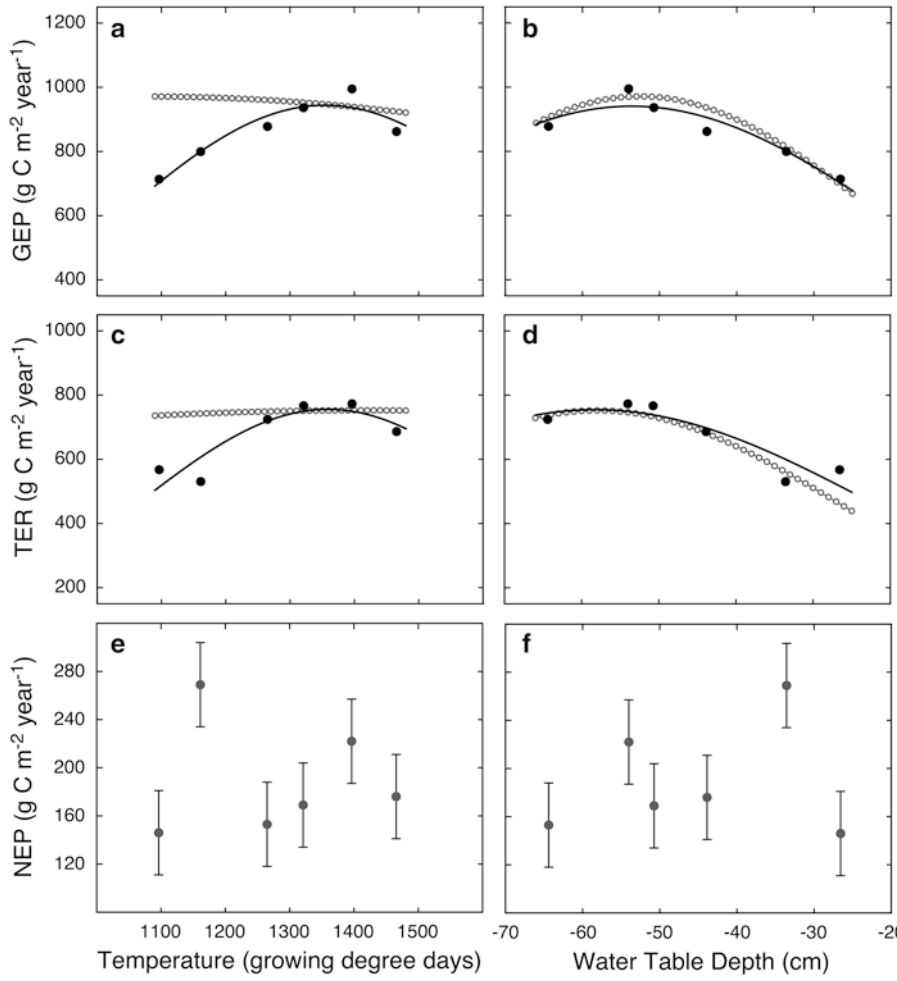


Fig. 14.6. Response of annual-integrated values of: (**a**, **b**) gross ecosystem photosynthesis (*GEP*); (**c**, **d**) total ecosystem respiration (*TER*); and (**e**, **f**) net ecosystem productivity (*NEP*), to variation in temperature (expressed as cumulative growing degree days, March-October) and water table depth (average value during May-October) at the La Biche River flux station in northern Alberta, Canada. The *solid lines* represent calculations done by assuming that variations in GEP and TER were only a result of changes caused by variation in temperature (**a**, **c**) or water table depth (**b**, **d**). The *dotted lines* represent calculations done by including the simultaneous effects of changes in both temperature and water table depth on GEP and TER, but the graphs (**a**–**d**) show the predicted response to due to changes in temperature (**a**, **c**) and water table depth (**b**, **d**) separately. The similarity of the *dotted* and *solid lines* responses to variation in water table depth indicated that inter-annual variation in water table depth controlled changes in GEP and TER, while inter-annual variation in temperature had virtually no effect. Error bars for the NEP measurements represent an estimate of total uncertainty in the eddy covariance measurements and associated data processing of ± 35 g C m^{-2} $year^{-1}$ (This figure is based on data published in Flanagan and Syed (2011)).

environmental factor at a time (solid lines) (Zhuang et al. 2004; Flanagan and Syed 2011). In the case of changes in water table depth, the fitted response considering both temperature and water table was almost identical to the fitted response considering only changes in water table depth. By contrast, for changes in temperature, the fitted response considering both temperature and water table depth was very different from the response that only included variation in temperature. The function that included consideration of

both environmental variables indicated that change in temperature had no significant effect (i.e. a flat-line response) on ecosystem photosynthesis and respiration. In other words, the reductions in photosynthesis and respiration observed in years with low temperature were not actually caused by low temperature, but were rather caused by the associated high water table that was present in those years. Our statistical analysis, therefore, strongly suggested that inter-annual variation in water table depth was the major cause of the variation we observed in GEP and TER (Flanagan and Syed 2011).

There was no significant correlation between annual NEP and either cumulative growing degree days ($r = -0.016$, $P > 0.05$) or average water table depth ($r = 0.189$, $P > 0.05$) (Fig. 14.6e, f). Inter-annual variation in NEP appeared to be associated with relatively subtle differences in the response of ecosystem photosynthesis and respiration to environmental conditions during the growing season. The ecosystem was a strong net sink for CO_2 with an average (± SD) NEP of 189 ± 47 g C m^{-2} $year^{-1}$ based on integrated eddy covariance measurements during the 6 year study.

The similar responses of ecosystem photosynthesis and respiration to warmer and drier conditions was contrary to previous predictions of peatland response to climate change which have suggested that respiration increases should override changes in photosynthesis and result in net losses of CO_2 as peatland ecosystems respond to warmer temperatures and lower water tables (Moore et al. 1998; Davidson and Janssens 2006; Tarnocai 2006). However, such predictions of a stronger response for respiration than photosynthesis may only occur if changes in temperature are the most important environmental change. If reduction in water table depth was the only significant environmental change, then more equitable responses of photosynthesis and respiration should be expected. In support of this suggestion, recent studies in a shrub-dominated wetland (Sulman et al. 2009) and in several fens (Sulman et al. 2010) have shown that reductions in the water table stimulated almost equal increases to ecosystem photosynthesis and respiration, so that there was no effect of water table changes on net ecosystem CO_2 exchange. Lower water tables can increase soil temperature, enhance oxygen supply to roots and improve nutrient availability, all factors that should stimulate both higher photosynthesis and respiration (Shaver et al. 1992; Larcher 1995). This has implications for future ecosystem responses to environmental change because draining of forested peatlands in Finland has resulted in continued net carbon sequestration in trees and soils for decades after the lowering of the water table (Minkkinen et al. 2002). In contrast, classic eco-physiological studies have shown that warmer temperatures alone do normally result in asymmetric responses of respiration and photosynthesis (Larcher 1995).

V. Peatland Succession and Implications for Historical and Future Carbon Sequestration

The average NEP value (189 g C m^{-2} $year^{-1}$) we measured via eddy covariance at the moderately-rich fen site was 3.8 times higher than the value (50 g C m^{-2} $year^{-1}$) predicted from peat core carbon accumulation and carbon losses associated with methane emission and organic and inorganic carbon in runoff (Flanagan and Syed 2011). In addition our NEP measurements were higher than values reported by other eddy covariance studies in several peatland types in Canada and Europe (Flanagan and Syed 2011). There are a number of factors that have contributed to the high rates of NEP we have measured over the last 6 years. First, our study site has a relatively high leaf area index (2.6) compared with many other peatland sites (range 0.4–2.3; Humphreys et al. 2006; Lund et al. 2010), and LAI is increasing with recent tree growth and tree density changes. Approximately 50 % of the peak summer leaf area was contributed by a broad-leaf shrub (*Betula pumila*) and the two tree species, and both *Betula* and *Larix laricina* have relatively high leaf-level

photosynthetic rates (Syed et al. 2006). The increasing woody plant biomass, with high C/N ratio, allows significant rates of carbon sequestration to occur even in a relatively nutrient limited peatland environment (Shaver et al. 1992, 2000). In addition, the nutrient availability at our study site was also likely higher than at many of the other peatlands reported in the literature, sites that are generally oligotrophic and mostly bogs (Flanagan and Syed 2011). Beyond these factors, the successional status of our moderately-rich fen site will strongly influence the observed current rates of ecosystem net carbon sequestration, as will be discussed more fully below.

A general pattern of successional development occurs in peatlands in continental, boreal regions of Canada (Kuhry et al. 1993). In this successional pattern a site develops through a series of stages from a pond, to marsh or open fen, to a treed rich fen, to a treed poor fen, to a forested dry bog. The terms "rich" and "poor" relate to species richness, not nutrient availability (Vitt et al. 1998). The transition from a rich fen to a poor fen is strongly correlated with a change in pH of the near-surface water from above pH 6 to below pH 5 (Kuhry et al. 1993). This temporal pattern of vegetation change is largely an internal (autogenic) process that is controlled by peat accumulation and a shift from early dominance of brown moss species (particularly *Drepanocladus*) to later dominance of *Sphagnum* moss, with the *Sphagnum* species responsible for an acidification of the water. Continued *Sphagnum* peat accumulation and acidification creates conditions for establishment and growth of *Picea mariana* trees and results in the surface of the peatland becoming elevated and separated from the mineral-rich ground water. Ultimately this process leads to the development of a forested bog where the surface vegetation relies strongly on precipitation input for water and nutrients (Kuhry et al. 1993).

Our study site is currently classified as a moderately-rich treed fen, but it appears to be near the stage of transition toward a poor fen. This suggestion is supported by the fact that a mixture of brown moss species and *Sphagnum* moss species are currently present

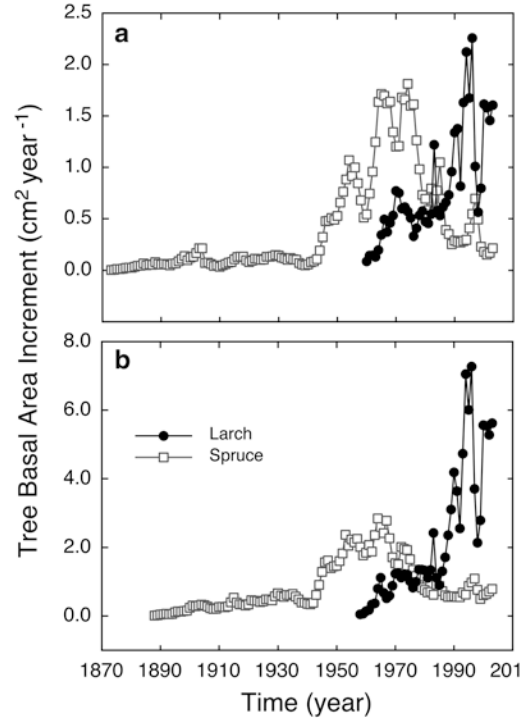


Fig. 14.7. Comparison of average basal area increment (*BAI*) of *Picea mariana* (Spruce) and *Larix laricina* (Larch) trees at the La Biche River flux station in northern Alberta, Canada. Tree cores were sampled in 2004. The calculated BAI was averaged for each year in the chronology for the five trees of a species. This was done separately for the two locations where the increment cores were collected: (**a**) approximately 100 m north of the flux tower; and (**b**) approximately 100 m south of the flux tower (This figure is reproduced from Flanagan and Syed (2011)).

at the site, but the surface water pH is still 6.2 (Syed et al. 2006). Analysis of increment cores of the oldest trees (Fig. 14.7) and examination of time sequences of air photographs of the site (data not shown) indicate that significant tree growth was not apparent until the early 1960s. The oldest *Picea mariana* trees at the site were established in approximately 1870, although peak basal area increment growth in these *Picea* trees did not occur until approximately 1960, the approximate time when the oldest *Larix laricina* trees became established at the site (Fig. 14.7). These data suggest that it was only about 50 years ago that peat accumulation reached the point where the peatland surface was

stable enough to support significant tree growth rates, although the majority of the larger trees present at the site still have stunted growth features consistent with the influence of water logging caused by a high water table (Syed et al. 2006). While the growth rate of oldest *Picea* trees has passed its peak (Fig. 14.7), there has been significant recruitment of a new cohort of *Picea* trees as shown by the proportionally high biomass (approximately 20 % of total above ground biomass) for small trees with a DBH less than 1 cm (Syed et al. 2006).

The significance of this information on peatland successional development and patterns of tree establishment is that the study site is currently changing from a relatively open fen with small stunted trees toward a poor fen with a greater density of *Picea* trees that, in the absence of external disturbance, will eventually form a closed canopy forested bog. The site is now in a phase of relatively rapid tree establishment and associated increase in LAI. An analogous mid-successional stage in upland forest ecosystems is a period when high rates of ecosystem NEP are usually apparent (Bond-Lamberty et al. 2006; Goulden et al. 2011). This has implications for comparisons between current rates of ecosystem NEP as measured by eddy covariance, with historical rates of net carbon accumulation measured in peat cores. In addition, the transition from rich fen to forested dry bog may require 50–350 years (Kuhry et al. 1993). As the succession occurs and above ground biomass accumulates, there should be an associated increase in ecosystem respiration and a decline in NEP until it reaches a near zero steady state value (Bond-Lamberty et al. 2006; Goulden et al. 2011). Therefore, in the absence of fire or other major disturbance, significant net carbon sequestration could continue for decades at this site and help to reduce the positive feedback of climate change on increasing atmospheric CO_2 concentration. However, climate change-induced warmer and drier conditions could also increase the risk of fire disturbance, which would release significant amounts of stored carbon and reset the succession to an early, less productive stage (Turetsky et al. 2002; Amiro et al. 2009; Goulden et al. 2011).

VI. Conclusions

Northern peatland ecosystems have been consistent carbon sinks for millennia, but it has been predicted that exposure to warmer temperatures and drier conditions associated with climate change will shift the balance between ecosystem photosynthesis and respiration providing a positive feedback to atmospheric CO_2 concentration. I suggest that this prediction is over simplified and ignores a number of important interacting biological and environmental factors that influence ecosystem photosynthesis, respiration and net carbon sequestration in peatland ecosystems. Peatland ecosystems exist in a wide variety of types with contrasting plant community composition associated with variation in water chemistry and hydrological features and water inputs. Because of contrasting dominant plant functional types in these different peatlands, the response of ecosystem CO_2 exchange to temperature and moisture variation can differ dramatically among peatland types. Biological succession can also result in significant changes in plant species composition over time that can influence patterns of ecosystem carbon sequestration for extended periods of time (e.g. potentially hundreds of years). Coupled to this complex biological variability are the strong interactions that occur between temperature and moisture effects on ecosystem photosynthesis and respiration. As shown here, if water table depth and soil moisture are the dominant environmental change influencing peatlands, initial reductions in water table depth may actually increase both photosynthesis and respiration because water tables currently tend to be higher than is optimal for supplying adequate root zone oxygen, which in turn is necessary for high levels of biological metabolism in peatlands. If trees are able to establish on peatland sites, net carbon sequestration in trees and peat soils may continue for decades after the

lowering of the water table, as long as the local hydrology does not change dramatically and the ecosystems are not exposed to severe soil moisture shortages. Prediction of the implications of future climate change effects on peatlands would be improved if global-scale models could include more details of the biological variability among peatlands (both spatial and temporal), with realistic parameterizations of the interacting responses of these ecosystems to variation in temperature, water table depth and soil moisture.

Acknowledgements

This research was supported by grants from the Natural Sciences and Engineering Research Council of Canada, the Canadian Foundation for Climate and Atmospheric Sciences, and Biosphere Implications of CO_2 Policy in Canada (BIOCAP). I thank Angela Adkinson, Peter Carlson, Aaron Glenn, Kamran Syed, and Eric Van Gaalen for their contributions to the research that is reviewed here.

References

Adkinson AC, Syed KH, Flanagan LB (2011) Contrasting responses of growing season ecosystem CO_2 exchange to variation in temperature and water table depth in two peatlands in northern Alberta, Canada. J Geophys Res 116:G01004. doi:10.1029/2010JG001512

Alm J, Schulman L, Walden J, Nykanen H, Martikainen PJ, Silvola J (1999) Carbon balance of a boreal bog during a year with an exceptionally dry summer. Ecology 80:161–174

Amiro BD, Cantin A, Flannigan MD, de Groot WJ (2009) Future emissions from Canadian boreal forest fires. Can J For Res 39:383–395

Arneth A, Kurbatova J, Kolle O, Shibistova OB, Lloyd J, Vygodskaya NN, Schulze E-D (2002) Comparative ecosystem-atmosphere exchange of energy and mass in a European Russian and a central Siberian bog II. Interseasonal and interannual variability in CO_2 fluxes. Tellus 54B:514–530

Aurela M, Riutta T, Laurila T, Tuovinen J-P, Vesala T, Tuittila E-S, Rinne J, Haapanala S (2007) CO_2 exchange of a sedge fen in southern Finland – the impact of a drought period. Tellus 59B:826–837

Baldocchi DD (2003) Assessing the eddy covariance technique for evaluating carbon dioxide exchange rates of ecosystems: past, present, and future. Glob Change Biol 9:479–492

Barr AG, Black TA, Hogg EH, Kljun N, Morgenstern K, Nesic Z (2004) Inter-annual variability in the leaf area index of a boreal aspen-hazelnut forest in relation to net ecosystem production. Agric For Meteorol 126:237–255

Bellisario LM, Moore TR, Bubier JL (1998) Net ecosystem CO_2 exchange in a boreal peatland, northern Manitoba. Ecoscience 5:534–541

Bond-Lamberty B, Gower ST, Goulden ML, McMillan A (2006) Simulation of boreal black spruce chronosequences: comparison to field measurements and model evaluation. J Geophys Res 11, G02014. doi:10.1029/2005JG000123

Bubier JL, Crill PM, Moore TR, Savage K, Varner RK (1998) Seasonal patterns and controls on net ecosystem CO_2 exchange in a boreal peatland complex. Global Biogeochem Cycles 12:703–714

Bubier JL, Bhatia G, Moore TR, Roulet NT, Lafleur PM (2003) Spatial and temporal variability in growing-season net ecosystem carbon dioxide exchange at a large peatland in Ontario, Canada. Ecosystems 6:353–367

Cai T, Flanagan LB, Syed KH (2010) Warmer and drier conditions stimulate respiration more than photosynthesis in a boreal peatland ecosystem: analysis of automatic chambers and eddy covariance measurements. Plant Cell Environ 33:394–407

Davidson EA, Janssens IA (2006) Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440:165–173

Desai AR, Richardson AD, Moffat AN, Kattge J, Hollinger DY, Barr A, Falge E, Noormets A, Papale D, Reichstein M, Stauch VJ (2008) Cross-site evaluation of eddy covariance GPP and RE decomposition techniques. Agric For Meteorol 148:821–838

Flanagan LB, Syed KH (2011) Stimulation of both photosynthesis and respiration in response to warmer and drier conditions in a boreal peatland ecosystem. Glob Change Biol 17:2271–2287

Glenn AJ, Flanagan LB, Syed KH, Carlson PJ (2006) Comparison of net ecosystem CO_2 exchange in two peatlands in western Canada with contrasting dominant vegetation, *Sphagnum* and *Carex*. Agric For Meteorol 140:115–135

Gorham E (1991) Northern peatlands: role in the carbon cycle and probable responses to climatic warming. Ecol Appl 1:182–195

Goulden ML, McMillan AMS, Winston GC, Rocha AV, Manies KL, Harden JW, Bond-Lamberty BP (2011) Patterns of NPP, GPP, respiration, and NEP during boreal forest succession. Glob Change Biol 17:855–871

Griffis TJ, Rouse WR, Waddington JM (2000) Interannual variability of net ecosystem CO_2 exchange at a subarctic fen. Global Biogeochem Cycles 14:1109–1121

Humphreys ER, Lafleur PM, Flanagan LB, Hedstrom N, Syed KH, Glenn AJ, Granger R (2006) Summer carbon dioxide and water vapor fluxes across a range of northern peatlands. J Geophys Res 111:G04011. doi:10.1029/2005JG000111

Johnson D, Kershaw L, MacKinnon A, Pojar J (1995) Plants of the western boreal forest and Aspen Parkland. Lone Pine, Edmonton

Kuhry P, Nicholson BJ, Gignac LD, Vitt DH, Bayley SE (1993) Development of *Sphagnum*-dominated peatlands in boreal continental Canada. Can J Bot 71:10–22

Lafleur PM, Roulet NT, Bubier JL, Frolking S, Moore TR (2003) Interannual variability in the peatland-atmosphere carbon dioxide exchange at an ombrotrophic bog. Global Biogeochem Cycles 17:1036. doi:10.1029/2002GB001983

Larcher W (1995) Physiological plant ecology: ecophysiology and stress physiology of functional groups, 3rd edn. Springer, Berlin

Lund M, Lafleur PM, Roulet NT, Lindroth A, Christensen TR, Aurela M, Chojnick BH, Flanagan LB, Humphreys ER, Laurila T, Oechel WC, Olejnik J, Rinne J, Schubert P, Nilsson MB (2010) Variability in exchange of CO_2 across 12 northern peatland and tundra sites. Glob Change Biol 16:2436–2448

Minkkinen K, Korhonen R, Savolainen I, Laine J (2002) Carbon balance and radiative forcing of Finnish peatlands 1900–2100 – the impact of forestry drainage. Glob Change Biol 8:785–799

Moffat AM, Papale D, Reichstein M, Hollinger DY, Richardson AD, Barr AG, Beckstein C, Braswell BH, Churkina G, Desai AR, Falge E, Gove JH, Heimann M, Hui D, Jarvis AJ, Kattge J, Noormets A, Stauch VJ (2007) Comprehensive comparison of gap-filling techniques for eddy covariance net carbon fluxes. Agric For Meteorol 147:209–232

Moncrieff JB, Jarvis PG, Valentini R (2000) Canopy fluxes. In: Sala OE, Jackson RB, Mooney HA, Howarth RW (eds) Methods in ecosystem science. Springer, New York, pp 161–180

Moore TR, Roulet NT, Waddington JM (1998) Uncertainty in predicting the effect of climatic change on the carbon cycling of Canadian peatlands. Clim Chang 40:229–245

Moore TR, Lafleur PM, Poon DMI, Heumann BW, Seaquist JW, Roulet NT (2006) Spring photosynthesis in a cool temperate bog. Glob Change Biol 12:2323–2335

National Wetlands Working Group (1988) Wetlands of Canada. Ecological land classification series no. 24. Sustainable Development Branch, Environment Canada/Polyscience Publications, Inc., Ottawa/Montreal

Parmentier FJW, van der Molen MK, de Jeu RAM, Hendriks DMD, Dolman AJ (2009) CO_2 fluxes and evaporation on a peatland in the Netherlands appear not affected by water table fluctuations. Agric For Meteorol 149:1201–1208

Shaver GR, Billings WD, Chapin FS, Giblin LF, Nadelhoffer KJ, Oechel WC, Rastetter EB (1992) Global change and the carbon balance of arctic ecosystems. Bioscience 42:433–441

Shaver GR, Billings WD, Chapin FS, Gurevitch J, Harte J, Henry G, Ineson P, Jonasson S, Melillo J, Pitelka L, Rustad L (2000) Global warming and terrestrial ecosystems: a conceptual framework for analysis. Bioscience 50:871–882

Shurpali NJ, Verma SB, Kim J (1995) Carbon dioxide exchange in a peatland ecosystem. J Geophys Res 100:14319–14326

Silvola J, Alm J, Ahlholm U, Nykanen H, Marikainen PJ (1996) CO_2 fluxes from peat in boreal mires under varying temperature and moisture conditions. J Ecol 84:219–228

Sonnentag O, van der Kamp G, Barr AG, Chen JM (2010) On the relationship between water table depth and water vapor and carbon dioxide fluxes in a minerotrophic fen. Glob Change Biol 16:1762–1776

Sulman BN, Desai AR, Cook BD, Saliendra N, Mackay DS (2009) Contrasting carbon dioxide fluxes between a drying shrub wetland in Northern Wisconsin, USA, and nearby forests. Biogeosciences 6:1115–1126

Sulman BN, Desai AR, Saliendra N, Lafleur PM, Flanagan LB, Sonnentag O, Mackay DS, Barr AG, van der Kamp G (2010) CO_2 fluxes at northern fens and bogs have opposite responses to interannual fluctuations in water table. Geophys Res Lett 37:L19702. doi:10.1029/2010GL044018

Syed KH, Flanagan LB, Carlson PJ, Glenn AJ, Van Gaalen KE (2006) Environmental control of net ecosystem CO_2 exchange and carbon balance in a treed, moderately rich fen in northern Alberta. Agric For Meteorol 140:97–114

Tarnocai C (2006) The effect of climate change on carbon in Canadian peatlands. Glob Planet Change 53:222–232

Turetsky MR, Weider RK, Halsey LA, Vitt DH (2002) Current disturbance and the diminishing peatland carbon sink. Geophys Res Lett 29:1526. doi:10.1029/2001GL014000

Turunen J, Tomppo E, Tolonen K, Reinikainen A (2002) Estimating carbon accumulation rates of undrained

mires in Finland – application to boreal and subarctic regions. Holocene 12:69–80

Vitt DH (1994) An overview of the factors that influence the development of Canadian peatlands. Mem Entomol Soc Can 169:7–20

Vitt DH, Halsey LA, Thormann MN, Martin T (1998) Peatland Inventory of Alberta. Phase 1: Overview of peatland resources in the natural regions and subregions of the province. Sustainable Forest Management Network, University of Alberta, Edmonton

Williams TG, Flanagan LB (1998) Measuring and modelling environmental influences on photosynthetic gas exchange in *Sphagnum* and *Pleurozium*. Plant Cell Environ 21:555–564

Zhuang Q, Melillo JM, Kicklighter DW, Prinn RG, McGuire AD, Steudler PA, Felzer BS, Hu S (2004) Methane fluxes between terrestrial ecosystems and the atmosphere at northern high latitudes during the past century: a retrospective analysis with a process-based biogeochemistry model. Global Biogeochem Cycles 18:GB3010. doi:10.1029/2004GB002239

Chapter 15

Physiological Ecology of Tropical Bryophytes

Sebastian Wagner and Maaike Y. Bader*
Department of Biology and Environmental Sciences, Functional Ecology of Plants, University of Oldenburg, 26111 Oldenburg, Germany

Gerhard Zotz
Department of Biology and Environmental Sciences, Functional Ecology of Plants, University of Oldenburg, 26111 Oldenburg, Germany

Smithsonian Tropical Research Institute, Balboa, Panama

Summary

Bryophytes in the tropics occur from cool alpine grasslands to warm lowland sites and from cloud forests to dry forests, varying markedly in abundance and diversity in these habitats. This chapter deals with the current knowledge of the ecophysiology of tropical bryophytes attempting to explain some of these abundance patterns, in particular the marked increase in bryophyte biomass with altitude in rain and cloud forests. As data are scarce, we include data on, physiologically rather similar, lichens in our account where appropriate. We focus

*Author for correspondence, e-mail: maaike.bader@uni-oldenburg.de

D.T. Hanson and S.K. Rice (eds.), Photosynthesis in Bryophytes and Early Land Plants, Advances in Photosynthesis and Respiration 37, pp. 269–289, DOI 10.1007/978-94-007-6988-5_15,

mostly on carbon relations, and water, nutrients, light, CO_2 and temperature are discussed as co-determinants of the carbon balance. In particular, we address the hypothesis that the surprisingly low bryophyte abundance in lowland rainforests is due to the limitation of net carbon gain by fast drying and low light levels during the day combined with moist and warm conditions at night, which promote high respiration rates. The timing of hydration is crucial in determining this diel balance between photosynthesis and respiration. Temperature is important in determining moisture loss rates and nocturnal carbon loss through respiration – if respiration does not acclimatize to higher temperatures. Since carbon balance precariously depends on daily hydration patterns, future climate change may pose a serious problem to tropical lowland bryophytes.

I. Introduction

Tropical forests harbor a particularly large diversity of bryophytes (mainly mosses and liverworts). The Neotropics alone are home to some 4,000 species of bryophytes, which represents about a quarter of the global species number (Gradstein et al. 2001). The majority of species grows epiphytically on live stems and branches of trees and shrubs, although a variety of other substrates are also used, -e.g. leaves (these epiphytes are referred to as epiphylls, Ruinen 1953), rocks, decaying wood, or termite mounds (Pócs 1982). Lowland rainforest floors are virtually devoid of bryophytes, probably due to high litter input, but subalpine forests can boast fully moss-covered soils (Wolf 1993) and terrestrial mosses can also be found in alpine bogs (Bosman et al. 1993) and in drier regions as a component of biological soil crusts (Belnap and Lange 2001; Godínez-Alvarez et al. 2012).

Bryophytes (and lichens) show a dramatic increase in abundance (e.g. Frahm 1990a; Wolf 1993) and diversity (e.g. Seifriz 1924; Pócs 1982; Gradstein and Pócs 1989) as one moves uphill away from the tropical lowlands. This increase is particularly noticeable above 1,000 m elevation and often reaches a maximum in upper montane forests around 3,000 m, while decreasing again above the treeline (Grau et al. 2007). For example, the "bryomass" in the Uluguru mountains in Tanzania increased from 1 kg ha^{-1} at 20 m elevation to 440 kg ha^{-1} in the 'mossy' forest at 2,100 m. (Frahm 1990a). Not surprisingly then, the impact of bryophytes on ecosystem function, particularly on hydrology (Pócs 1980; Hölscher et al. 2004) but also on nutrient cycling, is highest in montane systems (Hofstede et al. 1993; Nadkarni et al. 2004; Clark et al. 2005). For example, it has been extrapolated by Pócs (1980), that epiphytic bryophytes in an elfin forest may intercept more than 40,000 l of precipitation ha^{-1} $year^{-1}$. Leaching and decomposition of bryophyte organic material result in a pulsed release of nutrients after rehydration of dry mosses. Coxson (1991) estimated an efflux of 80 kg K ha^{-1} $year^{-1}$ in a tropical montane rainforest on Guadeloupe.

This ecological importance contrasts strongly with the availability of information on the ecophysiology of this plant group in the tropics. Just a handful of studies has addressed the ecophysiology of tropical lowland bryophytes, focusing on desiccation tolerance (Biebl 1964; Johnson and Kokila 1970), temperature tolerance (Biebl 1967), or photosynthetic light response and related functional traits (Waite and Sack 2010) and photosynthetic light, water, and temperature responses (Wagner et al. 2013). For bryophytes from the tropical montane zone the situation is somewhat better, with a number of published studies on CO_2 exchange (Frahm 1987a, b; Lösch et al. 1994; Zotz et al. 1997; Wagner et al. 2013), water relations (Biebl 1964; Proctor 2002) and temperature responses (Lösch and Mülders 2000). For upper montane cloud forest the information is very scarce again (Lösch et al. 1994; Lösch and Mülders 2000; Romero et al. 2006), though an interesting recent contribution addresses altitudinal patterns, including

lowland and upper-montane sites (300–2,200 m elevation), in various ecophysiological parameters ($\delta^{13}C$, N:P; Waite and Sack 2011a, b). We restrict this review to these moist forest types, simply because no ecophysiological data are available for bryophytes in other tropical ecosystems such as páramos, grasslands, dry forests or deserts. The dearth of information on mosses caused us to include some studies on tropical forest lichens in this review. This seems appropriate since both groups share major characteristics of their physiology, in particular their size and poikilohydric habit (Green and Lange 1994). Even after combining studies with lichens and mosses, the current data basis is thin, yet it is substantial enough to allow some major conclusions.

Several of the available studies focus on the same question: what is the physiological basis of the described gradient in diversity and abundance with altitude? Possible explanatory factors are reviewed in Frahm (1987a, b):

1. Increase of irradiance with increasing altitude
2. Decrease of temperature with increasing altitude
3. Increase of precipitation with increasing altitude
4. Direct influence of fog and dew in tropical montane forest

Tested in isolation, all these suggestions can be partly rejected. For example, shady locations in montane rainforests may still have lush bryophyte cover. On the other hand, even in wet tropical lowland rainforests where monthly rainfall always exceeds 100 mm, bryophytes are mostly rare. In fact, globally there is hardly any correlation between precipitation and bryophyte biomass (Lakatos 2011), as counterintuitive as this may be. On the other hand, air humidity correlates with moss cover within the tropical lowlands up to 650 m a.s.l. and highlands from 1,000 to 3,500 m a.s.l. (Karger et al. 2012). Also, in rare lowland situations where cool air accumulates at night and bryophytes are moistened by fog on most mornings, as in the newly described 'lowland cloud forest' in the Guyana Basin (Gradstein et al. 2010), bryophytes are quite abundant in the forest canopy. This *combination* of environmental conditions, also found in bryophyte-rich tropical montane forests and temperate rain forests, appears to be particularly favorable for bryophyte growth. Such observations lend support to an ecophysiological explanation for the lack of bryophytes in tropical lowland rainforests originally proposed by Richards (1984). He suggested that the low bryomass in the lowlands is due to high temperatures at night causing high respiration rates and large nocturnal carbon losses, which cannot be compensated during the day, when photosynthesis is restricted either by low light levels (during rainstorms and in the forest understory) or, in high-light conditions, by fast drying (Fig. 15.1).

In the following sections the current understanding of the physiological ecology of tropical bryophytes will be reviewed. We start with a brief description of particularities of the physical setting in the tropics and continue by discussing what is known about carbon relations of tropical bryophytes, or lichens if information on bryophytes is lacking completely. The problems described by Richards (1984) are a recurrent theme: the timing of hydration, light limitation and respiratory losses at high night temperatures. Considering the potentially dramatic impact of climatic changes on tropical ecosystems (e.g. Solomon et al. 2009; Wright et al. 2009; Lewis et al. 2011) we will end our contribution with a short outlook on the particular fate of tropical non-vascular epiphytes.

II. The Physical Setting

Physiologically, tropical bryophytes probably do not differ fundamentally from bryophytes elsewhere – or rather, the data basis is too thin to decide whether they do (Proctor 2002; Waite and Sack 2010). Yet the tropical environment sets particular limits and requirements for bryophyte functioning and growth. Tropical climates are not uniform, which is reflected in the range of vegetation types in the lowland tropics from, e.g., wet rain forests to savannahs to deserts. Mountains introduce elevational cooling trends, which also affect

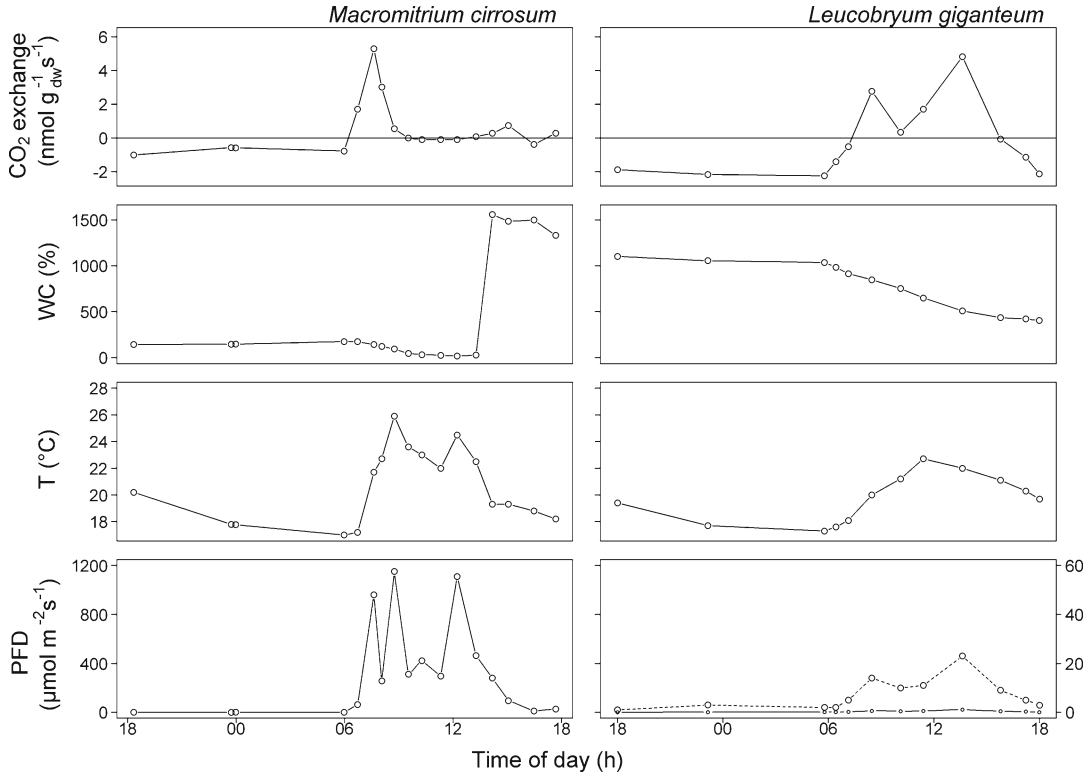


Fig. 15.1. Diel gas exchange courses of two bryophyte species of similar life form from a canopy (*left,* redrawn from Zotz et al. 1997) and a forest understory species (*right*, Westerman and Zotz unpublished data) in a lower montane rain forest (Fortuna, Panama, see section "The physical setting"). From *top to bottom*: net CO_2-exchange (NP, nmol g^{-1} s^{-1}), water content of the shoots (WC, $\%_{DW}$), air temperature (T, °C) and photon flux density (PFD, μmol m^2 s^{-1}). Note how in the understory the moss is active all day, with NP restricted by very low PFD, while in the canopy NP is restricted by low WC with no activity for several hours around noon. Activity after an afternoon rain is low (due to low PFD and possibly supersaturation). Note the 20-fold difference in the scales of the PFD axes in the lower right panel (*dashed line* scaled on right-hand y-axis)! Similar NP rates in spite of this are due to different maximum rates of the two species.

precipitation regimes, adding additional vegetation types. Tropical climates differ from all non-tropical climates in one respect: diurnal fluctuations in temperature are generally more pronounced than seasonal ones. Seasonal changes in precipitation, on the other hand, are common. Noteworthy, an extended dry season may exclude bryophyte taxa with limited desiccation tolerance (see sections "Effects of hydration and desiccation on carbon balance" and "Desiccation tolerance: desiccation duration and intensity"). Thus, a "tropical" site is not necessarily a wet and/or warm site, so that the local occurrence of a tropical bryophyte species may well be restricted by drought in a coastal desert or by frost in the páramo. Apart from these large-scale gradients in environmental conditions, there is predictable variation at a smaller scale, in particular along the vertical gradients of light, humidity, wind speed and temporal variability inside a forest. This temporal variability, daily oscillations in light and temperature in particular, is more pronounced in the upper strata of a forest than in the understory (Zotz and Winter 1994).

This chapter on tropical bryophytes focuses on wetter vegetation types, moist or wet lowland rainforests and montane rain or cloud forests. The following paragraph

summarizes the general physical setting of tropical wet climates (Richards 1996). The most striking feature of the tropics is the high humidity, which is generally 60–80 % (or even higher) during the day and 95–100 % during the night. Within 10° latitude of the equator the mean annual temperature range is between 24 and 28 °C in the lowlands, while temperatures over 35 °C are rare due to the cooling effect of evapotranspiration. The decline of temperature with altitude differs among regions. For example, in Costa Rica the decline is highest up to the cloud base with 1.2 °C per 100 m, lowest within the clouds and moderate above the cloud belt (0.6 °C per 100 m). By definition, annual precipitation in the wet tropics is high, ranging from 1,700 to >10,000 mm, with rainfall >3,000 mm, as found in western Colombia or New Guinea, considered as 'super wet'. In most cases there are two rainfall peaks annually, and north of 10° only one peak. The dry season (<100 mm $month^{-1}$) lasts typically less than 3 months and is irregular from year to year in the wet zones. In the super wet zone, dry periods are rare very short (less than a few weeks) and occur infrequently and irregularly.

Detailed climate data from lowland rainforests are readily available (e.g. from research stations like La Selva in Costa Rica or BCI in Panama), while data from montane habitats are rarer. For bryophyte ecophysiology, the most relevant information is on the microclimate directly at the bryophyte growing site and we will here present such data from a lower montane rainforest in western Panama (Fortuna, ca. 1,100 m; Zotz et al. unpublished). This is the locality where most of the available gas-exchange data for montane bryophytes and especially lichens have been collected (Zotz et al. 1998; Lange et al. 2000, 2004). The data presented here were recorded *in situ* for 14 days from September 18 to October 2, 1994.

Sunlight (measured as photosynthetic photon flux density, PFD) incident on the canopy exceeded 3,000 μmol m^{-2} s^{-1} on 5 of the 14 days, with an absolute maximum of 3,245 μmol m^{-2} s^{-1}. Daily integrated PFD above the forest canopy ranged from 15 to 48 mol m^{-2} day^{-1}, averaging 29 mol m^{-2} day^{-1}. Although surprisingly high at first glance, such values are not unique. For example, Körner et al. (1983) report peak values during sunny spells in overcast situations of >2,800 μmol m^{-2} s^{-1} from Pinadaunde Valley, New Guinea (ca. 3,500 m elevation), and maximum PFD values >2,500 μmol m^{-2} s^{-1} are also regularly reported from the lowland sites of La Selva (Costa Rica) and BCI (Panama): on 24 and 14 %, respectively, of all days during 1993–1995 (Sources: http://www.ots.ac.cr/en/laselva/metereological.shtml and http://stri.si.edu/sites/esp/). Not surprisingly, PFD within the forest differs drastically from such high values. In the understory in Fortuna, daily maxima exceeded 20 μmol m^{-2} s^{-1}only during brief light flecks (Fig. 15.1).

Diurnal changes in air temperature averaged 6.9 ± 1.3 °C, with night temperatures of 16–17 °C and peaks of up to 26 °C around noon. Air temperature gradients within the forest were negligible, but temperature is buffered inside the forest compared to the forest edge (Fig. 15.2).

Although no moss was measured, temperature measurements of a lichen thallus on a branch at ca. 3 m height at the forest edge during the same period should also be instructive for mosses. Maximum temperatures reached up to 36°, but averages were only slightly elevated compared to ambient (+1.2 °C).

While overall precipitation was quite high (ca. 100 mm in 14 days), rainless periods were rather long with average intervals between rain events (>1 mm) of 23 h. However, 'lateral' precipitation in the form of mist or fog, which is not captured by traditional rain gauges, occurred nearly every morning and sometimes during the day as well. Cavelier et al. (1996) found fog interception contributing between 2.4 and 61 % of the total water input depending on altitude in tropical Panama. Early-morning air was invariably saturated with water vapor and even around noon the relative humidity was still 75–80 %.

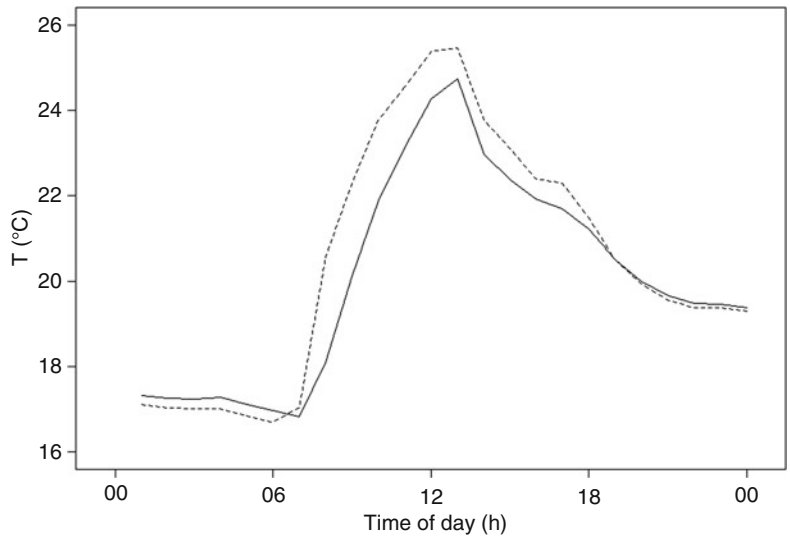


Fig. 15.2. Diel course of hourly temperature means at ca.2 m height in Fortuna, Panama, on July 7th 2011 (Wagner et al. unpublished data). *Dashed line*: forest edge. *Solid line*: inside forest.

Table 15.1. CO_2 concentrations (ppm) in the air and within the phylloplane of mosses, growing on living and dead wood in the lower parts of the forest measured before sunrise (5 am) and at 9 am on Sept. 30, 1994 in the lower montane rainforest of Fortuna, Panama (ca. 1,100 m a.s.l.)

Location	[CO_2] (ppm) 5:00 am	[CO_2] (ppm) 9:00 am	n
Air, forest edge	382	361	2, 2
Air, forest interior	393 ± 20	366 ± 32	3, 3
Moss phylloplane	486 ± 102	455 ± 96	10, 10

Data are averages ± SD, sample sizes at 5 am and 9 am are given in the last column. For methods see Tarnawski et al. (1992)

Growing close to living or decomposing substrate, CO_2 concentrations in the phylloplane of mosses and lichens may deviate substantially from ambient conditions (Tarnawski et al. 1992). This was also the case in Fortuna, where this difference amounted to about 100 ppm (Table 15.1). We also noticed diurnal variation, with predawn CO_2 concentrations [CO_2] in the air and in mosses being consistently higher, by ca. 30 ppm, than those measured in the morning (Table 15.1).

III. The Carbon Balance of Tropical Bryophytes

The most accepted explanation for the generally observed altitudinal trend in bryophyte abundance in the tropics involves environmental limitations for obtaining a positive carbon balance (Richards 1984; Frahm 1987b; Zotz 1999). Photosynthetic response curves and diel carbon balances have been determined for a small number of tropical bryophytes (see Table 15.2). For tropical lowland species, only a few published light response curves are available (Waite and Sack 2010), so that in the following discussion we also include the few data on lichens for the lowland situation (Zotz and Winter 1994; Zotz et al. 2003).

A. Growth

Information about growth and primary production of tropical bryophytes is scarce. There is only one study on growth and net production of tropical montane bryophytes (Clark et al. 1998). They divided bryophyte samples into two subgroups based on life forms (Mägdefrau

Table 15.2. Maximum rates of net photosynthesis (NP_{max}), rates of dark respiration (R_{Dmax}), and light compensation points (LCP) for a suite of tropical bryophytes. All measurements under optimum water content and average normal temperature of the study site.

Species	NP_{max} ($nmol g_{dw}^{-1} s^{-1}$)	R_{dmax} ($nmol g_{dw}^{-1} s^{-1}$)	NP_{max}/R_{dmax}	LCP ($\mu mol\ m^2 s^{-1}$)	Location
Dendropogonella rufescens[a]	4.9	7.0	0.7	11	Canopy
Holomitrium seticalycinum[b]	7.7	6.8	1.1	33	Branch
Pyrrhobryum pungens[b]	3.3	2.7	1.2	16	Tree trunk
Macromitrium cirrosum[c]	2.1	1.6	1.3	35	Exposed branch
Phyllogonium fulgens[a]	4.2	3.2	1.3	6	Inner canopy
Pilotrichella flexilis[a]	5.2	4.0	1.3	5	Canopy
Phyllogonium fulgens[c]	5.0	3.1	1.6	8	Canopy
Leucobryum antillarum[c]	6.1	3.1	2.0	19	Fallen log
Frullania convulata[a]	7.3	3.4	2.1	10	Exposed canopy
Campylopus hawaiicus[b]	5.1	2.3	2.2	23	Tree trunk
Holomitrium cf. terebellatum[c]	6.8	3.0	2.3	29	Fallen log
Leucubryum cf. seemannii[b]	3.9	1.3	3.0	17	Tree trunk
Acroporium fuscoflavum[b]	6.0	1.3	4.6	9	Tree trunk
Frullania mirabilis[c]	15.0	3.1	4.8	10	Canopy
Distichiphyllum freycinetii[b]	8.8	1.5	5.9	6	Terrestrial
Hookeria acutifolia[b]	14.0	2.3	6.1	5	Terrestrial
Fissiden spacificus[b]	9.2	1.1	8.4	6	Terrestrial
Macromitrium microstomum[b]	11.9	1.4	8.5	13	Branch
Macromitrium piliferum[b]	11.3	0.9	12.6	8	Branch

[a]*Romero et al.* (2006), *uppermontane* Quercus *forest*, *2,900 m*, *Costa Rica*
[b]*Waite and Sack* (2010), *lowland* Metrosideros *rainforest*, *Hawaii*
[c]*Zotz et al.* (1997), *lower montane mixed rain forest*, *1,200 m*, *Panama*

1969, 1982). Mass accumulation was slightly higher in turf-, weft- and mat-forming bryophytes (24.0 % $year^{-1}$ on average) compared to pendants, fan- and tail-forming species (15.5 % $year^{-1}$). Average net primary production was estimated at 122 g $m^{-2}year^{-1}$ for the pendant group and 203 g $m^{-2}year^{-1}$ for the turf group. These estimates were rather low compared to other ecosystems where bryophytes are abundant (summarized by Clark et al. 1998).

B. Maximum Carbon Exchange Rates

Light response curves of a range of tropical bryophyte species demonstrate rather low ratios of maximum net photosynthesis (NP_{max}) to dark respiration (R_{dmax}), on average 3.7. It ranged from <1 (i.e. dark respiration rates exceeding maximum photosynthesis rates) to >10 (Table 15.2). The NP_{max}/R_{dmax} ratios of leaves of vascular plants usually are around 10 (Larcher 2001). Although these relatively high respiration rates in bryophytes are not specific to the tropics (Green et al. 1998; Pannewitz et al. 2005), the consequences for diel carbon balances may be particularly pronounced in tropical lowlands because of the unfavorable timing of hydration and metabolic activity (see section "Effects of hydration and desiccation on carbon balance"). An NP_{max}/R_{dmax} ratio of c. 4means that even under the (unrealistic) assumption of saturating light conditions, optimal water content, and optimal temperature throughout the day one fourth of the carbon gain is lost during the 12 h of the night. This assumes that dark respiration at night equals dark respiration during the day, though this is not necessarily true in higher plants (Amthor 1995), while for bryophytes it has not been tested as far as we know. Since conditions are mostly far from optimal, as we will discuss below, even in montane forests (and for lowland lichens, see Zotz and Winter 1994),

observed diel carbon balances are often negative (Zotz et al. 1997). Logically, longer-term carbon balances have to be positive if species occur at all. The high relative losses imply, however, that balances are close to unity. It is critically important for the entire argument that R_d is not simply regulated by substrate availability, which could indeed be shown for lichens in the temperate zone (Lange and Green 2006): the nocturnal respiration of two chloro- and one cyanolichen studied in their natural habitat was not affected by preceding diurnal net photosynthesis, so the amount of carbohydrates available as substrate was less important than other environmental conditions.

C. Diel Balances

Diel carbon balances can be calculated from frequent gas exchange measurements on bryophytes *in situ* over a 24-h period. Variability between days can be substantial and to quantify longer-term carbon balance requires a very large number of measuring days (Bader et al. 2010). Still, even a limited set of days can indicate how diel balances depend on environmental conditions and can reveal typical patterns for a given climate zone (e.g. Lange et al. 2004).

Bryophytes in a lower montane rainforest (Fortuna, see section "The physical setting") lost, on average, about 60 % of the carbon gained on a given day during the subsequent night (Zotz et al. 1997). In the lowlands, proportional respiratory losses may be higher, but this assumption has not been tested for bryophytes. The foliose lichen *Leptogium azureum* from lowland Panama had a negative integrated diel carbon balance on 7 out of 13 days studied during the wet season (Zotz and Winter 1994). Two days were studied in the dry season and both were negative. A similar situation was found for another lowland lichen, *Parmotrema endosulphureum*, with only three positive diel balances out of seven measured days and an average nocturnal loss of 90 % of the daily gains (Zotz et al. 2003). This pattern was not due to absolutely low carbon gains, which were comparable to those of central European bryophytes in their most productive season (Zotz and Rottenberger 2001), but due to high nocturnal losses. In lower montane rainforest, lichens were found to lose less of their daily carbon gain at night (averages for 5–15 days between 59 and 94 % for different species), though negative diel carbon balances frequently occurred in some species (Lange et al. 2000, 2004). For comparison, the epilithic lichen *Lecanora muralis* from Europe had a negative diel carbon balance on 25 % of its active days measured in 1 year, and lost on average 57 % of the daily gains at night (Lange 2003).

IV. Effects of Hydration and Desiccation on Carbon Balance

As poikilohydric organisms bryophytes equilibrate more or less rapidly with external moisture conditions. Physiological activity ceases reversibly when shoots become too dry, whereas abundant external water limits photosynthesis, but not respiration, by increasing the diffusion resistance for CO_2 (Proctor 1981, 1990). Water relations thus prominently affect carbon relations, while also setting the requirements for desiccation tolerance. The effects of moisture regimes on bryophyte (and lichen) physiology include at least five dimensions (last four from Norris 1990): (1) time of day of hydration, (2) hydration duration (activity time), (3) desiccation-rehydration frequency, (4) desiccation duration, and (5) desiccation intensity. In the following sections we will apply this framework on bryophytes in tropical montane cloud forests and lowland rainforests. More in-depth reviews of bryophyte hydration dynamics and desiccation tolerance can be found in, e.g., Proctor et al. (2007a) or Green et al. (2011).

A. Timing of Hydration

The timing of hydration during a day has strong implications for the balance between photosynthesis and respiration. A "typical"

day in the tropical lowlands includes rain in the afternoon (continental regions) or late at night (maritime regions) (Richards 1996). For bryophytes this is unfavorable, because they are moist and physiologically active during much of the night. As a consequence much of the carbon gained during the preceding day is respired, especially since temperatures generally remain high during the night. If the following day is sunny, the bryophyte will then usually dry out before noon, thus missing out on the favorable light conditions (Zotz et al. 1997). In contrast, morning rain or fog increases the diel carbon balance, because high light levels at midday may coincide with optimal shoot water contents for photosynthesis. For the tropical lowland lichen *Parmotrema endosulphureum* the only day (out of seven) with a highly positive carbon balance occurred when thalli were dry all night and a rainstorm burst them into life after sunrise (Zotz et al. 2003). On the other hand, for corticolous lichens, a common life form in the lowland forest understory, late-morning dew on stems still cool from the night may be a significant extra water source and favorably timed relative to the midday light conditions (Lakatos et al. 2012).

Morning fog is the default situation in montane cloud forests, where rising air condenses into clouds while passing these forests. This allows carbon gain in the morning, although on sunnier days drying is often complete before the highest light conditions at midday, as found for a range of lichen (Lange et al. 2004) and bryophyte species (Zotz et al. 1997). Late-afternoon rain and fog are also common, hence nocturnal respiratory losses can be considerable in these ecosystems as well (Fig. 15.3), but may be lower than in the lowlands because of the lower temperatures (Zotz et al. 1997). Morning fog also appears to play an important role in maintaining a high bryomass in the rare tropical lowland cloud forest (Gradstein et al. 2010).

The timing of hydration may also be important in the context of intrinsic circadian rhythms in physiological processes. An internal clock exists in plants, including mosses, which causes diurnal fluctuations in net photosynthesis even when the plants are kept in constant light (Hennessey and Field 1991; Holm et al. 2010). Such fluctuations serve to increase metabolic efficiency by anticipating daily fluctuations in environmental conditions, in particular sunlight and potentially also hydration. The ecological relevance of such internal fluctuations, possibly interacting with fluctuating moisture conditions to affect carbon balances and stress responses, is unknown but may be worth investigating.

B. Activity Time

Many bryophytes spend most of their lives in a dry and inactive state. Carbon gain and growth are restricted to periods of sufficient hydration, and capturing and storing moisture are crucial abilities for bryophytes. Open life forms like pendants and fans are better suited for capturing moisture, while more compact forms like cushions, turfs and mats are generally better at water storage (Bates 1998; Kürschner et al. 1999). Pendant species are conspicuous in many tropical montane forests, where they can capture water from the frequent passage of clouds (Proctor 2002), although physiological activity before drying out again is short (in the order of a few hours, depending on conditions; Zotz et al. 1997, Wagner, unpublished data). In such species, carbon gain is obviously limited by low water content, in contrast to cushions in which carbon gain is frequently limited by supersaturation (Zotz et al. 1997; Fig. 15.3), so that a cushion reaches its highest rates of photosynthesis later than the pendant species (Fig. 15.3). A rain event in the afternoon can allow additional carbon gain in pendant species, while in cushions it may further reduce net CO_2 uptake due to supersaturation (Fig. 15.3).

Some bryophytes are able to hold large amounts of water; we have recently measured water contents ~7,000%$_{DW}$ in cushions of *Octoblepharum pulvinatum* directly after tropical rain events (T. Reich and S. Wagner,

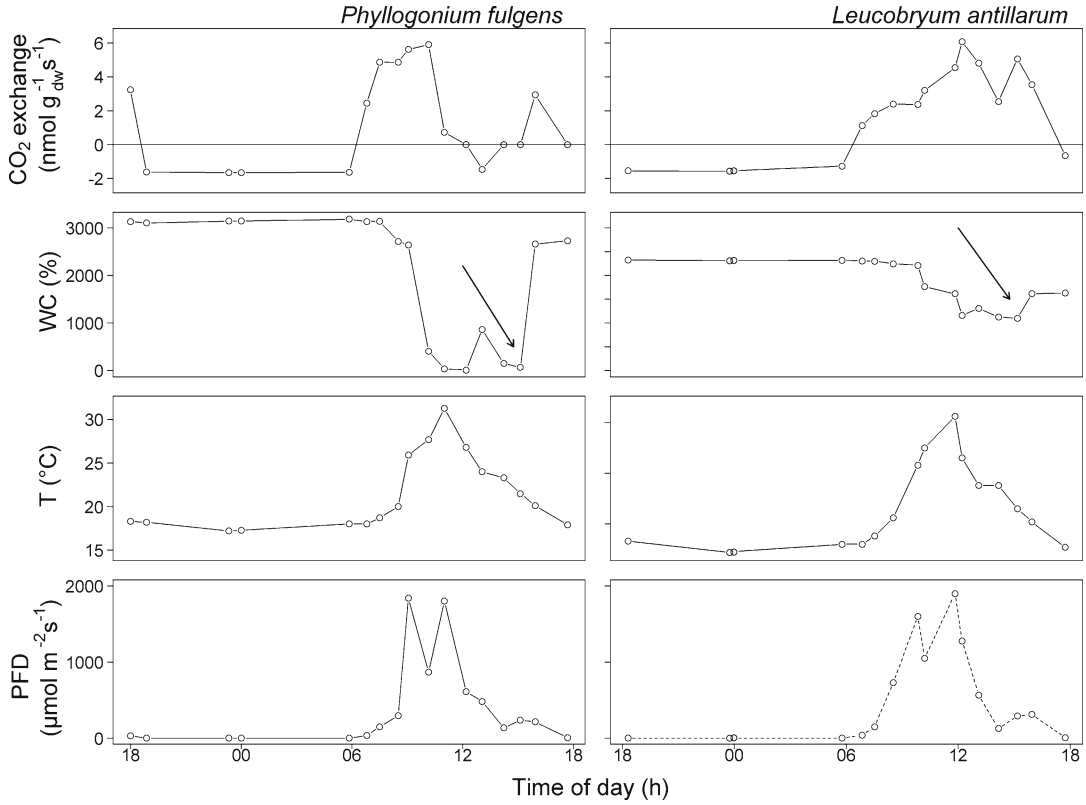


Fig. 15.3. Diel gas exchange courses (same day) of a pendant (*left*) and a cushion species (*right*), both from the forest edge in a lower montane rain forest (Fortuna, Panama, see section "The physical setting"). From *top* to *bottom:* net CO_2-exchange (NP, nmol g^{-1} s^{-1}), water content of the shoots (WC, $\%_{DW}$), air temperature (T, °C) and photon flux density (PFD, μmol m^2 s^{-1}). *Arrows* indicate a heavy rain event (Redrawn from Zotz et al. (1997)). Note the fast drying of the pendant species (*left*) and the supersaturation depressing NP in the cushion in the morning (*right*).

unpublished data). The pendant *Phyllogonium fulgens* can hold up to ~2,700%$_{DW}$ (Zotz et al. 1997), which agrees with reports from another pendant, *Pilotrichella ampullaceae* (Proctor 2002). The latter species can hold eight times as much water externally as internally (Proctor 2002). The external water has a water potential near zero and can evaporate fast without affecting the cell water balance (Zotz et al. 2000; Proctor 2004a). Intracellular water evaporates more slowly, but a strong evaporation barrier is lacking, so that all species eventually dry out if not remoistened. Still, if dense life forms grow in protected positions, e.g. in the forks of branches on accumulated humus, it may take days for them to dry out (Veneklaas et al. 1990).

High air humidity does not appear to activate carbon exchange in bryophytes (Lange 1969), although detectable fluorescence can be induced by air humidities above 95 % (Fig. 15.4 and M. Lakatos pers. com. 2011). For positive net photosynthesis relative humidity would probably have to be over 98 % (León-Vargas et al. 2006). The occurrence of such high humidity values could thus be a practical indicator of activity times in bryophytes.

Apart from environmental conditions and life forms, special morphological and anatomical structures that appear to affect water

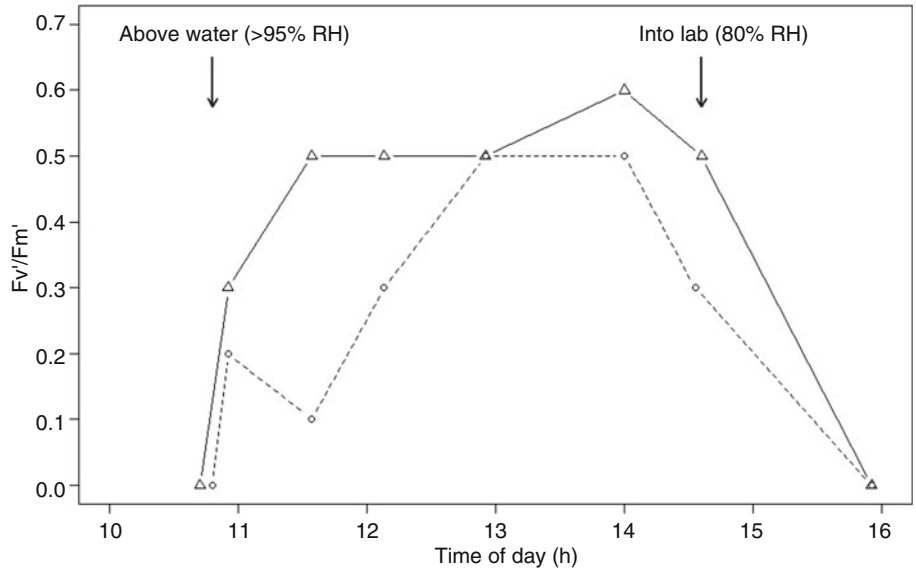


Fig. 15.4. Resumption of chlorophyll fluorescence activity after dry bryophyte samples of two species (in equilibrium with RH 80 %, water content 15 % resp. 16 % of dry weight) were transferred into moist air (in jar above tap water), and cessation of activity after transfer back into drier air (RH 80 % in air-conditioned lab). Water contents in moist air rose to 49 % of dry weight in both samples. *Dashed line*: *Zelometeoreum* sp. *Solid line*: *Orthostichopsis tetragona*.

storage or water loss may also increase activity times of bryophytes (Biebl 1964). A general altitudinal trend in such adaptations can be recognized in the tropics, with water storage structures being more common in the lowlands, while water capturing structures are more common at high altitudes (Kürschner et al. 1999). Examples of water storage structures are enlarged hyaline cells in the leaves of common tropical mosses in the Calymperaceae and Leucobryaceae (Castaldo et al. 1979; Frahm 1997), and water lobules and water sacs (their size decreasing plastically with increasing humidity in some species) in leafy liverworts in the Frullaniaceae, Lejeunaceae and Radulaceae (Kürschner et al. 1999). Examples of structures reducing water loss are leaf-tip hairs and imbricated phylloids (Romero et al. 2006). Structures for water capturing (e.g. 'fog stripping'), on the other hand involve increases in surface area, such as ciliate leaves, and usually present a trade-off with water storage.

C. Desiccation-Rehydration Frequency

When dry bryophytes are rehydrated, full recovery of photosynthetic rates can take up to several days, depending on the level of tolerance and the duration and intensity of the dry period (Proctor et al. 2007a). A higher cycling frequency has the advantage that no extended dry periods need to be survived (see section "Desiccation tolerance: desiccation duration and intensity" below), but it has several potential disadvantages for the carbon balance: direct carbon losses through resaturation respiration and carbohydrate leaching, reduced photosynthetic rates if recovery takes longer than the drying frequency, and an interference of repeated drying with this recovery (see also Chap. 9).

Immediately after rehydration, a so called 'resaturation burst' of respiration can be detected in gas-exchange measurements. A study on lichens in a lower montane rainforest revealed dark respiration rates four times higher than the steady state value 3 min after

rehydration (Zotz et al. 1998). NP as well as R_d recovered to steady state values after 15–20 min. This high respiration is related to the restoration of membrane integrity and re-establishment of protein synthesis (Proctor 2000a, b); it may take from minutes to a day for photosynthesis to exceed respiration (Proctor 2002). Nutrients and carbohydrates may be lost at each rehydration event through leaching. Coxson et al. (1992) calculated that it would take fewer than 30 wetting/drying cycles to exhaust the internal sugar- and polyol pools of tropical montane bryophytes. The quantitative importance of resaturation respiration and leaching for the bryophyte's carbon balance is largely unknown, but their influence would certainly increase with more frequent drying-wetting cycles.

Repeated cycles of hydration and dehydration caused greater loss of photosynthetic rates than continuous desiccation in Antarctic bryophytes from hydric habitats, while species from xeric habitats were better adapted to short periods of hydration (Davey 1997). For tropical species, we expect a similar relationship with continuous or highly intermittent moisture, but empirical evidence is lacking. The fastest hydration-rehydration cycles occur at exposed sites such as the outer canopy on days with intermittent precipitation. Such weather conditions are common in montane rainforests (see section "The physical setting"), occasionally allowing several cycles within a day (Norris 1990). Especially life forms with large surface areas, such as the pendant mosses so common in tropical montane forests, experience these rapid cycles and appear well adapted to this situation with fast recovery after rehydration (Zotz et al. 1997; Proctor 2002, 2004b).

D. Desiccation Tolerance: Desiccation Duration and Intensity

In non-seasonal rain- and cloud forests, bryophytes are hydrated nearly every day, so that species do not need adaptations to prolonged desiccation. Surprisingly then, Proctor (2002) found full recovery of two tropical pendant species even after 11 months of being stored dry (at 5 °C), which suggests a high degree of desiccation tolerance. Results of our own studies also indicate that desiccation tolerance is quite high in both lowland and lower montane rainforest species (>80 days for both, Bader et al. 2013). Even under changing precipitation regimes, desiccation tolerance as such is thus unlikely to limit future bryophyte distributions in the tropics.

Recovery from desiccation becomes slower and less complete as the desiccation period becomes longer, while recovery also depends on temperature and the intensity of desiccation (Proctor et al. 2007b). León-Vargas et al. (2006) measured compensation times, i.e. the time it takes for photosynthesis to compensate for respiratory carbon losses after rehydration, of *Meteorium nigrescens*, dry for 2–4 days. This species occurs either as a diffuse mass on palm trunks, or pendant on branches. The compensation time was nearly twice as long on the trunk (ca.100 min) as in the pendant form (60 min). This could be a form of acclimatization, as has been observed in colder ecosystems as well (Robinson et al. 2000), as the pendant experiences desiccation more often, for reasons already pointed out. Similarly, the pendant lower-montane moss *Pilotrichella ampullacea* showed compensation times of 30–60 min after 24 h dryness and 2–2.5 h after 6 days. This species recovered its Fv/Fm to normal values after 12 days of desiccation within ~40 h (Proctor 2002).

The intensity of desiccation, i.e. the water content of the desiccated moss, is generally not extreme in tropical rain forests, where relative air humidity rarely falls below 70 % (Biebl 1964, see section "The physical setting"). Bryophyte tolerances conform to these conditions: most epiphytic bryophytes studied (9/11) from a Puerto Rican montane forest did not survive 24 h at 52 % RH, 5/11 were damaged at 65 %, while most species from the forest floor (4/5) showed damage already at 75 % and some (2/5) even at 91 % (Biebl 1964). For comparison, bryophytes from xeric habitats can survive RHs <1 % (Höfler in Biebl 1962). The tolerance differences between species may also co-determine their local distribution within the forest.

Although desiccation damage generally increases with desiccation intensity (Biebl 1964; Proctor 2004b), mild desiccation can sometimes impose more damage than deep desiccation, especially in xeric species (Proctor 2001). Under mildly dry conditions some physiological processes, such as respiration, do not cease completely and can cause metabolic imbalances, while pathogens may provoke further damage (Proctor 2001; Proctor et al. 2007b). Due to the constantly high air humidity, bryophytes in tropical rainforests are exposed to mild desiccation only.

V. Effects of Light and CO_2 on Carbon Balance

Light conditions vary widely among bryophyte habitats, also in the tropics (see section "The physical setting"). Overall, the observed light responses of tropical mosses and hepatics are in the range of those observed at other latitudes (Larcher 2001). Published light compensation points (LCPs) range from 5 µmol m^{-2} s^{-1} in *Pilotrichella flexilis* to 35 µmol m^{-2} s^{-1} in *Macromitrium cirrosum* (Table 15.2). As expected, the lowest LCPs are exhibited by bryophytes from the forest floor where irradiation is lowest or in the inner crowns of trees. Highest LCPs are found in bryophytes growing on exposed branches or on logs where irradiance is high at least during parts of the day. Although photosynthesis saturates at rather low PFD (200–400 µmol m^{-2} s^{-1}; see Proctor 1982, 2004b; Zotz et al. 1997) light is limiting for bryophytes and lichens for most of the day even in the forest canopy (for comparison see Zotz and Winter 1994). The extremely high PFD levels reported in section "The physical setting" have little relevance for photosynthesis: light becomes limiting for photosynthesis especially during and after rain events, when bryophytes and lichens are wet and could potentially achieve high photosynthetic rates (Lange et al. 2004).

Light compensation points can shift to lower light levels if CO_2 levels are increased (Silvola 1985), although long-term effects of higher CO_2 tend to decrease through acclimatization (Tuba et al. 2011). Actual rates of photosynthesis increase with increasing CO_2 concentrations, and CO_2 concentrations in a bryophyte's microhabitat can be more than 100 ppm over concentrations in ambient air (Tarnawski et al. 1992, 1994; Pannewitz et al. 2005 and see section "The physical setting"). There is no information available of CO_2 responses for tropical bryophytes or lichens in particular. However, for bryophytes in general effects of elevated CO_2 can be substantial (Tuba et al. 1999, 2011). For example, Silvola (1990) reports that *Sphagnum fuscum* increases photosynthetic rates by 30–50 % when CO_2 concentration is raised from 300 to 500 ppm.

VI. Effects of Temperature on Carbon Balance

In all plants, the temperature response of photosynthesis typically follows an optimum curve, while dark respiration increases exponentially with increasing temperature (e.g. Lange 1980; Proctor 1982; Atkin and Tjoelker 2003). Temperature may affect respiration more strongly in colder climates like the Arctic (Q_{10} = 2.6) than in warmer environments like the tropics (Q_{10} = 2.1) (Atkin et al. 2005). As shown above, photosynthetic carbon gain in non-vascular plants is limited by various factors. Temperature-effects on photosynthesis rates are probably relatively unimportant in limiting carbon gain, but carbon loss due to high respiration rates at high night temperatures may pose a problem for tropical lowland bryophytes (Richards 1984). Surprisingly though, a recent study found no differences in the ratio of dark respiration to net photosynthesis rates between montane and lowland species under the respective temperature conditions at the altitudes of origin (Wagner et al. 2013). Higher temperatures may thus affect carbon balance more by increasing evaporation rates, i.e. by decreasing activity times, than by direct effects on metabolic rates.

Temperature optima for photosynthesis in arctic and temperate bryophytes generally range between 5 and 20 °C, though optima up to 24–30 °C have been reported even for Arctic species (summarized in Frahm 1990b; Larcher 2001). For tropical lowland and lower-montane bryophytes from Panama, temperature optima closely matched local temperature conditions, with optima shifting from ca. 26 to 20 °C between 0 and 1,200 m (Wagner et al. 2013). In contrast, the optimum temperature for the lowland lichen *Parmotrema endosulphureum* (Zotz et al. 2003) was rather low at 22 °C and similar to that of the montane lichens *Dictyonema glabratum* (Lange et al. 1994) and *Pseudocyphellaria aurata* (Lange et al. 2004), suggesting no special adaptation to high temperatures. Frahm (1990b) found optimum temperatures of 15 °C for a montane rainforest species (from 2,300 m) under high light conditions. He simulated lowland and highland temperatures with two different light levels. Consistent with Richards' (1984) notion, highland conditions (15 °C, 1,500 Lux) were associated with positive carbon balances for the investigated species, while under lowland conditions (30 °C, 300 Lux) respiration exceeded gross photosynthesis even during light periods (Frahm 1987b, 1990b). However, it is not unlikely that these results are related to *ex-situ* cultivating, which is rather difficult with tropical species (M.Y. Bader et al. personal observation).

The results of a single study are at odds with Richards' hypothesis (Lösch et al. 1994). This laboratory experiment with upper-montane bryophytes from Africa using Warburg manometry showed only a small effect of dark respiration on CO_2-exchange under lowland conditions. Temperature responses differed little between species from 800 and 3,200 m. Again, this surprising result could be an artifact of the high CO_2 concentrations supplied in the method used, or is similarly related to the fact that all species had been cultivated at the same temperature for 2 months before the measurements. In any case, these methodological concerns do not allow unambiguous conclusions.

A. Temperature Acclimation

A likely response to higher temperatures is metabolic acclimatization. Many vascular plants can down-regulate their dark respiration to acclimatize to a prolonged increase in temperature, but differences in this acclimatization potential between plant groups and climate zones are incompletely understood (Atkin et al. 2005). Seasonal acclimatization has been documented for temperate and polar bryophytes (Longton 1988). For example, Hicklenton and Oechel (1976) observed an acclimation of temperature optima after 72–120 h in experiments with *Dicranum fuscens*. In polar ecosystems, optimum-temperatures for bryophyte photosynthesis are seasonably variable, with optima varying up to 10 °C even within the summer season (Skre and Oechel 1981). However, tropical bryophytes normally experience much smaller changes in temperature and therefore their acclimatization potential might be smaller.

This acclimatization potential is of critical importance both for the question of the physiological basis of current altitudinal distribution patterns and for the likely responses of bryophytes to climate change. We quantified this potential in a transplant experiment by transporting cut branches covered with different bryophyte species from a lower montane rainforest (1,200 m) to sea level (Fig. 15.5). With acclimation we would expect a reduction of dark respiration after transplantation, but during the 16 days of the experiment no such acclimation was observed (means within or below confidence intervals at $t = 0$), while samples suffered obvious damage. Although suggesting a lack of acclimatization, this experiment cannot provide conclusive answers yet: longer-term monitoring, shorter transplantation distances (i.e. smaller temperature differences and lower stress levels) and larger sample sizes are necessary for stronger conclusions about the acclimatization potential of tropical bryophytes.

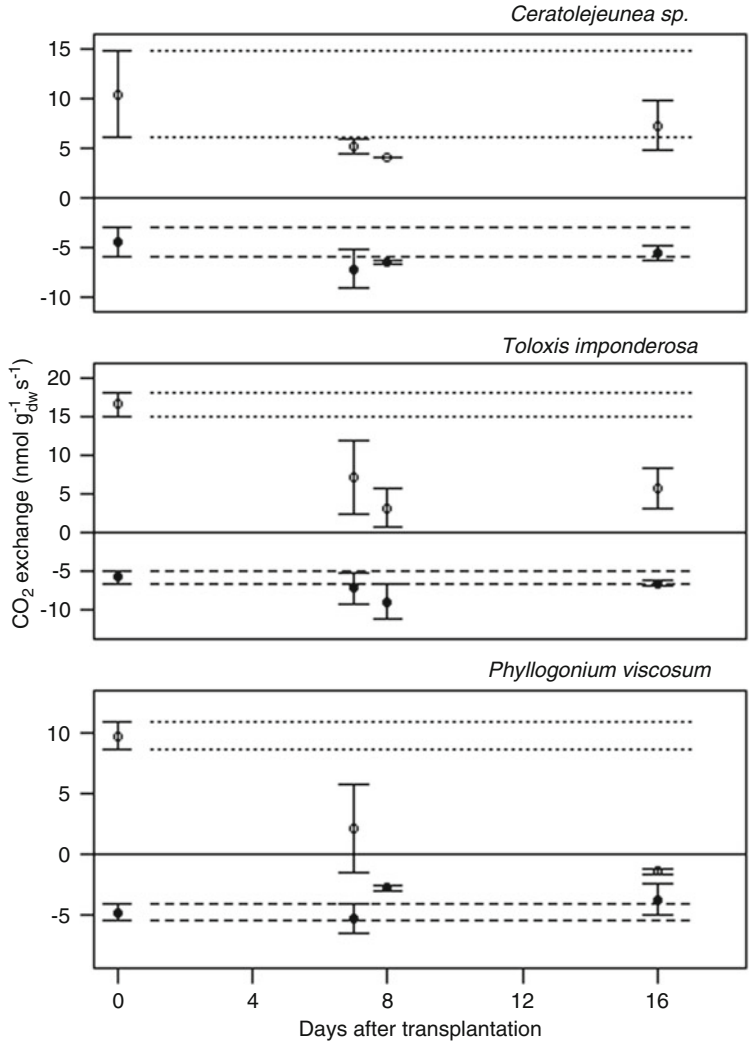


Fig. 15.5. Change of net photosynthesis (*open symbols*) and dark respiration (*filled symbols*) of three lower-montane bryophyte species (from Fortuna, ca. 1,100 m, Panama, see section "The physical setting", S. Rottenberger and G. Zotz unpublished data) after transplantation to the lowlands (at t = 0, first measurements were made directly after transplantation). Gas exchange rates were measured at optimum water content, cuvette temperature of 25 °C and PFD 600 μmol m^{-2} s^{-1} (photosynthesis) or in the dark (respiration). Values are means of 3–4 moss canopies, *error bars* indicate 95 % confidence intervals; the *dotted/dashed lines* continue the confidence intervals at t = 0. The lowering of net photosynthesis in *Toloxis imponderosa* and *Phyllogonium viscosum* probably indicates a general decline in health; all species showed clear evidence of damage after 2 weeks.

VII. Nutrients

Nutrient requirements in bryophytes are similar to those of vascular plants (Shaw and Goffinet 2000; Waite and Sack 2011a). So far we have discussed reasons why tropical epiphytes may be carbon limited, but nutrients could be limiting growth as well, although *in situ* growth does not appear to be nutrient limited in most mosses and liverworts (Oechel and Sveinbjörnsson 1978). In the tropics, where bryophytes mostly occur

as epiphytes, the situation may be very different from arctic and terrestrial systems, where most studies have been carried out (Oechel and Sveinbjörnsson 1978; Jauhiainen et al. 1998). A single study addresses nutrient limitation for bryophytes (and lichens) in a tropical system. Abundance and species richness of non-vascular epiphyte communities in Hawaii increased dramatically after experimental P (but not N) addition to the forest soil (Benner et al. 2007). Especially N-fixing cyanolichens profited, suggesting that at least these were P-limited before fertilization. Phosphorus is also commonly the most limiting nutrient for vascular epiphytes, although water is nearly always more limiting (Zotz 2004). In some cases this may be true also for non-vascular epiphytes (Benner et al. 2007), which also rely mainly on aerial nutrient sources (Clark et al. 1998, 2005; Tozer et al. 2005; Chen et al. 2010), though nutrients leached from the host tree or from decomposing plant parts can also be used (Goward and Arsenault 2000; Hietz et al. 2002). There is no indication, however, that nutrient inputs follow consistent altitudinal patterns around the tropics. So although tropical bryophytes are understudied in relation to nutrients, the major distributional patterns discussed in this review are hardly caused by differences in nutrient supply.

VIII. The Fate of Non-vascular Epiphytes under Global Change

Accepting the notion that tropical lowlands are already marginal habitats for bryophytes (and lichens) today due to high temperatures, the predicted increase of 2–5 °C for the tropics in the next 100 years (Solomon et al. 2007) may be particularly critical for this plant group. Climate change will certainly cause changes in species composition in general but the likelihood of extinction is difficult to predict unless fundamental temperature or moisture tolerances are exceeded (Wright et al. 2009). This may indeed be the case for bryophytes as a group.

In order to estimate the threat of the tropical lowlands becoming virtually devoid of bryophytes in the future, one approach is to model bryophyte carbon balances based on measured response curves and including potential losses through leaching and repair after desiccation. Such a model would allow us to identify the driving factors influencing bryophyte carbon balance in general and developments in the tropics under different climate change scenarios, in particular. Based on carbon exchange data and using a very simple model, Zotz and Bader (2009) estimated the theoretical carbon balance of the lowland lichen *Parmotrema endosulphureum* under climatic warming. With a temperature rise of 3 °C this lichen would have to increase its daily activity (at optimal rates) from 40 % of the light period to over 90 %, which is virtually impossible under tropical conditions. However, this model still needs refining and it needs actual data for tropical bryophytes in order to allow any predictions for this group.

Another approach to look into a warmer future is the experimental warming of existing species and communities. This can be achieved through transplantation to lower altitudes (Nadkarni and Solano 2002; Jácome et al. 2011), or through experimental warming *in situ*, though this has never been done for epiphytic mosses. We are currently initiating such a study which will pay particular emphasis to the interactive effects of temperature and CO_2.

Poikilohydric organisms like bryophytes and lichens could be threatened not only by a future warming but also by changes in precipitation regimes (Zotz and Bader 2009). Increased drought frequency, as reported for Amazonia recently (Lewis et al. 2011), might negatively affect non-vascular epiphytes. Although drought tolerance far exceeds the currently longest local droughts in a variety of both montane and lowland bryophyte species (Proctor 2002; Bader et al. 2013), tolerances are certainly species-specific. Community compositions may thus be

altered, even if communities do not collapse altogether (Hughes 2000), as was found in a transplantation experiment of montane bryophytes to a warmer and drier altitude (Jácome et al. 2011). By altered species composition or directly through effects of altered hydration regimes on the carbon balance, biomass and ecosystem functioning may also be affected by precipitation changes.

IX. Conclusions

The strong altitudinal gradient of bryophytes in the tropics is much-discussed but a so far little-studied phenomenon. Hypotheses explaining the paucity of bryophytes in the lowlands also have direct implications for the responses of tropical bryophytes to climatic changes and thus merit attention not only in the light of scientific curiosity.

Promising approaches for investigating these hypotheses include modeling of carbon balances, based on real input data (which thus need to be collected!) such as carbon-exchange responses to temperature, light, moisture, CO_2, as well as good estimates of these abiotic factors at the bryophytes' growing sites. Another approach is the experimental manipulation of hypothesized environmental influences. The most obvious candidate, of course, is temperature, while interesting interactions can also be expected with moisture and CO_2.

In conclusion, based on current knowledge it is likely, though as yet not unambiguously shown, that the globally observed altitudinal distribution patterns of tropical bryophytes have a rather simple physiological basis. We can exclude, though, that differences in drought tolerance play a major role in shaping these patterns. The delicate balance between carbon uptake and loss and the important role of temperature and precipitation regimes in determining this balance imply that predicted changes in global climate may have dramatic effects on bryophytes and lichens in the moist tropics.

Acknowledgements

We thank O. Lange, B. Büdel, A. Meyer and H. Zellner (Würzburg and Kaiserslautern, Germany) for permission to use unpublished microclimate data, and S. Rottenberger (Würzburg) for permission to used unpublished acclimatization data. Our own current studies are funded by the German Research Foundation, (Deutsche Forschungsgemeinschaft: BA 3843/3-1). Permission to work in Panama was granted by the local authorities (Autoridad Nacional del Ambiente; SC/P-7-11, SEX/P-62-11, SEX/P-7-10).

References

Amthor JS (1995) Higher plant respiration and its relationships to photosynthesis. In: Schulze ED, Caldwell MM (eds) Ecophysiology of photosynthesis. Springer, Berlin, pp 71–101

Atkin OK, Tjoelker MG (2003) Thermal acclimation and the dynamic response of plant respiration to temperature. Trends Plant Sci 8:343–351

Atkin OK, Bruhn D, Hurry VM, Tjoelker MG (2005) The hot and the cold: unravelling the variable response of plant respiration to temperature. Funct Plant Biol 32:87–105

Bader MY, Zotz G, Lange OL (2010) How to minimize the sampling effort for obtaining reliable estimates of diel and annual CO_2 budgets in lichens. Lichenologist 42:97–111

Bader MY, Reich T, Wagner S, González AS, Zotz G (2013) Differences in desiccation tolerance do not explain altitudinal distribution patterns of tropical bryophytes. J Bryol 35:47–56

Bates JW (1998) Is 'life-form' a useful concept in bryophyte ecology? Oikos 82:223–237

Belnap J, Lange OL (2001) Biological soil crusts: structure, function, and management. Springer, Berlin

Benner JW, Conroy S, Lunch CK, Toyoda N, Vitousek PM (2007) Phosphorus fertilization increases the abundance nitrogenase activity of the cyanolichen *Pseudocyphellaria crocata* in Hawaiian montane forests. Biotropica 39:400–405

Biebl R (1962) Protoplasmatische Ökologie der Pflanzen. Springer, Wien

Biebl R (1964) Austrocknungsresistenz tropischer Urwaldmoose auf Puerto Rico. Protoplasma 59: 277–297

Biebl R (1967) Temperaturresistenz tropischer Urwaldmoose. Flora 157:25–30

Bosman AF, Van Der Molen PC, Young R, Cleef AM (1993) Ecology of a paramo cushion mire. J Veg Sci 4:633–640

Castaldo R, Ligrone R, Gambardella R (1979) A light and electron microscope study on the phylloids of *Leucobryum candidum* (P. Beauv.) Wils. Rev Bryol Lichenol 45:345–360

Cavelier J, Solis D, Jaramillo MA (1996) Fog interception in montane forests across the Central Cordillera of Panamá. J Trop Ecol 12:357–369

Chen L, Liu WY, Wang GS (2010) Estimation of epiphytic biomass and nutrient pools in the subtropical montane cloud forest in the Ailao Mountains, south-western China. Ecol Res 25:315–325

Clark KL, Nadkarni NM, Gholz HL (1998) Growth, net production, litter decomposition, and net nitrogen accumulation by epiphytic bryophytes in a tropical montane forest. Biotropica 30:12–23

Clark KL, Nadkarni NM, Gholz HL (2005) Retention of inorganic nitrogen by epiphytic bryophytes in a tropical montane forest. Biotropica 37:328–336

Coxson DS (1991) Nutrient release from epiphytic bryophytes in tropical montane rain forest (Guadeloupe). Can J Bot 69:2129

Coxson DS, McIntyre DD, Vogel HJ (1992) Pulse release of sugars and polyols from canopy bryophytes in tropical montane rain forest (Guadeloupe, French West Indies). Biotropica 24:121–133

Davey MC (1997) Effect of continuous and repeated dehydration on carbon fixation by bryophytes from the martime Antarctic. Oecologia 110:25–31

Frahm J-P (1987a) Ökologische Studien über die epiphytische Moosvegetation in Regenwäldern NO-Perus. Beih Nova Hedwig 88:143–158

Frahm J-P (1987b) Which factors control the growth of epiphytic bryophytes in tropical rainforests? Symp Biol Hung 35:639–648

Frahm J-P (1990a) Bryophyte phytomass in tropical ecosystems. Bot J Linn Soc 104:23–33

Frahm J-P (1990b) The effect of light and temperature on the growth of the bryophytes of tropical rain forests. Nova Hedwig 51:151–164

Frahm J-P (1997) Welche Funktion haben die Hyalocyten in den Blättern der Dicranaceae? Cryptogam Bryol Lichénol 1997:235–242

Godínez-Alvarez H, Morín V, Rivera-Aguilar C (2012) Germination, survival and growth of three vascular plants on biological soil crusts from a Mexican tropical desert. Plant Biol 14(1):157–162

Goward T, Arsenault A (2000) Cyanolichen distribution in young unmanaged forests: a dripzone effect? Bryologist 103:28–37

Gradstein SR, Pócs T (1989) Bryophytes. In: Lieth H, Werger MJA (eds) Tropical rainforest ecosystems. Elsevier, Amsterdam, pp 311–325

Gradstein SR, Churchill SP, Salazar N (2001) Guide to the bryophytes of tropical America. Mem N Y Bot Gard 86:1–577

Gradstein SR, Obregon A, Gehrig C, Bendix J (2010) Tropical lowland cloud forest: a neglected forest type. In: Bruijnzeel LA, Scatena FN, Hamilton LS (eds) Tropical montane cloud forests. Cambridge University Press, New York, pp 130–133

Grau O, Grytnes J-A, Birks HJB (2007) A comparison of altitudinal species richness patterns of bryophytes with other plant groups in Nepal, Central Himalaya. J Biogeogr 34:1907–1915

Green TGA, Lange OL (1994) Photosynthesis in poikilohydric plants: a comparison of lichens and bryophytes. In: Schulze ED, Caldwell MM (eds) Ecophysiology of photosynthesis. Springer, Berlin, pp 319–341

Green TGA, Schroeter B, Kappen L, Seppelt RD, Maseyk K (1998) An assessment of the relationship between chlorophyll a fluorescence and CO_2 gas exchange from field measurements on a moss and lichen. Planta 206:611–618

Green TGA, Sancho LG, Pintado A (2011) Ecophysiology of desiccation/rehydration cycles in mosses and lichens. In: Lüttge U, Beck E, Bartels D (eds) Plant desiccation tolerance. Springer, Berlin/Heidelberg, pp 89–119

Hennessey TL, Field CB (1991) Circadian-rhythms in photosynthesis – oscillations in carbon assimilation and stomatal conductance under constant conditions. Plant Physiol 96:831–836

Hicklenton PR, Oechel WC (1976) Physiological aspects of the ecology of *Dicranum fuscescens* in the subarctic. I. Acclimation and acclimation potential of CO_2 exchange in relation to habitat, light and temperature. Can J Bot 54:1104–1119

Hietz P, Wanek W, Wania R, Nadkarni NM (2002) Nitrogen-15 natural abundance in a montane cloud forest canopy as an indicator of nitrogen cycling and epiphyte nutrition. Oecologia 131:350–355

Hofstede RGM, Wolf JHD, Benzing DH (1993) Epiphytic biomass and nutrient status of a Colombian upper montane rain forest. Selbyana 14:37–45

Holm K, Kallman T, Gyllenstrand N, Hedman H, Lagercrantz U (2010) Does the core circadian clock in the moss *Physcomitrella patens* (Bryophyta) comprise a single loop? BMC Plant Biol 10:14

Hölscher D, Köhler L, van Dijk A, Bruijnzeel LA (2004) The importance of epiphytes to total rainfall interception by a tropical montane rain forest in Costa Rica. J Hydrol 292:308–322

Hughes L (2000) Biological consequences of global warming: is the signal already apparent? Trends Ecol Evol 15:56–61

Jácome J, Gradstein SR, Kessler M (2011) Responses of epiphytic bryophyte communities to simulated climate change in the tropics. In: Tuba Z, Slack NG, Stark LR (eds) Bryophyte ecology and climate change. Cambridge University Press, Cambridge, pp 191–207

Jauhiainen J, Silvola J, Vasander H (1998) The effects of increased nitrogen deposition and CO_2 on *Sphagnum angustifolium* and *S. warnstorfii*. Ann Bot Fennici 35:247–256

Johnson A, Kokila P (1970) The resistance to desiccation of ten species of tropical mosses. Bryologist 73:682–686

Karger DN, Kluge J, Abrahamczyk S, Salazar L, Homeier J, Lehnert M, Amoroso VB, Kessler M (2012) Bryophyte cover on trees as proxy for air humidity in the tropics. Ecol Indic 20:277–281

Körner C, Allison A, Hilscher H (1983) Altitudinal variation of leaf diffusive conductance and leaf anatomy in heliophytes of montane New Guinea and their interrelation with microclimate. Flora 174:91–135

Kürschner H, Frey W, Parolly G (1999) Patterns and adaptive trends of life forms, life strategies and ecomorphological structures in tropical epiphytic bryophytes – a pantropical synopsis. Nova Hedwig 69:73–99

Lakatos M (2011) Lichens and bryophytes: habitats and species. In: Lüttge U, Beck E, Bartels D (eds) Plant desiccation tolerance. Springer, Berlin/Heidelberg, pp 65–87

Lakatos M, Obregón A, Büdel B, Bendix J (2012) Midday dew – an overlooked factor enhancing photosynthetic activity of corticolous epiphytes in a wet tropical rain forest. New Phytol 194:245–253

Lange OL (1969) CO_2-Gaswechsel von Moosen nach Wasserdampfaufnahme aus dem Luftraum. Planta 89:90–94

Lange OL (1980) Moisture content and CO_2 exchange of lichens. I. Influence of temperature on moisture-dependent net photosynthesis and dark respiration in *Ramalina maciformis*. Oecologia 45:82–87

Lange OL (2003) Photosynthetic productivity of the epilithic lichen *Lecanora muralis*: long-term field monitoring of CO_2 exchange and its physiological interpretation – III. Diel, seasonal, and annual carbon budgets. Flora 198:277–292

Lange OL, Green TGA (2006) Nocturnal respiration of lichens in their natural habitat is not affected by preceding diurnal net photosynthesis. Oecologia 148:396–404

Lange OL, Büdel B, Zellner H, Zotz G, Meyer A (1994) Field measurements of water relations and CO_2 exchange of the tropical, cyanobacterial basidiolichen *Dictyonema glabratum* in a Panamanian rainforest. Bot Acta 107:279–290

Lange OL, Büdel B, Meyer A, Zellner H, Zotz G (2000) Lichen carbon gain under tropical conditions: water relations and CO_2 exchange of three *Leptogium* species of a lower montane rain forest in Panama. Flora 195:172–190

Lange OL, Büdel B, Meyer A, Zellner H, Zotz G (2004) Lichen carbon gain under tropical conditions: water relations and CO_2 exchange of Lobariaceae species of a lower montane rain forest in Panama. Lichenologist 36:329–342

Larcher W (2001) Ökologie der Pflanzen. Ulmer, Stuttgart

León-Vargas Y, Engwald S, Proctor MCF (2006) Microclimate, light adaptation and desiccation tolerance of epiphytic bryophytes in two Venezuelan cloud forests. J Biogeogr 33:901–913

Lewis SL, Brando PM, Phillips OL, van der Heijden GMF, Nepstad D (2011) The 2010 Amazon drought. Science 331:554–554

Longton RE (ed) (1988) The biology of polar bryophytes and lichens. Cambridge University Press, Cambridge

Lösch R, Mülders P (2000) Heat and cold resistance of central African bryophytes. Bibl Lichenol 75:253–263

Lösch R, Mülders P, Fischer E, Frahm J-P (1994) Scientific results of the BRYOTROP expedition to Zaire and Rwanda. 3. Photosynthetic gas exchange of bryophytes from different forest types in eastern Central Africa. Trop Bryol 9:169–185

Mägdefrau K (1969) Die Lebensformen der Laubmoose. Veg Acta Geobot 16:285–297

Mägdefrau K (1982) Life-forms of bryophytes. In: Smith AJE (ed) Bryophyte ecology. Chapman & Hall, London/New York, pp 45–58

Nadkarni NM, Solano R (2002) Potential effects of climate change on canopy communities in a tropical cloud forest: an experimental approach. Oecologia 131:580–586

Nadkarni NM, Schaefer D, Matelson TJ, Solano R (2004) Biomass and nutrient pools of canopy and terrestrial components in a primary and a secondary montane cloud forest, Costa Rica. For Ecol Manage 198:223–236

Norris DH (1990) Bryophytes in perennial moist forests of Papua New Guinea: ecological orientation

and predictions of disturbance effects. Bot J Linn Soc 104:281–291

Oechel WC, Sveinbjörnsson B (1978) Primary production processes in arctic bryophytes at Barrow, Alaska. In: Tieszen LL (ed) Vegetation and production ecology of an Alaskan arctic tundra. Springer, New York, pp 269–298

Pannewitz S, Green TGA, Maysek K, Schlensog M, Seppelt R, Sancho LG, Turk R, Schroeter B (2005) Photosynthetic responses of three common mosses from continental Antarctica. Antarct Sci 17:341–352

Pócs T (1980) The epiphytic biomass and its effect on the water balance of two rain forest types in the Uluguru Mountains (Tanzania, East Africa). Acta Bot Acad Sci Hung 26:143–167

Pócs T (1982) Tropical forest bryophytes. In: Smith AJE (ed) Bryophyte ecology. Chapman & Hall, London/New York, pp 59–104

Proctor MCF (1981) Physiological ecology of bryophytes. Adv Bryol 1:79–166

Proctor MCF (1982) Physiological ecology: water relations, light and temperature responses, carbon balance. In: Smith AJE (ed) Bryophyte ecology. Chapman & Hall, London/New York, pp 333–381

Proctor MCF (1990) The physiological basis of bryophyte production. Bot J Linn Soc 104:61–77

Proctor MCF (2000a) The bryophyte paradox: tolerance of desiccation, evasion of drought. Plant Ecol 151:41–49

Proctor MCF (2000b) Physiological ecology. In: Shaw AJ, Goffinet B (eds) Bryophyte biology. Cambridge University Press, Cambridge, pp 225–247

Proctor MCF (2001) Patterns of desiccation tolerance and recovery in bryophytes. Plant Growth Regul 35:147–156

Proctor MCF (2002) Ecophysiological measurements on two pendulous forest mosses from Uganda, *Pilotrichella ampullacea* and *Floribundaria floribunda*. J Bryol 24:223–232

Proctor MCF (2004a) How long must a desiccation-tolerant moss tolerate desiccation? Some results of 2 years' data logging on *Grimmia pulvinata*. Physiol Plant 122:21–27

Proctor MCF (2004b) Light and desiccation responses of *Weymouthia mollis* and *W. cochlearifolia*, two pendulous rainforest epiphytes from Aust N Z. J Bryol 26:173

Proctor MCF, Ligrone R, Duckett JG (2007a) Desiccation tolerance in the moss *Polytrichum formosum*: physiological and fine-structural changes during desiccation and recovery. Ann Bot 99:75–93

Proctor MCF, Oliver MJ, Wood AJ, Alpert P, Stark LR, Cleavitt NL, Mishler BD (2007b) Desiccation-tolerance in bryophytes: a review. Bryologist 110:595–621

Richards PW (1984) The ecology of tropical forest bryophytes. In: Schuster RM (ed) New manual of bryology. The Hattori Botanical Laboratory, Nichinan, pp 1233–1270

Richards PW (1996) The tropical rain forest – an ecological study. Cambridge University Press, Cambridge

Robinson SA, Wasley J, Popp M, Lovelock CE (2000) Desiccation tolerance of three moss species from continental Antarctica. Aust J Plant Physiol 27:379–388

Romero C, Putz FE, Kitajima K (2006) Ecophysiology in relation to exposure of pendant epiphytic bryophytes in the canopy of a tropical montane oak forest. Biotropica 38:35–41

Ruinen J (1953) Epiphytosis. A second view on epiphytism. Ann Bogor 1:101–157

Seifriz W (1924) The altitudinal distribution of lichens and mosses on Mt. Gedeh, Java. J Ecol 12:307–313

Shaw AJ, Goffinet B (eds) (2000) Bryophyte biology. Cambridge University Press, Cambridge

Silvola J (1985) CO_2 dependence of photosynthesis in certain forest and peat mosses and simulated photosynthesis at various actual and hypothetical CO_2 concentrations. Lindbergia 11:86–93

Silvola J (1990) Combined effects of varying water content and CO_2 concentration on photosynthesis in *Sphagnum fuscum*. Holarct Ecol 13:224–228

Skre O, Oechel WC (1981) Moss functioning in different taiga ecosystems in interior Alaska. I. Seasonal, phenotypic, and drought effects on photosynthesis and response patterns. Oecologia 48:50–59

Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL (eds) (2007) II TEIL climate change 2007: the physical science basis. Contribution of working group I to the fourth assessment report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge

Solomon S, Plattner G-K, Knutti R, Friedlingstein P (2009) Irreversible climate change due to carbon dioxide emissions. Proc Natl Acad Sci 106: 1704–1709

Tarnawski MG, Melick D, Roser D, Adamson E, Adamson H, Seppelt RD (1992) *In situ* CO_2 levels in cushion and turf forms of *Grimmia antarctici* at Casey Station, East Antarctica. J Bryol 17:241–249

Tarnawski MG, Green TGA, Büdel B, Meyer A, Zellner H, Lange OL (1994) Diel changes of atmospheric CO_2 concentrations within, and above, cryptogam stands in a New Zealand temperate rainforest. N Z J Bot 32:329–336

Tozer WC, Hackell D, Miers DB, Silvester WB (2005) Extreme isotopic depletion of nitrogen in New Zealand lithophytes and epiphytes; the result of diffusive uptake of atmospheric ammonia? Oecologia 144:628–635

Tuba Z, Proctor MCF, Takács Z (1999) Desiccation-tolerant plants under elevated air CO_2: a review. Z Naturforsch [C] 54:788–796

Tuba Z, Ötvös E, Jócsák I (2011) Effects of elevated air CO_2 concentration on bryophytes: a review. In: Tuba Z, Slack NG, Stark LR (eds) Bryophyte ecology and climate change. Cambridge University Press, Cambridge, pp 55–70

Veneklaas EJ, Zagt RJ, Van Leerdam A, Van Ek R, Broekhoven AJ, Van Genderen M (1990) Hydrological properties of the epiphyte mass of a montane tropical rain forest, Colombia. Vegetatio 89:183–192

Wagner S, Zotz G, Salazar Allen N, Bader MY (2013) Altitudinal changes in temperature responses of net photosynthesis and dark respiration in tropical bryophytes. Ann Bot 111(3):455–465

Waite M, Sack L (2010) How does moss photosynthesis relate to leaf and canopy structure? Trait relationships for 10 Hawaiian species of contrasting light habitats. New Phytol 185:156–172

Waite M, Sack L (2011a) Does global stoichiometric theory apply to bryophytes? Tests across an elevation x soil age ecosystem matrix on Mauna Loa, Hawaii. J Ecol 99:122–134

Waite M, Sack L (2011b) Shifts in bryophyte carbon isotope ratio across an elevation x soil age matrix on Mauna Loa, Hawaii: do bryophytes behave like vascular plants? Oecologia 166:11–22

Wolf JHD (1993) Diversity patterns and biomass of epiphytic bryophytes and lichens along an altitudinal gradient in the northern Andes. Ann Mo Bot Gard 80:928–960

Wright SJ, Muller-Landau HC, Schipper J (2009) The future of tropical species on a warmer planet. Conserv Biol 23:1418–1426

Zotz G (1999) Altitudinal changes in diversity and abundance of non-vascular epiphytes in the tropics – an ecophysiological explanation. Selbyana 20:256–260

Zotz G (2004) The resorption of phosphorus is greater than that of nitrogen in senescing leaves of vascular epiphytes from lowland Panama. J Trop Ecol 20:693–696

Zotz G, Bader MY (2009) Epiphytic plants in a changing world: global change effects on vascular and non-vascular epiphytes. Prog Bot 70:147–170

Zotz G, Rottenberger S (2001) Seasonal changes in diel CO_2 gas exchange of three Central European cushion mosses: a one-year *in situ* study. Plant Biol 3:661–669

Zotz G, Winter K (1994) Photosynthesis and carbon gain of the lichen, *Leptogium azureum*, in a lowland tropical forest. Flora 189:179–186

Zotz G, Büdel B, Meyer A, Zellner H, Lange OL (1997) Water relations and CO_2 exchange of tropical bryophytes in a lower montane rain forest in Panama. Bot Acta 110:9–17

Zotz G, Büdel B, Meyer A, Zellner H, Lange OL (1998) *In situ* studies of water relations and CO_2 exchange of the tropical macrolichen, *Sticta tomentosa*. New Phytol 139:525–535

Zotz G, Schweikert A, Jetz W, Westerman H (2000) Water relations and carbon gain are closely related to cushion size in the moss *Grimmia pulvinata*. New Phytol 148:59–67

Zotz G, Schultz S, Rottenberger S (2003) Are tropical lowlands a marginal habitat for macrolichens? Evidence from a field study with *Parmotrema endosulphureum* in Panama. Flora 198:71–77

Chapter 16

Physiological Ecology of Dryland Biocrust Mosses

Kirsten K. Coe* and Jed P. Sparks
Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, NY 14853, USA

Jayne Belnap
U.S. Geological Survey, Southwest Biological Center, Canyonlands Research Station, Moab, UT 84532, USA

Summary

Soil biocrusts are assemblages of cyanobacteria, lichens, and mosses ubiquitous to arid and semi-arid (dryland) systems that offer an array of ecosystem services. Soil crust mosses are taxonomically diverse, account for up to 30 % of crust cover, and offer large contributions to crust biogeochemical functionality, yet remain the least understood component of the community. Because of selective pressures of their growth environment, such species are highly desiccation tolerant, with the ability to withstand the loss of most cellular water for extended periods of time, during which metabolism is suspended. Biocrust mosses can also tolerate larger ranges of temperature, light, and cellular water content than mesic species, yet still remain sensitive to certain aspects of environmental alteration. For one, changes in precipitation regime are likely to heavily influence survival in dryland mosses. Rainfall, occurring as discrete periods of hydration in dryland systems, causes mosses to undergo

*Author for correspondence, e-mail: kkc32@cornell.edu; kryder.coe@gmail.com

D.T. Hanson and S.K. Rice (eds.), Photosynthesis in Bryophytes and Early Land Plants, Advances in Photosynthesis and Respiration 37, pp. 291–308, DOI 10.1007/978-94-007-6988-5_16,

wet-dry cycles that result in either a positive or a negative carbon balance. Carbon balance can be used as a measure of performance during individual rainfall events, and is a metric for long-term viability. Recent work suggests rainfall event magnitude plays a large role in carbon balance, as does the frequency and seasonality with which events fall. Biocrust mosses are stimulated by elevated CO_2, yet may not acclimate photosynthetically to long-term enrichment. Interestingly, elevated CO_2 may favor stress tolerance at the expense of growth in biocrust moss, particularly at high temperatures. Finally, despite low annual growth rates, nitrogen appears to place physiological limitations on reproductive biology of biocrust mosses. High levels of nitrogen deposition, however, have been shown to cause toxicity, competitive exclusion by vascular plants, and can reduce cyanosymbioses.

I. Introduction

Soil biocrusts, also known as cryptobiotic crusts or microbiotic crusts, are assemblages of organisms living amongst soil particles within the top few centimeters of soil and at the soil surface. Crusts are composed primarily of cyanobacteria, lichen, and moss (Rosentreter et al. 2007), and these components exist in various proportions depending on microclimate and disturbance regime of the soil environment. In dryland ecosystems, crusts increase the water holding capacity of soils, reduce erosion (Belnap 2003; Belnap et al. 2006), and influence seedling establishment of grasses and shrubs (Clair and Johansen 1993). Biocrusts also influence elemental cycling in drylands (Housman et al. 2006), as they (a) contain a large proportion of photosynthesizing organisms and supply organic carbon to underlying soil; (b) contain free-living and lichenized cyanobacteria that fix atmospheric nitrogen (N_2) into a biologically available form (NH_4^+) (Evans and Belnap 1999); and (c) secrete compounds that increase phosphorous availability within soil (Harper and Pendleton 1993). Biocrusts occur in all dryland regions of the world and on every continent, including polar regions, and have been observed on nearly all soil types (Belnap et al. 2001). Crusts are found in the spaces between and under vascular plants, and in some regions where persistence of biocrust communities can be disproportionately favored (hot deserts or cool/cold drylands) can occupy 70 % or more of the living ground cover (Belnap 1995; Fig. 16.1a).

Although biocrusts have received significant attention in recent research in dryland ecosystems, bryophytes remain the poorest understood component of the community. In drylands where soil-dwelling mosses can persist, moss can account for 2 % to over 30 % cover in crusts (Thompson et al. 2005), often depending on crust maturity, and are a taxonomically diverse functional group (Brinda et al. 2007). Many common crust moss genera (e.g. *Syntrichia*, *Tortula*, *Pterogonium*, *Crossodium*, *Didymodon*) belong to the family Pottiaceae, and are typically slow-growing acrocarps with annual (normally short mosses <0.5 mm in height) as well as perennial (0.5 – several cm in height) life history strategies (Rosentreter et al. 2007).

Evidence suggests that while mosses offer significant contributions to overall biocrust function, they can be sensitive to environmental alterations, from discrete soil disturbance events to direct and subtle aspects of climate change. Moss removal from a biocrust has negative consequences for structure and composition of the crust as well as nutrient cycling in soils below (Reed et al. 2012). Therefore, understanding the physiological determinants of performance and survival of biocrust mosses is important for understanding

Abbreviations: RWC – relative water content; PAR – photosynthetically active radiation; NPQ – non-photochemical quenching; FACE – Free-Air CO_2 Enrichment; SSS – Spatial segregation of the sexes

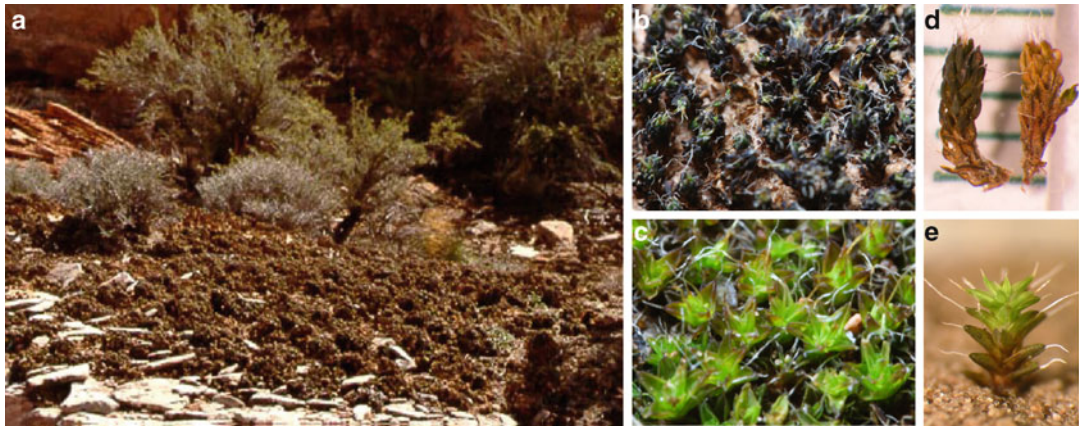


Fig. 16.1. (**a**) Moss in an intact biocrust from the Colorado Plateau in the Western United States; (**b**) the common biocrust species *Syntrichia caninervis* at ~6x magnification in the desiccated state, and (**c**) in the hydrated state; (**d**) dry *S. caninervis* shoots exhibiting normal (*left*) and reduced (*right*) pigmentation as a result of rapid wet-dry cycles (Photo credit: Lloyd Stark); and (**e**) architecture and new growth of an emerging *S. caninervis* shoot (Photo credit: Lloyd Stark).

dryland ecosystem ecology in general. Biocrust mosses possess a suite of adaptations to cope with environmental variability in dryland systems, and the physiological ecology of dryland mosses has received recent research interest, mainly in terms of the response of these organisms to environmental change but also to the ecological role of moss as part of crust communities. This chapter will review the current knowledge of biocrust moss ecophysiology in dryland systems, and will concentrate on water relations, temperature and light tolerance, nutrient status, and responses to elevated CO_2 with respect to photosynthetic performance, reproduction, biomass accumulation, and stress tolerance.

II. Desiccation Tolerance, Precipitation Pulses, and Carbon Balance

The growth environment of dryland biocrust mosses is often characterized by extended dry periods interspersed with small precipitation events, resulting in intermittent pulses of resource availability. This, along with high temperatures and exposed soil microclimates results in rapid drying and prolonged periods of desiccation. Because of these factors, there is thought to have been strong evolutionary pressure and selection for a desiccation tolerant strategy among biocrust species (Oliver et al. 2000a, b, 2005; Proctor et al. 2007).

All mosses are poikilohydric organisms where the water content of cells is in equilibrium with the environment and the control of tissue water content is passive. Moss gametophytes are often exposed to a range of environmental water availabilities, and as a consequence must possess the ability to withstand an extreme range of cellular water contents. In drylands, cell water content can often be very low for extended periods. Most, though not all, dryland mosses therefore display some degree of desiccation tolerance. Although cellular water potentials at full turgor are typically between −1.0 and −2.0 MPa, many mosses remain photosynthetically active over water potentials ranging from −0.5 to −10.0 MPa (Dilks and Proctor 1979; Proctor 2008). The limits of tolerance for most mosses are cellular water potentials of −20 to −40 MPa (Proctor and Pence 2002; Oliver et al. 2005; Proctor et al. 2007); the degree of this tolerance depends largely on the growth environments that species are adapted.

Mosses with the highest degrees of desiccation tolerance often represent the endpoints of these spectra. While highly desiccation-tolerant mosses occur in dry microclimates (on substrates such as exposed rock, sand, or bark) in all biomes, the diversity and abundance of such species is highest in dryland ecosystems.

Desiccation tolerance represents a strategy to suspend metabolism during dry intervals, and restrict physiological activity to periods of sufficient hydration. During desiccated periods, shoot tissues lose virtually all liquid water and can dry to 5–10 % dry mass and water potentials of −100 MPa in the most desiccation tolerant species (Proctor et al. 2007; Fig. 16.1b). A significant degree of molecular packaging mechanisms involving sugars (Smirnoff 1992) as well as proteins (Buitink et al. 2002; Oliver et al. 2005) are likely involved in the protection of macromolecules in the absence of water as well as maintenance of spatial relationships in cells. Mechanisms of antioxidant production to neutralize reactive oxygen species as well as photoprotection to dissipate excess light (Marschall 2004) also appear to be important as cells transition to the desiccated state. Because it influences the degree to which tissues are preserved and energy is invested in preservation, the rate at which tissues dry is an important determinant of survival in the desiccated state as well as recovery potential for many species, and in general drying speed is inversely correlated with future performance (Oliver et al. 2000a, b, 2005). When compared to shorter dry times, numerous measures of regeneration potential such as cell ultrastructure, pigmentation, electrolyte efflux, and photosynthetic performance have all been shown to be higher in mosses exposed to longer drying periods (Schonbeck and Bewley 1981a, b; Oliver and Bewley 1984; reviewed in Oliver et al. 2000a, b). This relationship is due to the ability of cells to initiate and complete preservation procedures for macromolecules and organelles prior to entering the desiccated state. In general, the more preserved tissues are while desiccated, the higher their recovery potential is given sufficient hydration.

Among mosses, dryland species display the highest levels of desiccation tolerance recorded, both in terms of the length of time a shoot can remain desiccated before full physiological recovery as well as the lowest relative water content (RWC) from which tissues can recover. Dryland biocrust species can survive in the desiccated state (e.g., 5–10 % dry mass in *Syntrichia*) for months and recover full photosynthetic function within <24 h (Tucker et al. 1975; Oliver et al. 1993; Proctor and Pence 2002). In the extreme, mosses have been observed to remain desiccated for >100 days in the Mojave Desert (Stark 2005). In the desiccated state, shoots can withstand significantly more variability in temperature and light levels, including those that can damage tissues when hydrated. Indeed, because of the stressful environments commonly occupied by biocrusts, many dryland crust mosses may actually *require* periods of desiccation for survival under temperature and light extremes.

From the perspective of a moss, when rainfall does occur in dryland systems it occurs as a pulse between two desiccation periods. This results in a wet-dry cycle and a characteristic response with periods of net carbon loss (where carbon loss through respiration is greater than carbon gain through photosynthetic fixation) and net carbon gain (when fixation outweighs respiration) (Fig. 16.2). Respiration recovers more rapidly than photosynthesis (due to a lag time in reinstatement of the Calvin cycle), thus the onset of hydration is characterized by a period of net carbon loss as respiratory energy is used to reinstate metabolism, repair membranes, and reconfigure and reorganize cellular components (Hinshiri and Proctor 1971; Tuba et al. 1994; Proctor et al. 2007). Because the photosynthetic apparatus remains intact during desiccation, recovery of photosynthetic function in biocrust mosses is often rapid (only slightly lagging behind respiration), and net carbon fixation can be reached in 10–30 min following hydration in some desiccation

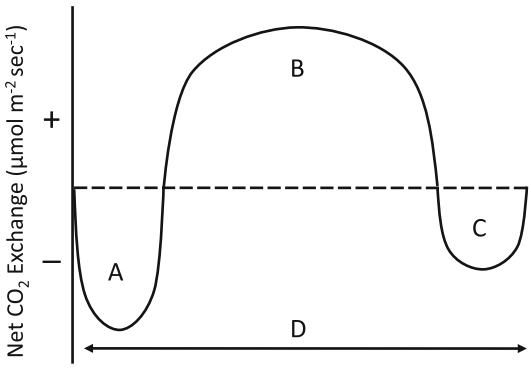


Fig. 16.2. Characteristic pattern of net carbon fixation in desert moss over the course of a wet-dry cycle (of length *D*) initiated by a rainfall event: an initial period of net carbon loss when respiratory costs are high (*A*) followed by a period of net carbon gains when tissues are hydrated and photosynthesis is higher than respiration (*B*; length varies as a function of *D*) and a period of subsequent carbon loss as photosynthesis ceases and tissues prepare for desiccation again (*C*). Following an individual rainfall event, carbon balance is equivalent to gains from net carbon fixation (area under *B*) minus carbon loss from respiration (areas under $A + C$) (Adapted from Coe et al. 2012a).

tolerant species (e.g. *Tortula* (*Syntrichia*) spp.; Bewley 1979; Tuba et al. 1996; Proctor and Smirnoff 2000; Reed et al. 2012; Li et al. 2010). Once photosynthesis outpaces respiration, a phase of net carbon gain occurs, the length of which is determined by the duration of tissue hydration. As tissues dry, photosynthetic rates diminish, and as energy is used to repackage cellular contents for another bout of desiccation, the cycle often ends with another brief phase of net carbon loss (Mishler and Oliver 2009; Reed et al. 2012). Depending on the relative magnitude of the periods of carbon gain and loss over the course of the wet-dry cycle, dryland mosses exhibit either an overall carbon balance that is positive or negative. The carbon balance of biocrust mosses during discrete events, when compounded over long periods, is a strong determinant of performance, growth, and long-term survival. Interestingly, this phenomenon appears to apply to mesic mosses as well and has been shown to be the case in tropical bryophytes exposed to discrete hydration events (see Chap. 15).

III. Water Relations

In spite of passive water regulation over the gametophyte surface, *cell* water relations of mosses are nearly identically to that of other dryland plants with respect to physiological parameters such as water potential (ψ_w) and its relationship to RWC. As is true in mosses from other environments, the RWC of dryland biocrust species is a strong determinant of photosynthetic performance (also see Chap. 5). Maximum rates of net carbon fixation are typically reached between 40 and 70 % RWC (Tuba et al. 1996; K.K. Coe, unpublished) as photosynthesis is inhibited at higher water contents by limited CO_2 diffusion and at lower water contents by low intracellular ψ_w. However, many dryland mosses possess a greater range of suitable hydration levels for photosynthesis and can withstand cell water potentials (when not physiologically active) well below those of species from mesic environments. Some dryland species (e.g. *S. ruralis*) can withstand cellular drying to a ψ_w of <-100 MPa when desiccated and photosynthesis and respiration have been detected in tissues with ψ_w as low as −10 and −20 MPa, respectively (Dilks and Proctor 1979). Sustained physiological functioning at such water potentials possibly owes to higher cell wall thickness relative to lumen, high cell wall extensibility, and/or low RWC at full turgor (Proctor et al. 1998; Proctor and Tuba 2002).

Dryland mosses possess a suite of morphological and physiological adaptations to maximize photosynthetic performance under dry conditions as well as to take advantage of water when it does become available. At the macro-scale, biocrust species almost always grow within the laminar boundary layer directly above the soil surface, thus minimizing convective water loss. In dryland mosses, cuticular waxes deposited on leaf surfaces play a role in restricting water loss (Xu et al. 2009a, b), as do hair points at the ends of leaves (e.g. *Grimmia*, *Syntrichia*) that serve to extend the height of the boundary layer and increase albedo (Valko 2003; Bowker et al. 2010). Shoots of biocrust mosses are

also typically short and densely packed in a characteristic clonal cushion or carpet habit, which behaves as a smooth object on the soil surface when exposed to wind (Proctor 2000), and maximizes extracellular water storage between adjacent shoots in capillary spaces.

Capillary water is an essential component of the physiological ecology of dryland mosses for several reasons. First, although some water does move through cells and cell walls, most water conduction is external and water held or moving within capillary spaces between shoots, in sheathing leaf bases and in rhizoid tomenta acts as a direct source of water for the plant. Second, capillary water can be a major component of the water associated with a shoot, can outweigh symplastic water by a factor of five or more (Proctor 2008), and can thus act as a primary source of water storage for moss. Indeed, water stored in this manner is directly related to the length of time shoots remain wet and photosynthetically active and can often assist dryland moss species in bridging gaps in water availability by extending the hydrated period provided by a rain event. Finally, capillary water content can vary widely without influencing the water status of plant cells in a turgid state, but when it is exhausted, cells dry rapidly. One hypothesized function of capillary water in *Tortula ruralis* is to maintain cells at full turgor until a cytological switch initiates rapid desiccation, whereby cells spend little time at intermediate water contents thought to impose damage (Gaff 1997; Proctor 2000). This hypothesis has not been fully explored in different species, however, and may conflict with the widely demonstrated relationship between drying speed and recovery potential (see section "Desiccation tolerance, precipitation pulses, and carbon balance" of this chapter).

Mosses must reconcile maximizing water conduction and storage and the need for gas exchange between the atmosphere and the interior of the leaf for photosynthesis. When shoots possess external water, exchange of CO_2 must occur through films of water where diffusion resistance for gas is four orders of magnitude higher than through air.

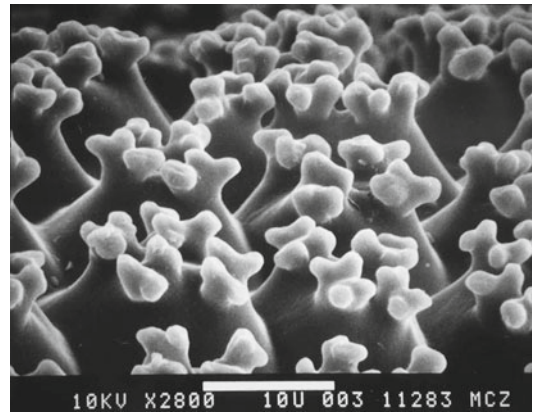


Fig. 16.3. Scanning electron micrograph of papillae on the surface of cells in *Syntrichia:* when cell surfaces are wet, carbon fixation occurs in the tips of papillae where chloroplasts are concentrated, while interstices serve as external conducting channels for water (Photo credit: Brent Mishler).

Typical adaptations to minimize such trade-offs include specialized leaves that perform either water conduction *or* gas exchange or the use of the outer surfaces of stem-sheathing leaves for gas exchange while employing inner surfaces to provide a capillary channel around the stem (Proctor 2008). Some additional adaptations are present in dryland genera and often involve the division of water conducting and gas exchange surfaces in the same structure or section of leaf on microscopic scales. For example, the genus *Syntrichia* (*Tortula*) is characterized by specialized papillae or mammillae extruding from the cellular surface (Fig. 16.3) that serve two simultaneous functions: first, to create a continuous network of interstices for water adhesion and transport in microcapillary spaces between papillae, and, second, to allow sustained photosynthesis at high water contents in the tips of papillae (also shown to contain proportionally higher chloroplast densities) even when the rest of cells are covered with a sheet of water that impedes CO_2 diffusion (Tucker et al. 1975; Proctor 1979; Xu et al. 2009a, b).

Irrespective of adaptations to limit water loss in dryland mosses, overall environmental water availability (primarily through alterations in precipitation regime) is likely

to influence performance and survival. This is because such alterations result in changes in carbon balance following rainfall events, and biocrust mosses are dependent on the maintenance of positive carbon balances from discrete rainfall events for biomass accumulation and long-term viability. Much of the recent research on this topic has been conducted in western North America, where climate models predict changes in mean annual rainfall as well as alterations in intra-annual precipitation parameters including individual event magnitude, timing (frequency) at which events fall, and the time of year precipitation occurs (Lioubimtseva 2004; Meehl et al. 2007; Seager et al. 2010). Rainfall event magnitude is one of the largest determinants of carbon balance in the common biocrust species *Syntrichia caninervis*, because increased rainfall amounts result in shoots that remain hydrated (thus remain in the phase of net carbon fixation; Fig. 16.2) longer, and small events result in limited gains from carbon fixation that cannot compensate for respiratory costs during the event (Barker et al. 2005; Coe et al. 2012a; Reed et al. 2012). Recovery of the cell cycle as well as reassembly of cytoskeletal elements can take >24 h in *Syntrichia*, thus brief periods of hydration may not be sufficient for full recovery of physiological function. Further, several studies indicate a rainfall event size threshold for carbon balance in dryland mosses of 2–3 mm, below which moss will, on average, enter carbon deficit (Fig. 16.1d; Barker et al. 2005; Stark 2005; Coe et al. 2012a). Given that >70 % of rainfall events in drylands are <5 mm effective size (Sala and Lauenroth 1982; Loik et al. 2004; Reynolds et al. 2004) and within that, the majority are less than half of that (Huxman et al. 2004), it is probable that small carbon deficits are quite common and dryland mosses are reliant on the presence of larger events for net carbon gain. The frequency with which events fall also influences carbon balance, and Coe et al. (2012a) suggest that increasing the dry interval between events reduces carbon balance during subsequent events. This likely results from increased cost of recovery during hydration following periods of increased desiccation intensity (Hinshiri and Proctor 1971; Dilks and Proctor 1974; Proctor and Pence 2002; Proctor 2003).

Most dryland biocrust species depend on precipitation during the cooler months of the year for annual growth because ambient temperature and humidity permit water from rainfall events to remain available for sustained periods. During the warmer months, meteorological conditions restrict water availability for biocrust species, and mosses appear to exhibit changes in physiological state over the course of the year that influence response to rainfall. For example, when measured under identical laboratory conditions, mosses collected in the winter display higher responsiveness to rainfall and higher average carbon balances when given same event size that caused significantly smaller carbon balances (and sometimes loss) in mosses collected in the summer (Coe et al. 2012a). Several climate models for North American drylands suggest winter rainfall will be reduced, while summer will exhibit more frequent small events and/or an increase in monsoonal systems (Castro et al. 2010; Meehl et al. 2007). The consequences of such changes could be severe, and it has been suggested that (a) more frequent <2 mm events during the summer may cause rapid moss decline and reduced nitrogen availability to crust organisms (Reed et al. 2012) and (b) an increase in monsoonal activity could have negative consequences for sex expression and associations with nitrogen fixing cyanobacteria in *Syntrichia* (Stark et al. 2011a, b). Finally, reductions in winter rainfall will be detrimental to many biocrust mosses that rely on cumulative carbon gains during these times of year to compensate for carbon losses during warmer, unfavorable periods.

IV. Temperature Relations

In dryland systems, air temperatures have wide diurnal and annual fluctuations. Biocrust mosses grow in close proximity to the soil surface, and shoot temperatures can exceed air temperature by 19 °C or more (Hearnshaw

and Proctor 1982). Under these conditions, selective pressures to tolerate such extremes are high, and as a consequence, the range of temperatures under which dryland biocrust mosses can survive greatly exceeds that of species from other biomes. Survival in the desiccated state in these species has been noted in temperatures ranging from <0 °C (for months) up to 100 °C (for minutes) (Hearnshaw and Proctor 1982) and dry biocrust mosses on sun-exposed substrates can frequently reach tissue temperatures >60 °C (Proctor 2008). The ability to withstand high temperatures in the desiccated state in particular contributes to the ability of dryland mosses to colonize and persist in unfavorable microclimates from which other less-tolerant species may be excluded (Kidron et al. 2000). Most biocrust species have perennial gametophytes, thus viability while desiccated enables shoots to persist throughout the entire year and bridge gaps in hydration occurring during the warmer months where growth cannot occur.

Temperature tolerance while hydrated in biocrust moss taxa, however, is similar to the range of many other bryophytes. Hydrated shoots are far less tolerant of temperature extremes, and tissue damage occurs at both the high and low ends of the temperature spectrum. When shoots are moist, tissue injury generally begins to occur when temperatures exceed 40 °C (Larcher 2003), with lethal high temperatures between 42 and 51 °C, depending on species and exposure time (Meyer and Santarius 1998; Proctor and Pence 2002). Less is known about responses to cold temperatures and although many dryland biocrust species survive and actively photosynthesize and grow throughout the winter, freezing (<−10 °C) of hydrated shoots may cause irreversible photoinhibition if repair mechanisms are not present (Lovelock et al. 1995). Production of isoprene (2-methyl-1, 3 butadiene) under conditions of temperature stress has been shown to have protective functions in some mosses that experience wide temperature fluctuations in nature (Hanson et al. 1999). Although thermoprotection via isoprene production has not yet been demonstrated in desert mosses, evidence for the evolution of its function in several early land plant lineages (Sharkey and Yeh 2001; Jobson and Qiu 2010) suggests it is worth considering its potential presence in this functional group.

Temperature exerts strong control on net carbon fixation in dryland species because of (a) biochemical limitations such as compromised membrane stability and enzyme denaturation, (b) increased respiration rates, and (c) reductions in environmental water availability due to increased rates of evaporation. Optimal temperatures for photosynthesis occur between 10 and 20 °C (Furness and Grime 1982; Alpert and Oechel 1987; K. K. Coe unpublished), and suppression of photosynthesis has been observed to occur at temperatures between 25 and 35 °C, depending on geographic locality and crust moss species (Grote et al. 2010; Coe et al. 2012b). At these temperatures, declines have been observed in net photosynthesis (Grote et al. 2010), carboxylation capacity, maximum rates of electron transport through photosynthetic membranes (Coe et al. 2012b), and efficiency of conversion of light energy to chemical energy (Hamerlynck et al. 2002; Coe et al. 2012b). Declines in photosynthetic performance are likely due to induced structural deficiencies such as enzyme denaturation, damage to chloroplast membranes and damage to the photosynthetic pigment apparatus (Larcher 2003), changes in membrane permeability associated with high heat while hydrated (Meyer and Santarius 1998; Liu et al. 2003), and/or offsets in the optimal temperature for photosynthesis as compared to respiration (Grote et al. 2010).

Because carbon fixation in dryland mosses is constrained at high temperatures, limited energy is available for physiological functioning, growth, stress tolerance, and reproduction. As a consequence, mosses growing in environments characterized by thermal stress are faced with a corresponding suite of energetic trade-offs. Male and female shoots in many biocrust moss taxa differ by an order of magnitude in energetic allocation to reproductive structures (Lackner 1939;

Paolillo 1979; Bowker et al. 2000); based on dry biomass, antheridia cost approximately six times as much as archegonia to produce (Stark et al. 2000). Therefore, male shoots are constrained by the cost of their reproductive structures, and in stressful conditions, are forced to reduce proportional carbon allocation to growth or stress tolerance. Females, on the other hand, can allocate proportionally more carbon to growth and stress tolerance while continuing to produce reproductive structures. To illustrate, leaf growth and regeneration in female shoots occurred twice as fast and was more complete than in males exposed to incremental increases in temperature from 25 to 45 °C for 60 min (Stark and McLetchie 2006). These differences cause overall differences in stress tolerance abilities in male and female gametophytes, and have resulted in two common patterns in the field. First, dryland mosses exhibit sex ratios in nature that differ substantially from 1:1. Ratios are almost always female biased, with male shoots occurring <30 % of the time (or as low as 0 %) at the population level (Stark and McLetchie 2006). Such female-skewed ratios are commonly observed in *Bryum* (Stark et al. 2010), *Didymodon* (Ochyra and Zander 2002), *Syntrichia* (Mishler and Oliver 1991; Stark et al. 2005), and in an array of biocrust species from the Mojave Desert in North America (Brinda et al. 2007). Such patterns are directly related to the inability of males to grow and produce reproductive parts in environments characterized by thermal stress (Stark and McLetchie 2006). Second, dryland mosses exhibit spatial segregation of the sexes (SSS) based on gradients of environmental stress, where females are more common in thermally stressed environments such as plant interspaces, and often males are restricted to shrub understories (Bowker et al. 2000; Stark et al. 2005, 2010).

When female shoots do produce sporophytes (though it is very uncommon in dryland species due to SSS and low water availability for sperm transfer to archegonia), the cost of production and maintenance of these structures is much higher than production of archegonia alone. Abortion of sporophytes is an extremely common occurrence in dryland biocrust taxa for two reasons. First, the energetic cost of maintaining a sporophyte (due to investment of structural materials as well as maintenance of cellular machinery) is high, and places additional stress on the female gametophyte (Stark et al. 2000; Stark 2001). Second, sporophytes are less tolerant of thermal stress than gametophytes (illustrated in *Microbryum* heat shock experiments; McLetchie 2006). An understanding of the steps leading to sporophyte abortion is confounded by the fact that the sporophytes are connected to and dependent on gametophytes, making mechanistic inferences into the process and reasons for abortion difficult. Nonetheless, female shoots appear to be more stress tolerant and possess higher growth rates than males under conditions of thermal stress, unless they produce sporophytes.

In dryland systems, thermal stress, skewed sex ratios, and SSS result in rates of sexual reproduction that are low to absent (Bowker et al. 2000). Although high surface temperatures and large variation in temperatures impose significant amounts of stress on carbon acquisition and physiological functioning, correlates of temperature (such as water and nutrient availability or light levels) along environmental gradients can play a large role in biocrust moss physiological ecology and reproductive biology. For example, water availability is shown to correlate with temperature along a canopy – interspace gradient (Stark et al. 2010), and has been shown to influence sex expression in *S. caninervis* due to males' inability to tolerate energy loss associated with repeated wet-dry cycles in plant interspace regions (Benassi et al. 2011).

V. Response to Variation in Light

Bryophytes as a group have high variation in light responses as well as seasonal and plastic variability (Proctor 2000). Dryland crust mosses may represent some of the extremes

in plasticity of response as they typically occur in plant interspaces that experience high light conditions (PAR > 1,000 μmol m^{-2} s^{1}), yet also can occupy habitats directly under low desert shrubs. When biocrust mosses are desiccated (and not physiologically active), they display higher tolerance of high light than wet shoots (Seel et al. 1992). Mosses that can persist in areas where they are exposed to extreme conditions (e.g. high irradiance plant interspaces) often, but not always, do so because they can survive in the desiccated state when the growth environment is extreme. Biocrust species are not always dry under conditions of high irradiance though, particularly following short rainstorms, thus still must be able to perform photosynthetically in and tolerate high light conditions while hydrated.

Most mosses fall into the category of shade plants based on their photosynthetic physiology (Valanne 1984). Their photosynthetic apparatuses saturate at low irradiances, and express low chlorophyll a:b ratios (Martin and Churchill 1982; Marschall 2004). Some biocrust taxa (e.g. *Bryum*) fall into this category and are often restricted to crusts under significant plant cover. Such mosses growing in lower light environments such as under shrubs exhibit higher photosynthetic rates at lower irradiances than those growing in exposed environments (Alpert and Oechel 1987). Further, even in biocrust taxa, photosynthetic activity typically occurs under <20 % full sun following rainfall events where cloud cover reduces PAR to 50–250 μmol m^{-2} s^{-1} (Marschall 2004). Some of the more common species (e.g. *Syntrichia ruralis*, *S. caninervis*, *Rhacometrium spp.*), however, saturate at higher irradiance levels. In open sun environments, *S. ruralis* exhibits 95 % saturation at PAR levels of 832–935 μmol m^{-2} s^{-1} and full saturation of *S. ruralis* (Proctor 2008) as well as *S. caninervis* (K. K. Coe unpublished) occur at PAR levels >1,000 μmol m^{-2} s^{-1}.

Adaptations to protect tissues from excess light energy that are present in dryland biocrust mosses include photoprotection as well as energy dissipation mechanisms, though there is still some debate as to which mechanism is more important in these species (see Marschall 2004). Photoprotection by xanthophyll cycle carotenoids to scavenge free radicals appears to be important in both dry and wet tissues (Hamerlynck et al. 2002; Marschall 2004). Energy dissipation via non-photochemical quenching (NPQ) has been estimated using chlorophyll fluorescence techniques, and appears to also play an important role in response to high light. When exposed to irradiances >1,000 μmol m^{-2} s^{-1}, *S. ruralis* displays NPQ levels that exceed that of more mesic species (Proctor 2000), and sun-exposed desiccation tolerant species tend to display NPQ values >2 times higher than more mesic mosses (Proctor and Smirnoff 2000; Hamerlynck et al. 2002; Proctor and Smirnoff 2011). However, NPQ levels diminish rapidly following several minutes in the dark and appear to be suppressed by violaxanthin inhibitors (Proctor and Smirnoff 2000). The current body of work (also see Chap. 7) suggests that photoprotection in biocrust mosses include strategies to dissipate excess energy as heat and regulation of xanthophyll-mediated protection appears to act as a control point that governs overall responses.

VI. Response to Elevated CO_2

For all photosynthetic organisms, the concentration of CO_2 at sites of carboxylation and, hence, responses to changes in CO_2 concentration, depends upon the atmospheric CO_2 concentration and diffusion rates to the point of carboxylation. Mosses differ from more commonly studied higher plants in that the diffusional pathway does not include stomata and is very sensitive to the saturation level of tissues in the diffusion path. However, biocrust mosses are similar to higher plants in that they are both responsive to both short-term and long-term elevated CO_2 treatments, and responses have been studied in experiments that include laboratory fumigation treatments in chambers, open-top

chambers in the field, and free air CO_2 enrichment (FACE) rings.

Biocrust mosses display CO_2 compensation points (the CO_2 concentration at which net carbon assimilation becomes positive) that fall within the typical range for C_3 plants (Dilks 1976), and short-term (<1 year) exposure to elevated CO_2 can result in a stimulation of CO_2 uptake by 30 % or more (Tuba et al. 1998) because of increased substrate for photosynthesis. Assimilation rates in *S. ruralis* were shown to increase by 30–35 % after initial exposure and remain significantly (~20 %) higher than ambient-grown mosses after growth for 5 months in a CO_2 enriched environment (700 ppm; Tuba et al. 1998), yet starch and sugar content remain unchanged compared to ambient-grown mosses after this length of time (Csintalan et al. 1997). This suggests that photosynthesis is stimulated in the short term, that photosynthetic acclimation may not occur within the first year of fumigation, and that additional assimilated carbon is immediately used for energy rather than stored.

Following longer term (>1 year) exposure to elevated CO_2, carbon content of shoots is altered in biocrust moss, and this change appears to influence physiological trade-offs among allocation to growth, sex expression, and stress tolerance. Research on *S. caninervis* collected from the Nevada FACE facility after 10 years exposure to CO_2 enrichment has shown that increased photosynthetic efficiency results in increased percent carbon per unit mass of shoots (Brinda et al. 2011; Coe et al. 2012b), but it is also appears that this does not always translate into increased shoot growth, and shoot growth in elevated CO_2 can even be significantly shorter compared to ambient-grown shoots if they express sex (Brinda et al. 2011). This may be because dryland biocrust species such as *S. caninervis* are slow growing stress tolerant species where growth is often sacrificed for other physiological processes, thus they may not respond to CO_2 enrichment with a marked increase in structural biomass, and fixed carbon may be allocated to physiological functions or energetic requirements other than growth. Further, in arid systems, larger plants may even be at a disadvantage due to higher rates of moisture loss.

It is currently unclear if biocrust mosses will acclimate photosynthetically to long-term CO_2 enrichment. Several studies have suggested that photosynthesis is downregulated after long-term exposure, and that reductions in Rubisco may occur (Long et al. 2004). However, Coe et al. (2012b) demonstrate that mosses after 10 years of CO_2 enrichment display higher photosynthetic rates at field growth concentrations and no reductions in either supply of CO_2 to carboxylation sites or rates of RuBP regeneration. The results from the Coe et al. study suggest that, because bryophytes possess neither the capacity to alter CO_2 supply via stomatal conductance nor the limitations imposed by reduced sink strength in phloem transport, photosynthetic performance remains unaltered in the long term.

Carbon allocation patterns do appear to change after long-term elevated CO_2 exposure, and may influence the tolerance of environmental stress and sex expression. Desiccation tolerance appears to be enhanced in biocrust moss grown in elevated CO_2 and likely mechanisms include: (a) increased protection of cellular components with sugars and starch while in the desiccated state; and/or (b) increased regeneration potential of protonema following a desiccation event (Brinda et al. 2011) due to allocation of stored energy reserves to repair or to cellular processes that work to mitigate damage due to desiccation.

Growth in elevated CO_2 appears to influence sex expression in biocrust species. Brinda et al. (2011) found that compared to shoots grown in ambient conditions, *S. caninervis* exposed to 10 years of CO_2 enrichment displayed accelerated sexual maturation, and sex expression in shoots was twice as likely.

The thermotolerance of photosynthesis in biocrust mosses appears to be enhanced by long-term exposure to elevated CO_2, and *Syntrichia* grown in elevated CO_2 and exposed to high (35–40 °C) temperatures exhibited increased CO_2 assimilation (Hearnshaw and Proctor 1982), increased

conversion efficiency of light energy into chemical energy in the photosynthetic light reactions, increased electron transport rates during photosynthesis, and increased availability of CO_2 at sites of carboxylation as compared to shoots grown at ambient CO_2 (Coe et al. 2012b). Photosynthetic thermotolerance can be enhanced by a number of means, and two commonly cited mechanisms are enhanced membrane stability at high temperatures and increased Rubisco activase activity under stress (Sharkey et al. 2001; Sharkey and Schrader 2006). Either or both of these possibilities could account for elevated CO_2-induced thermotolerance in desert mosses. Based on estimates of electron transport efficiency and diffusion of CO_2 through photosynthetic membranes, Coe et al. (2012b) suggest enhanced membrane stability is the most parsimonious explanation for thermotolerance in *S. caninervis* exposed to elevated CO_2 for 10 years. The role of Rubisco activase, however, in thermotolerance of biocrust mosses has received very little attention, yet could be equally important in protecting photosynthesis under high heat conditions. It is probable, based on work in other dryland plants (Sharkey et al. 2001) that increased amounts of Rubisco activase as a function of elevated CO_2 exposure could lead to less diminished photosynthetic rates under high temperatures. In spite of multiple possible mechanisms, elevated CO_2-induced photosynthetic thermotolerance is particularly important for biocrust mosses as shoots are exposed to a wide range of temperatures in dryland systems and are likely to experience increasingly heightened extremes in temperature in the future (Meehl et al. 2007).

In sum, stress tolerance is often favored at the expense of growth for dryland biocrust mosses, and elevated CO_2 appears to accentuate this trade-off. Carbon supplementation influences allocation to growth, stress tolerance, and sex expression in many species, and excess carbon from a more efficient photosynthetic process is preferentially allocated towards processes such as thermotolerance of photosynthesis and desiccation tolerance of shoots, although productivity and biomass may not change (or even decline) under CO_2 enrichment. This suggests that although growth rates may become even slower in these already slow-growing plants, biocrust mosses may be among the plants that exhibit sustained persistence in a CO_2-enriched future atmosphere in dryland systems due to enhanced performance under environmental stress.

VII. Nutrient Relations

Second only to water, nitrogen often strongly limits primary production in arid regions (Vitousek and Howarth 1991; Smith et al. 1997). Mosses (including biocrust species) rely heavily on nutrient uptake from atmospheric deposition compared to uptake from soil. Therefore, they are often buffered from belowground nutrient limitation and increased nitrogen deposition from atmospheric sources is likely to influence productivity of dryland mosses and their relative abundance in crusts. Because biocrusts influence cycling of trace elements and are important in regional and global budgets (Zaady et al. 2000), such effects may extend to influence ecosystem biogeochemistry.

Nitrogen appears to be a limiting factor for certain aspects of reproductive biology in crust mosses. Sex expression in *S. caninervis* has been shown to be stimulated following 4 years fertilization with 10 kg N ha^{-1} $year^{-1}$ and then suppressed under a higher treatment of 40 kg N ha^{-1} $year^{-1}$ (Stark et al. 2011a, b) suggesting that reproduction is nitrogen limited, but excess levels can have detrimental effects. In this same study, productivity and regeneration vigor were unaffected by the high nitrogen treatment, suggesting some growth parameters were decoupled from environmental nitrogen levels. At larger spatial scales, increased nitrogen to mosses appears to alter certain ecological dynamics due to interactions with other members of dryland communities. In general, exposure to supplemental nitrogen has a negative fertilization effect on mosses due to tissue toxicity or competitive exclusion by vascular plants (van der Wal et al. 2005), and the latter effect

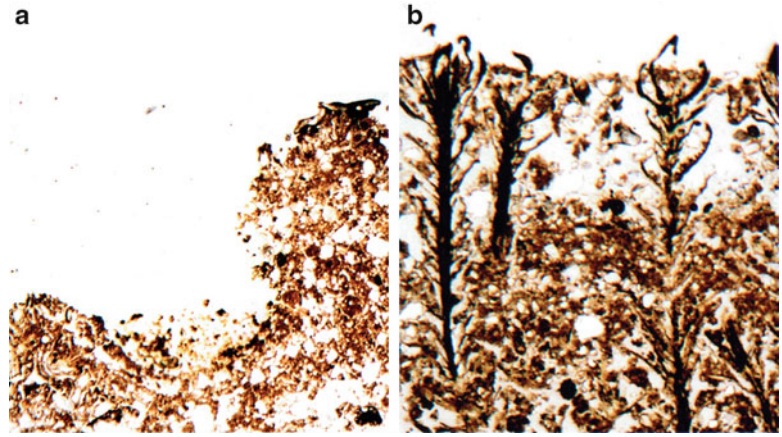


Fig. 16.4. Sections through intact biocrusts reveal the relative ability of crusts without mosses (**a**) and with mosses (**b**) to capture atmospheric dust and particulate matter, which often serve as an important nutrient source in drylands.

has been partially implicated in facilitation of Cheatgrass (*Bromus tectorum*) invasions in western North America (Schwinning et al. 2005). Finally, there may be variation in levels of cyanosymbiosis in dryland biocrust species (with the cyanobacterial genera *Microcoleus* and/or *Nostoc*) under changes in nitrogen availability. Changes in levels of such associations are likely to influence nitrogen fixation rates and soil fertility on ecosystem scales.

It is important to point out that while nitrogen availability plays a role in certain ecophysiological responses of biocrust moss, water availability overwhelms these effects under most circumstances. Experiments that have altered water availability and nitrogen levels simultaneously (e.g. Stark et al. 2011a, b, and ongoing work in the Mojave Desert) show that productivity and sex expression are influenced to a greater degree by water than nitrogen. Additionally, compared to hydrated controls, intermittent hydration periods resulting in repeated wet-dry cycles reduces nitrogen uptake potential in crust moss (Bates 1997), suggesting that water relations and desiccation tolerance at the shoot level also influence response to environmental nitrogen levels.

Though nitrogen may be the most limiting nutrient for biocrust mosses, other elements such as phosphorus, potassium, and trace metals (Mg, Na, Ca, and Mo) appear to place constraints on growth and development (Belnap et al. 2001; Bowker et al. 2005). Atmospheric dust inputs significantly increase deposition of all of these bio-essential nutrients in Western North America (Belnap et al. 2001; Reynolds et al. 2001) and consequently enrich biocrusts and underlying soils (Belnap 2003). Mosses increase the surface roughness of biocrusts, and enhance their capacity to capture dust (Belnap 2003; Fig. 16.4). Augmented dust inputs due to moss presence thus may serve to (a) enrich moss through direct uptake of deposited nutrients onto shoots, and (b) enrich crusts and soil from throughfall and enhanced nutrient turnover.

VIII. Distributions and Ecological Roles of Biocrust Moss in a Future Climate

Biocrust mosses influence crust structure and function through their effects on surface texture and nutrient cycling. Climate change and physiology are likely to interact to affect these ecological roles of mosses in dryland systems. Mosses control carbon flux into crusts and soil through CO_2 fixation, and photosynthetic rates are influenced primarily by water avail-

ability, but also by changes in CO_2 concentration and temperature. Carbon cycling in crusts will probably be influenced on shorter (1–5 year) timescales by alterations in the magnitude and timing of precipitation, and on longer (>5 year) timescales by concomitant increases in average temperatures and atmospheric CO_2. Nitrogen cycling in dryland crusts and soils is likely to be influenced by foliar uptake by moss shoots and the degree of cyanosymbiosis present, both of which will be influenced by future atmospheric deposition rates. Yet perhaps more importantly, the presence of moss influences microclimate biogeochemistry, and has been shown to control belowground nitrogen availability and cycling within crusts (Reed et al. 2012). Changes in biomass and moss percent cover due to alterations in intra-annual precipitation parameters may therefore exert more control on nitrogen cycling in aridlands compared to shoot-level physiological changes alone.

Distributions of dryland mosses are likely to be altered under future climate scenarios as well. Increases in average surface temperatures and in the frequency of extreme temperature events will place restrictions on suitable microhabitats of mosses within biocrusts and may exacerbate limited sexual reproduction due to spatial separation of the sexes. Rates of net carbon fixation and growth are likely to be suppressed under increased temperatures, limiting distributions in sun-exposed areas, yet elevated CO_2 may offset these limitations by enhancing stress tolerance. Finally, sun-exposed microhabitats that intensify water stress and reduce effective rainfall event size due to evaporation are likely to show reduced moss growth and establishment of young shoots, and may show some degree of replacement over time by habitats under shrubs where water and nutrient availabilities are higher.

IX. Conclusions

Evolutionary pressure in desert ecosystems favors stress tolerance at the expense of growth for many organisms, and many of the adaptations present in biocrust moss and ecophysiological responses to environmental alterations reflect this trade-off. Owing to their growth environment, dryland biocrust mosses are generally small, clonally growing species with a high level of desiccation tolerance. This strategy ensures that tissues can be protected from temperature and light extremes during extended dry periods, and restricts periods of physiological function, photosynthesis, and growth to intermittent periods of hydration. Changes in intra-annual precipitation patterns will likely heavily influence performance of dryland mosses because of changes in carbon balance, which when compounded over long time scales has a large influence on survival and presence within crusts. Elevated CO_2 is likely to interact with changes in temperature and rainfall to influence future performance in biocrust mosses by favoring stress tolerance and some aspects of sexual reproduction at the expense of growth.

As biocrusts are important in dryland regions for nutrient cycling and soil stability, and mosses play a key role in crust structure and function, the current body of knowledge contributing to our understanding of the physiological ecology of biocrust moss is essential in future ecosystem process modeling in dryland systems. We currently have little knowledge on cyanosymbiosis and its relation to nutrient status in dryland biocrust species, and only have limited information on the influences of CO_2 on time scales longer than 1 year, thus future work should focus on these aspects of crust moss ecophysiology.

Acknowledgements

We wish to thank Lloyd Stark for important discussions and insightful comments related to the information presented in this chapter, as well as use of photographs of *Syntrichia caninervis*. We also wish to acknowledge the members of the 2011–2012 Sparks Lab at Cornell University for helpful advice on previous versions of this chapter.

References

Alpert P, Oechel W (1987) Comparative patterns of net photosynthesis in an assemblage of mosses with contrasting microdistributions. Am J Bot 74:1787–1796

Barker D, Stark L, Zimpfer J, Mcletchie N, Smith S (2005) Evidence of drought-induced stress on biotic crust moss in the Mojave Desert. Plant Cell Environ 28(7):939–947

Bates J (1997) Effects of intermittent desiccation on nutrient economy and growth of two ecologically contrasted mosses. Ann Bot 79:299–309

Belnap J (1995) Surface disturbances: their role in accelerating desertification. Environ Monit Assess 37:39–57

Belnap J (2003) The world at your feet: desert biological soil crusts. Front Ecol Environ 1(4):181–189

Belnap J, Budel B, Lange OL (2001) Biological soil crusts: characteristics and distribution. In: Belnap J, Lange OL (eds) Biological soil crusts: structure, function and management, vol 150, Ecological studies. Springer, Berlin/Heidelberg, pp 3–30

Belnap J, Phillips S, Troxler T (2006) Soil lichen and moss cover and species richness can be highly dynamic: the effects of invasion by the annual exotic grass *Bromus tectorum*, precipitation, and temperature on biological soil crusts in SE Utah. Appl Soil Ecol 32(1):63–76

Benassi M, Stark L, Brinda J, Mcletchie D, Bonine M, Mishler B (2011) Plant size, sex expression and sexual reproduction along an elevation gradient in a desert moss. The Bryologist 114(2):277–288

Bewley JD (1979) Physiological aspects of desiccation tolerance. Annu Rev Plant Phys 30:195–238

Bowker M, Stark L, Mcletchie DN, Mishler B (2000) Sex expression, skewed sex ratios, and microhabitat distribution in the dioecious desert moss *Syntrichia caninervis* (Pottiaceae). Am J Bot 87(4):517–526

Bowker M, Belnap J, Davidson D, Phillips S (2005) Evidence for micronutrient limitation of biological soil crusts: importance to arid-lands restoration. Ecol Appl 15(6):1941–1951

Bowker M, Maestre F, Escolar C (2010) Biological crusts as a model system for examining the biodiversity-ecosystem function relationship in soils. Soil Biol Biochem 42(3):405–417

Brinda J, Stark L, Shevock J, Spence J (2007) An annotated checklist of the bryophytes of Nevada, with notes on collecting history in the state. The Bryologist 110(4):673–705

Brinda JC, Fernando C, Stark LR (2011) Ecology of bryophytes in Mojave Desert biological soil crusts: effects of elevated CO_2 on sex expression, stress tolerance, and productivity in the moss *Syntrichia caninervis* Mitt. In: Tuba Z, Slack N, Stark LR (eds) Bryophyte ecology and climate change. Cambridge University Press, Cambridge, pp 169–191

Buitink J, Hoekstra FA, Leprince O (2002) Biochemistry and biophysics of tolerance systems. In: Black M, Pritchard HW (eds) Desiccation and survival in plants: drying without dying. CAB International, Wallingford, pp 293–318

Castro C, McKee T, Pielke R Sr (2010) The relationship of the North American monsoon to tropical and North Pacific sea surface temperatures as revealed by observational analyses. J Clim 14:4449–4473

Clair L, Johansen J (1993) Introduction to the symposium on soil crust communities. West N Am Nat 53(1):1–4

Coe KK, Belnap J, Sparks JP (2012a) Precipitation-driven carbon balance controls survivorship of desert biocrust mosses. Ecology 93(7):1626–1636

Coe KK, Belnap J, Grote EE, Sparks JP (2012b) Physiological ecology of the desert moss *Syntrichia caninervis* after ten years exposure to elevated CO_2: evidence for enhanced photosynthetic thermotolerance. Physiol Plant 144(4):346–356

Csintalan Z, Takacs Z, Tuba Z, Proctor MCF, Smirnof N, Grace J (1997) Desiccation tolerance of grassland cryptograms under elevated CO_2: preliminary findings. Abstr Bot 21:309–315

Dilks TJK (1976) Measurement of the carbon dioxide compensation point and the rate of loss of $^{14}CO_2$ in the light and dark in some bryophytes. J Exp Bot 27:98–104

Dilks TJK, Proctor MCF (1974) The pattern of recovery of bryophytes after desiccation. J Bryol 8:97–115

Dilks TJK, Proctor MCF (1979) Photosynthesis, respiration and water content in bryophytes. New Phytol 82:97–114

Evans R, Belnap J (1999) Long-term consequences of disturbance on nitrogen dynamics in an arid ecosystem. Ecology 80(1):150–160

Furness S, Grime J (1982) Growth rate and temperature responses in bryophytes: II. A comparative study of species of contrasted ecology. J Ecol 70:525–536

Gaff DF (1997) Mechanisms of desiccation tolerance in resurrection vascular plants. In: Basra AS, Basra RK (eds) Mechanisms of environmental stress resistance in plants. Harwood Academic Publishers, London, pp 43–58

Grote EE, Belnap J, Housman D, Sparks JP (2010) Carbon exchange in biological soil crust communities under differential temperatures and soil water contents: implications for global change. Glob Change Biol 16(10):2763–2774

Hamerlynck E, Csintalan Z, Nagy Z, Tuba Z, Goodin D, Henebry G (2002) Ecophysiological consequences of contrasting microenvironments on the desiccation tolerant moss *Tortula ruralis*. Oecologia 131(4):498–505

Hanson D, Swanson S, Graham L, Sharkey T (1999) Evolutionary significance of isoprene emission from mosses. Am J Bot 86(5):634–639

Harper K, Pendleton R (1993) Cyanobacteria and cyanolichens: can they enhance availability of essential minerals for higher plants? West N Am Nat 53(1):59–72

Hearnshaw GF, Proctor MCF (1982) The effect of temperature on the survival of dry bryophytes. New Phytol 90:221–228

Hinshiri H, Proctor M (1971) The effect of desiccation on subsequent assimilation and respiration of the bryophytes *Anomodon viticulosus* and *Porella platyphylla*. New Phytol 70:527–538

Housman D, Powers H, Collins A, Belnap J (2006) Carbon and nitrogen fixation differ between successional stages of biological soil crusts in the Colorado Plateau and Chihuahuan Desert. J Arid Environ 66(4):620–634

Huxman T, Snyder K, Tissue D, Leffler A, Ogle K, Pockman W, Sandguist D, Potts D, Schwinning S (2004) Precipitation pulses and carbon fluxes in semiarid and arid ecosystems. Oecologia 141(2):1–15

Jobson RW, Qiu Y-L (2010) Amino acid compositional shifts during streptophyte transitions to terrestrial habitats. J Mol Evol 72(2):204–214

Kidron G, Barzilay E, Sachs E (2000) Microclimate control upon sand microbiotic crusts, western Negev Desert, Israel. Geomorphology 36(1–2):1–18

Lackner L (1939) Uber die Jahresperiodizitat in der Entwicklung der Laub-moose. Planta 29:534–616

Larcher W (2003) Physiological plant ecology: ecophysiology and stress physiology of functional groups, 4th edn. Springer, Berlin

Li Y, Wang Z, Xu T, Tu W, Liu C, Zhang Y, Yang C (2010) Reorganization of photosystem II is involved in the rapid photosynthetic recovery of desert moss *Syntrichia caninervis* upon rehydration. J Plant Physiol 167(16):1390–1397

Lioubimtseva E (2004) Climate change in arid environments: revisiting the past to understand the future. Prog Phys Geogr 28(4):502–530

Liu Y, Cao T, Glime J (2003) The changes of membrane permeability of mosses under high temperature stress. The Bryologist 106(1):53–60

Loik M, Breshears D, Lauenroth W, Belnap J (2004) A multi-scale perspective of water pulses in dryland ecosystems: climatology and ecohydrology of the western USA. Oecologia 141(2):269–281

Long S, Ainsworth E, Rogers A, Ort D (2004) Rising atmospheric carbon dioxide: plants FACE the future. Annu Rev Plant Biol 55(1):591–628

Lovelock C, Jackson A, Melick D, Seppelt R (1995) Reversible photoinhibition in Antarctic moss during freezing and thawing. Plant Physiol 109(3):955–961

Marschall M (2004) Are bryophytes shade plants? Photosynthetic light responses and proportions of chlorophyll a, chlorophyll b and total carotenoids. Ann Bot 94(4):593–603

Martin CE, Churchill SP (1982) Chlorophyll concentrations and a/b ratios in mosses collected from exposed and shaded habitats in Kansas. J Bryol 12(2):297–304

Mcletchie D (2006) Sporophyte and gametophyte generations differ in their thermotolerance response in the moss microbryum. Ann Bot 97(4):505–511

Meehl GA, Stocker TF, Collins WD, Friedlingstein P, Gaye AT, Gregory JM, Kitoh AJ, Knutti R, Murphy JM, Noda A, Raper SCB, Watterson IG, Weaver AJ, Zhao Z-C (2007) Global climate projections. In: Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL (eds) Climate change 2007: the physical science basis. Contribution of working group I to the fourth assessment report of the Intergovernmental Panel on Climate Change (IPCC). Cambridge University Press, Cambridge/New York

Meyer H, Santarius K (1998) Short-term thermal acclimation and heat tolerance of gametophytes of mosses. Oecologia 115(1):1–8

Mishler BD, Oliver MJ (1991) Gametophytic phenology of *Tortula ruralis*, a desiccation-tolerant moss, in the Organ Mountains of southern New Mexico. The Bryologist 94(2):143–153

Mishler BD, Oliver MJ (2009) Putting *Physcomitrella patens* on the tree of life: the evolution and ecology of mosses. Annu Plant Rev 36:1–15

Ochyra R, Zander R (2002) The genera *Didymodon* and *Bryoerythrophyllum* (Pottiaceae) in Antarctica. J Bryol 24(1):33–44

Oliver MJ, Bewley JD (1984) Plant desiccation and protein synthesis V. stability of Poly(A)− and Poly(B)+ RNA during desiccation and their synthesis upon rehydration in the desiccation tolerance moss *Tortula ruralis* and the intolerant moss *Cratoneuron filicinum*. Plant Physiol 74:917–922

Oliver MJ, Mishler BD, Quidenberry JE (1993) Comparative measures of desiccation-tolerance in the *Tortula ruralis* complex. I. Variation in damage control and repair. Am J Bot 80(2):127–136

Oliver MJ, Velten J, Wood AJ (2000a) Bryophytes as experimental models for the study of environmental stress tolerance: *Tortula ruralis* and desiccation tolerance in mosses. Plant Ecol 151:73–84

Oliver MJ, Tuba Z, Mishler BD (2000b) The evolution of vegetative desiccation tolerance in land plants. Plant Ecol 151:85–100

Oliver MJ, Velten J, Mishler BD (2005) Desiccation tolerance in bryophytes: a reflection of the primitive strategy for plant survival in dehydrating habitats? Integr Comp Biol 45:788–799

Paolillo DJ (1979) On the lipids of the sperm masses of three mosses. The Bryologist 82:93–96

Proctor MCF (1979) Structure and eco-physiological adaptation in bryophytes. In: Clarke GCS, Duckett JG (eds) Bryophyte systematics. Systematics Association special volume 14. Academic, London

Proctor MCF (2000) The bryophyte paradox: tolerance of desiccation, evasion of drought. Plant Ecol 151(1):41–49

Proctor MCF (2003) Experiments on the effect of different intensities of desiccation on bryophyte survival, using chlorophyll fluorescence as an index of recovery. J Bryol 25(3):201–210

Proctor MCF (2008) Physiological ecology. In: Shaw AJ, Goffinet B (eds) Bryophyte biology. Cambridge University Press, Cambridge

Proctor MCF, Pence VC (2002) Vegetative tissues: bryophytes, vascular resurrection plants and vegetative propagules. In: Black M, Pritchard HW (eds) Desiccation and survival in plants: drying without dying. CAB International, Wallingford, pp 207–239

Proctor MCF, Smirnoff N (2000) Rapid recovery of photosystems on rewetting desiccation-tolerant mosses: chlorophyll fluorescence and inhibitor experiments. J Exp Bot 51(351):1695–1704

Proctor MCF, Smirnoff N (2011) Ecophysiology of photosynthesis in bryophytes: major roles for oxygen photoreduction and non-photochemical quenching? Physiol Plant 141:130–140

Proctor MCF, Tuba Z (2002) Poikilohydry and homoihydry: antithesis or spectrum of possibilities? New Phytol 156:327–349

Proctor MCF, Nagy Z, Csintalan Z, Takács Z (1998) Water-content components in bryophytes: analysis of pressure-volume relationships. J Exp Bot 49(328):1845–1854

Proctor MCF, Oliver MJ, Wood A, Alpert P, Stark LR, Cleavitt N, Mishler B (2007) Desiccation-tolerance in bryophytes: a review. The Bryologist 110(4):595–621

Reed SC, Coe KK, Sparks JP, Housman DC, Zelikova TJ, Belnap J (2012) Changes to dryland rainfall result in rapid moss mortality and altered soil fertility. Nat Clim Change 2:752–755

Reynolds R, Belnap J, Reheis M, Lamothe P, Luiszer F (2001) Aeolian dust in Colorado Plateau soils: nutrient inputs and recent change in source. Proc Natl Acad Sci 98(13):7123–7127

Reynolds J, Kemp P, Ogle K, Fernandez R (2004) Modifying the pulse-reserve paradigm for deserts of North America: precipitation pulses, soil water, and plant responses. Oecologia 141(2):1–17

Rosentreter R, Bowker M, Belnap J (2007) A field guide to biological soil crusts of Western U.S. drylands. U.S. Government Printing Office, Denver

Sala O, Lauenroth W (1982) Small rainfall events: an ecological role in semiarid regions. Oecologia 53(3):301–304

Schonbeck MW, Bewley JD (1981a) Responses of the moss *Tortula ruralis* to desiccation treatments. I. Effects of minimum water content and rates of dehydration and rehydration. Can J Bot 59:2698–2706

Schonbeck MW, Bewley JD (1981b) Responses of the moss *Tortula ruralis* to desiccation treatments. II. Variations in desiccation tolerance. Can J Bot 59:2707–2712

Schwinning S, Starr B, Wojcik N, Miller M, Ehleringer J, Sanford R Jr (2005) Effects of nitrogen deposition on an arid grassland in the Colorado plateau cold desert. Rangel Ecol Manage 58(6):565–574

Seel WE, Hendry GAF, Lee JA (1992) The combined effects of desiccation and irradiance on mosses from xeric and hydric habitats. J Exp Bot 43(8):1023–1030

Sharkey TD, Schrader SM (2006) High temperature stress. In: Madhava Rao KV, Raghavendra AS, Reddy KJ (eds) Physiology and molecular biology of stress tolerance in plants. Springer, Dordrecht, pp 101–130

Sharkey TD, Yeh S (2001) Isoprene emission from plants. Annu Rev Plant Physiol Plant Mol Biol 52:407–436

Sharkey TD, Badger MR, von Caemmerer S, Andrews TJ (2001) Increased heat sensitivity of photosynthesis in tobacco plants with reduced Rubisco activase. Photosynth Res 67:147–156

Smirnoff N (1992) The carbohydrates of bryophytes in relation to desiccation tolerance. J Bryol 17:185–198

Smith SD, Monson RK, Anderson JE (1997) Physiological ecology of North American desert plants. Springer, Berlin/Heidelberg/New York

Stark LR (2001) Widespread sporophyte abortion following summer rains in Mojave Desert populations of *Grimmia orbicularis*. The Bryologist 104(1):115–125

Stark LR (2005) Phenology of patch hydration, patch temperature and sexual reproductive output over a four-year period in the desert moss *Crossidium crassinerve*. J Bryol 27(3):231–240

Stark LR, McLetchie D (2006) Gender-specific heat-shock tolerance of hydrated leaves in the desert moss *Syntrichia caninervis*. Physiol Plant 126(2):187–195

Stark LR, Mishler BD, Mcletchie DN (2000) The cost of realized sexual reproduction: assessing patterns of reproductive allocation and sporophyte abortion in a desert moss. Am J Bot 87(11):1599–1608

Stark LR, McLetchie D, Mishler B (2005) Sex expression, plant size, and spatial segregation of the sexes across a stress gradient in the desert moss *Syntrichia caninervis*. The Bryologist 108(2):183–193

Stark LR, McLetchie D, Eppley S (2010) Sex ratios and the shy male hypothesis in the moss *Bryum argenteum* (Bryaceae). The Bryologist 113(4):788–797

Stark LR, Brinda JC, McLetchie DN (2011a) Effects of increased summer precipitation and N deposition on Mojave Desert populations of the biological crust moss *Syntrichia caninervis*. J Arid Environ 75:457–463

Stark LR, McLetchie DN, Smith SD, Oliver MJ (2011b) Responses of a biological soil crust moss to increased monsoon precipitation and nitrogen deposition in the Mojave Desert. In: Tuba Z, Slack N, Stark LR (eds) Bryophyte ecology and climate change. Cambridge University Press, Cambridge, pp 149–169

Thompson D, Walker L, Landau F, Stark L (2005) The influence of elevation, shrub species, and biological soil crust on fertile islands in the Mojave Desert, USA. J Arid Environ 61(4):609–629

Tuba Z, Lichtenthaler HK (2011) Ecophysiology of homoiochlorophyllous and poikilochlorophyllous desiccation-tolerant plants and vegetations. In: Luttge U, Beck E, Batrels D (eds) Plant desiccation tolerance, vol 215, Ecological studies. Springer, Berlin/Heidelberg, pp 157–185

Tuba Z, Lichtenthaler H, Csintalan Z, Nagy Z, Szente K (1994) Reconstitution of chlorophylls and photosynthetic CO_2 assimilation upon rehydration of the desiccated poikilochlorophyllous plant *Xerophyta scabrida* (Pax) Th. Dur. et Schinz. Planta 192(3):414–420

Tuba Z, Csintalan Z, Proctor MCF (1996) Photosynthetic responses of a moss, *Tortula ruralis*, *ssp. ruralis*, and the lichens *Cladonia convoluta* and *C. furcata* to water deficit and short periods of desiccation, and their ecophysiological significance: a baseline study at present-day CO_2 concentration. New Phytol 133:353–361

Tuba Z, Csintalan Z, Szente K, Nagy Z, Grace J (1998) Carbon gains by desiccation-tolerant plants at elevated CO_2. Funct Ecol 12(1):39–44

Tucker E, Costerton J, Bewley J (1975) The ultrastructure of the moss *Tortula ruralis* on recovery from desiccation. Can J Bot 53(2):94–101

Valanne N (1984) Photosynthesis and photosynthetic products in mosses. In: Dyer AJ, Duckett JG (eds) The experimental biology of bryophytes. Academic, London, pp 257–273

Valko PG (2003) Monitoring biological soil crusts using hyperspectral remote sensing: determination of cyanobacteria, lichen, and moss contribution to spectral indices and observing community compositional changes due to global climate change. Senior thesis, George Washington University, St. Louis

Vitousek P, Howarth R (1991) Nitrogen limitation on land and in the sea: how can it occur? Biogeochemistry 13(2):87–115

Wal R, Pearce I, Brooker R (2005) Mosses and the struggle for light in a nitrogen-polluted world. Oecologia 142(2):159–168

Xu S, Jiang P, Wang Z, Wang Y (2009a) Crystal structures and chemical composition of leaf surface wax depositions on the desert moss *Syntrichia caninervis*. Biochem Syst Ecol 37:723–730

Xu S, Liu C, Jiang P, Cai W, Wang Y (2009b) The effects of drying following heat shock exposure of the desert moss *Syntrichia caninervis*. Sci Total Environ 407(7):2411–2419

Zaady E, Kuhn U, Wilske B, Sandoval-Soto L, Kesselmeier J (2000) Patterns of CO_2 exchange in biological soil crusts of successional age. Soil Biol Biochem 32(7):959–966

Chapter 17

Dominating the Antarctic Environment: Bryophytes in a Time of Change

Jessica Bramley-Alves, Diana H. King, and Sharon A. Robinson*
Institute for Conservation Biology and Environmental Management, University of Wollongong, Wollongong, NSW 2522, Australia

Rebecca E. Miller
Institute for Conservation Biology and Environmental Management, University of Wollongong, Wollongong, NSW 2522, Australia
School of Biological Sciences, Monash University, Clayton, VIC 3800, Australia

Summary

Polar ecosystems, and particularly Antarctica, are one of the few environs in which bryophytes dominate the flora. Their success in these regions is due to bryophytes' ability to withstand an array of harsh conditions through their poikilohydric lifestyle. However, the unique conditions that allow bryophytes to proliferate over other forms of vegetation also create considerable limitations to growth and photosynthetic activity. High latitude areas are

*Author for correspondence, e-mail: sharonr@uow.edu.au

D.T. Hanson and S.K. Rice (eds.), Photosynthesis in Bryophytes and Early Land Plants, Advances in Photosynthesis and Respiration 37, pp. 309–324, DOI 10.1007/978-94-007-6988-5_17,

already experiencing some of the most pronounced and rapid climatic change, especially in the Arctic, the Sub-Antarctic Islands and Maritime Antarctica, and these are predicted to continue over the next century. This climatic change is already impacting the flora of the polar regions both via direct and/or indirect impacts on plant species. Water availability and temperature are undoubtedly the most influential factors that determine bryophyte productivity in the Antarctic, but the ozone hole is also having an impact either directly via increased ultraviolet-B radiation and/or indirectly through the increasing wind speeds associated with ozone depletion. In a time of shifting climate the dominance of bryophytes in these regions may be threatened.

I. Introduction

A. Antarctic Climate and Flora

Bound by the thermally isolating Antarctic Circumpolar Current, Antarctica sits in a frozen state between latitudes 60°S and 90°S (Fig. 17.1). The Antarctic continent is faced with large temperature extremes and seasonal fluctuations in water availability and solar radiation. It is, by definition, the driest and windiest continent in the world, making life in this frozen desert incredibly difficult (Robinson et al. 2003). Plants that inhabit Antarctica typical deal with subzero temperatures, limited ice free areas, moisture loss, due to high winds and little or no available water during winter months (Kappen 1993). In summer they have continuous light and in winter 24 h darkness. In addition over the past 30 years, anthropogenic ozone depletion has resulted in the continent experiencing a rapid increase in tropospheric ultraviolet-B (UV-B) radiation.

As a result of these harsh conditions and the extremely short summer growing season, the Antarctic flora is dominated by a diversity of cryptogams (bryophytes, lichens and algae), comprising more than 300 species. This diversity contrasts with the two vascular plants that are restricted to the comparatively mild Antarctic Peninsula (Table 17.1; Lewis Smith 1984; Longton 1988; Bednarek-Ochyra et al. 2000; Øvstedal and Lewis Smith 2001, 2004; Ochyra et al. 2008). It is one of the few environs in which bryophytes predominate.

Abbreviations: DW – Dry weight; GRACE – Gravity Recovery and Climate Experiment; SAM – Southern Annular Mode; UV – ultraviolet; UVAC – ultraviolet absorbing compounds

B. Surviving the Freezer: Bryophytes Freeze Dry to Survive

The success of cryptogams in this region is undoubtedly due to their poikilohydric existence i.e. ability to equilibrate with the water status of their surroundings (Raven 1995; Schlensog et al. 2004). Due to morphological, biochemical and anatomical adaptations poikilohydric organisms can desiccate to a suspended metabolic state, where most of the protoplasmic water is lost and only a very small amount of tightly bound water remains in the cell. This typically occurs during times of adverse climatic conditions such as during the cold, dry Antarctic winter months (Proctor et al. 2007). In the desiccated state bryophytes can survive extremely cold conditions. In addition, their ability to freeze and thaw repeatedly is also essential during summer when temperatures are often below zero (e.g. Fig. 17.2). Surviving desiccation is made possible through the presence of compounds, including soluble carbohydrates (Smirnoff 1992) and lipids (Oliver et al. 2005), which protect membrane structure and function. When the environment becomes favorable poikilohydric organisms are able to then reactivate metabolism without major damage (Kappen and Valladares 1999).

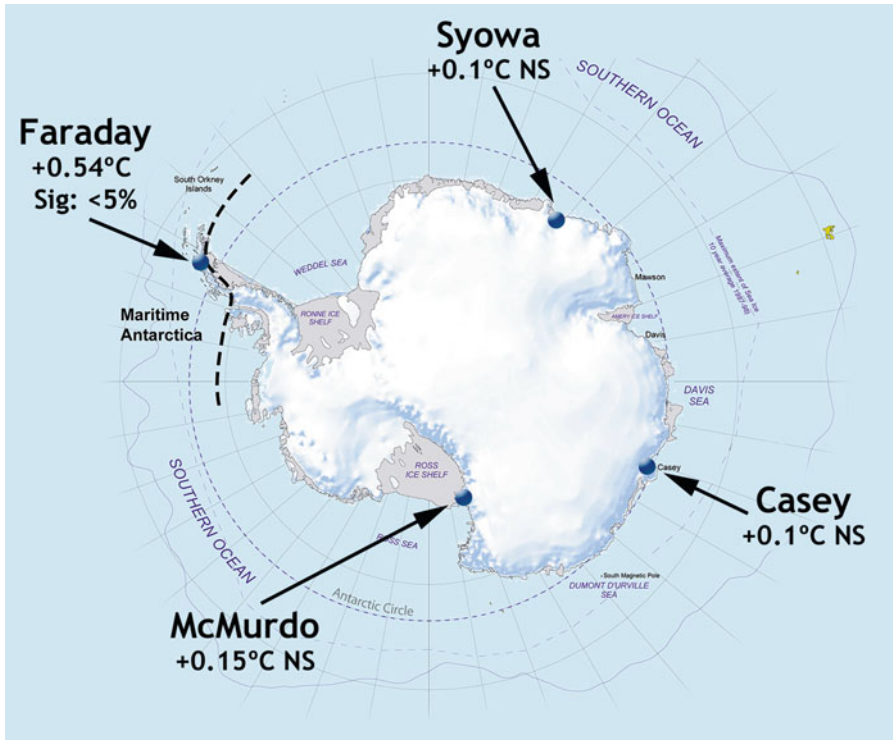


Fig. 17.1. Schematic map of Antarctica with the trends in mean annual air temperatures (degrees per decade) over the last 50 years (1951–2011 at selected research stations (Map adapted from Turner et al. (2013)). *Sig* significance value given, *NS* not significant.

Table 17.1. Estimated numbers of plant groups in Antarctica continental and maritime locations.

Region	Angiosperms	Mosses	Liverworts	Lichens
Continental	–	24	1	92
Peninsula	2	109	25	269
Total	2	111	27	393

Adapted from Lewis Smith (1984), Øvstedal and Lewis Smith (2001, 2004); Ochrya et al. (2008) and Bednarek-Ochyra et al. (2000)

This trait enables bryophytes to survive in a number of extreme habitats, ranging from the dry heat of deserts to the tops of mountains and the cold dry continent of Antarctica (Bewley 1979; Kappen 2000).

C. Climate Change in Antarctica

High latitude areas are predicted to experience some of the most pronounced climatic changes over the next century (Anisimov et al. 2001; Turner et al. 2013) with major changes already clearly apparent for the second half of the twentieth century, both on sub-Antarctic islands and across the Antarctic peninsula (Turner et al. 2013). Such changes include, rapid regional warming of the peninsula (Vaughan et al. 2003), increases in UV-B radiation (McKenzie et al. 2011) and associated increases in wind speeds (Marshall 2003; Turner et al. 2005; Hodgson et al. 2006; Perlwitz et al. 2008; Son et al. 2010; Ding et al. 2011), all of which are likely to have direct and/or indirect impacts on plant species in this area. Understanding current determinates of performance and

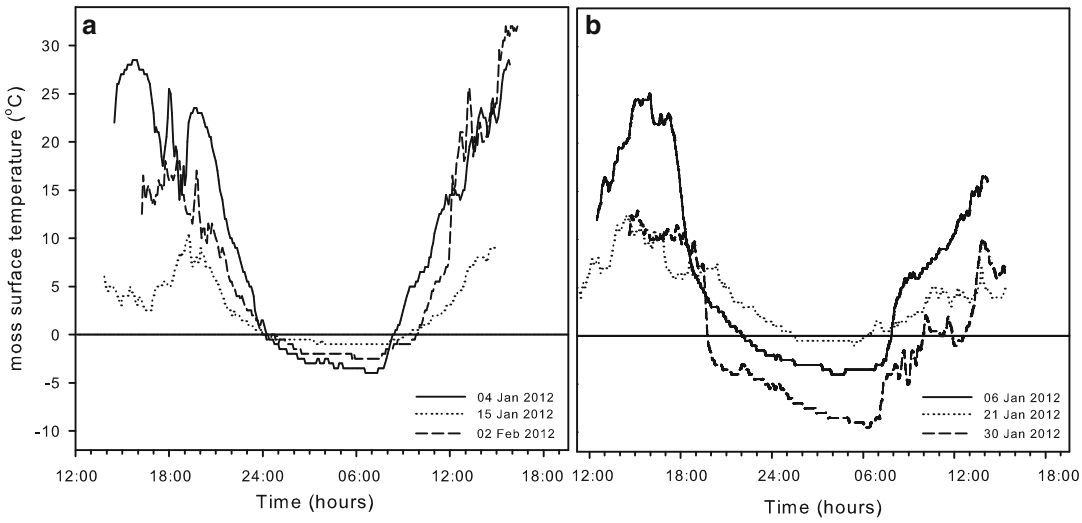


Fig. 17.2. Diurnal moss surface temperatures at two sites in the Windmill Islands, (**a**) Robinson's Ridge and (**b**) ASPA 135. At each site, temperatures were recorded at the same location over three 24 h periods, under varying weather conditions during the 2012 summer season. At Robinson's Ridge (**a**), two traces are for predominantly clear days (4–5 Jan, min/max air T: −4.0/1.7 °C and 2–3 Feb, min/max air T: −6.0/1.3 °C), and one is for an overcast day (15–16 Jan, min/max air T: −2.1/1.6 °C). At the ASPA (**b**), again the least variation in moss surface T (range 13.5 °C) was recorded on an overcast day (20–21 Jan, min/max air T: −1.6/2.8 °C), while despite different minima and maxima, moss surface temperatures ranged more widely over 21 and 29 °C, respectively, on two mostly clear days (30–31 Jan, min/max air T: −10.3/−1.1 °C and 6–7 Jan, min/max air T: −6.3/0.7 °C).

survival in Antarctic bryophytes is therefore important in order to predict how these ecosystems will respond to changes in the future.

II. Water Availability

A. Water Relations in a Frozen Desert

In climates that support extreme cold and/or dry conditions, water availability is one of the main factors determining plant growth and carbon gain (Melick and Seppelt 1994; Lenne et al. 2010). Antarctic mosses appear well adapted to high water availability and have relatively broad ranges of water content over which net photosynthesis is near maximal, for example 390–470 % for *Bryum subrotundifolium* and 245–1,400 % (g H_2O g^{-1} DW) for *Bryum pseudotriquetrum* (Pannewitz et al. 2005), 100–600 % for *Ceratodon purpureus* and 200–1,200 % (g H_2O g^{-1} DW) for *Schistidium antarctici* (Robinson et al. 2000). Therefore areas that have access to regular melt water during the summer months (November to March; Fig. 17.3a, b, e) typically support greatest moss biomass. In some regions of Antarctica bryophytes form moss pillars in fresh water, summer lakes (Fig. 17.3c; Kudoh et al. 2009). Mosses are often found in conjunction with algae species and cyanobacteria that also rely heavily on available water (Fig. 17.3b, c; Melick et al. 1997) and the surface of moss turfs will sometimes support lichen growth (Fig. 17.3f). However lichens grow more readily on rock surfaces or by creating endolithic communities within rock crevices (Fig. 17.3g). In some of the driest regions hypolithic communities dominated by mosses flourish under opaque rocks (Fig. 17.3h; Cowan et al. 2011). Lake and hypolithic habits allow bryophytes to escape the worst stresses and extremes of the Antarctic environment, and to thrive in these relatively stable, lower radiation environments which probably reduce the

Fig. 17.3. Typical examples of Antarctic bryophyte flora. (**a**, **b**) Mosses thrive in areas with free water such as these turfs in and around melt lakes in the Windmill Islands East Antarctica and (**c**) moss pillars that occur in deeper freshwater lakes such as Hotoke-ike, Sôya Coast, East Antarctica. (**d**) In more exposed locations moss buttons are a common form (seen here with a bird quill). (**f**) Where areas are drying lichen grows above moss turfs and (**g**) endolithic lichen communities are commonly found in rock crevices. (**h**) Hypolithic communities where flora exists under or within rocks are common in Antarctica (*h2 is the underside of a rock which was originally above h1*) and such communities are often dominated by mosses. Mosses can photosynthesise at low temperatures in Antarctica; (**e**) the water surface is covered with a thin layer of ice but the moss is still producing O_2 bubbles; (**i**) moss lines an icy melt stream at Robinson Ridge, Windmill Island East Antarctica (taken at 0900 local solar time) (Photographs by Sharon Robinson and Satoshi Imura).

chance of freeze-thaw damage, desiccation, photobleaching and disturbance (Cowan et al. 2011).

On the Antarctic continent all species of moss display some degree of desiccation tolerance. The ability to desiccate enables moss to survive in Antarctica, although the process of desiccation itself directly affects metabolism, and as a result photosynthetic capacity is reduced when moss water content declines below the optimum for net photosynthesis (Van Gaalen et al. 2007). Desiccation tolerance was investigated in three East Antarctic moss species (*S. antarctici*, *C. purpureus* and *B. pseudotriquetrum*) by Wasley et al. (2006b). The study showed that these species lost optimal photosynthetic efficiency (measured as the chlorophyll fluorescence parameter, F_v/F_m) when the water content of the mosses reached between 50 and 200 % (g H_2O g^{-1} DW), but that all three study species were able to survive desiccation and recover photosynthetic activity within an hour of rehydration (Wasley et al. 2006b). Within this broad desiccation tolerance, there were interspecific differences in regard

to photosynthetic functioning during desiccation, with *S. antarctici* showing the least tolerance of desiccation, *C. purpureus* the most and *B. pseudotriquetrum* intermediate tolerance (Robinson et al. 2000). This suggests that Antarctic bryophytes vary in their desiccation tolerance and shows that at least some species can acclimate to varying degrees of water availability.

Antarctic mosses appear to show plasticity in their response to desiccation depending on the moisture availability of their growth environment. This has been demonstrated for all three species of east Antarctic moss described above (Robinson et al. 2000). Similarly, Kappen and Schroeter (2002) showed that differences in the optimal water content for net photosynthesis were related to the water availability at the site of moss growth for Antarctic species. For example, xeric forms of the Antarctic moss species *Hennediella heimii* displayed optimal net photosynthesis at relative water contents of 200–300 % compared to over 500 % (g H_2O g^{-1} DW) in hydric forms. Furthermore, an earlier study by Kappen et al. (1989) found that the physiological response of the endemic species *S. antarctici* differed greatly between mesic and xeric environments, with a higher chlorophyll content, a lower light compensation point, a wider temperature range of positive net photosynthesis, and greater productivity in mesic rather than in xeric forms under similar conditions. Likewise, Davey (1997) found that regardless of other changing environmental factors (such as irradiance and temperature) there was a clear trend towards increasing photosynthetic performance in a range of Signy Island Antarctic bryophytes from xeric to mesic to hydric habitats. They concluded that water, rather than temperature, is the most important factor governing photosynthesis in this region. By contrast, an earlier study by Convey (1994), using a similar set of species from Signy Island, found no relationship between habitat wetness and productivity. It is also important to note that photosynthetic efficiency can decline at the highest tissue water contents (Robinson et al. 2000) and tolerance of complete submergence depends on the species (see Fig. 17.3f and Wasley et al. 2006b). In addition, moss at wet sites tend to freeze at higher temperatures than that at dry sites (Melick and Seppelt 1994). Desiccation prior to exposure to freezing temperatures is an important factor in the survival of Antarctic bryophytes and there is probably a trade-off between optimum water availability for photosynthesis and risk of freezing damage. If climate change produces more freeze-thaw events in summer this is likely to have negative effects on bryophyte productivity in the Antarctic (Lovelock et al. 1995a, b).

B. Climate Change and Future Water Availability

While it is not yet fully understood how climate change will affect biologically accessible water in Antarctic, rising temperatures are likely to augment melt, and therefore, have a short term positive effect on productivity, although if water becomes more available, nutrients may then become a more limiting factor (Robinson et al. 2003; Wasley et al. 2006a). Given that the summer growing season is so short it is probable that the length of availability of free water will be the critical factor. Thus a more rapid and extreme melt, accelerating run off, may potentially result in a shorter growing season. Studies of changes in the stable isotope ratio of carbon ($\delta^{13}C$) along the length of moss shoots have shown that several sites in the Windmill Islands have become drier in recent decades (Clarke et al. 2012) supporting predictions of a drier future for this region (Hodgson and Sime 2010). Long-term predictions of water availability are complex but tend to point towards increased aridity across the continent, especially in the biologically rich coastal regions (Krinner et al. 2007). The long-term effects of losing previously permanent water sources, which are already receding due to increased melt (Vaughan et al. 2003; Chen et al. 2008, 2009), are assumed to be negative. Recent satellite studies known as the Gravity Recovery and Climate Experiment (GRACE) have detected a loss in the polar ice-sheet

mass balance of up to 19,077 Gt per year, with a rapid loss of ice mass in coastal regions of East Antarctica since 2006 (Chen et al. 2009). Future precipitation, although predicted to increase in areas of Antarctica in the twenty-first century (Krinner et al. 2007), will need to be substantially higher than average in order to replenish these reserves (Robinson et al. 2003; Wasley et al. 2006a; Christensen et al. 2007). Furthermore, an increase in wind speed, due to the positive phase of the dominant weather system over Antarctica, the Southern Annular Mode (SAM), is likely to cause shifts to a negative water balance through evaporation. Evidence of this is already apparent in a number of coastal East Antarctic lakes (Hodgson et al. 2006) and moss beds (Clarke et al. 2012), as well as in soil moisture content in the McMurdo dry valleys (Doran et al. 2002). Since these increased wind speeds and associated evaporative drying are linked to ozone depletion as well as increased greenhouse gases, they are likely to continue until at least mid century (Perlwitz et al. 2008; United Nations Environment Programme 2012).

The reduction of permanent water sources, coupled with the already observed drying effect of increased wind speeds, will subject Antarctic vegetation to longer periods of desiccation. This implies that a number of species are in danger of reduced distribution, with sensitive endemic species, such as *S. antarctici*, particularly threatened (Wasley et al. 2006a). Preliminary results from a recently developed State of the Environment Indicator for continental Antarctic vegetation (Robinson et al. 2009) suggested a remarkable decline in the dominant moss species between 2003 and 2008 with a simultaneous increase in dead moss (King 2009). The trend suggests the sensitive endemic species, *S. antarctici*, is being overgrown by more desiccation resilient species, such as *C. purpureus* (Robinson et al. 2000; Wasley et al. 2006b), with recent drying in the region the major driver of this change. If these drying trends outweigh extra water inputs from increased temperature and precipitation then the resulting decreased water availability is likely to have a predominantly detrimental biological affect; however, this is clearly an area in which more research on both climate and resulting bryophyte carbon balance models is needed to predict the direction of change.

III. Temperature

A. Temperature Relations

Temperature in Antarctica is undoubtedly challenging to life. The continent is cold and strongly seasonal with yearly temperatures in coastal regions ranging from below −40 °C during winter to over 0 °C during summer months (Convey and Smith 2006). These low extremes are thought to be a primary limiting factor, both directly and through their influence on water availability to vascular plant growth in the region (Block et al. 2009). The shortness of the summer season, the few months when temperatures are close to or just above 0 °C, is a major factor in determining the flora of the continent since the cumulative number of days where temperatures are above zero and water can melt are critical for bryophyte productivity. A study by Davey and Rothery (1997) found that in Signy Island moss species, *Andreaea depressinerais*, *Chorisodontium aciphyllum* and *Brachythecium austrosalebrosum*, there were significant seasonal changes in the maximum rates of photosynthesis, associated with differences in the summer maxima. Furthermore, field measurements of net photosynthesis in East Antarctic moss species, *C. purpureus* and *B. pseudotriquetrum*, found the maximum rate of net photosynthesis to be only 4 μmol CO_2 m^{-2} s^{-1} at saturating radiation intensity and at an optimum temperature of 10 °C (Ino 1990). The severity of the winter months restricts the growth of cryptogams, which congregate to sites that maintain a relatively high level of solar radiation (Seppelt and Ashton 1978) such as on North facing sides of rocks, sheltered from the wind.

Temperature at ground surface level is, however, strongly influenced by both radiative inputs and the boundary layer effect

(Geiger 1965). Moss cushions conform to black body solar radiation (Newsham 2010) and therefore have been found to reach temperatures above 40 °C during the summer months if situated in sun-exposed but wind-sheltered sites (Lewis Smith 1988). The button, turf and hypolith habits of Antarctic bryophyte communities (see Fig. 17.3d, b, h) are all effective at reducing wind chill as is their location in sheltered valleys, small depressions and upwind from rocks or in rock crevices. For example, studies by Schenker and Block (1986) recorded soil surface temperatures between 3.7 and 10.7 °C warmer than air temperature, and a study by Lewis Smith (1995) identified an increase in ground surface level temperature of between 5 and 25 °C compared to air temperatures. A study on the Antarctic Peninsula by Schlensog and Schroeter (2000) reported the diurnal thermal cycle within a cushion of *Andreaea gainii* to range between −2 and 52 ° C during the summer months. Whilst on the continent during a sunny day in January, Lewis Smith (1988) recorded a diurnal temperature cycle of between 9.2 and 42.8 °C just a few millimeters beneath the surface of a cushion of *S. antarctici*. At this same location in the Windmill Islands, moss surface temperatures were more than 30 °C above maximum air temperature on a sunny day, and even on an overcast day, the maximum moss surface temperature was more than 9 °C above the maximum air temperature (Fig. 17.2).

In summer, night-time temperatures can drop to −20 °C potentially exposing actively growing moss to a 40–60 °C daily range (Lovelock et al. 1995a, b). Such large ranges are most likely to occur on clear, sunny days, when maximum heating of moss turfs occurs through the day, but when cooling is more rapid during the cloudless low light night. For example, the surface temperature of the same moss ranged from −2.5 to 32 °C over a 24 h period with mostly clear skies in January in the Windmill Islands, but ranged from −1.0 to 10.5 °C on an overcast day the same month (Fig. 17.2). Fig. 17.3g shows a typical example of a moss lined icy melt stream taken on a sunny morning in the Windmill Islands region of East Antarctica. Such conditions potentially expose moss to high photosynthetically active and UV radiation whilst the plants are cold, and would be expected to produce photoinhibition in less tolerant plants (see Chap. 7).

These large fluctuations between the extreme cold temperatures of winter to the warm temperatures of summer mean that Antarctic moss must possess a much greater range of temperature tolerance (~100 °C) than most equivalent species in other global biomes. Whilst the winter cold extremes will occur when the mosses are freeze dried and metabolically inactive, temperatures can rise above zero in mid winter potentially rehydrating moss and exposing it to freeze-thaw damage (Lenne et al. personal communication).

The two dangers of low temperatures for bryophyte growth and development are, firstly, a reduction in physiological activity due to cold, and secondly the more immediate danger of tissue freezing (Lovelock et al. 1995a, b; Lenne et al. 2010). For polar plants the ability to perform photosynthesis at low temperatures it is vital to compensate for the very short summer in which production is possible (Kennedy 1993) although as discussed above, it seems likely that the bulk of photosynthesis actually occurs when the bryophytes are warmed by solar radiation. Consistent with this, temperature optima for photosynthesis for the Antarctic mosses that have been measured are between 5 and 25 °C (Table 17.2). The more pressing danger is to avoid damage caused by ice formation in living tissue and to recover quickly from such damage in order to be able to respond opportunistically to the small window of production during the summer months (Lenne et al. 2010). Polar bryophytes have developed various biochemical, physiological and morphological mechanisms to limit such damage (e.g., Robinson et al. 2000; Wasley et al. 2006b; Block et al. 2009). Kappen (1993) demonstrated the ability of polar bryophytes to withstand prolonged periods of burial by snow and ice but resume normal photosynthesis within a few hours after exposure to extreme (and non physiologically

Table 17.2. Temperature optimum for photosynthesis for a range of Antarctic bryophytes measured under field and laboratory conditions.

Species	Temperature optimum for photosynthesis (°C)	Method and location of measurements (Field or laboratory)	References
Bryum subrotundifolium	13.7	Field laboratory NP	Pannewitz et al. (2005)
Bryum pseudotriquetrum	10–12.0	Field laboratory NP	Pannewitz et al. (2005), Ino (1990)
	≥20	Lab O_2 evolution	Lewis Smith (1999)
Bryum argenteum	15	Field laboratory NP	Green et al. (2000)
	≥20	Lab O_2 evolution	Lewis Smith (1999)
	25	Lab O_2 evolution	Rastorfer (1970)
Ceratodon purpureus	6.6	Field laboratory NP	Pannewitz et al. (2005)
	≥20	Lab O_2 evolution	Lewis Smith (1999)
Schistidium antarctici	5–10	Lab NP	Kappen et al. (1989)
Cephaloziella varians	≥20	Lab O_2 evolution	Newsham (2010)

Net photosynthesis (*NP*) was measured as CO_2 assimilation. Laboratory measurements were performed using an O_2 electrode system (Rastorfer 1970)

relevant cold; −196 °C) conditions. Several studies have demonstrated that continental Antarctic bryophyte species can survive repeat freeze-thaw events (Melick and Seppelt 1992; Lovelock et al. 1995a, b), but there are costs to such protection and increased frequency of such events in future could be detrimental (Lovelock et al. 1995a; Lenne et al. 2010).

B. Climate Change and Future Temperature

Discerning recent temperature trends for the Antarctic continent as a whole is challenging, and much debated. Past studies by Raper et al. (1984) claimed that Antarctica, in its entirety, had been warming significantly by 0.29 °C per decade since the 1950s, whereas later studies by Doran et al. (2002) claimed a net cooling of the continent over this same period. More recent studies, such as by Turner et al. (2002), have exposed the invalidity of such studies due to limited data and overly large extrapolations, and since this time the Reference Antarctic Data for Environmental Research (READER) project has been implemented to provide an improved data set for use in climate change studies (Turner et al. 2013).

While the bulk of East Antarctica has experienced little significant change in temperature over the last 50 years (Fig. 17.1; Turner et al. 2013), recent studies suggest that West Antarctica has warmed by over 0.1 °C per decade (Steig et al. 2009b; Ding et al. 2011). The most significant change in temperature has occurred over the Antarctic Peninsula, where the accelerated rate of warming has seen this area classified as one of the fastest warming regions on Earth (Vaughan et al. 2003). Temperatures rose on the west and northern parts of the peninsula by 0.56 °C per decade from 1951 to 2000 (Turner et al. 2013) with the greatest rates of warming during the winter months (King and Harangozo 1998). The changing Southern Annular mode (SAM) has played a key role in driving warming in this region (Marshall et al. 2006, 2011; Fogt et al. 2009), mainly through generating stronger winds that bring relatively warm maritime air masses across the peninsula (Mayewski et al. 2009). In contrast, East Antarctica has shown regional differences, with Turner et al. (2005) proposing a gradual cooling to the area as a whole since the 1980s. Although there is no clear evidence of warming from station meteorological records in the region,

recent studies of ice-sheet mass balance have shown accelerated ice loss since 2006 from the East Antarctic sheet in the vicinity of Casey Station (Chen et al. 2009). Further, infrared satellite data suggest that contrary to previous reports, East Antarctica has warmed by 0.1 °C per decade since 1957 (Steig et al. 2009a, b).

Increasing temperature and precipitation in polar regions due to climate change (Chen et al. 2009) were predicted to result in increased bryophyte growth rates through increases in water availability and length of the growing season (Robinson et al. 2003). Even though temperature patterns in this region remain unclear, a shift to either warmer or cooler conditions could have serious consequences for Antarctic vegetation. The majority of bryophyte species respond positively to warmer temperatures, suggesting that a rise in temperatures would generate more productivity and vice versa. Studies on both vascular and non-vascular Antarctic plants have shown an increase in the maximum rate of gross photosynthesis in conjunction with temperature increases within the range of 0–20 °C (Xiong et al. 1999). Lewis Smith (1999) found net photosynthesis increased with temperature (tested up to 20 °C) for a range of Antarctic bryophytes including *Bryum argenteum*, *B. pseudotriquetrum* and *C. purpureus*. Likewise, a field study on vascular Antarctic tundra by Day et al. (2008) found that warming led to greater aboveground plant biomass, as well as greater mass of the litter layer and organic soil horizon.

On the other hand, too high a rise in temperature has been demonstrated to reduce bryophyte productivity. This is also apparent in Antarctic vascular plants as demonstrated by Xiong et al. (1999) who found that net photosynthesis was depressed above 20 °C in both *Deschampsia antarctica* and *Colobanthus quitensis*, but remained high at temperatures greater than 10 °C. This was consistent with the work of Vining et al. (1997), who found a pronounced decline in net photosynthesis in the same species at temperatures greater than 12 °C, with negligible photosynthesis at 35 °C. Furthermore, low temperatures appear to be important for some species in order to achieve positive net carbon balance. For example, in the maritime moss *Sanionia uncinata* photosynthesis remains low over a temperature range of 0–20 °C but dark respiration steadily increases (Nakatsubo 2002) suggesting that increasing temperatures may reduce carbon gain through increasing respiratory losses.

IV. The Ozone Hole and Increased Ultraviolet Radiation

Stratospheric ozone depletion, resulting from anthropogenic, atmospheric pollution, has occurred since the 1980s, with an ozone hole (defined as the area with an ozone thickness of <220 DU) developing each austral spring (September–November) over Antarctica (Roy et al. 1994; NASA 2012). The largest ozone hole was recorded in September 2006 (NASA 2012) and full recovery of the ozone layer is not expected until 2050 (McKenzie et al. 2011). Depletion of stratospheric ozone, has led to increased ultraviolet (UV) radiation at the Earth's surface, as well as a spectral shift to the more biologically damaging shorter wavelengths, especially over Antarctica (Frederick and Snell 1988).

A. Protection from Ultraviolet Radiation in Antarctic Bryophytes

The ozone hole has resulted in Antarctic plant communities being exposed to a rapid change in UV-B exposure over the past four decades. A meta-analysis of the impact of this increase in UV-B suggests that Antarctic bryophytes respond to increasing UV-B radiation in a similar way to vascular plants, with increases in UV absorbing compounds (UVAC), reductions in aboveground biomass and plant height and increased accumulation of DNA damage (Searles et al. 2001; Newsham and Robinson 2009). There was little evidence of consistent impacts on photosynthesis, optimum photosynthetic efficiency (F_v/F_m) or chlorophyll pigments

from this meta-analysis, but Antarctic plants responded to increased UV-B radiation by increasing their carotenoid concentrations by 17 % whilst Arctic plants did not show this response. As detailed in Chap. 7, UV-B radiation is implicated in direct damage to PSII, and photoprotective carotenoids, such as zeaxanthin and β-carotene, can mitigate against such damage through the dissipation of excess energy as heat thus reducing the formation of reactive oxygen species (ROS) as well as scavenging any ROS that are produced.

Studies have shown impacts on photosynthetic pigmentation for particular species; for example, decreases in chlorophyll and increases in zeaxanthin and β-carotene in *Schistidium antarctici* exposed to UV-B radiation (Robinson et al. 2005). Total carotenoids also increased with increasing UV-B/PAR in both the leafy liverwort, *Cephaloziella varians* and the mosses, *Sanionia uncinata* and *Andreaea regularis* (Newsham et al. 2002, 2005) but in contrast to *S. antarctici*, chlorophyll pigments were unaffected in these species. Acclimation of shade to sun forms of *C. purpureus* and *Bryum subrotundifolium* was achieved in as little as 6 days and sun forms of the two mosses exhibited enhanced UV-A shielding as measured using a UV-PAM flurometer (Green et al. 2005).

Increasing UVAC in response to increasing UV-B radiation have been found in the liverwort, *Cephaloziella varians* and the mosses; *S. uncinata* (Newsham et al. 2002), *A. regularis* (Newsham 2003), *B. pseudotriqetrum* (Dunn and Robinson 2006) and *B. argenteum* (Ryan et al. 2009). In contrast two other Antarctic bryophyte species (*C. purpureus* and *S. antarctici*) have been shown to contain UVAC that are not particularly responsive to changes in UV-B radiation (Lovelock and Robinson 2002; Dunn and Robinson 2006). Both these mosses have since been shown to accumulate UVAC in their cell walls and it remains to be seen if these cell wall UVAC are responsive to changing UV-B radiation or produced constitutively (Clarke 2008; Chap. 7 this volume). Given that UV-B radiation dose has only been measured around Antarctica for the last 30 years, bryophytes and bryophyte spores that contain UVAC that respond to UV-B radiation could be used to determine historic levels of UV-B radiation (Lomax et al. 2008; Ryan et al. 2009).

DNA damage has been detected in several Antarctic bryophyte species under naturally varying UV radiation (Turnbull and Robinson 2009) and was induced by UV supplementation in the lab (Turnbull et al. 2009) and the field (Lud et al. 2003). Desiccated bryophytes accumulated fewer DNA photoproducts suggesting that either DNA is better stabilized in desiccated mosses or that screening is more effective (Turnbull et al. 2009). Most studies seem to suggest that DNA damage that accumulates under natural UV exposure is rapidly repaired (Lud et al. 2003).

B. Climate Change and Future Ultraviolet Radiation

As a result of the Montreal Protocol, recovery of the ozone layer to pre 1980s levels is expected by mid century and climate models suggest that by 2100 UV-B radiation over Antarctica should be lower than it was prior to ozone depletion (Newman et al. 2007; McKenzie et al. 2011). With the closing of the ozone hole in coming decades, any additional UV-B radiation effects on Antarctic bryophytes should thus disappear.

Of more concern for the future is the influence of ozone depletion and increasing greenhouse gases on the jet stream. Ozone depletion has been implicated in a southwards shift of the jet stream, bringing stronger westerly winds to the Antarctic continent (Son et al. 2010; Perlwitz 2011). These winds are responsible for both warming of the Antarctic Peninsula and evaporative drying around the coast of East Antarctica (see sections above). Currently whilst both greenhouse gases and ozone depletion contribute to this jet stream shift, ozone depletion is the major driver. As the ozone hole recovers, the extent to which these winds continue to lash the Antarctic coast will depend on the levels of greenhouse gases in the atmosphere

(Perlwitz 2011). Since temperature and water appear to have more dramatic impacts on Antarctic bryophytes than increasing UV-B radiation (Clarke et al. 2012), these indirect changes are potentially of more concern for the future.

V. Conclusions

Water availability and temperature are undoubtedly the most influential factors that determine current bryophyte productivity in the Antarctic and are likely to remain the major drivers in the future. Whilst mean temperatures are a key factor, extremes, especially those that initiate unseasonable freezing, can be particularly damaging. The extreme Antarctic climate explains the current success of poikilohydric organisms including bryophytes, but a changing climate could be threatening their dominance.

Acknowledgements

JBA and DK are in receipt of Australian Postgraduate Awards. Funding was provided by Australian Research Council DP110101714 and Antarctic Science Grants 3129 and 3042.

References

Anisimov O, Fitzharris BB, Hagen JO, Jefferies B, Marchant H, Nelson F, Prowse T, Vaughan D (2001) Polar regions (Arctic and Antarctic). In: McCarthy JJ, Canziani OF, Leary NA, Dokken DJ, White KS (eds) Climate change 2001: impacts, adaptation, and vulnerability. Contribution of Working Group II to the third assessment report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, pp 801–841

Bednarek-Ochyra H, Vána J, Ochyra R, Lewis Smith RI (2000) The liverwort flora of Antarctica. Polish Academy of Sciences, Institute of Botany, Cracow

Bewley J (1979) Physiological aspects of desiccation tolerance. Annu Rev Plant Physiol 30:195–238

Block W, Smith RIL, Kennedy AD (2009) Strategies of survival and resource exploitation in the Antarctic fellfield ecosystem. Biol Rev 84:449–484

Chen JL, Wilson CR, Tapley BD, Blankenship D, Young D (2008) Antarctic regional ice loss rates from GRACE. Earth Planet Sci Lett 266:140–148

Chen JL, Wilson CR, Blankenship D, Tapley BD (2009) Accelerated Antarctic ice loss from satellite gravity measurements. Nat Geosci 2:859–862

Christensen J, Hewitson B, Busuioc A, Chen A, Gao X, Held I, Jones R, Kolli R, Kwon W, Laprise R, Magaña Rueda V, Mearns L, Menéndez C, Räisänen J, Rinke A, Sarr A, Whetton P (eds) (2007) Regional climate projections. Climate change 2007: the physical science basis. Contribution of working group I to the fourth assessment report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge/New York

Clarke L (2008) Resilience of the Antarctic moss *Ceratodon pupureus* to the effects of elevated UV-B radiation and climate change. Doctor of Philosophy thesis, Biological Sciences, University of Wollongong, Wollongong

Clarke LJ, Robinson SA, Hua Q, Ayre DJ, Fink D (2012) Radiocarbon bomb spike reveals biological effects of Antarctic climate change. Glob Change Biol 18:301–310

Convey P (1994) Photosynthesis and dark respiration in Antarctic mosses – an initial comparative study. Polar Biol 14:65–69

Convey P, Smith RIL (2006) Responses of terrestrial Antarctic ecosystems to climate change. Plant Ecol 182:1–10

Cowan D, Pointing S, Stevens M, Cary C, Stomeo F, Tuffin M (2011) Distribution and abiotic influences on hypolithic microbial communities in an Antarctic Dry Valley. Polar Biol 34:307–311

Davey MC (1997) Effects of short-term dehydration and rehydration on photosynthesis and respiration by Antarctic bryophytes. Environ Exp Bot 37:187–198

Davey MC, Rothery P (1997) Interspecific variation in respiratory and photosynthetic parameters in Antarctic bryophytes. New Phytol 137:231–240

Day TA, Ruhland CT, Xiong FS (2008) Warming increases aboveground plant biomass and C stocks in vascular-plant-dominated Antarctic tundra. Glob Change Biol 14:1827–1843

Ding QH, Steig EJ, Battisti DS, Kuttel M (2011) Winter warming in West Antarctica caused by central tropical Pacific warming. Nat Geosci 4:398–403

Doran P, Priscu J, Lyons B, Walsh J (2002) Antarctic climate cooling and terrestrial ecosystem response. Nature 415:517–520

Dunn JL, Robinson SA (2006) Ultraviolet B screening potential is higher in two cosmopolitan moss species than in a co-occurring Antarctic endemic moss: implications of continuing ozone depletion. Glob Change Biol 12:2282–2296

Fogt RL, Perlwitz J, Monaghan AJ, Bromwich DH, Jones JM, Marshall GJ (2009) Historical SAM variability. Part II: Twentieth-century variability and trends from reconstructions, observations, and the IPCC AR4 models. J Clim 22:5346–5365

Frederick JE, Snell HE (1988) Ultraviolet radiation levels during the Antarctic spring. Science 241:438–440

Geiger R (1965) The climate near the ground. Harvard University Press, Cambridge, MA, US

Green TGA, Schroeter B, Seppelt R (2000) Effect of temperature, light and ambient UV on the photosynthesis of the moss *Bryum argenteum* hedw. In continental Antarctica. In: Davison W, Howard-Williams C, Broady P (eds) Antarctic ecosystems: models for wider ecological understanding. Canterbury University, Christchurch, pp 165–170

Green TGA, Kulle D, Pannewitz S, Sancho LG, Schroeter B (2005) UV-A protection in mosses growing in continental Antarctica. Polar Biol 28:822–827

Hodgson DA, Sime LC (2010) Palaeoclimate: Southern westerlies and CO_2. Nat Geosci 3:666–667

Hodgson D, Roberts D, McMinn A, Verleyen E, Terry B, Corbett C, Vyverman W (2006) Recent rapid salinity rise in three East Antarctic lakes. J Paleolimnol 36:385–406

Ino Y (1990) Field measurement of net photosynthesis of mosses at Langhovde, East Antarctica. Ecol Res 5:195–205

Kappen L (1993) Plant activity under snow and ice, with particular reference to lichens. Arctic 46:297–302

Kappen L (2000) Some aspects of the great success of lichens in Antarctica. Antarct Sci 12:314–324

Kappen L, Schroeter B (2002) Plants and lichens in the Antarctic, their way of life and their relevance to soil formation. In: Beyer L, Bölter M (eds) Geoecology of Antarctic ice-free coastal landscapes. Springer, Berlin, pp 327–373

Kappen L, Valladares F (1999) Opportunistic growth and desiccation tolerance: the ecological success of poikilohydrous autotrophs. In: Pugnaire F, Valladares F (eds) Handbook of functional ecology. Dekker, New York, pp 9–80

Kappen L, Lewis Smith RI, Myer M (1989) Carbon dioxide exchange of two ecodomes of *Shistidium antarctici* in continental Antarctica. Polar Biol 9:415–422

Kennedy A (1993) Photosynthetic response of the Antarctic moss *Polytrichum alpestre* Hoppe to low temperatures and freeze-thaw stress. Polar Biol 13:271–279

King D (2009) Rapid change in continental Antarctic vegetation communities. BEnvSci Honours thesis, Environmental Science, University of Wollongong, Wollongong

King J, Harangozo S (1998) Climate change in the western Antarctic Peninsula since 1945: observations and possible causes. Ann Glaciol 27:571–575

Krinner G, Magand O, Simmonds I, Genthon C, Dufresne J (2007) Simulated Antarctic precipitation and surface mass balance at the end of the twentieth and twenty-first centuries. Clim Dyn 28:215–230

Kudoh S, Tanabe Y, Matsuzaki M, Imura S (2009) In situ photochemical activity of the phytobenthic communities in two Antarctic lakes. Polar Biol 32:1617–1627

Lenne T, Bryant G, Hocart C, Huang C, Ball M (2010) Freeze avoidance: a dehydrating moss gathers no ice. Plant Cell Environ 33:1731–1741

Lewis Smith RI (1984) Terrestrial plant biology of the sub-Antarctic and the Antarctic. In: Laws RM (ed) Antarctic ecology. Academic, London

Lewis Smith RI (1988) Recording bryophyte microclimate in remote and severe environments. In: Glime JM (ed) Methods in bryology. Hattori Botanical Laboratory, Nichinan, pp 275–284

Lewis Smith RI (1995) Colonization by lichens and the development of lichen-dominated communities in the maritime Antarctic. Lichenologist 27:473–483

Lewis Smith RI (1999) Biological and environmental characteristics of three cosmopolitan mosses dominant in continental Antarctica. J Veg Sci 10:231–242

Lomax BH, Fraser WT, Sephton MA, Callaghan TV, Self S, Harfoot M, Pyle JA, Wellman CH, Beerling DJ (2008) Plant spore walls as a record of long-term changes in ultraviolet-B radiation. Nat Geosci 1:592–596

Longton RE (1988) Biology of polar bryophytes and lichens. Cambridge University Press, Melbourne

Lovelock CE, Robinson SA (2002) Surface reflectance properties of Antarctic moss and their relationship to plant species, pigment composition and photosynthetic function. Plant Cell Environ 25:1239–1250

Lovelock CE, Osmond C, Seppelt RD (1995a) Photoinhibition in the Antarctic moss *Grimmia antarctici* card when exposed to cycles of freezing and thawing. Plant Cell Environ 18:1395–1402

Lovelock CE, Jackson AE, Melick DR, Seppelt RD (1995b) Reversible photoinhibition in Antarctic moss during freezing and thawing. Plant Physiol 109:955–961

Lud D, Buma AGJ, Moerdijk TCW, Huiskes AHL (2003) DNA damage and photosynthesis in *Prasiola crispa ssp antarctica* and *Sanionia uncinata* in response to manipulated UV-B radiation. In: Antarctic biology in a global context: proceedings of the VIIIth SCAR international biology symposium, Amsterdam, The Netherlands, pp 69–73

Marshall G (2003) Trends in the southern annular mode from observations and reanalyses. J Clim 19:4134–4143

Marshall GJ, Orr A, van Lipzig NPM, King JC (2006) The impact of a changing Southern Hemisphere annular mode on Antarctic Peninsula summer temperatures. J Clim 19:5388–5404

Marshall G, Di Battista S, Naik S, Thamban M (2011) Analysis of a regional change in the sign of the SAM-temperature relationship in Antarctica. Clim Dyn 36:277–287

Mayewski PA, Meredith MP, Summerhayes CP, Turner J, Worby A, Barrett PJ, Casassa G, Bertler NAN, Bracegirdle T, Naveira Garabato AC, Bromwich D, Campbell H, Hamilton GS, Lyons WB, Maasch KA, Aoki S, Xiao C, van Ommen T (2009) State of the Antarctic and Southern Ocean climate system. Rev Geophys 47:RG1003

McKenzie RL, Aucamp P, Bais A, Björn L, Ilyas M, Madronich S (2011) Ozone depletion and climate change: impacts on UV radiation. Photochem Photobiol Sci 10:182–198

Melick DR, Seppelt R (1992) Loss of soluble carbohydrates and changes in freezing point of Antarctic bryophytes after leaching and repeated freeze-thaw cycles. Antarct Sci 4:399–404

Melick DR, Seppelt R (1994) The effect of hydration on carbohydrate levels, pigment content and freezing point of *Umbilicaria decussata* at a continental Antarctic locality. Cryptogam Bot 4:212–217

Melick DR, Hovendon M, Seppelt R (1997) Vegetation patterns in relation to climatic and endogenous changes in Wilkes Land, continental Antarctica. J Ecol 85:43–56

Nakatsubo T (2002) Predicting the impact of climatic warming on the carbon balance of the moss *Sanionia uncinata* on a maritime Antarctic island. J Plant Res 115:99–106

NASA (2012) Ozone hole watch. http://ozonewatch.gsfc.nasa.gov/. 09 Jan 2012

Newman PA, Daniel JS, Waugh DW, Nash ER (2007) A new formulation of equivalent effective stratospheric chlorine (EESC). Atmos Chem Phys 7:4537–4552

Newsham KK (2003) UV-B radiation arising from stratospheric ozone depletion influences the pigmentation of the Antarctic moss *Andreaea regularis*. Oecologia 135:327–331

Newsham KK (2010) The biology and ecology of the liverwort *Cephaloziella varians* in Antarctica. Antarct Sci 22:131–143

Newsham KK, Robinson SA (2009) Responses of plants in polar regions to UV-B exposure: a meta-analysis. Glob Change Biol 15:2574–2589

Newsham KK, Hodgson DA, Murray AWA, Peat HJ, Smith RIL (2002) Response of two Antarctic bryophytes to stratospheric ozone depletion. Glob Change Biol 8:972–983

Newsham KK, Geissler P, Nicolson M, Peat HJ, Lewis Smith RI (2005) Sequential reduction of UV-B radiation in the field alters the pigmentation of an Antarctic leafy liverwort. Environ Exp Bot 54:22–32

Ochyra R, Lewis Smith RI, Bednarek-Ochyra H (2008) The illustrated moss flora of Antarctica. Cambridge University Press, Cambridge

Oliver MJ, Velten J, Mishler B (2005) Desiccation tolerance in bryophytes: a reflection of the primitive strategy for plant survival in dehydrating habitats? Integr Comp Biol 45:788–799

Øvstedal DO, Lewis Smith RI (2001) Lichens of Antarctica and South Georgia: a guide to their identification and ecology. Cambridge University Press, Cambridge

Øvstedal DO, Lewis Smith RI (2004) Additions and corrections to the lichens of Antarctica and South Georgia. Cryptogam Mycol 25:323–331

Pannewitz S, Green TGA, Maysek K, Schlensog M, Seppelt R, Sancho LG, Turk R, Schroeter B (2005) Photosynthetic responses of three common mosses from continental Antarctica. Antarct Sci 17:341–352

Perlwitz J (2011) Atmospheric science: tug of war on the jet stream. Nat Clim Change 1:29–31

Perlwitz J, Pawson S, Fogt RL, Nielsen JE, Neff WD (2008) Impact of stratospheric ozone hole recovery on Antarctic climate. Geophys Res Lett 35:L08714

Proctor MCF, Oliver MJ, Wood AJ, Alpert P, Stark LR, Cleavitt NL, Mishler BD (2007) Desiccation-tolerance in bryophytes: a review. Bryologist 110:595–621

Raper S, Wigley T, Mayes PR, Jones PD, Salinger MJ (1984) Variations in surface air temperature. Part 3. The Antarctic 1957–82. Mon Weather Rev 112:1341–1353

Rastorfer JR (1970) Effects of light intensity and temperature on photosynthesis and respiration of two East Antarctic mosses, *Bryum argenteum* and *Bryum antarcticum*. Bryologist 73:544–556

Raven J (1995) The early evolution of land plants: aquatic ancestors and atmospheric interactions. Bot J Scotl 47:151–175

Robinson SA, Wasley J, Popp M, Lovelock CE (2000) Desiccation tolerance of three moss species from continental Antarctica. Funct Plant Biol 27:379–388

Robinson SA, Wasley J, Tobin AK (2003) Living on the edge – plants and global change in continental and maritime Antarctica. Glob Change Biol 9:1681–1717

Robinson SA, Turnbull JD, Lovelock CE (2005) Impact of changes in natural ultraviolet radiation on pigment composition, physiological and morphological characteristics of the Antarctic moss, Grimmia antarctici. Glob Change Biol 11:476–489

Robinson S, Wasley J, King D (2009) Indicator 72 – windmill Islands terrestrial vegetation dynamics. http://data.aad.gov.au/aadc/soe/display_indicator.cfm?soe_id=72 (Online database)

Roy CR, Gies HP, Tomlinson DW (1994) Effects of ozone depletion on the ultraviolet radiation environment at the Australian stations in Antarctica. Ultraviolet radiation in Antarctica: measurements and biological effects. Antarct Res Ser 62:1–15

Ryan KG, Burne A, Seppelt RD (2009) Historical ozone concentrations and flavonoid levels in herbarium specimens of the Antarctic moss *Bryum argenteum*. Glob Change Biol 15:1694–1702

Schenker R, Block W (1986) Micro-arthropod activity in three contrasting terrestrial habitats on Signy Island, maritime Antarctic. Br Antarct Surv Bull 71:31–43

Schlensog M, Schroeter B (2000) Poikilohydry in Antarctic cryptogams and its influence on photosynthetic performance in mesic and xeric habitats. In: Davison W, Howard-Williams C, Broady P (eds) Antarctic ecosystems: models for wider ecological understanding. Caxton Press, Christchurch, pp 175–182

Schlensog M, Pannewitz S, Green TGA, Schroeter B (2004) Metabolic recovery of continental Antarctic cryptogams after winter. Polar Biol 27:399–408

Searles PS, Flint SD, Caldwell MM (2001) A meta-analysis of plant field studies simulating stratospheric ozone depletion. Oecologia 127:1–10

Seppelt R, Ashton D (1978) Studies on the ecology of the vegetation at Mawson Station, Antarctica. Aust J Ecol 3:373–388

Smirnoff N (1992) The carbohydrates of bryophytes in relation to desiccation tolerance. J Bryol 17:185–191

Son SW, Gerber EP, Perlwitz J, Polvani LM, Gillett NP, Seo KH, Eyring V, Shepherd TG, Waugh D, Akiyoshi H, Austin J, Baumgaertner A, Bekki S, Braesicke P, Brühl C, Butchart N, Chipperfield MP, Cugnet D, Dameris M, Dhomse S, Frith S, Garny H, Garcia R, Hardiman SC, Jöckel P, Lamarque JF, Mancini E, Marchand M, Michou M, Nakamura T, Morgenstern O, Pitari G, Plummer DA, Pyle J, Rozanov E, Scinocca JF, Shibata K, Smale D, Teyssèdre H, Tian W, Yamashita Y (2010) Impact of stratospheric ozone on Southern Hemisphere circulation change: a multimodel assessment. J Geophys Res 115:D00M07

Steig EJ, Schneider DP, Rutherford SD, Mann ME, Comiso JC, Shindell DT (2009a) Corrigendum: warming of the Antarctic ice-sheet surface since the 1957 International Geophysical Year. Nature 460:766

Steig EJ, Schneider DP, Rutherford SD, Mann ME, Comiso JC, Shindell DT (2009b) Warming of the Antarctic ice-sheet surface since the 1957 International Geophysical Year. Nature 457:459–463

Turnbull JD, Robinson SA (2009) Accumulation of DNA damage in Antarctic mosses: correlations with ultraviolet-B radiation, temperature and turf water content vary among species. Glob Change Biol 15:319–329

Turnbull JD, Leslie SJ, Robinson SA (2009) Desiccation protects two Antarctic mosses from ultraviolet-B induced DNA damage. Funct Plant Biol 36:214–221

Turner J, King J, Lachlan-Cope T, Jones P (2002) Climate change (communication arising): recent temperature trends in the Antarctic. Nature 418:291–292

Turner J, Colwell SR, Marshall GJ, Lachlan-Cope TA, Carleton AM, Jones PD, Lagun V, Reid PA, Iagovkina S (2005) Antarctic climate change during the last 50 years. Int J Clim 25:279–294

Turner J, Adams B, Arthern R, Atkinson A, Barbante C, Bargagli R, Bergstrom D, Bertler N, Bindschadler R, Bockheim J, Boutron C, Bromwich D, Chown S, Comiso J, Convey P, Cook A, Prisco GD, Fahrbach E, Fastook J, Forcarda J, Gili J-M, Gugliemin M, Gutt J, Hellmer H, Hennion F, Heywood K, Hodgson D, Holland D, Hong S, Huiskes A, Isla E, Jacobs S, Jones A, Lenton A, Marshall G, Mayewski P, Meredith M, Metzl N, Monaghan A, Naveira-Garabato A, Newsham K, Orejas C, Peck L, Pörtner H-O, Rintoul S, Robinson S, Roscoe H, Rossi S, Scambos T, Shanklin J, Smetacek V, Speer K, Stevens M, Summerhayes C, Trathan P, Veen KVD, Vaughan D, Verde C, Webb D, Wiencke C, Woodworth P, Worby T, Worland R, Yamanouchi T (2009) The instrumental period. In: Turner J, Bindschadler R, Convey P, Prisco G, Fahrbach E, Gutt J, Hodgson D, Mayewski P, Summerhayes C (eds) Antarctic climate change and the environment. Scientific Committee on Antarctic Research, Cambridge, pp 183–298

Turner J, Barrand NE, Bracegirdle TJ, Convey P, Hodgson DA, Jarvis M, Jenkins A, Marshall G, Meredith MP, Roscoe H, Shanklin J, French J, Goosse H, Guglielmin M, Gutt J, Jacobs S, Kennicutt MIC, Masson-Delmotte V, Mayewski P, Navarro F, Robinson S, Scambos T, Sparrow M, Summerhayes C, Speer K, Klepikov A (2013) Antarctic climate change and the environment: an update. Polar Record FirstView:1–23

United Nations Environment Programme EEAP (2012) Environmental effects of ozone depletion and its interactions with climate change: progress report, 2011. Photochem Photobiol Sci 11:113–127

Van Gaalen E, Flanagan L, Peddle DR (2007) Photosynthesis, chlorophyll fluorescence and spectral reflectance in *Sphagnum* moss at varying water contents. Oecologia 153:19–28

Vaughan DG, Marshall GJ, Connolley WM, Parkinson C, Mulvaney R, Hodgson DA, King JC, Pudsey CJ, Turner J (2003) Recent rapid regional climate warming on the Antarctic Peninsula. Clim Change 60:243–274

Vining E, Crafts-Brandner S, Day TA (1997) Photosynthetic acclimation of Antarctic hair grass (*Deschampsia antarctica*) to contrasting temperature regimes. Bull Ecol Soc Am 78:327

Wasley J, Robinson SA, Lovelock CE, Popp M (2006a) Climate change manipulations show Antarctic flora is more strongly affected by elevated nutrients than water. Glob Change Biol 12:1800–1812

Wasley J, Robinson S, Lovelock C, Popp M (2006b) Some like it wet – an endemic Antarctic bryophyte likely to be threatened under climate change induced drying. Funct Plant Biol 33:443–455

Xiong F, Ruhland C, Day TA (1999) Photosynthetic temperature response of the Antarctic vascular plants *Colobanthus quitensis* and *Deschampsia antarctica*. Physiol Plant 106:276–286

Chapter 18

Opportunities in Bryophyte Photosynthesis Research

Steven K. Rice*
Department of Biological Sciences, Union College, Schenectady, NY 12308, USA

David T. Hanson
Department of Biology, University of New Mexico, Albuquerque, NM 87131, USA

Summary

The synthesis of important concepts in bryophyte and early land plant photosynthesis provided by the authors in this volume compiles a foundation of knowledge for new and experienced scientists interested in the field. In this prospective chapter, we highlight some areas where additional research is needed.

*Author for correspondence, e-mail: rices@union.edu

D.T. Hanson and S.K. Rice (eds.), Photosynthesis in Bryophytes and Early Land Plants, Advances in Photosynthesis and Respiration 37, pp. 325–332, DOI 10.1007/978-94-007-6988-5_18,

I. Introduction

The functional syndrome of contemporary bryophytes and their early land plant ancestors, which includes small stature, poikilohydry, external water conduction and the reliance on external boundary layers to resist drying, presented opportunities for bryophytes and their ancestors to expand into novel habitats during the early terrestrialization of land and to continue to exploit early successional habitats like bare rock and sterile mineral soil as well as those of high environmental stress. These traits evolved into a successful strategy to achieve positive carbon balance over wet-dry cycles and also led to ecological success in more favorable conditions in microsites not occupied by rooted, larger plants like on bark, in disturbed or saturated soils and in intermittent streams. As described in the chapters in this book, these characteristics place constraints on the photosynthetic and respiratory physiology of bryophytes. High external resistance to CO_2 uptake, the respiratory demands of desiccation tolerance, dense canopies that attenuate light quickly and photoprotective mechanisms to avoid or tolerate high light are among the physiological characteristics common among bryophytes.

As evidenced in the chapters of this volume, there is uneven knowledge about these processes, their significance to individual bryophyte species and their contributions to the water and carbon dynamics of whole ecosystems. We conclude this book by highlighting some areas that hold promise for future research and by directing the reader to resources that may be of use for those transitioning to bryophytes as study organisms and as well as for researchers already familiar with the area.

Abbreviations: IWCB – integrated water-driven carbon budget

II. Opportunities in Bryophyte Photosynthesis Research

A. Link Photosynthesis and Production

Given the poikilohydric strategy of bryophytes, production in the field is often affected by plant water status. In part, this recognition led to the integrated water-driven carbon budget (IWCB) model proposed by Mishler and Oliver (2009) and implemented by Coe and associates (Coe et al. 2012; Chap. 16). Although photosynthetic characteristics affect the carbon gains achieved when a plant is hydrated, the model emphasizes the importance of respiration to whole plant carbon balance, especially during respiratory bursts associated with dry to wet and wet to dry transitions. Indeed, one outcome of the IWCB model is the recognition that understanding of photosynthesis alone is insufficient to predict production in the field. This will require a better understanding of the physiological responses associated with desiccation cycles and how environmental variables and their interaction with tissue performance and acclimation affect those responses. We believe that applying this concept more broadly with more species across a greater range of environments will lead to better predictive models that link short-term measurements of photosynthesis with long-term estimates of production.

The IWCB model also applies to wetland species like *Sphagnum*, which experience fluctuations in water content (Chap. 13) that affect production in the field (Granath et al. 2009). However, understanding short-term water and carbon dynamics alone may be inadequate to predict differences in production. Granath et al. (2012) grew three *Sphagnum* species under different N treatments, but under constant moisture conditions in a greenhouse. Although production and maximum net photosynthesis both ranged approximately by a factor of three, there was no association between the two. Differences in respiration were discounted, as there were no differences observed in dark respiration. The authors

suggest that P limitation under high N loads may not affect photosynthetic carbon gain yet cause a shift in allocation to growth. Understanding the importance and distribution of such mechanisms will be important to help link short-term to long-term physiological processes.

B. Apply Modern Genomics Tools

The genomic era is bringing extremely powerful tools for use by biologists interested in bryophyte photosynthesis and respiration, potentially with greater impact than any other group of plants. One reason for such promise is that the wealth of characters in genomic data can resolve the deep phylogenetic branching patterns of these ancient land plant lineages (Kugita et al. 2003; Sugiura 2003; Rensing et al. 2008; Li et al. 2009; Forrest 2011). A second is the ability to examine whole gene families and gene networks for understanding complex traits that have evolved over nearly half a billion years (Alboresi et al. 2008, 2010, 2011; Danielson and Johanson 2008; Pitsch et al. 2010; Polyakov et al. 2010; Gerotto et al. 2011). The opportunities for studying the evolution of photosynthesis and respiration through the use of entire genome comparisons is still in its infancy, especially when examining whole genome expression in response to environmental stimuli. However, the most promising and unique feature among plants that bryophytes bring to the table is their ease of genetic manipulation (Chap. 11). Targeted gene replacement, knocking out whole gene families or individual members of a gene family (Hofmann et al. 1999) to understand gene networks could uncover fundamental ways that regulation of plant metabolism has evolved. The potential is hard to overstate and the field is wide open.

C. Understand Mixotrophy: Mechanisms and Ecological Significance

Although the available evidence favors CO_2 derived from the atmosphere or from soil or peat respiration as a primary plant carbon source in bryophytes (Chaps. 6 and 13), bryophytes and some algal ancestors have been shown to use externally derived organic carbon (Chap. 2). Such mixotrophic effects may benefit bryophytes that experience reduced CO_2 availability due to the presences of external water films. In *Sphagnum compactum*, Graham et al. (2010) found that dissolved glucose, fructose and sucrose applied in less than 2 % solutions stimulated growth by more than 20 times. Indeed, *Sphagnum* and other bryophytes also take up more complex organic compounds like amino acids, which would benefit their N as well as C economy (Krab et al. 2008). Understanding the significance of exogenous carbon utilization in natural habitats and in the terrestrialization of land will require combining genomic, biochemical, cellular, physiological and ecological approaches. Given the observed stimulation of growth from simple sugars, this area represents an exciting target for further research.

D. Symbioses and Interactions with Other Organisms

Modern bryophytes are known to form symbioses with both fungi (Wickett and Goffinet 2008; Davey et al. 2009; Pressel et al. 2010; U'ren et al. 2010) and cyanobacteria (Meeks and Elhai 2002; Villarreal and Renzaglia 2006; Adams and Duggan 2008), though the line between mixotrophy within a community and symbiosis is easily blurred. Regardless of the exact type of interaction, the movement of carbon and nutrients between mosses and their surrounding community is clear and an exciting area of research (De Deyn et al. 2011). These processes may have been key to the success of early land plants and their subsequent radiation by providing important resources such as nitrogen and phosphorous to support photosynthesis and growth. A clearer picture of these complex interactions will improve our understanding of how plants have used carbon derived from photosynthesis to conquer terrestrial environments.

E. Scale Across Levels of Organization

Given their small size, studies of function in bryophytes normally focus on shoots and shoot systems, which are convenient for gas exchange and fluorescence measurements (Chap. 5). In ecosystems where bryophytes contribute significantly to whole system function, it will be essential to develop methods to evaluate bryophyte performance at larger, more ecologically relevant spatial scales. Eddy covariance techniques allow for measurements of mass and energy fluxes from ecosystems and when combined with finer-scale physiological studies, such methods show promise for evaluating the contribution of bryophytes and their response to present and future environmental conditions (Chap. 14).

Imaging-based methods that evaluate physiological performance using reflected visible and invisible radiation also show promise to transition from small-scale to large-scale understanding in bryophytes. Graham et al. (2006) demonstrated that variation in reflected light from *Syntrichia princeps*, which can be monitored using a camera-based system, was sufficient to determine the state of hydration and generate estimates of CO_2 exchange rate. In *Sphagnum*, more detailed spectral analysis of reflected light shows relationships with water content, xanthophyll cycling pigments and photosynthetic performance across species and water contents in laboratory settings, and shows promise for up-scaling to airborne and satellite-based platforms (Harris 2008; Harris and Bryant 2009; Neta et al. 2011). For ecosystems with 1–1,000 m^2 habitat patches, a scale appropriate for many bryophyte applications, unmanned aerial vehicles have been developed for use with various optical sensors. For moss beds in Antarctica, such a system has been deployed and used to create fine-resolution elevation maps (Lucieer et al. 2011; Turner et al. 2012) that may be combined with functional imaging (S Robinson, personal communication) to develop fine-grained mapping of environment—performance relationships, which can be monitored regularly.

F. Explore Canopy Dynamics

Although the bryophyte canopy is often studied as a functional unit, variation in developmental and physiological processes within and integration among elements within bryophyte canopies remains largely unknown (Chap. 5). Canopy models that apply to crop plants or deciduous vascular plant species may not apply to bryophytes (Rice et al. 2011) and focus on the dynamics of tissue senescence, physiological acclimation and within-canopy environmental gradients as they apply to bryophytes will be important (Chap. 9). In addition, simulations of bryophyte canopy water and carbon dynamics suggests that the degree of lateral or vertical integration in capillary water among canopy elements may strongly affect canopy carbon balance. Modeling by Rice (2012) indicated that increased lateral transfer of water among shoot systems within a canopy led to elevated rates of water loss and reduced net carbon gain in rough canopies. This result contrasts with that found in vascular plants, where physiological integration within colonies often benefits overall growth. More research into the mechanisms and consequences of resource sharing within bryophyte canopies should help further our understanding of the costs and benefits of physiological integration.

G. Focus on Respiratory Processes

Bryophytes express high rates of respiration relative to photosynthesis and have relatively few cells that are not photosynthetic when compared to other land plants. Therefore, respiration in the light may be regulated more like algae than in other land plants. In addition, the response of respiration relative to photosynthesis during drying and rehydration plays a large role in the over-all carbon balance of desiccation tolerant bryophytes. However, respiration in illuminated photosynthetic

tissues is poorly understood in all organisms due to the inherent difficulty associated with measuring gross rather than net CO_2 fluxes. Modern isotopic methods that can non-destructively separate photosynthesis from respiration (similar to measurements of $^{13}CO_2$ flux described in Chap. 6) should be able to address this question. Even respiration in darkened tissues needs better characterization, especially during stress as respiration and photosynthesis have different stress tolerances. Lastly, due to their small stature and C_3 photosynthetic physiology, bryophytes are especially influenced by the respiration rates of their substrates. The respired CO_2 from soils and decaying organic matter can substantially increase the CO_2 partial pressure available for uptake by photosynthetic cells (Chap. 4), yet the gross and isotopic effects of this remain poorly characterized. Without a full understanding and good models for the respiratory CO_2 supply from substrates it is hard to adequately describe the environments where many bryophytes thrive and therefore may lead to erroneous assertions about adaptive radiation in the past and in response to future climate change.

H. Characterize Range of Functional Diversity

Contemporary bryophytes represent over 16,000 lineages and studies of photosynthetic performance have been restricted to a very limited number. Just as targeted studies are sorting out the range and variation of C_3, C_4 and C_3-C_4 intermediates in vascular plants, similarly relevant functional variation of a different type may also exist in bryophytes. Often, studies focus on common species with ecological relevance or on species that tolerate environmental extremes and mechanistic studies across a wider range of species are likely to provide yet undiscovered variants that may inform our understanding of the performance of extant bryophytes as well as provide insight into early land plant evolution. Genomic or phylogenetic methods may be employed to help guide species choice to find possible variants.

III. Bryophyte Biology and Related Resources

The following is a listing of a few books and websites that the editors have found useful during their professional development. The list is by no means comprehensive, but the items listed here should quickly guide individuals to a wide spectrum of valuable bryological resources.

A. Books

Black M, Pritchard HW (eds) (2002) Desiccation and survival in plants: drying without dying. CABI Publishing, New York

Glime JM (2007) Bryophyte ecology. Volume 1. Physiological ecology. E-book sponsored by Michigan Technological University and the International Association of Bryologists. Accessed 6 Nov 2012 http://www.bryoecol.mtu.edu/

Hemsley AR, Poole I (eds) (2004) The evolution of plant physiology: from whole plants to ecosystems. Linnean Society symposium series number 21. Elsevier Academic Press, London

Malcom B, Malcom N (2006) Mosses and other bryophytes: an illustrated glossary. Micro-Optics Press, Nelson

Rydin H, Jeglum J (2006) The biology of peatlands. Oxford University Press, Oxford

Shaw AJ, Goffinet B (eds) (2000) Bryophyte biology. Cambridge University Press, Cambridge

Tuba Z, Slack NG, Stark L (eds) (2011) Bryophyte ecology and climate change. Cambridge University Press, Cambridge

Vanderpoorten A, Goffinet B (2009) Introduction to bryophytes. Cambridge University Press, Cambridge

Wood AJ, Oliver MJ, Cove DJ (eds) (2004) New frontiers in bryology: physiology, molecular biology and functional genomics. Springer, Dordrecht

B. Websites

Society Websites: Three great places to start exploring bryophyte biology are the websites

of the three major bryological societies (in alphabetical order): The American Bryological and Lichenlogical Society http://www.abls.org/, the British Bryologcial Society http://www.britishbryologicalsociety.org.uk/, and the International Association of Bryologists http://iab-bryologists-website.blogspot.com/. Each of these sites has extensive and regularly updated lists of bryophyte resources.

Bryophyte Ecology: Also listed under books above, Professor Janice Glime has published and maintains an E-book sponsored by Michigan Technological University and the International Association of Bryologists (http://www.bryoecol.mtu.edu/). It is very accessible and a great place to start looking for primary literature on a wide range of topics.

Photographs: A few books (such as the book by Bill and Nancy Malcom above) and guides have a selection of bryophyte photographs. However, it is often very convenient to obtain bryophyte images by purchase CDs of digital images, for example those by Michael Lüth www.milueth.de/Moose

A Perspective Oriented Guide for the Identification of North American Bryophyte Genera:

Malcolm Sargent (http://www.life.illinois.edu/plantbio/People/Faculty/Sargent.htm), in collaboration with Diane Lucas, has produced a useful online guide for the identification of bryophyte genera in North America. See: http://www.life.illinois.edu/moss-guide/. We recommend this site to the readers of this book since it is user friendly and highly useful to researchers on Bryophytes. The guide is simple; it uses first the characters visible to the eye, followed by those seen by the hand lens, by the dissecting microscope, and lastly by the compound microscope. We note, in particular, that this site is also a good teaching tool.

IV. Conclusions

Pursuit of the questions posed in this chapter will require contributions from investigators who focus mainly on bryophyte function as well as from those whose primary interest is in other plant groups. Our present understanding of bryophyte photosynthesis results from such combined efforts and we expect this to continue. Above, we list general resources that may be helpful for those interested in bryophyte photosynthesis, understanding that more specific references can be found within the chapters of this book. We hope that these will be useful for those transitioning to the study of bryophytes and for students and practitioners of bryophyte functional biology alike.

References

Adams DG, Duggan PS (2008) Cyanobacteria-bryophyte symbioses. J Exp Bot 59:1047–1058

Alboresi A, Caffarri S, Nogue F, Bassi R, Morosinotto T (2008) In silico and biochemical analysis of Physcomitrella patens photosynthetic antenna: identification of subunits which evolved upon land adaptation. PLoS One 3:e2033

Alboresi A, Gerotto C, Giacometti GM, Bassi R, Morosinotto T (2010) *Physcomitrella patens* mutants affected on heat dissipation clarify the evolution of photoprotection mechanisms upon land colonization. Proc Natl Acad Sci USA 107:11128–11133

Alboresi A, Gerotto C, Cazzaniga S, Bassi R, Morosinotto T (2011) A red shifted antenna protein associated to photosystem II in *Physcomitrella patens*. J Biol Chem 286:28978–28987

Coe KK, Belnap J, Sparks JP (2012) Precipitation-driven carbon balance controls survivorship of desert biocrust mosses. Ecology 93:1626–1636

Danielson JAH, Johanson U (2008) Unexpected complexity of the aquaporin gene family in the moss *Physcomitrella patens*. BMC Plant Biol 8:45

Davey ML, Nybakken L, Kauserud H, Ohlson M (2009) Fungal biomass associated with the phyllosphere of bryophytes and vascular plants. Mycol Res 113:1254–1260

De Deyn GB, Quirk H, Oakley S, Ostle N, Bardgett RD (2011) Rapid transfer of photosynthetic carbon through the plant-soil system in differently managed species-rich grasslands. Biogeosciences 8:1131–1139

Forrest LL (2011) Deep sequencing of *Ptilidium* (Ptilidiaceae) suggests evolutionary stasis in liverwort plastid genome structure. Plant Ecol Evol 144:29–43

Gerotto C, Alborese A, Giacometti GM, Bassi R, Morosinotto T (2011) Role of PSBS and LHCSR in

Physcomitrella patens acclimation to high light and low temperature. Plant Cell Environ 34:922–932

Graham EA, Hamilton MP, Mishler BD, Rundel PW, Hansen MH (2006) Use of a networked digital camera to estimate net CO_2 uptake of a desiccation-tolerant moss. Int J Plant Sci 167:751–758

Graham LE, Kim E, Arancibia-Avila P, Graham JM, Wilcox LW (2010) Evolutionary and ecophysiological significance of sugar utilization by the peatmoss *Sphagnum compactum* (Sphagnaceae) and the common charophycean associates *Cylindrocystis brebissonii* and *Mougeotia* sp. (Zygnemataceae). Am J Bot 97:1485–1491

Granath G, Strengbom J, Breeuwer A, Heijmans MMPD, Berendse F, Rydin H (2009) Photosynthetic performance in *Sphagnum* transplanted along a latitudinal nitrogen deposition gradient. Oecologia 159:705–715

Granath G, Strengbom J, Rydin H (2012) Direct physiological effects of nitrogen on *Sphagnum*: a greenhouse experiment. Funct Ecol 26:353–364

Harris A (2008) Spectral reflectance and photosynthetic properties of *Sphagnum* mosses exposed to progressive drought. Ecohydrology 1:35–42

Harris A, Bryant RG (2009) A multi-scale remote sensing approach for monitoring northern peatland hydrology: present possibilities and future challenges. J Environ Manage 90:2178–2188

Hofmann AH, Codón AC, Ivascu C, Russo VE, Knight C, Cove D, Schaefer DG, Chakhparonian M, Zrÿd JP (1999) A specific member of the Cab multigene family can be efficiently targeted and disrupted in the moss *Physcomitrella patens*. Mol Gen Genet 261:92–99

Krab EJ, Cornelissen JHC, Lang SI, van Logtestijn RSP (2008) Amino acid uptake among wide-ranging moss species may contribute to their strong position in higher-latitude ecosystems. Plant Soil 304:199–208

Kugita M, Kaneko A, Yamamoto Y, Takeya Y, Matsumoto T, Yoshinaga K (2003) The complete nucleotide sequence of the hornwort (*Anthoceros formosae*) chloroplast genome: insight into the earliest land plants. Nucleic Acids Res 31:716–721

Li L, Wang B, Liu Y, Qiu Y-L (2009) The complete mitochondrial genome sequence of the hornwort *Megaceros aenigmaticus* shows a mixed mode of conservative yet dynamic evolution in early land plant mitochondrial genomes. J Mol Evol 68:665–678

Lucieer A, Robinson S, Turner D (2011) Unmanned aerial vehicle (UAV) remote sensing for hyperspatial terrain mapping of Antarctic moss beds based on structure from motion (SfM) point clouds. In: Proceedings of the 34th international symposium on remote sensing of environment, Sydney, Australia

Meeks JC, Elhai J (2002) Regulation of cellular differentiation in filamentous cyanobacteria in free-living and plant-associated symbiotic growth states. Microbiol Mol Biol Rev 66:94–121

Mishler BD, Oliver MJ (2009) Putting *Physcomitrella patens* on the tree of life: the evolution and ecology of mosses. In: Knight C, Perroud P-F, Cove D (eds) The Moss *Physcomitrella*, vol 36, Annual plant reviews. Wiley-Blackwell, West Sussex, pp 1–15

Neta T, Cheng Q, Bello RL, Hu B (2011) Development of new spectral reflectance indices for the detection of lichens and mosses moisture content in the Hudson Bay Lowlands, Canada. Hydrol Processes 25:933–944

Pitsch NT, Witsch B, Baier M (2010) Comparison of the chloroplast peroxidase system in the chlorophyte *Chlamydomonas reinhardtii*, the bryophyte *Physcomitrella patens*, the lycophyte *Selaginella moellendorffii* and the seed plant *Arabidopsis thaliana*. BMC Plant Biol 10:133. doi:10.1186/1471-2229-10-133

Polyakov NB, Slizhikova DK, Izmalkova MY, Cherepanova NI, Kazakov VS, Rogova MA, Zhukova NA, Alexeev DG, Bazaleev NA, Skripnikov AY, Govorun VM (2010) Proteome Analysis of chloroplasts from the moss *Physcomitrella patens* (Hedw.) BSG. Biochemistry (Mosc) 75:1470–1483

Pressel S, Bidartondo MI, Ligrone R, Duckett JG (2010) Fungal symbioses in bryophytes: new insights in the Twenty First Century. Phytotaxa 9:238–253

Rensing SA, Lang D, Zimmer AD, Terry A, Salamov A, Shapiro H, Nishiyama T, Perroud PF, Lindquist EA, Kamisugi Y, Tanahashi T, Sakakibara K, Fujita T, Oishi K, Shin-I T, Kuroki Y, Toyoda A, Suzuki Y, Hashimoto SI, Yamaguchi K, Sugano S, Koharo Y, Fujiyama A, Anterola A, Aoki S, Ashont N, Barbazuk WB, Barker E, Bennetzen JL, Blankenship R, Cho SH, Dutcher SK, Estelle M, Fawcett JA, Gundlach H, Hanada K, Heyl A, Hicks KA, Hughes J, Lohr M, Mayer K, Melkozernov A, Murata T, Nelson DR, Pils B, Prigge M, Reiss B, Renner T, Rombauts S, Rushton PJ, Sanderfoot A, Schween G, Shiu SH, Stueber K, Theodoulou FL, Tu H, Van de Peer Y, Verrier PJ, Waters E, Wood A, Yang L, Cove D, Cuming AC, Hasebe M, Lucas S, Mishler BD, Reski R, Grigoriev IV, Quatrano RS, Boore JL (2008) The *Physcomitrella* genome reveals evolutionary insights into the conquest of land by plants. Science 319:64–69

Rice SK (2012) The cost of capillary integration for bryophyte canopy water and carbon dynamics. Lindbergia 35:53–62

Rice SK, Neal N, Mango J, Black K (2011) Relationships among shoot tissue, canopy and photosynthetic characteristics in the feathermoss *Pleurozium schreberi*. Bryologist 114:367–378

Sugiura C (2003) Complete chloroplast DNA sequence of the moss *Physcomitrella patens*: evidence for the loss and relocation of rpoA from the chloroplast to the nucleus. Nucleic Acids Res 31:5324–5331

Turner D, Lucieer A, Watson C (2012) An automated technique for generating georectified mosaics from ultra-high resolution unmanned aerial vehicle (UAV) imagery, based on structure from motion (SfM) point clouds. Remote Sens 4:1392–1410

U'ren JM, Lutzoni F, Miadlikowska J, Arnold AE (2010) Community analysis reveals close affinities between endophytic and endolichenic fungi in mosses and lichens. Microb Ecol 60:340–353

Villarreal JC, Renzaglia KS (2006) Structure and development of *Nostoc* strands in *Leiosporoceros dussii* (Anthocerotophyta): a novel symbiosis in land plants. Am J Bot 93:693–705

Wickett NJ, Goffinet B (2008) Origin and relationships of the myco-heterotrophic liverwort Cryptothallus mirabilis Malmb. (Metzgeriales, Marchantiophyta). Bot J Linn Soc 156:1–12

Subject Index

D.T. Hanson and S.K. Rice (eds.), Photosynthesis in Bryophytes and Early Land Plants, Advances in Photosynthesis and Respiration 37, pp. 333–342, DOI 10.1007/978-94-007-6988-5,