PHYSIOLOGICAL ECOLOGY OF BIOCRUST MOSSES UNDER GLOBAL CHANGE:
IMPLICATIONS FOR DRYLAND BIOGEOCHEMISTRY

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by
Kirsten Kryder Coe
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Increased atmospheric CO$_2$ is changing the energy balance of the Earth and altering global precipitation. Drylands cover >40% of the terrestrial surface and may be among the most responsive ecosystems to these changes. My dissertation addresses the influences of global change on biocrust mosses, a group of plants instrumental to biogeochemistry in drylands.

First, I provide an overview of the physiological ecology of biocrust mosses. These plants are adapted to pulse-dynamic water and resource availability in drylands because they are desiccation tolerant, which (1) enables carbon fixation during precipitation events, and dormancy (including protection from light and temperature extremes) between them, but (2) places constraints on performance owing to the costs of responding to precipitation, resulting in vulnerability to changes in precipitation regime.

Second, I examine the physiological effects of 10 years elevated CO$_2$ and high temperature events on biocrust mosses. I show that, unlike vascular plants, mosses do not acclimate photosynthetically to elevated CO$_2$. High temperatures reduce photosynthetic performance, but elevated CO$_2$ exposure partially alleviates this stress, potentially reflecting reallocation of nitrogen to cellular components that offer photosynthetic thermotolerance.

Third, I study the influence of changes in precipitation event size, frequency, and seasonality on moss carbon balances from precipitation events. Precipitation event size was
positively correlated with (and was the strongest driver of) carbon balance because mosses remain photosynthetically active longer during large events. Rainfall frequency was negatively correlated with carbon balance because the cost to regain functional capacity during a precipitation event is higher after longer dry periods. Finally, carbon balance varied over the course of the year, even for events of identical magnitude, indicating that intrinsic physiological differences are present across seasons than influence response to precipitation.

Lastly, I combine physiology with meteorological records from Western North America to create a predictive model for biocrust moss functioning in the next 100 years. Using simulations, I show that subtle shifts in intra-annual precipitation can drive long-term performance, rare large precipitation events can change the trajectory of survival, and most projected dryland scenarios will result in reduced performance in biocrust mosses.
BIOGRAPHICAL SKETCH

Kirsten Kryder Coe was born in October 1982 in Baltimore, Maryland, where she grew up in Windsor Hills, a neighborhood that borders one of the largest urban parklands in the United States, Gwynns Falls Leakin Park, and harbors a diversity of socially and environmentally conscious citizens.

Kirsten was introduced to the natural world at an early age, and spent much of her youth hiking in the Appalachian Mountains, and traveling with her family to National Parks in the Southwestern United States. Kirsten attended a Quaker school in Baltimore from kindergarten through grade 12, during which time the important values of simplicity, patience, and cooperation were (albeit not too subtly) instilled, and still carry great importance in her life today. As a high school senior, Kirsten elected to double-up on her science requirement and signed up for an Ecology class, during which she discovered she had a penchant for plant taxonomy and field identification. Although plant ecology came extremely naturally to Kirsten, it wasn’t until several years later that it developed into her career path.

In 2000, Kirsten began college at Syracuse University, rapidly majored in Geology, began work in the Syracuse University Stable Isotope Lab, and even won an undergraduate Earth Science academic excellence award as a freshman, before transferring to SUNY College of Environmental Science and Forestry (ESF) for the remainder of her degree. ESF became a perfect academic home for Kirsten because it was comprised of students and professors that deeply cared about biology, education, and environmental stewardship. Two classes in particular shaped both Kirsten’s time at ESF, and her future as an academic. In The Ecology of Mosses, Kirsten was introduced to an extraordinary, miniature world of plants from which myriad
ecological questions seemed to arise, many of which were still unanswered! In The Deserts of Southern Africa, Kirsten traveled to South Africa and Namibia where she learned about the diverse adaptations of dryland organisms to withstand harsh environmental conditions, and completed an independent study on the leaf physiology of the ancient desert gymnosperm *Welwitschia mirabilis* in the Namib Desert. Her experiences during this course led her to take a summer research position at the University of Kwazulu Natal in South Africa, where she worked in the field on projects examining stress physiology in bush babies and elephant shrews. Before graduating *summa cum laude* from ESA in December 2003, Kirsten, unable to stray too far from bryophytes, completed another independent study on habitat partitioning of calciphilic mosses on limestone outcrops in central New York.

Before beginning her graduate program at Cornell University, Kirsten first spent a summer working for the National Park Service near Anchorage, AK, and also worked as a field assistant on a University of Alaska project examining the impacts of nitrogen deposition on feathermoss productivity in the boreal forest understory. Kirsten then stayed in Anchorage for an additional year during which time she was employed in commercial construction, working long hours and developing useful engineering skills that would later come to be invaluable in the design of specialized field equipment for ecophysiology research. Then, in 2005, Kirsten worked in Botswana as the manager of a safari lodge in the Okavango Delta. The remote lodge was located inside one of the largest intact wilderness areas in Africa and was only accessible by small plane. Because the lodge was unfenced, it was not uncommon to run into elephant, baboon, and giraffe on the grounds by day, and find the tracks of lion, leopard, and hippo that had walked through the camp by night. The experiences and life skills Kirsten gained while living immersed
in another culture in Botswana (and being responsible for the health, welfare and safety of the on-site camp staff as well as tourists) were extremely rewarding and unforgettable.

Kirsten began her PhD research with Dr. Jed Sparks at Cornell in fall 2006. During her time as a graduate student, Kirsten learned an immense amount about designing and conducting ecological research, but learned an equal amount about herself as a person, teacher, and academic. Kirsten’s PhD thesis topic, the influence of global change on the physiological ecology of dryland biocrust mosses, seemed to develop seamlessly and logically from all of her meaningful past research and coursework experiences. She relished the opportunity to conduct research in the gorgeous, vast, and grand Southwestern U.S., working with colleagues at the USGS “crust lab” in Moab, UT. Over the years at Cornell, Kirsten’s research questions expanded in scope, and through developing an understanding of the physiological mechanisms underling response to global change as well as the ecosystem-scale consequences of such changes, she developed a truly holistic understanding of global change in dryland systems.

One of the most rewarding aspects of Kirsten’s time as a PhD student was the vast array of opportunities to gain teaching experience and expertise. Kirsten was a TA for eight different biology courses, and was awarded the CALS Teaching Award twice. Kirsten also spent two summers teaching Ecology to prisoners Auburn Maximum Security Prison with the Cornell Prison Education Program (CPEP). Kirsten designed and taught the first general Ecology course offered by CPEP, and also worked to create the first ever educational garden on prison grounds to be used by incarcerated students for field experimentation. She will soon publish an article based on her experiences at Auburn titled “Ecology behind bars: A teaching garden cultivates free minds” in the journal Radical Teacher.
Upon finishing her PhD, Kirsten will work as a postdoctoral associate with Dr. Ted Schuur, at the University of Florida, where she will be examining the consequences of permafrost thaw in the Alaskan arctic on terrestrial carbon cycling and feedbacks to global change. On the longer term, she looks forward to thoughtfully building a fulfilling career as an ecophysiologist and ecosystem scientist, and starting a family with her partner, Petra.
DEDICATION

I dedicate this dissertation to my mother, Julee Hemington Kryder-Coe
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This work would not have been possible without the generous support of family, friends, colleagues, and mentors.

First and foremost, Jed Sparks has been a fantastic advisor throughout the duration of my time at Cornell. Jed has been committed to my success as a researcher, student, teacher, and academic from the very beginning. I am thankful for the freedom he always gave me to pursue my research goals independently, the helpful reminders of the broader context of my work, and the assistance in articulating my work in written as well as presentation formats. I also very much appreciate Jed as a person; he has a good heart and it showed in his interactions with me.

The entire Sparks lab over the years has been a great community in which to learn and grow as a scientist. It has been very stimulating and challenging to be a part of a group that has shared interests and also takes on a broad array of research topics. The interactions I have had with my lab group have helped make me a confident plant physiological ecologist and biogeochemist.

My other Ph.D. committee members were also incredibly helpful throughout this process. Barbara Bedford always helped me see the place my work fit within the overlapping scientific communities of biogeochemistry, plant ecology, and ecophysiology. Barbara was also one of my most important mentors at Cornell, and I am grateful for her advice during the difficult parts of this journey. Tom Whitlow has encouraged me to think outside the box in my research, and has shown me how scientific tools, methodologies, and theories can be useful across disciplines.

There have been other Cornell professors that served as mentors throughout this process as well. Harry Greene has always opened his office door to me for conversations about my
research, teaching, folk music, or his travel and hunting adventures, and I have appreciated these moments very much. Harry has also been one of the most encouraging people for me through all of ups and downs of graduate school, and I think of him as a true friend. Steve Ellner was an instrumental part of the execution of the beginning stages of the modeling work presented in Chapter 4. I also have to thank Rob Raguso and Anurag Agrawal for keeping me well rounded as an ecologist with many stimulating conversations on a variety of topics over the years.

Lloyd Stark (University of Nevada Las Vegas) has been a fantastic colleague, friend, and fellow bryologist nerd over the years. His knowledge of dryland moss ecology is unmatched, and he has been an exceptional resource for me in my research.

The USGS Canyonlands Research Station (the “Crust Lab”) in Moab, UT was the site where the majority of my Ph.D. research was conducted. Jayne Belnap, the head P.I. for the facility throughout my time there, has been an amazing collaborator on my work. She introduced me to my research topic by showing me the effects of changing precipitation on biocrust mosses, and the dramatic effects of moss removal on biocrust structure. Jayne, in addition to Jed, has also continually provided the important perspective of a true desert-dweller on my work. My research would absolutely not have been possible without the incredible engineering skills of Ed Grote, who designed the gas exchange system I used at the Crust Lab to measure CO$_2$ exchange from mosses. I am grateful for his expertise, and his patient instruction as I learned the ropes. Sasha Reed, also at the Crust Lab, has been there for countless insightful conversations about plant-soil interactions, and it has been a true joy working with her in recent years coupling my plant ecophysiology work to soil biogeochemistry. Finally, the numerous research technicians at the lab, especially Hilda Smith and Adam Kind, truly made this work possible by being trustworthy assistants during the prep phases of my experiments.
As an undergraduate at SUNY College of Environmental Science and Forestry, I was heavily influenced as an academic by two professors. First, Robin Kimmerer introduced me to the awe-inspiring field of bryology, and I am indebted to her for encouraging me to pursue my own research to fill in the current gaps of knowledge within that scientific field. Second, Scott Turner introduced me to dryland systems through a field course in southern Africa. I am thankful to still be in touch with him and feel his continued support as my academic journey moves forward.

One of the most rewarding and valuable aspects of my time as a graduate student at Cornell was the opportunity to teach biology to undergraduates. I am so thankful to all of the students in the eight different courses in which I served as a teaching assistant. Collectively, they helped me become a better and better teacher over the years. In addition to teaching Cornell undergraduates, I also spent two summers teaching an Ecology course that I developed to inmates at Auburn Maximum Security Prison through the Cornell Prison Education Program. It is impossible to overestimate the importance of this experience for me as a socially conscious academic. The men I taught at Auburn were some of the most inspiring and respectful students I have ever had, and the challenges of working in a carceral institution forced me to be an adaptive and creative teacher. These collective experiences have shaped me into the instructor I am today, and have helped form my vision of myself as an academic in a research institution.

My friendships, particularly with other Cornell graduate students, have been a wonderful part of my time as a graduate student. Without these relationships, my Ph.D. experience would have been far less colorful, fun, amusing, and meaningful. Also, I am grateful to have learned so much about their own research topics over our years of friendship! My officemates, Sara DeLeon
and Marjorie Weber, have been particularly fantastic friends and sounding boards for ideas and venting sessions alike on a +/- daily basis.

In my early years, my mother (Julee), father (John), and brother (Justin) and I spent much of our free time as a family out of doors. My father was a passionate naturalist, adept mountaineer, and great teacher about the natural world, and I have fond memories of riding on his shoulders through the woods as a child. My mother thoughtfully planned our numerous trips to national parks in the Western US, the English windswept countryside, and the island of St. John, all of which exposed all of my senses to new environments and enhanced my understanding of natural systems. I think my mother truly believed I could do anything I wanted to do, and her encouragement for my academic and professional goals matched that sentiment. Both of my parents were also politically active, informed citizens who worked for social equality throughout their lives, and have taught and inspired me to do the same. Although I lost them too early in life, their life’s passions still inspire me to learn enthusiastically, teach thoughtfully, and be environmentally and socially conscious as I move forward in my career as an academic.

Petra, my incredibly loving partner and best friend, has been the one thing I could truly count on every single day. Her sincere supportiveness of my research and teaching has been an instrumental part of my successes in both of these areas, and I am happy to be forever in her dept for those gifts. She is also one of the most inspiring people, naturalists, thinkers, and scientists I have ever known, and she has helped me become a more informed and thoughtful ecologist as well as a better person overall. I am so grateful to be able to spend my life with such a brilliant and beautiful soul.

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CHAPTER 1

Physiological Ecology of Dryland Biocrust Mosses

Abstract

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 wet-dry cycles that result in either a positive or a negative carbon balance. Carbon balance is 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.
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; 2006), and influence seedling establishment of grasses and shrubs (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 & Pendelton 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 (Budel et al. 2003). 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. 1.1a).

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
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).

**Figure 1.1:** (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).

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, in review). Therefore, understanding the physiological determinants of performance and survival of biocrust mosses is important for understanding 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.

**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. 2000; Oliver et al. 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 & Proctor 1979; Proctor 2008). The limits of tolerance for most mosses are cellular water potentials of -20 to -40 MPa (Proctor & 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 1.1b). A significant degree of molecular packaging mechanisms involving sugars (Smirnoff 1992) as well as proteins (Oliver et al. 2005, Buitink et al. 2002) 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 & Proctor 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. 2000; Oliver et al. 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 & Bewley 1981
a, b; Oliver & Bewley 1984; reviewed in Oliver et al. 2000). 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.

**Figure 1.2:** 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. 2012b *Ecology*, also see Ch. 3 in this dissertation).

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 & 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 an initial period 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. 1.2). 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 reinitiate metabolism, repair membranes, and reconfigure and reorganize cellular components (Hinshiri & Proctor 1971; Tuba et al. 2004; 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 minutes following hydration in some desiccation tolerant species (e.g. *Tortula (Syntrichia)* spp.; Bewley 1979; Tuba *et al.* 1996; Deltorro *et al.* 1998; Proctor & Smirnoff 2000; Reed *et al.* in review; 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 & Oliver 2009; Reed *et al.* in review). 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.

**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 ($\psi_w$) 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. Maximum rates of net carbon fixation are typically reached between 40 and 70% RWC (Tuba et al. 1996; Coe, unpublished) as photosynthesis is inhibited at higher water contents by limited CO$_2$ diffusion and at lower water contents by low intracellular $\psi_w$. 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 $\psi_w$ of $<-100$ Mpa when desiccated and photosynthesis and respiration have been detected in tissues with $\psi_w$ as low as -10 and -20 Mpa, respectively (Dilks & 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 & 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 2009), 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.

Figure 1.3: 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).

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 CO₂ must occur through films of water where diffusion resistance for gas is four orders of magnitude higher than through air. 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. 1.3) 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 CO₂ diffusion (Tucker et al. 1974; Proctor 1979a; Xu et al. 2009).

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 et al. 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. 1.2) 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.*
al. in review). Recovery of the cell cycle as well as reassembly of cytoskeletal elements can take >24 hr 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. 1.1d; Stark 2005; Barker et al. 2005; Coe et al. 2012a). Given that >70% of rainfall events in drylands are <5 mm effective size (Sala & 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. (in press) 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 & Proctor 1971; Dilks & Proctor 1974; Proctor & 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) compared to those 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. 2001; 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. in review) 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. 2011). 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.

**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 & Proctor 1982). Under these conditions, selective pressures to tolerate such extremes are high, and as a consequence, the range of temperature 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 & Proctor 1982) and dry biocrust mosses on sun-exposed substrates can frequently reach tissue temperatures >60 °C (Proctor 2010). 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, however, in biocrust moss taxa 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 42-51 °C, depending on species and exposure time (Meyer & Santarius 1998; Proctor & 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 emission has not yet been demonstrated in desert mosses, evidence for the evolution of its function in several early land plant lineages (Sharkey & Yeh 2001; Jobson & Qiu 2011) 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 10-20 °C (Furness et al. 1982; Alpert & Oechel 1987; Coe, unpublished data), 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 & 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 minutes (Stark & 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 & McLetchie 2006). Such female-skewed ratios are commonly observed in *Bryum* (Stark *et al.* 2010), *Didymodon* (Ochyra & Zander 2007), *Syntrichia* (Mishler & 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 & 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; Stark *et al.* 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 & Stark 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, environmental 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).

**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 > 1000 µmolm⁻² sec⁻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 & Churchill 1982; Marshall & Proctor 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 & 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\(^{-2}\) sec\(^{-1}\) (Marschall & Proctor 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\(^{-2}\) sec\(^{-1}\) and full saturation of *S. ruralis* (Proctor 2008) as well as *S. caninervis* (Coe, unpublished) occur at PAR levels >1000 µmol m\(^{-2}\) sec\(^{-1}\).

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 Marshall & Proctor 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 & Proctor 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 $>1000 \, \mu\text{mol m}^{-2}\text{sec}^{-1}$, *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 & Smirnoff 2000; Hamerlynck et al. 2002; Proctor & Smirnoff 2010). However, NPQ levels diminish rapidly following several minutes in the dark and appear to be suppressed by violaxanthin inhibitors (Proctor & Smirnoff 2000). The current body of work 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.

**Responses 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 CO₂ can result in a stimulation of CO₂ 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 five months in a CO₂ 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 CO₂, 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ enrichment. Several studies have suggested that photosynthesis is downregulated after long-term exposure (Otvos & Tuba 2005), and that reductions in Rubisco may occur (Long et al. 2004). However, Coe et al. (2012b) demonstrate that mosses after 10 years of CO₂ enrichment display higher photosynthetic rates at field growth concentrations and no reductions in either supply of CO₂ 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 CO₂ 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 CO₂ exposure, and may influence the tolerance of environmental stress and sex expression. Desiccation tolerance appears to be enhanced in biocrust moss grown in elevated CO₂ 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 CO₂ 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 CO₂ 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 CO₂, and Syntrichia grown in elevated CO₂ and exposed to high (35-40 °C) temperatures exhibited increased CO₂ assimilation (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\textsubscript{2} at sites of carboxylation as compared to shoots grown at ambient CO\textsubscript{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 & Schrader 2006). Either or both of these possibilities could account for elevated CO\textsubscript{2}-induced thermotolerance in desert mosses.

Based on estimates of electron transport efficiency and diffusion of CO\textsubscript{2} through photosynthetic membranes, Coe et al. (2012b) suggest enhanced membrane stability is the most parsimonious explanation for thermotolerance in \textit{S. caninervis} exposed to elevated CO\textsubscript{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\textsubscript{2} exposure could lead to less diminished photosynthetic rates under high temperatures. In spite of multiple possible mechanisms, elevated CO\textsubscript{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\textsubscript{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.

**Nutrient relations**

Second only to water, nitrogen often strongly limits primary production in arid regions (Vitousek & Howarth 1991; Smith et al. 1997). Mosses (including biocrust species) primarily receive nutrients from atmospheric deposition (Bates 2000) and do not rely on 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 four years fertilization with 10 kg N ha$^{-1}$ yr$^{-1}$ and then suppressed under a higher treatment of 40 kg N ha$^{-1}$ yr$^{-1}$ (Stark et al. 2011) 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 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. 2011, 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. 2008). 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. 1.4). 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.

**Figure 1.4:** 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. Photo credit: Belnap, J.

**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 availability, 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. in review). 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.

Conclusion

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 CO₂ 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 CO₂ on time scales longer than one year, thus future work should focus on these aspects of crust moss ecophysiology.

References


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CHAPTER 2

Physiological ecology of desert biocrust moss following 10 years exposure to elevated CO$_2$: evidence for enhanced photosynthetic thermotolerance$^2$

$^2$ Originally published as Coe, K.K., Belnap, J., Grote, E. & Sparks, J. (2012) Physiological ecology of the desert moss Syntrichia caninervis after ten years exposure to elevated CO$_2$: evidence for enhanced photosynthetic thermotolerance. Physiologia Plantarum 144(4), 346-356
Abstract

In arid regions, biomes particularly responsive to climate change, mosses play an important biogeochemical role as key components of biocrusts. Using the biocrust moss *Syntrichia caninervis* collected from the Nevada Desert Free Air CO$_2$ Enrichment Facility, we examined the physiological effects of 10 years of exposure to elevated CO$_2$, and the effect of high temperature events on the photosynthetic performance of moss grown in CO$_2$-enriched air. Moss exposed to elevated CO$_2$ exhibited a 46% decrease in chlorophyll, a 20% increase in carbon, no difference in either nitrogen content or photosynthetic performance, and no evidence for photosynthetic acclimation. However, when subjected to high temperatures (35–40°C), mosses from the elevated CO$_2$ environment showed higher photosynthetic performance and photosystem II (PSII) efficiency compared to those grown in ambient conditions, potentially reflective of a shift in nitrogen allocation to components that offer a higher resistance of PSII to heat stress. This result suggests that mosses may respond to climate change in markedly different ways than vascular plants, and observed CO$_2$-induced photosynthetic thermotolerance in *S. caninervis* will likely have consequences for future desert biogeochemistry.

Introduction

Non-vascular plants (mosses and their relatives) are an important component of global net primary productivity (Campioli et al. 2009, Lindo and Gonzalez 2010), biogeochemistry (Turetsky 2003) and biodiversity (Molau and Alatalo 1998, Shaw et al. 2005). Because they are photosynthetically similar to their vascular counterparts, it is often assumed that these plants will
likely be similarly responsive to elevated atmospheric CO₂. However, mosses do not possess the same regulatory mechanisms associated with gas exchange and sugar transport that define vascular plant responses to long-term CO₂ fumigation, so their response to a future atmosphere is likely to be different.

In vascular plants grown in elevated CO₂, the increased substrate for photosynthesis has a threefold impact on physiological ecology: photorespiration is reduced and carboxylation efficiency increases (Sage et al. 1989, Bazzaz 1996, Cousins et al. 2001, Long et al. 2004), stomatal aperture is reduced and as a consequence plant water use efficiency increases (Bazzaz 1990, Long et al. 2004, Ainsworth and Rogers 2007) and sucrose production is enhanced in leaves (Long et al. 2004). Therefore, vascular plant response to short-term (1–3 years) enrichment includes stimulation of photosynthesis, productivity and biomass accumulation (Norby et al. 1999, Nowak et al. 2004, Ainsworth and Long 2005, Saxe et al. 2008). In the long term (>8 years), however, initial increases in production are usually offset by the acclimation of photosynthetic rates to align with source-sink carbon (C) relations (downstream limitation in phloem-linked networks) and nutrient availability (Sage et al. 1989, Long et al. 2004). Because of the reduced demand for cellular mechanisms involved in C capture and processing under long-term CO₂ enrichment, this response also usually leads to a decrease in the function and amount of Rubisco as well as an overall decrease in nitrogen (Long et al. 2004, Ainsworth and Long 2005, Leakey et al. 2009).

Most moss gametophytes, possessing neither stomata nor complex vascular tissue, are likely to respond to elevated CO₂ differently. First, gas exchange is not actively regulated via the stomatal aperture and instead CO₂ concentration within chloroplasts (cₐ) depends on diffusion of air through the boundary layer, external capillary water, water-saturated cell walls and plasma
membranes to points of carboxylation. Similar to vascular plants, $c_c$ in mosses is heavily influenced by photosynthetic rate, but because water limits the diffusion of CO$_2$, the primary environmental controls on $c_c$ in mosses tend to be shoot water content (Williams and Flannagan 1998) that varies with microclimate water availability. Second, mosses lack phloem, and although transport distances are short and cellular export of sugars is likely (Ligrone et al. 2000), mosses possess neither the extensive transport mechanisms necessary to translocate sugars over long distances nor the ability to regulate carbohydrate production based on sink strength or phloem-loading characteristics. Therefore, response to prolonged elevated CO$_2$ cannot include active suppression of the Calvin cycle or differences in water use in a manner analogous to vascular plants.

To date, moss responses to CO$_2$ enrichment have been measured in several peatland species, where biomass production has been observed to either be increased (Tuba et al. 1998, Heijmans et al. 2001) or not influenced (Berendse et al. 2001, Toet et al. 2006) after 1–3 years of treatment, and in one dryland species (Brinda et al. 2011) where sexual reproduction was enhanced and improved desiccation tolerance was observed. Soluble sugars in *Sphagnum* spp. have been shown to increase by 20–30% after short-term CO$_2$ exposure, and nitrogen (N) content either declines or remains constant (Tuba et al. 1998, Berendse et al. 2001, Heijmans et al. 2001). With the exception of recent work by Brinda et al. (2011), no work to date has examined responses beyond three years of elevated CO$_2$ treatment, and we currently possess very limited knowledge on responses of non-mesic mosses.

In arid and semi-arid systems, mosses often play a disproportionately large ecological role due to their presence in biocrust communities (composed of mosses, lichens and cyanobacteria) that are essential in the biogeochemical cycling of nutrients in these regions.
Crusts can account for up to 70% of ground cover in deserts of the Southwestern United States where they fix substantial amount of C and N (Evans and Lange 2003, Housman et al. 2006), can act as the primary source of N to plant and soil communities (Evans and Ehleringer 1993), aid in seedling establishment (Su et al. 2007), increase water retention of soil, and decrease erosion (Belnap and Gillette 1998, Belnap 2003). Mosses within biocrusts appear to be particularly sensitive to climate change, and declines in moss have been correlated with reduced overall desert biocrust functionality (Belnap, unpublished data).

Deserts of the Southwestern United States, in addition to experiencing the global 250–500 ppb increase in CO₂ within the next century (Meehl et al. 2007), are predicted to be subjected to dramatic (4–6°C) average temperature increases as well as more frequent extreme high temperature events (Bazzaz 1996, Wagner 1996, Meehl et al. 2007). As these regions are likely to simultaneously experience elevated CO₂ and high temperatures in the foreseeable future (Meehl et al. 2007), it is likely that these factors will act in concert to impact plant physiology and functional ecology. Understanding how desert plants, particularly biocrust mosses, growing in an enriched atmosphere will respond to high temperature events will therefore be instrumental in predictions for arid ecosystem function.

Some recent work has suggested that short-term exposure to elevated CO₂ enhances temperature tolerance of photosynthesis in plants (Faria et al. 1996, Huxman et al. 1998, Hamerlynck et al. 2000a, 2000b, Taub et al. 2000, Wang et al. 2008). Based on the climate change scenarios, such thermotolerance mechanisms may be particularly important for desert plants. The ability to withstand high temperatures during physiological functioning is already evolutionarily adaptive and present in this biome (Berry and Bjorkman 1980, Downton et al. 1984, Loik and Harte 1996); therefore, mechanisms involved in photosynthetic thermotolerance
are most likely a component of general function (Knight and Ackerly 2002, 2003). Desert mosses are a likely candidate for possession of elevated CO₂-induced thermotolerance of photosynthesis, with clear consequences for biocrust functioning in this region, yet the presence of such mechanisms have to be identified in this plant functional group.

In this study, we examined the effects of long-term (10 years) elevated CO₂ exposure on the common desert moss *Syntrichia caninervis* (Mitten) collected from the Nevada Free Air CO₂ Enrichment (FACE) Facility in the Mojave Desert, and were interested in the physiological and photosynthetic responses to long-term CO₂ enrichment as well as the temperature tolerance of photosynthesis for plants grown in CO₂ enriched air. Specifically, we predicted that (1) desert moss exposed to 10 years of CO₂ enrichment will exhibit the stimulatory effects of increased C substrate (including higher photosynthetic rates and increased C uptake), yet will not downregulate biochemical fixation ability in the long-term because the limitations imposed by stomata and phloem transport are not present, and (2) desert moss, already possessing thermotolerance mechanisms due to their growth environment, will exhibit enhanced photosynthetic thermotolerance as a consequence of 10 years of elevated CO₂ exposure.

**Methods**

**Growth conditions and field collection**

Samples of *S. caninervis* were collected from the Nevada Desert FACE Facility (NDFF), located approximately 80 km north of Las Vegas, NV, USA. Constructed in 1997, the NDFF was designed to measure the effects of CO₂ enrichment on an intact Mojave Desert ecosystem. The NDFF is comprised of nine, 25-m diameter rings that enclose naturally occurring Mojave Desert
vegetation. Using attached blowers, each plot is continuously fumigated from the surrounding ring with either ambient air (approximately 360 ppm CO₂) or CO₂-enriched air (550 ppm CO₂; n = 3 rings per treatment), and an additional three rings were left unfumigated as a control. At the time of collection, moss had been subjected to treatments for 10 years (April 1997–April 2007) during which time the environmental conditions at the NDFF included annual temperatures between −10 and 45°C, and mean annual precipitation of approximately 140 mm. Additional details of the NDFF design and site characteristics are described in Jordan et al. (1999).

Samples of *S. caninervis* were collected on April 17 and 18, 2007, from 360 ppm control and 550 ppm treatment plots just after CO₂ treatments ceased at the NDFF. Moss samples (n = 72), and the underlying organic layer (<1 cm below the soil surface), were carefully lifted off the ground using a dissecting probe and placed in labeled herbarium packets documenting the ring number, latitude and longitude. Samples were collected from rings wherever patches were large enough (>5 cm²) and the number of samples collected in each ring differed slightly due to heterogeneity in moss distribution. Following collection, moss samples were immediately transported to the USGS Canyonlands Research Station in Moab, UT, USA, for analyses. All samples were collected and transported in the desiccated state to eliminate exposure to (and reaction with) ambient atmospheric conditions prior to experimentation.

**Sample pre-treatment**

A 2-cm diameter section of each sample was thoroughly rinsed with distilled water to remove soil and placed in a numbered gold-plated copper cap lined with filter paper. Each copper cap had small holes to allow drainage of excess water. Following rinsing and drainage, each sample was assumed to be at 100% relative water content (RWC). Groups of four samples
were placed in Petri dishes and incubated at 30°C for approximately 90 min prior to measurement. This controlled incubation served to (1) ensure sufficient rehydration time for commencement of physiological activity and (2) attain a standard RWC for photosynthesis measurements (which was approximately 50%; data not shown). These conditions are not typical for bryophyte pre-treatment (where samples are allowed to dry more slowly) and for this study represented a trade-off between drying samples to a level where optimum photosynthetic rates were reached, yet simultaneously minimizing exposure to ambient air. Due to the small amount of available plant material, we used samples primarily for gas exchange (18 samples consisting of four caps each) and chlorophyll fluorescence (30 caps analyzed prior to gas exchange). Elemental analysis and pigment information were gathered on samples following gas exchange measurements.

**Photosynthetic gas exchange**

We used laboratory-based CO₂ exchange analysis to examine the effect of 10 years of elevated CO₂ on photosynthetic performance of *S. caninervis* and to determine the effect of high temperatures on photosynthetic functioning. Traditionally, A–ci analyses, the leaf photosynthetic rate measured over a range of leaf-internal CO₂ concentrations, are used to examine diffusional and biochemical limitations to photosynthesis in higher plants (Farquhar et al. 1980). However, because mosses possess a different set of resistances to CO₂ diffusion compared to vascular plants, an A–cᵢ curve cannot be resolved to generate estimates of Vcmax (limitation to photosynthesis due to CO₂ diffusion) and J_max (limitation due to electron transport). First, cᵢ (thus cₑ) cannot be estimated in bryophytes simply from gas exchange and has been shown to vary primarily as a function of shoot water content (Williams and Flannagan 1998). Therefore, all
measurements presented in this study were made at a constant shoot water content. Additionally, after passing through the boundary layer, the resistance path of CO$_2$ into chloroplasts of mosses is exclusively a mesophyllic resistance because the diffusion path does not include stomata. Internal CO$_2$ concentration in vascular plants is often defined as the CO$_2$ concentration at sites of evaporation (i.e. the substomatal cavity surface) rather than the point of carboxylation; yet in mosses, the analogous evaporative surface is the entire gametophyte that is exposed to CO$_2$ in the surrounding air. Finally, boundary layer conductance to CO$_2$ was assumed to be large in this experiment (based on the construction of our cuvette and the uniform depth of green tissue in tightly packed moss colonies), therefore external CO$_2$ concentration ($c_a$) can be used to approximate CO$_2$ at the leaf surface ($c_s$). Thus, while we could not use the standard A–c$_i$ technique in this study, we performed a similar analysis by measuring CO$_2$ exchange (A) as a function of external CO$_2$ concentration ($c_a$) under constant light and humidity.

Since it is of the same supply–demand form as an A–c$_i$ curve, a photosynthesis-response curve for mosses at a constant water content created from an analysis of A as a function of $c_a$ produces two parameters that can be compared across treatments: initial slope ($m_i$; indicative of the limitation to photosynthesis imposed by the diffusional resistance imposed by the CO$_2$ pathway from the atmosphere to the site of carboxylation) and maximum rate ($f_{\text{max}}$; maximum CO$_2$ flux, indicative of the limitation imposed by inherent biochemistry at maximum CO$_2$ consumption – either as the rate of regeneration of RUBP through the Calvin cycle or as the activity of Rubisco). In this study, differences in the parameters, $m_i$ and $f_{\text{max}}$, across CO$_2$ and/or temperature levels thus reflect differences in photosynthetic performance imposed by treatments.

For gas exchange analysis, groups of four moss samples were placed in a nickel-plated, custom-built cuvette attached to a desktop gas exchange system. The gas exchange system
consisted of a gas mixer (Environics Series 4000; Environics Inc., Tolland, CT), humidifier, custom-built cuvette and an infrared gas analyzer (Li-6262; Li-COR Inc., Lincoln, NE) and was run with the program LabView (National Instruments Corporation, Houston, TX). Internal conditions of the cuvette were maintained at approximately 800 μmol m^{-2} s^{-1} photosynthetically active radiation and approximately 65% relative humidity. The cuvette was temperature controlled (±0.5°C) using a water bath (Fisher Isotemp 105; Thermo Fisher Scientific Inc., Waltham, MA) and a coil system built into the cuvette. We used thermocouples to continuously monitor air and moss temperature within the cuvette over the course of the experiment (the difference between air and moss temperatures never varied >1°C).

We measured maximum CO₂ assimilation rates for S. caninervis grown under ambient and elevated CO₂ treatments at the following sequence of CO₂ concentrations (cₐ; ppm): 375, 250, 150, 50, 150, 250, 375, 500, 800 and 1000, where cₐ is the CO₂ level exiting the chamber. Each group of four samples was allowed 5 min to acclimatize to each concentration prior to measurements at low CO₂ levels (<375 ppm) and 10–15 min to acclimate to higher concentrations (times previously determined by the stabilization of CO₂ levels in the chamber). Gross photosynthetic rate (A_{gross}) was calculated as the sum of maximum CO₂ assimilation (A_{net}) in light conditions and dark respiration values at each CO₂ level. Respiration rates did not differ between samples grown in enriched and ambient conditions; therefore, A_{gross} is proportionally equivalent to A_{net} for all measurements. Samples were kept at an RWC approximately 50% by weight (distilled water was added with a dropper to individual samples as needed) throughout all gas exchange measurements. Moss photosynthetic rate (A) was plotted as a function of CO₂ concentration (cₐ) to create response curves for moss samples from each treatment, and m, and f_{max} were estimated from each curve using curve fitting.
Gas exchange measures were conducted at three leaf temperatures: 20, 30 and 40°C, with the 40°C level designed to simulate temperatures that fall within the range of predicted values under future climate scenarios. Soil temperatures in the Mojave Desert often exceed 40°C in the summer. However, currently mosses in this system are inactive and desiccated during this time of the year. If summer rainfall increases in the future as predicted by some modeling efforts, then these temperatures would be experienced by mosses in a hydrated state. We chose 40°C as our maximum temperature treatment because (1) it falls just below a heat shock threshold at which photochemical destruction (Xu et al. 2009) and declines in reproductive success and growth (Stark and McLetchie 2006) occur in *S. caninervis* and (2) it represents a relevant temperature stress level under which mosses are likely to be hydrated and physiologically active under future climate scenarios.

**Chlorophyll fluorescence**

We used the chlorophyll fluorescence parameter, $F_v/F_m$, to examine differences in photosynthetic efficiency in *S. caninervis* from elevated and ambient CO$_2$ treatments. Following incubation procedures described above, 30 moss samples were dark-adapted for 15 min [previously shown to be sufficient to dissipate non photochemical quenching (NPQ)] and pulse amplitude-modulated (PAM) fluorescence was measured using a PAM-2000 fluorometer (H. Walz, Effeltrich, Germany). Baseline fluorescence ($F_o$) of samples exposed to an actinic light was measured prior to exposure to saturating pulses. Using a spacer to ensure constant sample distance of 1 cm from the light source, maximum fluorescence ($F_m$) was driven by a 0.8-s saturating pulse (3000 mol m$^{-2}$ s$^{-1}$ PPFD) propagated through a fiber optic cable. Maximum
photochemical efficiency of photosystem II (PSII) was calculated as $F_v/F_m = (F_m - F_o)/F_m$ (Shreiber 1998).

**Tissue analysis**

To assess changes in relative allocation of N to light capture for photosynthesis across treatments (a measure of acclimation to elevated CO$_2$), we measured pigmentation levels in moss from elevated and ambient CO$_2$ plots. Chlorophyll a and b, β-carotene, violaxanthin and lutein content of *S. caninervis* were quantified using high performance liquid chromatography (HPLC), as described by Garcia-Pichel and Castenholz (1991). Moss samples ($n = 58$) were removed from herbarium packets, thoroughly rinsed of soil and debris, left to dry overnight in the light, then mixed well using a mortar and pestle and weighed to the nearest 0.001 g. Each sample was ground using a mixer mill (Spex Certi-prep Inc., Metuchen, NJ) for 10 min at 27 Hz, then 1/2 ml of distilled water was added to the sample, followed by 10 ml of acetone. Solutions were vortexed daily and allowed to sit for two nights, filtered with Whatman GF/F glass-fiber filter paper, then evaporated down to 3 ml using a warm water bath and a mild stream of N prior to HPLC analysis. Concentrations for all pigments were quantified using peak areas integrated from a photodiode array data at 436 nm (Garcia-Pichel et al. 1992, Belnap et al. 2004). Results were expressed as mg pigment g$^{-1}$ moss tissue.

To determine the effect of long-term CO$_2$ enrichment on C and N levels in moss shoots, elemental analysis of *S. caninervis* tissue was conducted at the Cornell University Stable Isotope Laboratory. To prepare tissue for analysis, moss gametophytes were immersed in water and green portions were separated from brown (below-ground) sections. Green tissue samples were then pulverized with a spatula, weighed out to approximately 2.5 mg and then stored in a
desiccant jar prior to analysis. Samples (n = 20) were loaded into an NC2500 Carlo Erba elemental analyzer coupled to a Thermo Delta Plus isotope ratio mass spectrometer (Thermo Fisher Scientific Inc., Thermo Electron, Waltham, MA, USA), combusted at 1000°C and then passed through a chromium oxide column. Released NO\textsubscript{x} gases were reduced to N\textsubscript{2} using a copper column at 650°C, and the resultant CO\textsubscript{2} and N\textsubscript{2} were passed through a magnesium perchlorate trap and then separated on a GC column. Percent N and C values were derived from sample peaks (including peak area, amplitude and sample weight).

**Statistical analysis**

One-way ANOVA (JMP version 7.0, SAS Institute Inc., Cary, NC) was used to determine differences between percent C and N of *S. caninervis* grown in ambient (360 ppm) and elevated (550 ppm) CO\textsubscript{2} environments. These data were arcsine transformed prior to statistical analysis to meet ANOVA distribution assumptions (Zar 1999). Student's t-tests were used to determine differences in mean $F_v/F_m$ and in pigment concentration of samples grown in each of the CO\textsubscript{2} environments.

A two-way ANOVA was performed to analyze differences in $m_i$ and $f_{\text{max}}$ of samples from both CO\textsubscript{2} treatments under three different temperatures (20, 30 and 40°C) using CO\textsubscript{2} level and temperature as main factors in the model. Results are presented for the individual effects of temperature and CO\textsubscript{2} level on $m_i$ and $f_{\text{max}}$ as well as on the interactions between these factors. In addition, because we were specifically interested in the influence of CO\textsubscript{2} level on photosynthetic performance at high temperatures, we used a t-test assuming equal variance to analyze differences in $m_i$ and $f_{\text{max}}$ occurring during the simulated high-heat event (40°C). Results for
statistical analyses are shown for 95% confidence intervals and an $\alpha = 0.05$, unless otherwise noted.

**Figure 2.1:** Pigment concentrations (chlorophyll $a$, chlorophyll $b$, $\beta$-carotene, violaxanthin and lutein; mg g$^{-1}$) of *Syntrichia caninervis* following 10 years exposure to ambient (360 ppm) or elevated (550 ppm) CO$_2$ (n = 58). Error = ±1 se; **$P < 0.01$; ***$P < 0.001$; #P < 0.1.

**Results**

Mosses exposed to elevated CO$_2$ exhibited lower photosynthetic pigment levels when compared to samples grown under ambient conditions (Fig. 2.1). Chlorophyll levels decreased by approximately 46%; chlorophyll $a$ decreased from 0.248 to 0.134 mg g$^{-1}$ ($P < 0.01$) and chlorophyll $b$ was reduced to 0.0638 from 0.118 mg g$^{-1}$ ($P < 0.001$). $\beta$-Carotene levels were reduced from 0.052 to 0.035 mg g$^{-1}$ ($P < 0.01$) and lutein levels decreased from 0.084 to 0.048 mg g$^{-1}$ ($P < 0.001$). The concentration of violaxanthin in tissue decreased from 0.014 to 0.008 mg g$^{-1}$ in samples from the elevated CO$_2$ treatment, but this difference was not significant at an
α = 0.05 (P = 0.06). When compared to individuals grown under ambient conditions, *S. caninervis* from the elevated CO₂ treatment exhibited a 20% increase in carbon (C) content (F₁,₁₇ = 6.36, P < 0.05) and no change in N content (F₁,₁₇ = 0.10, P = 0.89; Fig. 2.2). This resulted in an increase in C:N from 15.2 in mosses exposed to ambient conditions to 17.7 in those exposed to elevated CO₂ (F₁,₁₇ = 7.13, P < 0.05).

Figure 2.2: Mean carbon (C) and nitrogen (N) content (±1 se) of *Syntrichia caninervis* shoots from ambient (360 ppm) and elevated (550 ppm) CO₂ plots (n = 20). A one-way ANOVA was used to determine differences between CO₂ treatments (asterisks indicate differences where P < 0.05). Values for percent tissue composition were arcsine transformed prior to statistical analysis to meet anova distribution assumptions.

Temperature had a significant overall effect on carboxylation capacity (m_i) and maximum rate of CO₂ flux (f_max) in *S. caninervis* (Table 2.2A). Moss measured at temperatures common during the growing season (20–30°C) displayed no difference in m_i or f_max for the two CO₂ environments (although values for both photosynthetic parameters were slightly higher at
30°C; Fig. 2.3, Table 2.1), thus the overall effect was driven by differences during the high temperature event. At 40°C, gross CO₂ assimilation rates were greatly reduced relative to the lower temperatures, with maximum values of approximately 3.5 µmol m⁻² s⁻¹ at 40°C compared to 8–10 µmol m⁻² s⁻¹ at 20–30°C (mᵢ: F₃,₁₄ = 18.01, P < 0.0001; fₘₐₓ: F₃,₁₄ = 15.03, P < 0.0001). Photosynthetic performance (mᵢ and fₘₐₓ) was also significantly reduced to <50% of levels from 20 to 30°C measurement temperatures during the high-heat event (P < 0.05; Table 2.1).

**Table 2.1**: Photosynthetic parameters estimated from CO₂-response curves, mᵢ (initial slope of curve; photosynthetic limitation due to CO₂ availability) and fₘₐₓ (final slope of curve; photosynthetic limitation due to biochemistry at maximum C fixation rate) conducted at three temperatures for *Syntrichia caninervis* grown under ambient (360 ppm) and elevated (550 ppm) CO₂ conditions (n = 3 per treatment combination). Asterisks indicate significant difference from ambient controls (P < 0.05).

<table>
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<th>CO₂ treatment (ppm)</th>
<th>CO₂ treatment (µmol s⁻¹ ppm CO₂⁻¹)</th>
<th>CO₂ treatment (µmol m⁻² s⁻¹)</th>
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</tr>
<tr>
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</tr>
<tr>
<td>40 °C</td>
<td>7.49</td>
<td>17.5*</td>
</tr>
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</table>

The overall effect of CO₂ level on photosynthetic performance (mᵢ and fₘₐₓ) was not significant over the entire range of temperatures nor was the interaction with temperature (Table 2.2A) because mᵢ and fₘₐₓ were not influenced by CO₂ at the 20–30°C level (Fig. 2.3, Table 2.1). However, analysis of these parameters during only the 40°C simulated event showed that CO₂ treatment strongly influenced photosynthetic performance under high temperature conditions (Fig. 2.3, Table 2.2B). Assimilation rates of moss from the elevated CO₂ treatment were not reduced to the extent of those grown at ambient levels (i.e. they displayed higher photosynthetic performance) and consequently exhibited mᵢ and fₘₐₓ values double that of moss grown under
ambient CO₂ conditions (F₁,₄ = 20.02, P = 0.011 and F₁,₄ = 9.62, P = 0.036, respectively).

Further, moss grown in elevated (550 ppm) CO₂ also exhibited a 15% increase in the efficiency of PSII when measured at 35°C (P < 0.05), shown by an average Fᵥ/Fₘ value of 0.578 (±0.01) compared to a value of 0.512 (±0.02) for moss from ambient (360 ppm) conditions.

**Table 2.2:** (A) Results from two-way ANOVA on the singular and interactive effects of CO₂ level (360 and 550 ppm) and temperature (20, 30 and 40°C) on the photosynthetic parameters, mᵢ and fₘₐₓ, for Syntrichia caninervis. Asterisks indicate significant effects (α = 0.05) of treatments on mᵢ and fₘₐₓ. (B) Analysis of the effect of CO₂ level on mᵢ and fₘₐₓ of S. caninervis during the simulated high-heat event (40°C) using an equal variance t-test. Asterisks indicate significant differences (α = 0.05) in either mᵢ or fₘₐₓ as a function of CO₂ treatment.

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**Figure 2.3:** Gross CO₂ assimilation (μmol m⁻² s⁻¹; ±1 se) as a function of cuvette air CO₂ concentration (cₐ) for Syntrichia caninervis grown at ambient (360 ppm, filled symbols) and elevated (550 ppm, open symbols) CO₂ at 20, 30, and 40°C (symbols represent mean values from 3 replicates; 18 replicates total). Due to instrument limitations, data are not presented for the 1000 ppm CO₂ level at 40°C because this concentration and temperature combination could not be maintained in the chamber.
Ambient (360 ppm) ◯ Elevated (550 ppm)

20°C

30°C

40°C

Gross CO₂ exchange (μmol m⁻² sec⁻¹)

External CO₂ concentration (cₐ; ppm)
Discussion

Exposure of the desert moss *S. caninervis* to 10 years of CO₂ enrichment altered the tissue composition and physiology of this species, both in terms of the form and function of the photosynthetic apparatus and with respect to tissue C:N.

We predicted that 10 years of elevated CO₂ treatment would increase C acquisition in *S. caninervis*, and we did observe increased C content (Fig. 2.2), but the photosynthetic performance of moss from elevated CO₂ plots was not different from moss grown under ambient conditions when measured at temperatures between 20 and 30°C (Fig. 2.3, Table 2.1). Long-term elevated CO₂ did not alter parameters associated with the supply of CO₂ or rates of carboxylation (mᵢ and fₘₐₓ) suggesting that this moss did not downregulate biochemical fixation ability in response to treatment. Direct numerical comparisons of mᵢ and fₘₐₓ to Vₖₘₐₓ and Jₖₘₐ₇ is challenging because of the differences in resistances to CO₂ diffusion parameters in vascular plants and mosses, yet both analyses are based on a supply and demand function focusing on the differences in initial and final slopes of the photosynthesis–CO₂ relationship, thus a relative comparison between the methods is reasonable. Similar photosynthetic performance (mᵢ and fₘₐₓ) in *S. caninervis* in treated and control plants after 10 years of CO₂ enrichment contrasts what is reported for most vascular plants, where acclimation to elevated CO₂ results in an average reduction of 13% in Vₖₘₐₓ and 5% in Jₖₘₐ₇ (Long et al. 2004). Further, declines in these photosynthetic parameters are normally accompanied by a concomitant decrease in N content (Long et al. 2004, Toet et al. 2006), but in the moss examined in this study, N contents were unchanged across treatments. These data taken together suggest that desiccation-tolerant mosses, such as *S. caninervis*, do not respond to elevated CO₂ in a way similar to higher plants and do not
display photosynthetic acclimation: mosses maintain tissue N and do not increase the diffusional resistance to CO₂. Part of this may simply be due to structure (e.g. a lack of dynamic stomata) and a reliance on ambient moisture conditions to control the diffusion rate of CO₂, although efficiency of CO₂-transporting aquaporins may also play a role (Borstlap 2002, Shelden et al. 2009). The reason for maintaining levels of tissue N, however, is less clear, and we outline one possible explanation below (an allocation of N to increased thermotolerance).

Temperature had a strong effect on the photosynthetic performance of *S. caninervis* (Table 2.2A) and when subjected to 40°C, photosynthetic rates, mᵢ and fₘₐₓ all declined sharply in both treatment and control plants. However, rates were depressed less in moss grown under elevated CO₂ (Fig. 2.3, Table 2.1). This suggests that the elevated CO₂ treatment in some way enhanced photosynthesis at high temperature, supporting our second hypothesis that long-term CO₂ enrichment would induce thermotolerance in *S. caninervis*.

A portion of the observed declines in performance at high temperature was likely a result of photorespiration. However, measurements were made at a constant temperature and CO₂ concentration in both CO₂-treated and control plants making it unlikely that the differences observed could be driven entirely by differential photorespiration or, if it were, it was due to CO₂-treated mosses maintaining a higher CO₂ concentration at the site of carboxylation compared to control mosses at the same ambient CO₂ concentration.

Higher fₘₐₓ at high temperatures in mosses exposed to elevated CO₂ compared to those under ambient conditions indicates higher rates of electron transport and augmented carboxylation capacity in mosses grown under elevated CO₂. This result would be consistent with any of the following mechanisms: (1) an increase in the rate of RUBP regeneration, (2) an increase in the activity of Rubisco (Campbell and Ogren 1990, Ruuska et al. 2000) or (3) an
increased supply of elemental phosphorus (Mohr et al. 1995). Electron transport slows with increased temperatures due to destabilization of phospholipid bilayers in which the electron transport chains reside; therefore, $f_{\text{max}}$ and the three above mechanisms associated with it would be expected to decrease as temperatures increase. Therefore, the differences in photosynthesis at high temperature observed in this study may have been driven by changes in membrane stability at high temperatures related to elevated CO$_2$ exposure.

PSII efficiency in *S. caninervis* (as quantified by $F_{\text{v}}/F_{\text{m}}$) was also 15% higher in plants grown under elevated CO$_2$ and is likely indicative of enhanced electron transport in plants exposed to high temperatures, despite the reduction in chlorophyll. Although $F_{\text{v}}/F_{\text{m}}$ values for moss in this study were approximately 25% lower than typically observed in healthy vascular plants, they fall within the typical range for *Syntrichia (Tortula)* species (Hamerlynck et al. 2000a, 2000b) and other mosses at approximately 50% RWC (Robinson et al. 2000). When considered together, $f_{\text{max}}$ and fluorescence analyses suggest that long-term exposure to CO$_2$ in *S. caninervis* resulted in increased rates of electron transport in thylakoid membranes, yet differences were only apparent at high temperature, a result strongly suggesting limitation of damage under conditions of stress.

The photosynthetic parameter, $m_i$, was also less diminished in moss exposed to long-term elevated CO$_2$ at high temperature suggesting that available CO$_2$ at the site of carboxylation was maintained at a higher level relative to moss under ambient conditions. We could not detect any changes in macrostructure (through measurements of shoot density and shoot length) that might indicate differences in boundary layer conductance or diffusional path length between treatments (data not shown), and samples were held at constant water content. Therefore, the influence of
the elevated CO₂ treatment on CO₂ supply is likely also reflective of stabilization of membranes that influenced the rate of CO₂ diffusion.

One suggested outcome of growing in an enriched-CO₂ atmosphere is that the photosynthetic rates are often higher at lower foliar N contents (i.e. less N is required for photosynthesis; Epron et al. 1996). In bryophytes, however, relationships between N, C and photosynthesis may not follow the same patterns as vascular plants (Rice et al. 2008). Foliar N content per unit mass of *S. caninervis* was not reduced under elevated CO₂, and if less of this N was allocated to photosynthetic function, it suggests that surplus tissue N may exist. N in excess of photosynthetic requirements could thus be allocated to other physiological functions including N-rich proteins and enzymes involved in membrane stability. Elevated CO₂ has previously been suggested to cause plants to repartition nutrients to fulfill physiological requirements (Sage 1994, Huxman et al. 1998) and change resource allocation to improve stress tolerance (Brinda et al. 2011), and the observed increased thermotolerance in *S. caninervis* may have been a result of changes in N allocation over the course of the 10-year CO₂ treatment. Under conditions where mosses have surplus substrate available for photosynthesis, N that was originally allocated to the photosynthetic apparatus could be re-allocated to protecting photosynthesis from heat damage. Elevated CO₂ may therefore influence the trade-off in N allocation between light harvesting/carboxylating mechanisms and thermotolerance of membranes in *S. caninervis*.

Moss grown in an elevated CO₂ atmosphere also exhibited a reduction in photosynthetic pigments when compared to moss grown under ambient conditions. Chlorophyll a and b levels decreased by 46%, and carotenoid (lutein and β-carotene) levels decreased by 44% on a dry weight basis (Fig. 2.1). Individually, pigment concentrations were reduced fairly uniformly across types (by 40% or more) in moss exposed to elevated CO₂. The reductions observed in this
study, especially in reference to chlorophyll, differ from the responses observed in a wide array of vascular plants from North American FACE experiments (reviewed by Long et al. 2004), where long-term growth under elevated CO$_2$ had no significant effect on chlorophyll content. Although little data exist on the effects of long-term exposure to elevated CO$_2$ on carotenoid levels, Houpis et al. (1988) noted reductions in such pigments after exposure to elevated CO$_2$ for 2.5 years in open-top chambers. Because carotenoids may screen UV light in addition to acting as antenna pigments, reductions in lutein and $\beta$-carotene in $S$. caninervis may reflect a reduction in ability to prevent photooxidative damage from UV in the future. On the other hand, reduced investment in antenna pigments could also eliminate excess light harvesting and could manifest in reduced damage when CO$_2$ assimilation drops at high temperatures.

In higher plants, short-term exposure to elevated CO$_2$ often results in the production of foliage with reduced chlorophyll content likely reflective of reduced demand for fixation in C$_3$ plants under conditions where there is increased substrate for C fixation (Houpis et al. 1988, Epron et al. 1996). However, this reduction is often not sustained (Long et al. 2004). Therefore, because of the slow growth rate of $S$. caninervis reduced pigmentation could have resulted from either a loss of pigments from existing tissues, reduced production of pigments in new tissues produced under CO$_2$ treatment, or a combination of both scenarios.

The ability of plants to maintain physiological function at high temperatures will be an important factor in determining future distributions of plants in arid regions (Nobel and Smith 1983, Nobel 1984), and deserts are expected to have some of the largest increases in temperature in the future. Previously, very little was known about the response of desert mosses to future atmospheric conditions. We have demonstrated that desert mosses may respond to long-term elevated CO$_2$ very differently than vascular plants. In particular, the lack of photosynthetic
acclimation and the potential for sustained photosynthesis under high temperature for *S. caninervis* grown under elevated CO\(_2\) suggest the opportunity for higher rates of survival than would have been predicted under future conditions of simultaneous warming and CO\(_2\) enrichment. Relative to present temperatures, C fixation rates will be reduced in *S. caninervis* during future high temperature scenarios, but our results suggest that CO\(_2\)-induced photosynthetic thermotolerance in this species may enable individuals to perform better than expected under these conditions. This may affect growth and biomass of *S. caninervis* within soil crust communities and because *S. caninervis* is the dominant moss in low elevation Mojave Desert biocrusts (Stark et al. 1998, Bowker et al. 2000), growth that is sustained under higher temperatures will have important ecological ramifications for C cycling in the Southwestern United States. Given that the distribution of this species extends throughout western North America (Flowers 1973) and in dry regions of Africa, Asia, the Middle East and Europe (Kramer 1980), the potential for impacts on global arid-region C cycling is likely if responses like the ones observed in this study are common across this species' broad distribution and in related biocrust moss taxa.

**Acknowledgements**

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Kind. Any use of trade names is for descriptive purposes only and does not imply endorsement by the US Government.

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CHAPTER 3

Precipitation-driven carbon balance controls survivorship of desert biocrust mosses

Abstract

Precipitation patterns including the magnitude, timing, and seasonality of rainfall are predicted to undergo substantial alterations in arid regions in the future, and desert organisms may be more responsive to such changes compared to shifts in only mean annual rainfall. Soil biocrust communities (consisting of cyanobacteria, lichen, and mosses) are ubiquitous to desert ecosystems, play an array of ecological roles, and display a strong sensitivity to environmental changes. Crust mosses are particularly responsive to changes in precipitation, and exhibit rapid declines in biomass and mortality following the addition of small rainfall events. Further, loss of the moss component in biocrusts leads to declines in crust structure and function. In this study, we sought to understand the physiological responses of the widespread and often dominant biocrust moss *Syntrichia caninervis* to alterations in rainfall. Moss samples were collected during all four seasons and exposed to two rainfall event sizes and three desiccation period (DP) lengths. A carbon balance approach based on single precipitation events was used to define the carbon gain or loss during a particular hydration period. Rainfall event size was the strongest predictor of carbon balance and the largest carbon gains were associated with the largest precipitation events. In contrast, small precipitation events resulted in carbon deficits for *S. caninervis*. Increasing the length of the DP prior to an event resulted in reductions in carbon balance, likely because of the increased energetic cost of hydration following more intense bouts of desiccation. The season of collection (i.e., physiological status of the moss) modulated these responses, and the effects of DP and rainfall on carbon balance were different in magnitude (and often sign) for different seasons. In particular, *S. caninervis* displayed higher carbon balances in the winter than in the summer, even for events of identical size. Overall, our results suggest
Annual carbon balance and survivorship in biocrust mosses is largely precipitation-driven, and because of the role mosses play in biocrusts, changes in intra-annual precipitation patterns can have implications for hydrology, soil stability, and nutrient cycling in dryland systems.

**Introduction**

Global climate models predict that anthropogenic warming will lead to a modest increase in mean annual rainfall within the next century (Folland et al. 2001; Stainforth et al. 2005; Held & Soden 2006; Meehl et al. 2007). However, alterations in intra-annual precipitation patterns (such as event magnitude, event frequency, and seasonality of rainfall) are predicted to change significantly for many terrestrial regions as well (Hereford et al. 2002; Meehl et al. 2007; Sun et al. 2007). On regional scales, the magnitude and direction of precipitation change is still not completely agreed upon (Meehl et al. 2007, Zhang 2007). Uncertainties are particularly apparent in predictions for arid regions (Weltzin et al. 2003), and in the case of Southwestern North America, are due to limited data and complex terrain (Hereford et al. 2002; Higgins et al. 2007).

In arid regions, water strongly limits primary productivity (Went, 1949; Noy-Meir 1973; Melillo et al. 1993; Whitford 2002; Housman et al. 2006a), and the majority of precipitation events occur as small (<5 mm) short duration events (Sala and Lauenroth 1982). Therefore, the majority of arid ecosystems exhibit a pulse-dynamic response to rainfall, where individual events offer brief pulses of resource availability for desert organisms (Huxman et al. 2004a,b) and individual rainfall events can exert a strong influence on structure and function of plant and microbial communities (Austin et al. 2004; Ogle & Reynolds, 2004; Cable et al. 2008). Some desert organisms will be relatively resilient to changes in precipitation regime because they are
adapted to extreme environments (Kleidon et al. 2000; Ward 2009). Others, already living near the physiological limits of tolerance, are likely to be particularly sensitive to changes in the features of individual events that characterize intra-annual precipitation patterns (Sala and Lauenroth 1982; Weltzin et al. 2003; Schwinning et al. 2004; Williams et al. 2006; Potts et al. 2006), regardless of the magnitude and direction of such changes.

A feature of most arid ecosystems is the presence of a biocrust living at the soil-atmosphere interface. Biocrusts are communities of cyanobacteria, lichens, and mosses (Rosentreter et al. 2007) that function collectively to increase soil stability (Belnap 2003), water holding capacity (Belnap & Lange 2001; Belnap 2003), nutrient availability (Harper & Pendelton 1993; Evans & Belnap 1999), elemental cycling (Housman et al. 2006b; Ebert et al. 2009; Grote et al. 2010), and seedling establishment of grasses and shrubs (Belnap et al. 2001; Li et al. 2005). Despite their low biomass relative to other primary producers, crust organisms have a disproportionally large influence on biogeochemistry in arid regions. Constituents of biocrusts are also extremely sensitive to environmental alteration (Belnap & Eldridge 2001; Belnap 2003; Barger et al. 2006; Ustin et al. 2009; Budel et al. 2009), and declines in diversity and biomass of crust constituent species can result in substantially reduced structural integrity of soil crusts (Belnap & Eldridge 2001) and altered soil nutrient availability (Yeager et al. 2004; Wu et al. 2009).

Biocrust mosses can play a large role in crust structure and function, and appear to be particularly sensitive to environmental change (Belnap & Eldridge 2001). For example, exposure to an increase in the frequency of small summer rainfall events has been shown to cause >90% mortality in mosses during a single growing season, and this loss of mosses led to dramatic declines in crust structure and altered biogeochemical cycling in soils (Reed et al. in press). The
sensitivity of mosses to changes in precipitation is related to the way these organisms respond to rainfall. Mosses are poikilohydric organisms for which the water content of cells is in equilibrium with the surrounding environment. Possessing neither vascular tissue nor roots, mosses are decoupled from belowground water sources, and cellular water status is determined by hydration from precipitation pulses. Physiological activity is limited to periods of hydration initiated by rainfall events, and, outside of these discrete periods of hydration, desert mosses exist in a desiccated state of suspended metabolism. Therefore, performance and growth during individual hydration events is a strong determinant of long-term persistence.

For biocrust mosses, each rainfall event induces a wet-dry cycle that is characterized by periods of net carbon (C) gain or loss (Fig. 3.1). The onset of hydration is characterized by a period of net C loss due to the respiratory costs of repair and reinstatement of metabolism. After the initiation of photosynthesis, C fixation rates surpass respiration leading to net C gains during a period of hydration. As shoots dry, a phase of C loss occurs as photosynthesis ceases and tissues are preserved for desiccation (Mishler & Oliver 2009). Therefore, in arid and semi-arid environments, each hydration event represents a balance between energy spent during rehydration and energy gained while hydrated, and ultimately results in an overall C balance that is either positive or negative. When compounded over longer time scales, C balances from individual events are directly related to long-term growth and survival.

Although intra-annual rainfall parameters such as event magnitude, frequency, and seasonality likely exert a considerable influence on the physiological ecology and survivorship of biocrust mosses, no work to date has examined how changes in such precipitation patterns influence C balance. Rainfall magnitude is typically a driver of resource availability and growth in pulse-dynamic systems and is very likely to exert a strong control on the C balance in desert
mosses. Similarly, rainfall frequency is likely to influence C balance because the timing of events is directly related to length of desiccation period between events, which is probably related to recovery cost. Further, the seasonality of rainfall likely modulates C balance because poikilohydric plants may vary in their ability to respond to hydration depending upon the season.

Figure 3.1: 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).

The objectives of this study were, first, to understand to what extent three intra-annual precipitation parameters (event magnitude, event frequency, and seasonality of precipitation) individually and synergistically influence C balance in the widespread and often dominant biocrust moss *Syntrichia caninervis*; and second, to measure and compare C balance values in *S. caninervis* across different rainfall event magnitudes, frequencies, and over the four seasons of
the year. Collectively, this information will allow for robust predictions of biocrust moss survivorship under future climate scenarios. We hypothesized that (1) rainfall magnitude would be the strongest predictor of C balance in *S. caninervis* because level of hydration is directly proportional to physiological activity; and (2) rainfall frequency and seasonality of rainfall would act as secondary controls on C balance. Further, we predicted that C balance values would be highest under conditions of large rainfall amounts occurring in high frequency during the winter months.

**Methods**

**Sample collection and rainfall treatments**

We used *S. caninervis* as a system in this study because: (a) it is the dominant biocrust moss on the Colorado Plateau; (b) it is widely distributed throughout drylands in the Northern hemisphere (Flowers 1973; Kramer 1980); and (c) due to morphological similarities and taxonomic relationships with other dryland mosses, physiological responses to precipitation are likely representative of other biocrust moss taxa. Mosses used in this study were collected from intact biocrusts approximately eight km South of Moab, UT on the Colorado Plateau. Here, climatic conditions include mean annual rainfall of 300 mm, mean maximum air temperatures for summer and winter of 35 and 10 °C, respectively, and an average elevation of 1550 m. Mosses were collected during spring (April 2009, May 2010) summer (July 2009, 2010) fall (October 2008, 2010) and winter (January 2008, 2009, 2010) by inserting a 2.5-cm gold-plated copper pipe cap into the moss mat and extracting a 2.5-cm diameter disc containing colonies of shoots. Due to travel logistics and available measurement time, multiple years were used for
sampling. Differences among years were tested and found to not be significant so mosses collected from multiple years in the same season were pooled for further analysis. After collection, each moss disc was rinsed of excess soil using DI water such that only soil contributing to the structural integrity of the colony remained. Discs were placed in individual 2.5-cm gold-plated copper pipe caps lined with filter paper (Whatman No. 4, Whatman/GE Healthcare corp.) and placed in labeled Petri dishes and immediately placed in a growth chamber (Heraeus Revco BK 3 m³ No. BOD10A15, Thermo Fisher Sci., Inc., Waltham, MA). Groups of four caps were necessary for reasonable gas exchange measurements and were permanently grouped as one experimental unit for all measurements. Therefore, the total number of samples (i.e., groups of four discs) were 27, 24, 22, and 35 collected in spring, summer, fall, and winter, respectively, for a total of 108.

The primary factor influencing gas exchange in mosses is degree and length of hydration. Although desert mosses typically regain physiological and photosynthetic function within minutes of hydration (Proctor & Smirnoff 2000), field collected samples often do not exhibit statistically comparable carbon balances without some standardized hydration period prior to analysis (Coe, unpublished data; Stark, L., Belnap, J., personal communication). Therefore, in order to make comparable gas exchange measurements we standardize samples before the implementation of treatments: all samples were held in a growth chamber for five days (Daytime conditions: Photosynthetically Active Radiation (PAR) ~250 μmol m⁻² sec⁻¹, 16.5 °C, RH ~ 45%, and a day length of ~ 9h; Nighttime conditions: PAR = 0, 14.5 °C and RH artificially increased by slightly ajar Petri lids placed over the samples) and watered 5 mm twice per day.

To simulate differences in rainfall frequency in the laboratory, we used the length of dry period prior to a hydration event (referred to as desiccation period; DP) as a proxy. Samples were
exposed to either a 1-day, 5-day, or 10-day DP (n = 28, 41, and 39, respectively) prior to the rainfall event used for analysis. To ensure moss dried to the desiccated state at a natural rate, growth conditions identical to those used during pre-treatment were maintained during drying, only watering was omitted. These conditions were held constant during experimental desiccation periods.

To quantify C balance of *S. caninervis* as a function of rainfall event magnitude, samples were given either an average-size rainfall event (5 mm; n = 57) or a ¼ average size event (1.25 mm; n = 51). These event magnitudes were determined following analysis of four years of climate data from Southeastern Utah (EPA hourly climate records from CAN 407 station, 2005-2009). Events were applied directly to moss using a dropper, and samples were stored in the growth chamber at constant humidity between gas exchange measurements.

**Gas exchange measurements and carbon balance analysis**

To measure the per hydration event C balance of moss, we used photosynthetic gas exchange analysis to measure net C fixation to and from moss shoots over the course of an entire dry-wet-dry cycle. Approximately 10 minutes following application of the simulated rainfall event, samples of *S. caninervis* were placed in a custom nickel-plated cuvette attached to a gas-exchange system that consisted of a gas mixer (Environics Series 4000, Environics Inc., Tolland, CT), humidifier, and an infrared gas analyzer (Li-6262; Li-COR Inc. Lincoln, Nebraska) and was run with the program LabView (National Instruments Corporation, Houston, TX). Internal conditions of the cuvette were maintained at ~ 800 µmol m$^{-2}$ s$^{-1}$ PAR and ~ 65% relative humidity. The cuvette was temperature controlled at 17±0.5 °C using a water bath (Fisher Isotemp 105, Thermo Fisher Scientific, Inc., Waltham, MA) and a coil system built into the
cuvette. We used thermocouples to continuously monitor air and moss temperature within the cuvette over the course of the experiment (these tracked each other and never differed by >1 °C). Net C fixation was measured at hourly intervals (30 min intervals at the onset of hydration when fluxes were most dynamic) until samples had dried completely and physiological activity ceased. Samples were kept in the growth chamber between measurements, and sample weight was measured prior to and following rain events to ensure samples were dry.

Net C fixation curves were constructed using measurements over the course of dry-wet-dry cycles from simulated rainfall events. The integrated area under these curves was used to estimate total C balance for the event.

Respiration analysis

We also measured dark respiration at all net C fixation measurement intervals by extinguishing the light source, covering the cuvette with an opaque cloth and allowing samples to equilibrate for 5-10 min in the dark prior to measurement. We constructed respiration curves in the manner described above for C balance curves, and compared integrated respiration values for individual events across treatments.

Statistical Analysis

In addressing the first objective in this study (understanding how rainfall amount, DP, and season influence carbon balance and respiration in *S. caninervis*), we used linear models to determine relationships between response and predictor variables. Data for C balance and respiration were log transformed prior to these analyses to meet assumptions of normality. Although we had no *a priori* reason to suspect inter-annual variability in responses or altered
results based on small variation in seasonal sample numbers, we performed analyses to
determine whether to include year (2008, 2009, 2010) as a fixed factor in our model and to
examine the influence of sample size variation in seasonal responses. Inclusion of year did not
significantly improve fit of C balance models and only improved Akaike Information Criterion
(AIC) scores by <1 point for respiration models, therefore we treated year as a random effect in
our analyses. Additionally, variation in samples sizes across seasons did not influence
conclusions drawn from results, therefore we included all collected samples in our analyses.

In order to determine which combinations of our three predictor variables (rainfall
amount [referred to in models as ‘rain’], DP, and season) and their interactions were best able to
account for the variance observed in C balance and respiration, we developed a set of candidate
models using a full factorial framework, with the explicit goal of selecting the model that best
represented trends in data. Our model selection criteria included AIC score analysis (a method
for model selection that takes into account both likelihood and parsimony) and an $r^2$ selection
method (based on the ability of models to explain the largest amount of variance in data).
Additionally, we used partial model residuals to determine the amount of variation explained by
each of the continuous predictors (rain and DP) after accounting for the effects of the other two
variables.

To understand the influence of season as a categorical modulator of C balance and
respiration, we analyzed these dependent variables as a function of rain and DP for each season
individually. We produced equations for linear models of C balance and respiration for each
season. Using differences in parameter estimates for the effects of rain, DP, and rain x DP
interactions, we determined how these factors acted differently to influence C balance and
respiration over the course of the year.
To address the second main objective in this study (comparing levels of carbon balance and respiration within different environmentally relevant treatment categories), we employed an analysis of variance (ANOVA). Our data, although continuous, were collected in a manner that allowed us to use a categorical ANOVA framework to examine differences in C balance and respiration values in *S. caninervis* across the four seasons, three desiccation periods, and two rainfall amounts. Additionally, we used Tukey’s HSD tests to examine differences in mean C balance and respiration values within DP and rainfall amount categories for each season. ANOVA and linear model construction were performed using JMP v. 9 (SAS Institute, Inc. Cary, NC), and models for partial residuals were completed using the open source platform *R* (Comprehensive R Archive Network; see cran.r-project.org).

**Results**

*Carbon Balance*

Increasing precipitation magnitude resulted in higher C balances following events, and increasing DP length resulted in a reduction in C balance during a subsequent event (Table 3.1). On average, C balance in *S. caninervis* changed from positive to negative when the event size decreased from average to ¼ average amounts (Table 3.1, Fig. 3.2a). Average C balance values decreased significantly as DP was increased from 1 day to 10 days, and exhibited a sign change between 5 and 10 days from weakly positive values (1.65 ±2.01) to substantially negative values (-6.59±2.06; *F*$_{2, 105}$ = 20.99; *P*<0.0001) (Table 3.1, Fig. 3.2a). Carbon balance also exhibited significant differences across seasons (*F*$_{3, 104}$ = 6.04; *P*<0.05) with the lowest values observed in
fall and summer (-2.90±2.71 and 0.47±2.61 µmol m⁻², respectively) and values up to 10 times higher in magnitude occurring in winter (10.05±2.71 µmol m⁻²; Table 3.1).

**Table 3.1:** Average carbon balance and respiration (µmol m⁻²±SE) of *Syntrichia caninervis* under different rainfall amounts, desiccation periods, and seasons (n=117 total). Different letters within treatments represent significant differences (P<0.05) in carbon balance and respiration as determined by 1-way ANOVAs and Tukey’s HSD tests within treatment categories.

<table>
<thead>
<tr>
<th>Rainfall Amount (mm)</th>
<th>Carbon Balance (µmol m⁻²)</th>
<th>Respiration (µmol m⁻²)</th>
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</thead>
<tbody>
<tr>
<td>5</td>
<td>11.41 ± 1.71 a</td>
<td>-30.06 ± 0.93 a</td>
</tr>
<tr>
<td>1.25</td>
<td>-5.33 ± 1.80 b</td>
<td>-7.79 ± 0.98 b</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Desiccation Period (days)</th>
<th>Carbon Balance (µmol m⁻²)</th>
<th>Respiration (µmol m⁻²)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>14.05 ± 2.41 a</td>
<td>-16.04 ± 1.31 a</td>
</tr>
<tr>
<td>5</td>
<td>1.65 ± 2.01 b</td>
<td>-16.29 ± 1.09 a</td>
</tr>
<tr>
<td>10</td>
<td>-6.59 ± 2.06 c</td>
<td>-24.45 ± 1.12 b</td>
</tr>
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<table>
<thead>
<tr>
<th>Season</th>
<th>Carbon Balance (µmol m⁻²)</th>
<th>Respiration (µmol m⁻²)</th>
</tr>
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<tbody>
<tr>
<td>Spring</td>
<td>4.54 ± 2.47 a</td>
<td>-12.66 ± 1.34 a</td>
</tr>
<tr>
<td>Summer</td>
<td>0.47 ± 2.61 a</td>
<td>-13.66 ± 1.42 a</td>
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<tr>
<td>Fall</td>
<td>-2.90 ± 2.71 a</td>
<td>-24.83 ± 1.48 b</td>
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<tr>
<td>Winter</td>
<td>10.05 ± 2.17 b</td>
<td>-24.56 ± 1.18 b</td>
</tr>
</tbody>
</table>

In the case of C balance, our best model was the one considering all three variables (rain, DP, and season) and the interactions between them, which explained 76% of the variance in C balance (P <0.0001; Table 3.2). Partial residual analyses indicated that rain was the most important predictor of C balance: rain amount accounted for 79% of variance in C balance after DP and season were accounted for (Table 3.2). On the other hand, DP only explained 17% of variance in C balance after rain amount and season were taken into account (Table 3.2). Linear model equations for C balance had strong predictive power for all seasons, with r²adj values ranging from 0.59 to 0.79 (P<0.0001; Table 3.3). These models differed across seasons of the year due to differing coefficients for the predictor variables DP, rain, and the DP x rain interaction. Rainfall event magnitude significantly and positively influenced C balance in every season, DP negatively influenced C balance in fall and winter. The rain x DP interaction was significant for C balance in summer (where it was positive) and fall (where it was negative).
Table 3.2: Results from linear models using season, desiccation period (DP), rainfall amount (rain), and the interactions between these parameters, in a full-factorial design to explain variance in (A) C balance, and (B) respiration in Syntrichia caninervis. Models using interactions between factors also include all individual factors. Adjusted $r^2$ values and Akaike Information Criterion (AIC) scores show relative model fit, and partial residual (PR) adjusted $r^2$ values for rain and DP show variance explained by predictors after accounting for the effects of the other two variables.

### A) Carbon Balance

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>$F$</th>
<th>$P$</th>
<th>$r^2_{adj}$</th>
<th>AIC</th>
<th>PR $r^2_{adj}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Season</td>
<td>3</td>
<td>1.593</td>
<td>13.55</td>
<td>&lt;0.0001</td>
<td>0.01</td>
<td>116.16</td>
<td>-</td>
</tr>
<tr>
<td>DP</td>
<td>1</td>
<td>3.237</td>
<td>82.66</td>
<td>&lt;0.0001</td>
<td>0.18</td>
<td>93.91</td>
<td>0.173</td>
</tr>
<tr>
<td>Rain</td>
<td>1</td>
<td>3.131</td>
<td>79.93</td>
<td>&lt;0.0001</td>
<td>0.24</td>
<td>85.53</td>
<td>0.791</td>
</tr>
<tr>
<td>Season x DP</td>
<td>3</td>
<td>3.476</td>
<td>29.58</td>
<td>&lt;0.0001</td>
<td>0.41</td>
<td>65.21</td>
<td>-</td>
</tr>
<tr>
<td>Season x Rain</td>
<td>3</td>
<td>0.97</td>
<td>8.23</td>
<td>&lt;0.0001</td>
<td>0.27</td>
<td>85.33</td>
<td>-</td>
</tr>
<tr>
<td>DP x Rain</td>
<td>1</td>
<td>0.082</td>
<td>2.10</td>
<td>0.1504</td>
<td>0.43</td>
<td>57.58</td>
<td>-</td>
</tr>
<tr>
<td>Season x DP x Rain</td>
<td>3</td>
<td>0.801</td>
<td>6.82</td>
<td>&lt;0.0001</td>
<td>0.76</td>
<td>19.47</td>
<td>-</td>
</tr>
</tbody>
</table>

### B) Respiration

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>$F$</th>
<th>$P$</th>
<th>$r^2_{adj}$</th>
<th>AIC</th>
<th>PR $r^2_{adj}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Season</td>
<td>3</td>
<td>13.00</td>
<td>47.19</td>
<td>&lt;0.0001</td>
<td>0.12</td>
<td>265.88</td>
<td>-</td>
</tr>
<tr>
<td>DP</td>
<td>1</td>
<td>4.18</td>
<td>45.57</td>
<td>&lt;0.0001</td>
<td>0.04</td>
<td>272.95</td>
<td>0.519</td>
</tr>
<tr>
<td>Rain</td>
<td>1</td>
<td>49.47</td>
<td>538.59</td>
<td>&lt;0.0001</td>
<td>0.64</td>
<td>166.53</td>
<td>0.823</td>
</tr>
<tr>
<td>Season x DP</td>
<td>3</td>
<td>3.28</td>
<td>11.91</td>
<td>&lt;0.0001</td>
<td>0.18</td>
<td>262.77</td>
<td>-</td>
</tr>
<tr>
<td>Season x Rain</td>
<td>3</td>
<td>0.43</td>
<td>1.55</td>
<td>0.207</td>
<td>0.79</td>
<td>116.80</td>
<td>-</td>
</tr>
<tr>
<td>DP x Rain</td>
<td>1</td>
<td>0.003</td>
<td>0.03</td>
<td>0.865</td>
<td>0.68</td>
<td>157.35</td>
<td>-</td>
</tr>
</tbody>
</table>

**Respiration**

On average, respiration during periods of hydration increased as a function of rainfall amount (Table 3.1, Fig. 3.2b). Respiration decreased when the event size was reduced, and values following 1.25 mm rainfall events were ~25% of the magnitude of those following 5 mm events ($P<0.05$), Table 3.1, Fig 3.2b). Respiration did not differ between 1 and 5 day DP treatments but did increase by ~50% when DP was increased to 10 days ($F_{2, 105} = 6.34; P<0.0001$) Table 3.1, Fig. 3.2b). Average respiration values did not differ between spring and summer or between fall and winter, but did significantly differ *between* these groups ($F_{3, 104} = 10.21$;
with fall and winter samples showing approximately a two-fold increase in respiratory C losses (Table 3.1).

**Table 3.3:** Equations, $r^2_{adj}$ values, and $P$ values for linear models describing C balance and respiration (both log transformed) as a function of rain, DP, and the rain x DP interaction for spring, summer, fall, and winter. Boldface indicates terms with significant effects at the $\alpha=0.05$ level.

### A) Carbon Balance

<table>
<thead>
<tr>
<th>Season</th>
<th>Linear Model Equation</th>
<th>$r^2_{adj}$</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring</td>
<td>C Balance = 3.34 – 0.01(DP) + 0.10(Rain) – 0.004 (DP*Rain)</td>
<td>0.63</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Summer</td>
<td>C Balance = 3.26 + 0.01(DP) + 0.06(Rain) + 0.01(DP*Rain)</td>
<td>0.59</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fall</td>
<td>C Balance = 3.75 – 0.12(DP) + 0.06(Rain) – 0.03 (DP*Rain)</td>
<td>0.78</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Winter</td>
<td>C Balance = 3.68 – 0.01(DP) + 0.17(Rain) – 0.003 (DP*Rain)</td>
<td>0.79</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

### B) Respiration

<table>
<thead>
<tr>
<th>Season</th>
<th>Linear Model Equation</th>
<th>$r^2_{adj}$</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring</td>
<td>Respiration = 1.01 + 0.01(DP) + 0.40(Rain) + 0.003 (DP*Rain)</td>
<td>0.92</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Summer</td>
<td>Respiration = 0.30 + 0.12(DP) + 0.41(Rain) + 0.014 (DP*Rain)</td>
<td>0.92</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fall</td>
<td>Respiration = 1.33 + 0.10(DP) + 0.34(Rain) + 0.004 (DP*Rain)</td>
<td>0.84</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Winter</td>
<td>Respiration = 1.88 + 0.01(DP) + 0.34(Rain) + 0.01 (DP*Rain)</td>
<td>0.77</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

In the case of respiration, our best model was also the one using all three factors (rain, DP and season) as well as the interactions between them. This model was able to account for 88% of variance in respiration, but was not significant at the $\alpha=0.05$ level ($P = 0.257$; Table 3.2). Rainfall amount was a strong predictor of respiration, and accounted for 64% of variance in the single factor model ($P<0.0001$). Partial residual analyses showed that rain was the most important predictor of respiration: rain amount accounted for 82% of variance in respiration after accounting for DP and season as predictors (Table 3.2). Length of DP was also a good predictor of respiration, however, and explained 52% of variance after rainfall and season were accounted for (Table 3.2).
Figure 3.2: (A) Carbon balance (μmol m⁻²) and (B) respiration (μmol m⁻²) of *Syntrichia caninervis* subjected to either average (5 mm) or ¼ average (1.25 mm) rainfall amounts, following a 1-day (white bars), 5-day (gray bars), or 10-day (black bars), desiccation period (DP) and collected in Spring, Summer, Fall, and Winter. Values presented are averages (± 1 SE) of five replicates each (n=108 total) and different letters represent significant differences (P<0.05) in carbon balance and respiration within each season after LS means contrasts using Tukey’s HSD analyses.
Seasonal linear model equations for respiration had very strong predictive power, and $r^2_{adj}$ values ranged from 0.77 (winter) to 0.92 (spring and summer; Table 3.3). Respiration was positively influenced by rain and DP over all seasons, yet this effect was only significant for DP in fall and winter (Table 3.3).

**Discussion**

The intra-annual precipitation parameters rainfall magnitude, frequency, and seasonality interact to strongly influence C balance in *S. caninervis*, suggesting that alterations in one or more of these parameters can have a large effect on the physiological ecology and survival of this biocrust species under future climate scenarios.

Rainfall magnitude was positively correlated with C balance, and reducing rainfall event size from 5 to 1.25 mm resulted in both a significant reduction in C balance and, on average, a shift from C gains to C losses. This observation was driven by small events hydrating moss shoots for shorter amounts of time compared to moss shoots experiencing larger precipitation events. Small rainfall events caused moss to either stay in the active, photosynthetic phase of the wet-dry cycle (Phase B in Fig. 3.1) for a reduced period, or not enter this phase at all. In contrast, larger (5 mm) precipitation events caused mosses to enter and remain within the photosynthetic phase for longer, resulting in C gains sufficient to compensate for the initial phase of respiratory C loss (Phase A in Fig. 3.1), and leading to a positive C balance following each event. Mosses receiving 1.25 mm events, on the other hand, produced diminutive C gains that were outweighed by respiratory losses, causing C deficits.
Our results suggest that there is a minimum rainfall event size required to sustain a positive C balance in *S. caninervis*, and that this value is between 1.25 and 5 mm. Rainfall events below 2 mm may not allow mosses to fully hydrate (Stark 2005) and can lead to pigment degradation if repeated (Barker et al. 2005), supporting the notion of a minimum event size for net C gain in these organisms. Given that >70% of events in drylands are < 5 mm effective size (Sala & Lauenroth 1982; Loik et al. 2004; Reynolds et al. 2004), small carbon deficits may actually be quite common for these organisms, and desert mosses are likely reliant on the presence of a few larger events each year for long term carbon gain.

Reducing rainfall frequency resulted in a reduction in C balance of *S. caninervis* during a subsequent rainfall event. Because respiration was negatively correlated with rainfall frequency and increased significantly as DP was lengthened from 1 day to 10 days, it is probable that increasing the dry interval between rainfall events creates conditions where recovery carries a higher C cost and is more metabolically expensive. Higher respiratory losses following longer desiccation periods are likely more difficult to overcome by energetic gains from C fixation later.

We show here that when DP length increases from 5 to 10 days, C losses from respiration increase by ±50% during a subsequent rainfall event, and mosses transition from net C gains to C losses (Table 3.1). These data suggest that above a threshold desiccation length (between 5 and 10 days) the respiratory cost of hydration increases to such an extent that mosses enter C deficit from a subsequent event. Intensity of desiccation may increase with desiccation length in these environments, and recovery from desiccation bouts of higher intensity or longer duration requires more time under favorable, hydrated conditions (Hinshiri & Proctor 1971; Proctor & Smirnoff 2000; Proctor et al. 2007). Our results suggest that more energy is also required for recovery from longer periods without rain, and that unless rainfall events are large enough to
provide compensatory gains from fixation, DPs ≥10 days will consistently result in C deficit in *S. caninervis*.

We also demonstrate that responses of C balance in *S. caninervis* to rainfall amount and frequency are modulated by season. To illustrate, event magnitudes that produced positive C balances in winter often resulted in negative C balances in summer, in spite of identical measurement and pre-treatment conditions (Fig. 3.2a). Such a result may be related to fluctuations in energetic allocation to photosynthesis (and associated enzyme activity levels) over the course of the year, which is known to occur in perennial plants (Katahata et al. 2007; Koh, 2009; Kuptz et al. 2011). The Colorado Plateau receives the majority of its annual rainfall in the cool season from October to April and to a certain extent during late summer/early fall monsoon (Hereford et al. 2002). Our results suggest that the largest range and highest maximum C balance values occur during the winter and fall (Fig. 3.2a), supporting the notion of upregulated physiological response to rainfall during these times of year. Further, in contrast to most summer months when high temperatures and evaporation rates constrain the effective size of rainfall events (and water that does arrive in the form of precipitation is often likely to disappear rapidly from the system), conditions of increased humidity and reduced evaporation present in the winter result in water from precipitation events that is likely to remain available in the environment longer. Higher energetic investment in response to winter precipitation would be favorable in this environment, and while we can only speculate, our results would be consistent with *S. caninervis* exhibiting such a strategy.

The relative influence of DP on C balance and respiration also varied over the course of the year, demonstrating that changes in the timing of events in different seasons can have markedly different effects. Reducing rainfall frequency by 20%, for example, is likely to cause a
reduction in C balance and an increase in respiration that is > 10-fold greater in the fall than in the spring (Table 3.3). These relationships may result from fluctuations in energy reserves in mosses that are related to seasonal aspects of carbon assimilation. Further, in addition to constitutive protection and repair, some desert mosses possess inducible aspects of desiccation tolerance (Oliver et al. 2000) based on environmental conditions that influence rate of recovery of photosynthesis (Mayaba et al. 2001). If present in S. caninervis, such mechanisms operating as a function of seasonal conditions could influence the balance between C lost and C gained during precipitation events, ultimately resulting in variation in response to rainfall over the course of the year.

In general, climate change driven shifts in precipitation are uncertain for arid regions (Weltzin et al. 2003). However, we can couple the physiological responses observed in this study with modeled future precipitation patterns to form qualitative and quantitative projections under different precipitation scenarios (summarized in Table 3.4). For the Southwestern United States, most climate models predict an overall drying trend in mean annual rainfall (Hereford et al. 2002; Seager et al. 2007) caused by reductions in winter precipitation by ~10% (Meehl et al. 2007; Seager & Vecchi 2010) and an overall shift throughout the year to larger events interspersed with longer intervening drought periods (Meehl et al. 2007; Schoof et al. 2010; Scenario 1 in Table 3.4). Based on the results from this study, a low frequency rainfall scenario such as this (increasing average desiccation period by 1-3 days) would cause ~10-30% reduction in C balance of S. caninervis, especially if the drying trend also includes a reduction in event magnitude. Corresponding reductions in biomass would likely occur if C deficits are compounded over longer time scales. Further, biocrust mosses such as S. caninervis likely are reliant on C gains from precipitation events in the cooler months to compensate for losses.
occurring during warmer periods, and predicted loss of winter precipitation coupled with longer
droughts may result in dramatic (30-40%) annual reductions in cumulative C balance.
Table 3.4: Qualitative and quantitative predictions for annual C balance of desert mosses based on four precipitation scenarios for the next century: two broad sets of projections (Scenarios 1-2), and two individual General Circulation Models (CGMs) with strong predictive power for Southwestern North America (Scenarios 3-4). Components of each scenario were summarized based on available literature, and predictions for C balance were made based on relationships with precipitation parameters and seasonal linear models from this study.

<table>
<thead>
<tr>
<th>Estimated Components</th>
<th>Annual Moss Carbon Balance Predictions</th>
<th>Integrative Prediction</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Scenario 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean annual precip 6-18%↓</td>
<td>10-20% reduction</td>
<td>Cumulative reduction</td>
<td>Hereford et al. 2002</td>
</tr>
<tr>
<td>Winter precip 10%↓</td>
<td>8-15% reduction</td>
<td>(driven by mean annual and winter precipitation)</td>
<td>Meehl et al. 2007</td>
</tr>
<tr>
<td>10-20% larger events</td>
<td>5-50% increase after these events, depending on season</td>
<td></td>
<td>Seager et al. 2007</td>
</tr>
<tr>
<td>15-30% longer droughts</td>
<td>5-25% decrease after these DPs, depending on season</td>
<td></td>
<td>Seager &amp; Vecchi 2010</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Schoof et al. 2010</td>
</tr>
<tr>
<td><strong>Scenario 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Annual precip frequency 10-17%↑</td>
<td>winter: 12-15% increase</td>
<td>No change or cumulative increase; cumulative decrease if summer frequency increases proportionally more</td>
<td>Higgins et al. 2007</td>
</tr>
<tr>
<td>Intensity of monsoonal rainfall↑</td>
<td>summer: 5-10% decrease small increase depending on event size</td>
<td></td>
<td>Schoof et al. 2010</td>
</tr>
<tr>
<td><strong>Scenario 3</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean winter precip 3-10%↑</td>
<td>5-20% increase</td>
<td>Cumulative reduction or no change, magnitude dependent on rainfall frequency</td>
<td>GCM: Hadgem1</td>
</tr>
<tr>
<td>Mean spring precip 10-25%↓</td>
<td>5-10% decrease</td>
<td></td>
<td>Garfin et al. 2010</td>
</tr>
<tr>
<td>Mean summer precip 30%↑</td>
<td>0-10% increase, depending on event sizes</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Scenario 4</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean winter precip 3-10%↑</td>
<td>5-20% increase</td>
<td>Cumulative reduction, magnitude dependent on rainfall frequency</td>
<td>GCM: Mpi_echam5</td>
</tr>
<tr>
<td>Mean spring precip 10-25%↓</td>
<td>5-10% decrease</td>
<td></td>
<td>Garfin et al. 2010</td>
</tr>
<tr>
<td>Mean summer precip 25-30%↓</td>
<td>5-20% decrease</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
In contrast to the modeling studies outlined above, historical analyses suggest that a ~5% increase in rainfall frequency has occurred over the last five decades in this region (Higgins et al. 2007). Several models predict a similar trend to continue, and forecast an additional 10-15 wet days per year (representing a 10-17% increase in rainfall frequency for the Colorado Plateau) and a slight increase in intensity of monsoonal events (Higgins et al. 2007; Schoof et al. 2010; Scenario 2 in Table 3.4). This higher frequency scenario would result in favorable growth conditions in the winter months for *S. caninervis*, as even small events can produce carbon gains during this time of year, particularly if they are separated by short desiccation periods. The same scenario in the summer, however, is likely to cause repeated C deficits and cumulative losses over the course of the season. This particular mechanistic response is likely to have caused the observed >90% mortality of mosses in experiments conducted on the Colorado Plateau (Reed et al. in press).

Finally, there are two individual General Circulation Models (GCMs) with strong predictive power for precipitation patterns in Southwestern North America: Hadgem1, and Mpi_echam5 (Garfin et al. 2010; Scenarios 3 and 4, respectively, in Table 3.4). These models are somewhat limited in that they do not take into account changes in precipitation frequency, yet both forecast that in the next century mean winter precipitation will increase and mean spring precipitation will decrease. Because precipitation is a very strong predictor of C balance in *S. caninervis*, these conditions would result in increases in cumulative C balance in desert mosses during the winter, and reductions during the spring. Where these two models differ is in projections for summer rainfall: Hadgem1 forecasts small increases in summer rainfall, which is likely to produce either diminutive gains or even C losses in mosses, while Mpi_echam5 predicts reductions in rainfall, a scenario very likely to result in cumulative C losses. In spite of
contrasting predictions for summer precipitation, integrative projections from our data suggest cumulative C losses for biocrust mosses under both of these scenarios in the next century.

In conclusion, the work presented here suggests that (1) intra-annual precipitation parameters are strong predictors of C balance in desert mosses and should be considered in future efforts working to understand biocrust response to climate change; (2) surpassing minimum rainfall magnitudes and maximum desiccation periods can mean the difference between C gains and C deficits in biocrust mosses, and such thresholds may function to constrain biocrust functionality under future climate scenarios; and (3) responses to rainfall magnitude and frequency differ based on season. Despite conventional thought that increased rainfall in deserts will produce positive (even linear) ecophysiological responses in desert organisms (Kawabata et al. 2001; O’Connor et al. 2001; reviewed in Ogle & Reynolds 2004), this work illustrates that actual behavior depends on how much rain falls, how often, and during what time of year. Additionally, we demonstrate that some of the most ecologically important desert organisms can respond dramatically and nonlinearly to subtler aspects of changes in precipitation.

Acknowledgements

We wish to thank the USGS technicians Hilda Smith, Adam Kind, and Phillip Adams for invaluable assistance in sample collection and pre-treatment procedures. We also thank Ed Grote for feedback, discussion of results and continued technical support with gas exchange operations. Francoise Vermelian and Steve Ellner also offered insights and advice on statistical analyses. Any use of trade names is for descriptive purposes only and does not imply endorsement by the US Government.
References


CHAPTER 4

Predictions for dryland biocrust moss performance under future precipitation scenarios
Abstract

Drylands are likely to experience shifts in intra-annual precipitation patterns over the next century, yet little is known about how these systems will respond ecologically to these changes. In particular, changes in precipitation are likely to influence persistence of soil biocrusts (assemblages of cyanobacteria, lichen and mosses). Because of the role biocrusts play in soil fertility and stability in drylands, changes in biocrust structure and function resulting from altered performance of crust members could affect dryland ecosystem function on large spatio-temporal scales. Using the biocrust moss *Syntrichia caninervis*, we constructed a predictive model for annual cumulative carbon balance (CCB; a proxy for biomass accumulation) based on meteorological data from Southwestern North America coupled with experimentally determined physiological responses to rainfall. Using the model, we explored the influence of changes in rainfall event size, frequency, seasonality, and the number of rare large precipitation events over the next 100 years. We also ran the model using four published precipitation scenarios for drylands. Reduced rainfall event sizes, increase drought duration, and combinations of these variables decreased CCB by up to 90%. Larger rainfall event sizes and increases in the number of rare large events appeared to increase CCB by up to 150% because the carbon gains from large events sustained mosses through unfavorable environmental periods. Changes in rainfall event sizes in spring and fall had the largest effect on CCB over time, suggesting seasonal redistributions of rainfall may also influence future performance. Finally, three of four precipitation scenarios predicted by climate models resulted in 30-85% reductions in CCB in the next century, suggesting that compounded effects of different types of intra-annual precipitation alteration will cause declines in performance and survival of biocrust mosses, and emphasizing
the importance of including intra-annual precipitation in models predicting dryland response to
global change.

Introduction

Increases in atmospheric greenhouse gas concentrations and subsequent warming of the
Earth’s surface has resulted in dramatic and ongoing changes in global precipitation patterns.
Alterations in mean annual rainfall are occurring across all biomes (Trenberth et al. 2003;
Stainforth et al. 2005; Alexander et al. 2006; Meehl et al. 2007; Zhang et al. 2007), and the
frequency of rare large rainfall events and droughts is increasing globally (Karl & Knight 1998;
Easterling et al. 2000; Alexander et al. 2006; Meehl et al. 2007). In addition, it is now understood
that changes to intra-annual precipitation patterns such as individual rainfall event size,
frequency at which events fall, and the seasonality of precipitation are occurring concomitantly
(Hereford et al. 2002; Alexander et al. 2006; Meehl et al. 2007; Sun et al. 2007). In some
ecosystems, such changes in intra-annual rainfall parameters may have larger effects on ecology
and biogeochemistry than changes to mean annual rainfall alone.

Drylands (semiarid and arid systems) typically exhibit a pulse-dynamic response to
precipitation where each rainfall event offers a discrete period of resource availability to
organisms (Austin et al. 2004; Huxman et al. 2004; Schwinning & Sala 2004). Therefore,
individual events and the features that define them are important for the physiological
performance of organisms as well as the overall function of dryland ecosystems (Ogle &
Reynolds, 2004; Schwinning & Sala 2004; Cable et al. 2008). While organisms living in
drylands are often stress tolerators that are resilient to fluctuating environmental conditions
(Ward 2009), changes in the features of individual rainfall events (such as magnitude and timing) is one aspect of environmental alteration to which they can be extremely responsive (Sala and Lauenroth 1982; Weltzin et al. 2003; Schwinning et al. 2004; Williams et al. 2006; Potts et al. 2006).

Soil biocrusts (communities of cyanobacteria, lichens, and mosses living at the soil-atmosphere interface) are a feature of dryland ecosystems that are essential for soil stability (Belnap 2003) and biogeochemistry (Harper & Pendelton 1993; Evans & Belnap 1999; Housman et al. 2006; Ebert et al. 2009; Grote et al. 2010), yet are also among the most responsive of communities to intra-annual precipitation change (Belnap et al. 2004; Budel et al. 2009; Ustin et al. 2009). Recent evidence suggests that mosses in particular may play an important role in both the structure and function of biocrusts, yet could be the most vulnerable member of biocrusts in the face of altered precipitation regimes (Belnap & Eldridge 2001). For example, an increased frequency of small rainfall events in the summer has been shown to cause mortality in biocrust mosses after a single growing season, with dramatic declines in overall soil structure and fertility (Reed et al. in press).

Features of individual precipitation events represent a significant point of control for biocrust mosses because of the way these poikilohydric organisms respond to individual rainfall events. Biocrust mosses are desiccation tolerant plants that only hydrate and become photosynthetically active during rainfall events. Each individual rainfall event results in an intrinsic carbon balance for the moss (Green & Sancho 2011; Coe et al. 2012), and based on the magnitude, frequency, and seasonality of rainfall, events can result in either a negative or positive carbon balance (Coe et al. 2012). Survival and persistence of mosses in the biocrust community is directly related to cumulative carbon balance (CCB) from many discrete rainfall
events over long time scales. Increases in CCB in the long-term result in sustained growth for mosses, while decreases in CCB lead to declines in biomass or mortality. Developing an understanding of how different intra-annual precipitation scenarios may operate to constrain CCB in biocrust mosses will improve overall understanding of dryland response to future precipitation scenarios.

Predictions for how rainfall event magnitude, frequency, and seasonality may change over the next century in dryland systems are uncertain and variable (Weltzin et al. 2003; Meehl et al. 2007). Previous efforts to model vegetation responses to precipitation in drylands include conceptual models using the pulse-dynamic paradigm (Noy-Meir 1973, Ogle et al. 2004) and modifications to this framework that incorporate physiological responses across plant functional types (Reynolds et al. 2004). Existing models for these responses are scarce, however, and none address the direct influences of global change. Until now, predictive models for dryland system response to changes in precipitation regime for the next century (2000-2100) have not included intra-annual precipitation factors in simulations, and have not yet included biocrust organisms.

We present here a predictive model for the performance of biocrust mosses under future precipitation scenarios, with particular attention to changes in intra-annual aspects of precipitation. As an exemplar system to address modeled effects on performance, we used the biocrust moss Syntrichia caninervis and the drylands of Western North America (one region in which S. caninervis is widespread and often dominant; Rosentreter & Belnap 2001). Our objectives were to: (1) create a baseline model for biocrust moss performance under current meteorological conditions using published physiological responses to rainfall coupled with climate records from drylands in Western North America; (2) perform a series of century-long simulations to determine which intra-annual precipitation parameters have the largest influence

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on future performance and survival in biocrust mosses; and (3) simulate and compare future
performance in biocrust mosses using four complete predicted precipitation scenarios for
Western North America.

Methods

Baseline model development

We coupled published data on the physiological responses of a biocrust moss to
alterations in precipitation event amount, timing, and seasonality (Coe et al. 2012) with
meteorological data from Southeastern Utah (EPA CAN 407 weather station) to form a baseline
model predicting annual CCB in biocrust moss. In *S. caninervis*, rainfall event-based carbon
balance is determined by the size of the rainfall event and the length of the desiccation period
(DP) occurring prior to the event, and carbon balance varies over the course of the year due to
physiological differences across seasons (Coe et al. 2012). Therefore, to develop the structure of
the model to generate CCB we used physiology-based equations derived from relationships
established by Coe et al. (2012) and calculated carbon balance as a function of (a) rainfall event
size, and (b) DP, for each season of the year. As an independent test of model performance, we
applied the carbon balance equations to rainfall event sizes and DP values that represented
historical annual precipitation conditions (based on compiled precipitation records from
Southeastern Utah). After simulating CCB in this manner for one year, values were compared to
predicted CCB values based on published annual growth rates in *S. caninervis* (Stark et al. 1998).
Second, we tested the ability of the model to correctly predict mortality in *S. caninervis* by
simulating meteorological conditions previously hypothesized to cause physiological stress and
death in this species (Barker et al. 2005). We simulated a four-year drought period where rainfall was reduced by 30%, and tested the ability of our model to project mortality. We compared the proportion of negative moss CCB values in 100 replicates after the four-year drought to the proportion of populations exhibiting bleaching and mortality in field observations published by Barker et al. (2005).

In order to use the model construct to simulate the future biological behavior of mosses, we developed a strategy to simulate future precipitation patterns. First, we determined the natural frequencies of rainfall events and DPs of particular sizes that occur during each season of the year based on records from Southeastern Utah from 2006 to 2010. From these data, we generated probability distributions for rainfall event sizes and DPs for each season. The probability of rainfall event sizes within each season was best represented by a gamma distribution. The probability of DP lengths was best represented by a gamma distribution for spring, summer and fall, and a normal distribution for winter. To produce simulations of DPs and rainfalls over the course of a year (or for multiple years) we drew values from the rainfall event and DP distributions for each season. To change simulated rainfall event sizes or DPs from our baseline model, as in the simulations described below, we altered the parameters that define the shapes of the distributions (shape and rate for the gamma distribution, and mean and standard deviation for the normal distribution) to reflect the simulated precipitation conditions.

**Simulations using individual precipitation parameters, rare large events, and projected scenarios**

To determine the relative effects of intra-annual precipitation parameters on performance of biocrust mosses in the future, we manipulated four variables (rainfall event size, DP,
seasonality, and the number of rare large events) in 38 independent 100-year simulations using the model. To simulate alterations in precipitation event sizes and the length of dry period between events, we increased and decreased annual rainfall event size and DP distributions by 10%, 20%, and 30%. We altered distribution parameters for these simulations by manipulating the original meteorological data from Southeastern Utah by the desired percent reduction or increase in event size or DP, and generated novel parameters (rate or, mean and standard deviation) to be used in distributions for each season of the year. To simulate altered rainfall event sizes and frequency in our model, we drew values from these novel distributions.

To simulate an increase in either rare large precipitation events or rare long drought periods, we ran the model where 5, 10, or 15 of the simulated rainfall events or DPs in a given year were replaced with a value four times the mean annual value.

To simulate seasonal variation in rainfall event size and DP length (and determine during what time of year rainfall alterations had the greatest effect) we manipulated rainfall event size and DP to be either +30% or -30% in a single season (Spring, Summer, Fall, Winter).

To focus our studies, we ran simulations that matched as closely as possible four future precipitation scenarios predicted by prognostic modeling: Scenario 1 (+20% DP, 10 rare large rainfalls per year; see Hereford et al. 2002, Meehl et al. 2007, Seager et al. 2007, Seager & Vecchi 2010), Scenario 2 (-20% DP, -20% rainfall event size; see Higgins et al. 2007, Schoof et al. 2010), Scenario 3 (-20% rainfall event size, 10 rare long DPs per year; see Hereford et al. 2002, Meehl et al. 2007, Seager et al. 2007, Seager & Vecchi 2010, Schoof et al. 2010), and Scenario 4 (rainfall event sizes: +10% in winter, -20% in spring, and +30% in summer; see Garfin et al. 2010, Hadgem1 GCM).
All simulations were run for 100 years, using 100 independent runs (replicates) each, and CCB was compiled at 25 year, 50 year, and 100 year time points for each run. Descriptive statistics (mean, standard deviation, 5th, 50th, and 95th percentiles) were recorded for CCB values for all time points for all simulations. Our aim in this study was to compare percent changes in CCB in each simulation with the baseline CCB for current meteorological conditions (and determine which simulated percent changes had the greatest proportional effects, compared to others). Therefore, in our analyses we focused on the percent change in CCB from baseline for each of the simulations performed.

It is likely that there are interactions between precipitation frequency, precipitation event size, and mean annual rainfall in dryland systems that can make it challenging to understand the causes of physiological responses to rainfall. For example, mean annual rainfall could be increased by (1) a decrease in average DP length annually (increasing rainfall frequency) because close temporal spacing of events could lead to an increase in the number of total events in the year, or (2) an increase in average rainfall event size. To overcome this challenge, our rainfall simulations were run with the same number of rainfall events per year, thus a decrease in average DP length over the course of a year in our model did not result in an increase in mean annual rainfall. This framework enabled us to manipulate DP independently of mean annual rainfall, and allowed us to determine the cause of changes in moss CCB on longer timescales. In terms of rainfall event size, this framework provided for environmentally relevant variation rainfall event sizes, but because the number of annual events was held constant, total annual rainfall has the possibility of being altered. This was an acceptable component of our model because, from the perspective of a moss, rainfall event size and mean annual rainfall are much more independent than for vascular plants: changes in these two variables, even if occurring in
the same direction, do not necessarily cause the same physiological effects. Overall, this method of modeling rainfall event sizes and rainfall frequency represented a compromise between running environmentally relevant simulations and the ability to determine causality of results. In spite of its caveats, this framework represents a strong step forward in modeling the effects of intra-annual precipitation in dryland systems.

**Statistical analysis**

Model construction, simulations, and statistical analyses were all performed using the program *R* (R Development Core Team, 2011). Percent change in CCB from baseline after 100 years was compared across the 38 simulation categories (rainfall manipulations=6, DP manipulations=6, rare large events=6, seasonal changes=16, and predicted precipitation scenarios=4). We used a one-way analysis of variance (ANOVA) to test for significant differences in percent change from baseline across simulation categories. We then used a Tukey’s HSD post-hoc multiple comparison of means with an overall 95% confidence interval to analyze differences between mean percent change values in CCB across simulation categories. Finally, a multiple linear regression was run on simulation results to determine the relative influence of individual intra-annual precipitation variables on percent change in CCB. We used an α level of 0.05 in analyses unless otherwise noted.

**Results**

*Tests of the initial model*
Our initial model (using season-specific equations to calculate carbon balance) predicted annual CCB values ranging from 50-450 µmol C m\(^{-2}\) (mean = 275 µmol C m\(^{-2}\)), which fell within 95% of CCB values predicted from published annual growth rates of *S. caninervis* (Stark et al. 1998). Further, the simulation of a four-year drought period known to have caused mortality in the field (simulated rainfall was reduced by 30% over this period) resulted in negative CCB values (up to -500 µmol C m\(^{-2}\)) in 30% of the simulations. This projected mortality of 30% was similar to field observations in Nevada after a 4-year drought where 68% of populations exhibited chlorosis ranging from 28-48% (Barker et al. 2005).

**Simulations**

Simulated manipulations of event size, DP length, rare large events, seasonal precipitation changes, and combinatorial scenarios caused significant divergences in CCB away from the CCB predicted by baseline meteorological conditions when simulated over 100 years (*P*<0.001; Fig. 4.1, Table 4.1A). All differences in CCB were apparent after the first 25 years of simulation, and differences became larger in magnitude as time increased. Overall, the model was more sensitive to rainfall event size compared to DP length (Table 4.1B), with rainfall event size accounting for 32% of the variance in CCB percent change. Increasing the average rainfall event size by 10-20% resulted in a 30-40% increase in CCB and increasing the average rainfall event size by 30% resulted in a 100% increase in CCB. In contrast, reducing average rainfall event size by 10-20% resulted in a 50% reduction in CCB and reducing average rainfall event size further to 30% resulted in a 90% reduction in CCB. Increasing DP length by 10-20% resulted in a 35% reduction in CCB and increasing DP length by 30% caused a 75% reduction in CCB. In contrast, 10-20% reductions in DP were
variable, and the increase in CCB ranged from 10% to 40%. Reducing DP length further to 30% resulted in a 65% increase in CCB (Fig. 4.1).

Manipulations of rainfall event size and DP by ±30% within individual seasons changed CCB in the 100-year simulation, and these changes were specific to season (Fig. 4.1). The model

**Figure 4.1:** Percent change in cumulative carbon balance (CCB) from baseline CCB (vertical dashed line) simulations of rainfall event size (±10, 20, and 30%), DP length (±10, 20, and 30%), rare large (4x average) rainfall events and rare long (4x average) DPs (+5, 10, or 15 additional per year), variation in rainfall event size and DP (±30%) across seasons (spring, SP; summer, SU; fall, FA; winter, WI), and four predicted scenarios for future precipitation patterns in Southwestern North America: Scenario 1 (+20% DP, 10 rare large rainfalls per year), Scenario 2 (-20% DP, 20% decrease in rainfall event size), Scenario 3 (-20% rainfall event size, 10 rare long DPs per year), and Scenario 4 (rainfall event sizes: +10% in winter, -20% in spring, and +30% in summer). Values presented are mean percent change in CCB (± 1 standard deviation) from baseline after 100 years of simulation.
CCB, percent change from baseline

Baseline

Scenario 1
Scenario 2
Scenario 3
Scenario 4

Dryland Projections
displayed the highest sensitivity to manipulations of spring rainfall, where CCB decreased 65% when spring rainfall event sizes were reduced 30%, and the least sensitivity to winter and summer rainfall, where CCB decreased <25% when event sizes were reduced by the same magnitude. When DP was increased by 30% in the spring and fall, the model showed a 30-50% increase in CCB. When DP was reduced by 30%, CCB was decreased by ~40% when manipulations took place in the spring and summer, and increased by 30-50% when changes occurred in the fall and winter.

Table 4.1: (A) Results from ANOVA comparing percent change in CCB from baseline across all simulation categories. (B) Results from linear regressions preformed on CCB as a function of the precipitation variables rainfall event size, DP, rare large rainfall events, and rare long DPs.

<table>
<thead>
<tr>
<th>Precipitation Variable</th>
<th>Linear Model Coefficient Estimate</th>
<th>P</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rainfall Event Size</td>
<td>3.034</td>
<td>&lt;0.001</td>
<td>0.32</td>
</tr>
<tr>
<td>DP</td>
<td>-1.900</td>
<td>&lt;0.001</td>
<td>0.06</td>
</tr>
<tr>
<td>Rare Large Rainfall Events</td>
<td>10.321</td>
<td>&lt;0.001</td>
<td>0.39</td>
</tr>
<tr>
<td>Rare Long DPs</td>
<td>-5.406</td>
<td>&lt;0.001</td>
<td>0.16</td>
</tr>
</tbody>
</table>

The incorporation of rare large precipitation events and rare long droughts into the model caused significant changes in CCB from baseline conditions during the 100-year simulation (P<0.001). The presence of rare large rainfall events accounted for over 30% of the variance in CCB percent change (Table 4.1B). As the number of rare large rainfall events were added to the model (5, 10, 15 events per year), CCB increased 15, 80 and 160%, respectively. As the number of rare long DPs were increased by the same increments, CCB decreased ~60% with the addition
of 5 and 10 rare long DPs, and further decreased 100% with 15 rare long DPs added to the model.

Three of the four scenarios of precipitation change based on climate projections for Western North America predicted declines in CCB in the next 100 years. Scenario 3 (-20% rainfall event size + 10 rare long DPs per year) caused the greatest reduction in CCB (-83%), and scenarios 2 and 4 showed 40-60% reductions in CCB. Scenario 1 (+20% DP + 10 rare large rainfall events) was the only scenario that produced an increase in CCB (+70%) over the next 100 years.

Discussion

From the perspective of instantaneous responses to individual rainfall events, current experimental evidence suggests that changing precipitation patterns will influence the physiology of biocrust mosses (Coe et al. 2012; Reed et al. in press). By extrapolating this physiological understanding over historical time using precipitation records, we created a model that successfully predicted growth and mortality in moss. The model was then used to predict future performance of mosses.

Simulations manipulating intra-annual precipitation characteristics had a large impact on moss viability. In most 100-year precipitation manipulations, changes in performance occurred in the first 25 years, indicating rapid responses to change.

Changes in rainfall event size and rainfall frequency had large effects on performance and biomass of mosses. Simulations indicate that a 30% reduction in average rainfall event size will cause a 100% decline in moss CCB in the next 100 years, and a 30% increase in average DP...
will result in a 70% decline (Fig. 4.1). These two variables also largely drove the overall response to complete scenarios predicted by climate modeling for dryland systems, highlighting their importance for the physiological performance of mosses.

Rainfall event size and desiccation period are strong controlling factors of long-term CCB. However, the presence of a few rare large wet or dry events per year can override these effects and dramatically change moss CCB. Across all of the simulations, changing these precipitation extremes induced the largest (>100%) changes in CCB after 100 years. In terms of rare large (4x average) rainfall events, this appears to be due to large rainfall events driving sizeable carbon gains in desert mosses (Coe et al. 2012), thus increases in function to sustain mosses through unfavorable time periods. For example, in simulations one or two rare large rainfall events each summer enables persistence overcoming compounded carbon deficits incurred during multiple small precipitation events. On the other hand, rare long (4x average) DPs place large constraints on CCB on both short and long time scales. Longer periods of drought can lead to carbon starvation through the cost of recovery from intense periods of desiccation (Proctor 2001; Coe et al. 2012). If droughts of long duration become more common in the future, they are likely to drive declines in CCB even if rainfall event sizes do not change.

A strong emergent property of the model is that the magnitude of change in moss performance in the next century will be strongly related to the season in which precipitation patterns change. Manipulating rainfall event size in the spring or the fall had a large effect on CCB (>40 % change), but changes to rainfall event size in summer or winter had very little effect. Based on the physiological responses reported by Coe et al. (2012), this appears to be most related to: a) the seasonal physiological state of moss that influences the magnitude of carbon gains and losses from individual precipitation events; or b) the importance of the carbon
gain during rainfall events in spring and fall to compensate for losses during the summer and, to a lesser extent, the winter. The time of year that rain falls (or does not fall) therefore can have a large influence on current and future performance and biomass of biocrust mosses.

While changes to rainfall event size were more important in certain times of year than others, changes to rainfall frequency were equally important in all seasons. An increase in rainfall frequency in fall, winter, or spring led to 30-50% increases in CCB over the next 100 years, but increases during the summer resulted in 35% declines in CCB, and our data suggest that a critical rainfall frequency threshold exists for moss viability in the summer of approximately two <3 mm events per week. During the summer, experimental evidence shows that most precipitation events result in net carbon losses (Coe et al. 2012) and densely spaced events where there are more instances of loss can rapidly lead to mortality (Reed et al. in press). If future rainfall frequencies increase beyond this threshold, there are likely to be dramatic declines in performance and biomass of biocrust mosses.

Scenarios suggested by prognostic modeling for precipitation changes in North American drylands resulted in substantial changes in performance of biocrust mosses within the next century, and three out of four scenarios caused declines in viability. Scenario 1 (+20% DP, 10 rare large rainfalls per year) was the only scenario that produced a positive change in CCB. In this scenario, drought conditions imposed by the increase in DP that would ordinarily cause reductions in CCB were offset by the presence of additional rare large rainfall events per year. This provides further evidence for the importance of extremes in precipitation for long-term performance of biocrust organisms in dryland systems. An increase in number of large rainfall events, even if relatively rare, can change the trajectory of long-term performance in this species, in spite of water-limiting conditions that may co-occur.
Scenario 2 (-20% DP, -20% rainfall event size) resulted in a 48% reduction in moss CCB. In simulations altering only DP, decreased DP resulted in increased CCB likely due to reduced recovery costs from short dry periods, but in this scenario reductions in rainfall event sizes appear to have counteracted these benefits. Even if dry intervals between events are reduced, reductions in the size of rainfall events can lead to large reductions in long-term biomass accumulation. It is very likely that the results of Scenario 2 were strongly influenced by summer precipitation when most rainfall events cause carbon loss, and reductions in DP cause a reduction in CCB by compounding these deficits (see above). The results of the simulations reported here are similar to the experimental results reported by Reed et al. (in press) where the addition of small rainfall events during the summer led to moss mortality.

Scenario 3 (-20% rainfall event size, 10 rare long DPs per year) produced the largest percent decline (83%) in CCB in mosses for the next century. Reduced rainfall event size and increased frequency of extreme droughts exerted considerable influence on CCB as independent simulations. This scenario combined these variables to produce conditions that greatly limit water availability, resulting in compounded effects and dramatic declines. Collectively, the manipulation of these two parameters likely caused large reductions in CCB because (a) carbon reserves used for recovery from prolonged droughts must be replenished during rainfall events; and (b) rainfall events of insufficient size for positive carbon balance prevent this from occurring.

Scenario 4 (rainfall event sizes: +10% in winter, -20% in spring, and +30% in summer) resulted in a 40% reduction in CCB, and illustrates the importance of seasonal redistributions of precipitation on the performance of biocrust mosses. Annually, rainfall events increased in size in this scenario, but seasonally they were not increased in the time of year when biocrust mosses
are most responsive to rainfall. Therefore, increases in rainfall event size in winter and summer were insufficient to compensate for reductions in spring rainfall event sizes with respect to long-term performance. This scenario shows that alterations to the seasonality of precipitation can result in changes, and in this case, declines in performance, even if there is an overall increase in mean annual rainfall.

Overall, the model predicts that the most detrimental precipitation scenarios for biocrust moss functioning in the next century include those that reduce rainfall event size, increase drought duration, or include the combined effects of these variables (e.g., Scenario 2). Under conditions where water becomes more limiting, carbon uptake is constrained annually, leading to compounded reductions in CCB on longer time scales. In contrast, global change scenarios that drive increases in rainfall event size in dryland systems will result in higher performance and survival of biocrust moss. These scenarios could include increases in the average size of individual events, the effects of which will be most important in the spring and fall, or the presence of rare large events throughout the year. We also show that regardless of the direction of the change, responses to alterations in intra-annual rainfall can occur rapidly (<25 years) and continue on the same trajectory for a century or more.

Although predictions for changes in precipitation for drylands in North America and elsewhere globally currently come with varying degrees of uncertainty, most point to scenarios that result in reduced water availability for organisms, either through augmented drought conditions, reductions in rainfall event sizes, or seasonal redistribution of rainfall. Based on our data, such conditions are likely to result in reductions in biomass of biocrust mosses and lead to corresponding reductions in crust function. Globally, in areas with mature biocrusts that contain mosses, such changes are likely to have strong consequences for dryland ecology. In addition to
producing quantitative projections for biocrust moss functioning in a future climate, we demonstrate here that prognostic modeling of dryland biocrust function will be unable to adequately predict biocrust responses to changing precipitation regimes without an explicit consideration of the details of intra-annual precipitation patterns.

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References


