Climate change and habitat fragmentation in a boreal forest bryosphere experiment

Jonathan Arthur Whiteley

Doctor of Philosophy

Department of Biology

McGill University
Montréal, Québec, Canada
2012-12-14

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Doctor of Philosophy

DEDICATION

For Mike, Zak & Sam

ABSTRACT

Climate change encompasses not only global changes in temperature, but also changes in precipitation, variability, and large-scale shifts in conditions to higher latitudes and altitudes. Many species respond by following suitable environmental conditions to new locations, but the necessary dispersal may not be possible on landscapes fragmented by anthropogenic land-use. This non-additive interaction is poorly understood, particularly in the boreal forest, whose extensive circumpolar distribution and large pool of soil carbon have the potential to feedback to global climate. These forests take up atmospheric carbon through primary production, which is often limited by nitrogen, and release carbon through decomposition, which may be sensitive to changes in temperature, precipitation, or biotic communities. Nitrogen-fixing cyanobacteria in symbiotic association with feather mosses may reduce nitrogen-limitation, but the environmental and biotic factors controlling them have only recently begun to be explored.

I used a two-year field experiment near the northern limit of the boreal forest in northern Québec, Canada, to assess the impacts of habitat fragmentation and simulated climate change treatments on model moss ecosystems. I measured treatment effects on microarthropod and symbiotic cyanobacteria communities associated with the feather moss *Pleurozium schreberi*, as well as ecosystem processes of nitrogen-fixation, moss growth, and decomposition within the bryosphere (comprising the moss layer and associated biota). The experiment showed that N-fixation was positively affected by moisture conditions, but negatively affected by available nitrogen. N-fixation was only weakly related to cyanobacteria density, which was unaffected by experimental treatments. Moss growth stopped by the second year of drought, leading to net biomass loss, due to rates of decomposition exceeding moss productivity. Microarthropod abundance and richness also declined under drought conditions, but only in isolated patches, suggesting that dispersal is able to maintain populations in the face of environmental stress. This reveals the predicted synergistic effects of climate change and fragmentation: the combined effects are greater than the sum of individual effects. The results of this long-term field experiment highlight the overall importance of

water availability in the bryosphere, and the strength of environmental controls on ecosystem processes, even in such a biodiverse system.

RÉSUMÉ

Les changements climatiques incluent non-seulement les changements de température au niveau planétaire, mais également les changements au niveau des précipitations et variabilité, ainsi que le déplacement à grande échelle des conditions aux altitudes et latitudes élevées. Plusieurs espèces ont comme réaction à ces modifications de suivre les conditions environnementales plus propices à de nouvelles locations. Mais les déplacements nécessaires peuvent être impossibles sur des paysages divisés par l'utilisation anthropique des terres. Cette interaction non-additive demeure incomprise, particulièrement dans la forêt boréale dont sa répartition circumpolaire étendue et sa grande quantité de carbone dans le sol créent un potentiel de rétroaction au climat planétaire. Ces forêts absorbent le carbone atmosphérique par la production primaire, laquelle est souvent limitée par la disponibilité en azote. Elles relâchent également le carbone par un processus de décomposition, lequel est influencé par les changements de température, de précipitation et les communautés biotiques présentes. Les cyanobactéries fixatrices d'azote en symbiose avec les hypnes peuvent réduire les limites induites par l'azote. Cependant, les facteurs environnementaux et biotiques les contrôlant sont étudiés depuis peu.

Afin d'examiner les effets de la fragmentation des habitats et des traitements simulés des changements climatiques sur des modèles d'écosystème de mousse, j'ai effectué une expérience de terrain d'une durée de deux ans, qui s'est déroulée près de la limite nordique de la forêt boréale au Québec. J'ai évalué la réponse des communautés de microarthropodes et de cyanobactéries symbiotiques, associées à *Pleurozium schreberi*, à ces traitements, ainsi que les processus écosystémiques de fixation de l'azote et la croissance et la décomposition à l'intérieur de la bryosphère (incluant la couche de mousse et le biote associé). L'expérience a démontré que la fixation de l'azote est positivement influencée par les conditions d'humidité, mais négativement influencée par la disponibilité de l'azote. La fixation de l'azote n'est que faiblement reliée à la densité et diversité des cyanobactéries, lesquelles n'ont pas été perturbées par les traitements expérimentaux. La croissance des mousses s'est arrêtée à la deuxième année de sécheresse, menant à une perte nette

de biomasse causée par un taux de décomposition excédentaire à la production de mousse. L'abondance et la richesse des microarthropodes diminuent également sous des conditions de sécheresse, mais seulement à des endroits isolés. Cela suggère que la dispersion est apte à maintenir les populations même lors de stress environnementaux. Cela confirme également les effets synergiques prédits des changements climatiques et de la fragmentation des habitats : les effets combinés sont plus amples que la somme des effets individuels. Les résultats de cette expérience à long terme soulignent l'importance de la disponibilité de l'eau dans la bryosphère ainsi que l'influence des contrôles environnementaux sur les processus environnementaux, et cela même dans un environnement aussi diversifié.

PREFACE

Acknowledgements

This research was supported by a post-graduate scholarship from the Natural Sciences and Engineering Research Council of Canada (NSERC), a Tomlinson Science Award from McGill University, and financial support from Andrew Gonzalez. I received travel support from the Northern Scientific Training Program from what was Indian and Northern Affairs Canada at the time.

I thank my supervisor and collaborator on this project, Andrew Gonzalez, and the rest of my supervisory committe: Gregor Fussmann and Michel Loreau. Zoë Lindo collaborated with me on aspects of this project, published elsewhere, and helped with field work, sample collection, as well as showing me how to work with and identify mesostigmatid mites and collembola. Zoë provided editorial advice on some manuscripts, shared many insights into soil and moss ecology, and was an academic inspiration on many levels. Mitran Mehta helped count cyanobacteria, providing valuable data I wouldn't have had time to include on my own. Heather McIntosh cut moss stems into 1 cm sections, weighed each one individually, and took meticulous notes. Without her data, conversion of moss growth to productivity estimates would not have been possible, and I am forever grateful for her time and efforts. Oksana Choulik prepared dinners and helped with logistics at the McGill Subarctic Research Station in Schefferville, Quebec. She manages to do a lot to keep the station running, with very little funding, and I thank her for her dedication. Dr. Tim Moore, in the department of Geography at McGill University, provided helpful advice on methods of measuring decomposition, and helped me try to find out details of the fire history at my study site.

I want to thank everyone who participated in the department's R / statistics workshops, for keeping it active, and giving me a chance to learn more than I expected. Their support and discussions helped renew my love of statistics, and appreciate data analysis in a whole new way. All the members of the Gonzalez lab have been supportive and helpful, sharing many thoughts, condolences, and merciless feedback on practice talks: Xoxo (Georgina) O'Farrill, Pradeep Pillai,

Michael Pedruski, Matthew Mitchell, Patrick Thompson, Edward Wong, and Bronwyn Rayfield. Many graduate students in the department have been sources of inspiration, advice, and generally engaging discussions: I thank you all.

Last, but not least, I thank my family and my partner Mike, for emotional and material support when I needed it most. You believed in me even when I didn't.

Thesis format

This is a manuscript-based thesis, with connecting statements between manuscript chapters. Each manuscript has been submitted, or is planned for submission, to an academic journal, and has been written to stand alone. A general introduction provides a general review of the academic context that motivated the research, followed by an overview of a large two-year multifactorial field experiment, designed to combine habitat fragmentation, warming, and drought. Each manuscript presents results from a different subset of the data collected from this experiment, and discusses their implications:

1. Nitrogen-fixation and cyanobacteria responses to habitat fragmentation and simulated climate change.

2. Net moss biomass accumulation or loss under drought conditions, based on estimates of moss growth and decomposition.

3. Environmental controls over N-fixation and moss growth, including cyanobacteria abundance, moisture, warming, and available nitrogen.

4. Microarthropod community response to habitat fragmentation and simulated climate change.

Because all chapters are derived from the same experiment, there is some repetition in methodology across all manuscripts, such that each can stand alone. In general, greater detail is provided in earlier chapters, and referred to in subsequent chapters. The thesis concludes with a summary of the main findings and general conclusions in the context of recent research on biodiversity and ecosystem function under the combined effects of climate change and habitat fragmentation.

Author Contributions

Andrew Gonzalez and I conceived and designed the field experiment. I set-up the experiment, collected data, performed analyses, and wrote the manuscripts, with advice and supervision from Andrew Gonzalez. Zoë Lindo assisted with sample collection during June 2009, helped me with microarthropod identification, and provided analytical and editorial advice as a collaborator on chapter 6 (Microarthropod community response to fragmentation and simulated climate change). Each manuscript notes additional contributions from specific individuals regarding sample or data collection, in the acknowledgements.

Statement of Originality

This is the first experiment to combine habitat fragmentation and climate change conditions in a factorial manner, while measuring responses in biotic communities and relevant ecosystem processes. This experiment also examines such effects on symbiotic interactions between moss and nitrogen-fixing cyanobacteria; Most studies of environmental change have focused on competitive or trophic communities, while the importance of positive interactions is usually ignored. I developed a novel method for high volume measurement of cyanobacteria density, which is often inferred from measurements of nitrogen-fixation, rather than measured directly, limiting our understanding of mechanisms affecting real N-fixation rates in nature. The results of this experiment provide insight into the combined effects of relevant controls on nitrogen-fixation, moss growth, and decomposition in boreal forest moss. These results are relevant to improving predictions of boreal forest responses to climate change, and confirm the synergistic effects of drought and isolation on microarthropod communities. My results also address the relative importance of environmental and biotic controls over ecosystem processes, an unresolved question in the field of biodiversity and ecosystem function.

TABLE OF CONTENTS

List of Figures

List of Tables

CHAPTER 1

General Introduction

> "Biological diversity" means the variability among living organisms from all sources including, *inter alia*, terrestrial, marine and other aquatic systems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems.
>
> United Nations Environment Programme (Heywood, 1995, p. 8)

Biodiversity is essential for ecosystem function. This seems intuitive, given that ecosystems include living organisms by definition, as well as their abiotic environment. Many ecological communities are dominated by a few species, however, which has led ecologists to ask whether the "extra" species contribute anything substantial to ecosystems (Chapin *et al.*, 1997; Hooper *et al.*, 2005). Phrased another way, "how much diversity do we really need" for ecosystems to continue to function and provide benefits that humans rely on for food, raw materials, and a safe living environment? Research has recently begun to quantify the effect of biodiversity *per se* on aggregate ecosystem-level processes, revealing the importance of both the richness and composition of phenotypes, whether they are species within an ecological community, or individuals within populations (Loreau, 2000; Norberg *et al.*, 2001).

Biodiversity and ecosystem function experiments, primarily in grassland systems, have demonstrated that increasing species richness also increase levels of aggregate community properties such as productivity, nutrient cycling or material stocks (Naeem *et al.*, 1994; Hooper and Vitousek, 1997; Tilman *et al.*, 1997; Loreau, 2000). Large-scale experiments have found the same general relationship at sites around the world (Hector *et al.*, 1999; Loreau and Hector, 2001; Maestre *et al.*, 2012). A positive relationship between biodiversity and ecosystem processes is

viewed as an outcome of two mechanisms acting together (Loreau, 2000; Loreau *et al.*, 2001): (1) *selection effects*, whereby having more species in a community increases the probability of including a single highly productive, dominant species that disproportionately increases levels of ecosystem function (O'Connor and Crowe, 2005; Cardinale *et al.*, 2006); and (2) *niche complementarity*, whereby a larger variety of species coexisting within a community are able to partition resources more efficiently, and thus the community as a whole outperforms even the best monoculture (Chapin *et al.*, 1997; Loreau and Holt, 2004; Hooper *et al.*, 2005). In the second case, such 'overyielding' can also occur as a result of positive interactions, including facilitation or mutualisms (Cardinale *et al.*, 2002).

Long-term experiments have revealed that the effect of biodiversity on ecosystem processes increases with time (Tilman *et al.*, 2001; Reich *et al.*, 2012). The analyses of ever-growing data sets also reveals that the importance of biodiversity increases as more locations, times, environmental changes, or measures of ecosystem function are considered (Zavaleta *et al.*, 2010; Cardinale *et al.*, 2011; Isbell *et al.*, 2011; Maestre *et al.*, 2012). Virtually any biodiversity loss therefore translates into a reduction of overall ecosystem multifunctionality (Hector and Bagchi, 2007; Gamfeldt *et al.*, 2008).

The contribution of biodiversity to ecosystem processes may not always be apparent over the short-term, or at small scales. Even in communities where ecosystem processes are dominated by a few species at a time, apparently redundant species may contribute over the long-term, particularly under changing environmental conditions (Naeem, 1998; Yachi and Loreau, 1999). The relationship between variability of population abundances and aggregate community properties, in the context of species coexistence, has been the subject of a long-standing "diversity-stability" debate (MacArthur, 1955; May, 1974; Hughes and Roughgarden, 2000; McCann, 2000; Ives and Hughes, 2002). Aggregate ecosystem properties may be less variable over time, as a result of variance-averaging over many species (Doak *et al.*, 1998; Tilman *et al.*, 1998; Ives and Hughes, 2002), or compensatory dynamics of species with relative abundances that are not perfectly correlated over time (Tilman, 1996; Yachi and Loreau, 1999; Cottingham *et al.*, 2001; Ives and Hughes, 2002; Gonzalez and Loreau, 2009).

Changes in ecosystem properties are generally assumed to be the result of changes in species abundances, either numerical or biomass (Yachi and Loreau, 1999). Environmental change, whether gradual, sudden, or fluctuating, can drive changes in relative abundance of species that differ in relative fitness under different

conditions. Variation in phenotypic response to the environment offers both a method of coexistence for multiple species (Chesson, 2000; Descamps-Julien and Gonzalez, 2005), and a mechanism that accounts for insurance effects of such diversity for the long-term maintenance of ecosystem properties (Yachi and Loreau, 1999; Hughes and Roughgarden, 2000; Ives *et al.*, 2000; Norberg *et al.*, 2001; Ives and Hughes, 2002; Loreau *et al.*, 2003a; Gonzalez *et al.*, 2009).

A diversity of environmental responses among taxa contributing to the same ecosystem process is also critical to ecological resilience, allowing ecosystems to quickly recover from perturbations (Holling, 1973; Chapin *et al.*, 1997; Peterson *et al.*, 1998; Chapin *et al.*, 2000; Elmqvist *et al.*, 2003) Conversely, complex dynamics within ecosystems can also lead to large shifts in response to small environmental changes (Scheffer *et al.*, 2001; van Nes and Scheffer, 2004; Scheffer *et al.*, 2009). The extent to which the environment controls both biodiversity and ecosystem function in real ecosystems has been questioned (Cardinale *et al.*, 2000; Huston *et al.*, 2000; Loreau, 2000; Hooper *et al.*, 2005) and remains an area of active research in the field (Houlahan *et al.*, 2007; Cardinale *et al.*, 2011).

Overall, biodiversity can contribute to ecosystem properties over the short-term via selection and complementarity, but even short-term redundancy provides an insurance against long-term changes in species abundance (Isbell *et al.*, 2011; Reich *et al.*, 2012). Biodiversity may therefore regulate ecosystem properties over the long-term, reducing variability, and increasing the long-term average and reliability of ecosystem properties (Naeem and Li, 1997; Naeem, 1998; Rastetter *et al.*, 1999; Loreau, 2000; Loreau *et al.*, 2003a; Gonzalez *et al.*, 2009).

1.1 Biodiversity loss and community disassembly

Unfortunately, biodiversity is disappearing globally at rates far in excess of long-term historical averages (Millennium Ecosystem Assessment, 2005; Cardinale *et al.*, 2012). The biodiversity crisis has heightened concerns regarding the implications of biodiversity loss for ecosystems and the people who rely on them (Chapin *et al.*, 2000; Cardinale *et al.*, 2012). Previous research had focused on general relationships between ecosystem processes and random assemblages of different numbers of species (Loreau *et al.*, 2001), but concern over biodiversity loss shifted the focus onto ecosystem processes under different scenarios of community *disassembly*, distinct from community *assembly* (Solan *et al.*, 2004; Gross and Cardinale, 2005).

Many experiments that combine random species at different levels of richness implicitly include processes of community assembly. Such experimental communities are not the result of removing species from a community of long-associated species, but rather species additions, often simultaneous, to an environment that is usually depopulated of any unwanted species. Random assembly experiments may represent possible scenarios of random extinction, suggesting an accelerating decline in ecosystem function with biodiversity loss (Loreau *et al.*, 2001; Gross and Cardinale, 2005). Redundancy among species contributing to a particular ecosystem process corresponds to the popular 'rivet model' of ecosystem response to biodiversity loss: losing a few species from a community of many reduces ecosystem function much less than losing the same number when there are only a few remaining, resulting in rapid loss of function below a critical number of species (Ehrlich and Ehrlich, 1981; Cardinale *et al.*, 2011).

Random assembly experiments may not appropriately take into account species interactions as species are removed rather than gained, as well as the secondary indirect effects of extinctions on community structure and ecosystem properties (Cardinale *et al.*, 2002, 2011). Fukami and Morin (2003) demonstrated that the history of community assembly — the order in which species were added to a community – had an effect on eventual ecosystem structure and function. If so, then the converse might also be true. How are ecosystem properties affected by the order of species extinctions? Are species extinctions likely to be random, or ordered in some predictable manner?

The degree to which species extinctions are random ultimately depends on whether the probability of extinction is equal for all species in a community, and the correlation between particular species traits and extinction risk (Petchey, 2000; Gross and Cardinale, 2005). Larger body size, higher trophic level, sensitivity to environmental stress, longevity, and ultimately small population size all tend to increase the probability of extinction under environmental change (Gilbert *et al.*, 1998; Raffaelli, 2004; Gross and Cardinale, 2005; Dobson *et al.*, 2006). Specific perturbations or environmental changes may select against certain traits more than others, but small populations are generally much more likely to go extinct within a given period of time, due to stochasticity alone. Smaller populations also tend to have less genetic and phenotypic variation, leaving little opportunity for selection or adaptation, and leaving the entire population sensitive to the same stressors.

Several ecological processes and community characteristics might influence

how ecosystem properties are affected by extinction scenarios (Raffaelli, 2004; Solan *et al.*, 2004; Gross and Cardinale, 2005). If we know what determines extinction risk, then knowledge gained from random assembly experiments may in fact inform predictions about ecosystem responses to biodiversity loss (Gross and Cardinale, 2005). Theoretical studies show that the relationship between biodiversity and ecosystem processes can depend on how closely extinction risk is tied to species contributions to ecosystem function.If functional traits are positively associated with extinction risk, then effects of biodiversity loss could be more pronounced than under random extinction scenarios (Solan *et al.*, 2004; Gross and Cardinale, 2005).

Differences in species responses to environmental change and perturbation create the potential for compensation, allowing changes in relative abundance of functionally redundant species to buffer changes in environmental conditions. The same type of redundancy also reduces effects of biodiversity loss, so long as functionally redundant species do not share the same risk of extinction. Nevertheless, mounting evidence of the multifunctional importance of biodiversity calls into question the level of overall ecosystem redundancy that exists in 'natural' ecosystems (Gamfeldt *et al.*, 2008; Cardinale *et al.*, 2011; Isbell *et al.*, 2011).

Any amount of global biodiversity loss is likely to reduce ecosystem function, resulting in fewer benefits to humans, such as food, raw materials, employment and recreation opportunities, or regulating services (Millennium Ecosystem Assessment, 2005). These negative effects of biodiversity loss may not be apparent, depending on the scale, time, or properties measured. Available evidence suggests that the current biodiversity crisis is one of humanity's own making, the result of anthropogenic activities that have reduced available habitat, resources, and changed aspects of the earth's physical environment at global scales, triggering changes in ecosystem structure and function across many scales (Millennium Ecosystem Assessment, 2005; Rockstrom *et al.*, 2009). Habitat fragmentation and climate change are of particular concern, not only due to their strong individual effects, but also because of potential synergistic interactions that could limit the ability of biodiversity and ecosystems to maintain ecosystem properties, or recover from additional perturbations.

1.2 Habitat fragmentation and climate change: the dynamic duo of global change

Climate change shifts environmental conditions in space and time

Global climate change models forecast changes in average temperature and precipitation, as well as their variability in space and time (Wigley *et al.*, 1998; IPCC, 2002; Logan *et al.*, 2011). Temperatures are predicted to increase, more so in sensitive environments closer to the poles (Pearson and Dawson, 2003; Thomas *et al.*, 2004). Precipitation patterns will change, with some areas receiving more, others less (IPCC, 2002; Logan *et al.*, 2011). For both temperature and precipitation, variation is expected to increase and become more temporally autocorrelated (larger, less frequent changes), leading to an increase in extreme weather events, including heat waves, droughts, floods, and storms (Wigley *et al.*, 1998; IPCC, 2002; Soja *et al.*, 2007; Hansen *et al.*, 2012; Bellard *et al.*, 2012). As temperatures increase, climate conditions generally shift to higher altitudes and latitudes, while seasonal events shift in time (Parmesan and Yohe, 2003; Thomas *et al.*, 2004; Bellard *et al.*, 2012). Many species will soon find themselves in habitats and locations with environmental conditions that are no longer suitable for their growth and reproduction. Global changes in patterns of temperature and precipitation have profound effects on species abundances, distributions, and community structure, which have consequences for ecosystem processes (McLaughlin *et al.*, 2002; Walther *et al.*, 2002; Parmesan and Yohe, 2003; Chen *et al.*, 2011; Bellard *et al.*, 2012).

Species can respond to climate change by: adapting to new conditions locally (physiologically within generations, or by evolution across generations), dispersing to find suitable environments elsewhere, persisting in a maladaptive state at low abundance (perhaps on a slow decline toward extinction), declining in abundance to extinction, or perhaps not responding at all (Watkinson and Gill, 2002; Bellard *et al.*, 2012). Dispersal to new locations with suitable environmental conditions can occur over a range of scales. Small-scale dispersal may allow species to find suitable microclimates in nearby habitats, such as slopes of different aspect or incline, proximity to water bodies, or shade from solar radiation (Watkinson and Gill, 2002). As climate averages shift pole-ward, species may also move at continental scales to track suitable conditions.

Species distributions are often associated with a "bioclimate envelope" of characteristic temperature and moisture conditions (Pearson and Dawson, 2003).

As these conditions shift to higher altitudes and latitudes with climate change, many species are therefore expected to follow, leading to general poleward shifts in species distributions (Pearson and Dawson, 2003; Thomas *et al.*, 2004). Such shifts have already been observed in a range of taxa (Walther *et al.*, 2002; Parmesan and Yohe, 2003; Parmesan, 2006; Chen *et al.*, 2011). For many more, however, the dispersal necessary to colonize locations newly suitable under climate change could be prevented by a lack of habitat connectivity (Watkinson and Gill, 2002).

Habitat fragmentation limits dispersal

Habitat area is one of the most important factors that promote population sizes, and thus biodiversity (Rosenzweig, 1996; Gaston, 2000; Hodgson *et al.*, 2009). Habitat loss, primarily due to land-use change and anthropogenic disturbance, is therefore seen as a principal driver of biodiversity loss (Sala *et al.*, 2000; Hanski, 2005; Millennium Ecosystem Assessment, 2005). Nevertheless, for a given amount of habitat, the spatial arrangement and connectivity among patches affect dispersal rates and thus determine biodiversity at multiple scales (With and King, 1999; Cumming, 2007; Doerr *et al.*, 2011).

Habitat fragmentation is the decline in connectivity among habitat patches, independent of habitat loss, even though the two processes often occur together (Fahrig, 2003). Fragmentation can reduce biodiversity by lowering dispersal rates between habitat patches, preventing species from accessing a sufficient quantity of suitable habitat to support viable population sizes. Connectivity allows dispersal of individuals among habitat patches, increasing the total effective area available to a population, thus promoting persistence of populations (Bascompte and Solé, 1996). More populations able to persist and coexist across a landscape translates to higher species richness and biodiversity at local and regional scales (Loreau *et al.*, 2003a; Mouquet and Loreau, 2003).

Dispersal is thus a fundamental ecological process that directly affects biodiversity and species distributions (e.g., colonization) and abundance (e.g., immigration, emigration, source-sink dynamics and mass effects) (MacArthur and Wilson, 2001; Leibold *et al.*, 2004; Vellend, 2010). The metapopulation framework captures the role of dispersal and spatial structure in population dynamics, by conceptualizing populations as a collection of sub-populations linked by dispersal (Hanski and Gilpin, 1991; Hanski, 1999). Extending this framework to incorporate multiple interacting species has given rise to the metacommunity concept (Leibold *et al.*, 2004; Holyoak *et al.*, 2005), while incorporating material flows has led

to a metaecosystems perspective (Loreau *et al.*, 2003b; Gravel *et al.*, 2010). A metacommunity perspective views landscapes as habitat areas with varying levels of dispersal, giving rise to a range of processes and patterns (Leibold *et al.*, 2004).

The metacommunity framework is well-suited to studying the effects of habitat fragmentation and connectivity on biodiversity and ecosystems, by controlling dispersal rates among discrete habitat patches, throughout networks of habitat patches connected by movement corridors, or across landscapes of different habitat types (Holyoak *et al.*, 2005). Model ecosystems have been used to demonstrate biodiversity loss in experimentally fragmented networks, explicitly designed as metacommunities (Gonzalez *et al.*, 1998; Davies *et al.*, 2001; Staddon *et al.*, 2010).

Climate change and fragmentation are potentially synergistic

Just as multiple phenotypes may outperform the sum of their constituent parts, drivers of biodiversity loss and change can be more powerful in combination, particularly when species' tolerance to stressors are negatively correlated (Vinebrooke *et al.*, 2004). The potential non-additive effects of climate change and habitat loss are of particular concern because habitat fragmentation can block dispersal, preventing species from tracking environmental conditions that are shifting as a result of climate change (Davis and Shaw, 2001; IPCC, 2002; Opdam and Wascher, 2004). Simulation studies suggest that range-shifting over large scales requires higher levels of connectivity than would be needed for metapopulation persistence without climate change (Travis, 2003; McInerny *et al.*, 2007).

Climate change may drive species extinct due to changes in environmental conditions too rapid for species to track by dispersal or adaptation (Davis and Shaw, 2001; Burrows *et al.*, 2011), whereas fragmentation already presents a challenge to species that require dispersal throughout a habitat network to persist (Gilbert *et al.*, 1998; Grant *et al.*, 2010). Species that may be tolerant of one change may be more sensitive to the other, leading to additive extinctions (Vinebrooke *et al.*, 2004). Extinctions of species that may be able to withstand either change on its own but not both together represent non-additive, synergistic impacts.

For example, a species may be unaffected by surrounding fragmentation if it is able to persist under current environmental conditions. As climate changes, such a species may be intrinsically capable of adequate dispersal to track shifting environmental conditions, but is prevented from doing so by now-relevant habitat fragmentation. Conversely, species may be able to tolerate environmental change

across part of their range, with sufficient population size and access to habitat area. A reduction in connectivity and associated dispersal could reduce population sizes below levels that are resistant to environmental change, or prevent immigration of adapted phenotypes capable of rescuing what have become sink populations (Gonzalez and Holt, 2002; Holt *et al.*, 2003; Matthews and Gonzalez, 2007).

The bioclimate envelope approach to predicting species' responses to climate change ignores non-climate limits to species distributions, particularly species interactions, which can profoundly alter the extent and direction of species range shifts (Davis *et al.*, 1998a,b; Pearson and Dawson, 2003; Botkin *et al.*, 2007). Species interactions can also shape community-level responses to climate change (Gilman *et al.*, 2010; Norberg *et al.*, 2012). Faster rates of climate change can disrupt otherwise stable coexistence dynamics and lead to sudden species extinctions, depending on species interactions and dispersal abilities (Brooker *et al.*, 2007). To further complicate matters, climate changes may alter the connectivity of habitat itself, by changing patterns of disturbance (Opdam and Wascher, 2004). Climate conditions may also shift too fast for species to keep up with by conventional dispersal mechanisms (Pearson and Dawson, 2005), which can be problematic for other species at the limits of their range that depend on physical habitat provided by slow-moving species, such as forest trees.

Climate change alone presents a suite of components changing together, including temperature, precipitation, CO_2 concentration, as well as patterns of these variables in space and time. Each of these changes can have different effects when considered independently or in combination. The potential for interactive effects among climate change components themselves has led to a burst of *multifactor* experiments, explicitly designed to consider more than one type of environmental change concurrently in a factorial design that allows independent and interactive effects to be measured separately (Mikkelsen *et al.*, 2008; Villalpando *et al.*, 2009; Kardol *et al.*, 2011).

Predicting net effects of climate change on biodiversity in fragmented ecosystems is one of the greatest challenges currently facing ecology.This challenge requires accounting for non-additive combinations of environmental change, and integrating increasingly complex processes at several spatial scales and levels of organization (Opdam and Wascher, 2004). I will explore the direct and indirect effects of both climate change and habitat fragmentation on biodiversity and ecosystem function within a framework that takes into account direct and indirect effects of both drivers on the relationship between biodiversity and ecosystem

function (Figure 1.1).

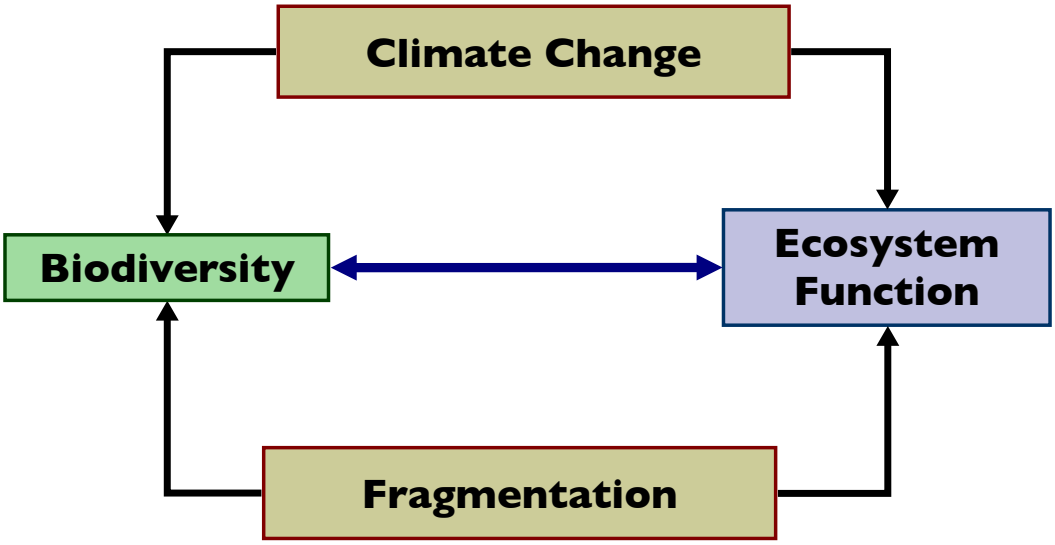

Figure 1.1 *Conceptual framework for the thesis.*

1.3 Environmental change in the boreal forest

Both climate and land-use change are expected to be major drivers of biodiversity change in the boreal region, in addition to nitrogen deposition (Sala *et al.*, 2000). Because boreal forests can contribute significantly to carbon cycling and global climate, there is great potential for feedbacks between climate change and boreal forest dynamics.

Boreal forests cover at least 10% of the Earth's land surface, across circumpolar subarctic latitudes in the northern hemisphere (Taggart and Cross, 2009). Dominated by coniferous spruce trees (primarily members of the *Pinaceae* family), the boreal forest is darker in colour than tundra to the north, so its distribution can affect planetary albedo and the amount of solar radiation absorbed as heat (Eugster *et al.*, 2000; Bernier *et al.*, 2011). In addition, slow decomposition rates in boreal soils and wetlands has led to a steady accumulation of organic matter, including carbon, in boreal forest soils: the largest pool of soil carbon in the world (Davidson and Janssens, 2006). Therefore, changes in boreal forest distribution, or carbon cycling across its current extensive circumpolar distribution, will have consequences for global climate.

Boreal forests may continue to be an important carbon sink, or they may become a net source of carbon emissions, depending on the relative rates of primary production (carbon uptake), and processes that contribute to carbon emission, such as fires, deforestation and decomposition (Bonan and Van Cleve, 1992; Peng and Apps, 1999; O'Donnell *et al.*, 2009). Decomposition has been historically lower than primary production, leading to an accumulation of organic matter and carbon in boreal forest soils (Davidson and Janssens, 2006). Warmer temperatures combined with changes in precipitation patterns, and plant composition associated with climate change are expected to increase decomposition, although the combined effects, including changes in the decomposer community and litter quality, remain unclear (Davidson and Janssens, 2006; Jackson *et al.*, 2010). Although fire frequency is expected to increase under climate change in the boreal forest, with profound implications for carbon cycling (O'Donnell *et al.*, 2009), fire dynamics are beyond the scope and focus of this thesis.

Despite increasing concentrations of atmospheric CO_2, rates of primary production in boreal forests may not increase under climate change, due to nitrogen-limitation (Lindahl *et al.*, 2002; Hungate *et al.*, 2003; Janssens and Luyssaert, 2009; Norby *et al.*, 2010). Although nitrogen (N) is more abundant in soils following fires, as stands age, most available nitrogen becomes immobilized in standing stocks of trees and recalcitrant litter in soil humus (DeLuca *et al.*, 2007, 2008). Most nitrogen throughfall in mature boreal forest stands arrives on the forest floor, which is dominated by bryophytes such as feather mosses.

Bryophytes are extremely efficient at absorbing available nutrients over their entire surface, and recycling it internally during senescence, acting like a "nutrient sponge" (Chapin *et al.*, 1987; Turetsky, 2003). Bryophyte productivity can be as high as tree productivity, dominating ground cover in most boreal forest stands (Turetsky, 2003; Lindo and Gonzalez, 2010). Bryophytes can also regulate microclimate conditions within the space surrounding tightly packed shoots, and in the upper soil layers below them. The discovery of epiphytic nitrogen fixing cyanobacteria associated with boreal forest mosses has added yet another mechanism by which bryophytes affect nitrogen cycling within boreal forest ecosystems (DeLuca *et al.*, 2002; Zackrisson *et al.*, 2004; Lagerström *et al.*, 2007).

1.4 Nitrogen-fixation and boreal forest moss

Although nitrogen is an important element needed in many proteins and other molecular components of living cells, atmospheric nitrogen is not directly accessible to most organisms, because breaking the bond between atoms in molecular nitrogen requires a lot of energy, and specialized molecular tools. The actual process of nitrogen-fixation involves the reduction of atmospheric N_2 into NH_4^+, which is performed by the aptly-named *nitrogenase*, an enzyme (protein) unique to prokaryotes (Böhme, 1998; Bothe *et al.*, 2007). The act of "fixing" nitrogen refers to converting it from an atmospheric gas to a non-gaseous state suitable for biochemical reactions in living cells. Fixed nitrogen is easily converted to other nitrogen-based organic compounds, and represents an important resource for eukaryotic life, which is incapable of fixing nitrogen on its own (Böhme, 1998; Bothe *et al.*, 2007).

Cyanobacteria found in association with boreal forest mosses are all filamentous heterocystous taxa, meaning that they grow in multicellular colonies with differentiated cells, often forming long filaments (Rippka *et al.*, 1979). Specialized cells called *heterocysts* forego photosynthesis in order to maintain the anoxic conditions necessary for the process of nitrogen-fixation (Böhme, 1998). The large energy requirements of N-fixation and its value as a source of limiting nutrients for plants, makes heterocystous cyanobacteria prime candidates for symbiotic associations where host plants provide a suitable environment and energy in the form of photosynthetic products, in exchange for fixed nitrogen (in the form of glutamine) from the cyanobacterial symbionts (Böhme, 1998; Bergman *et al.*, 2007). N-fixation rates tend to be higher for most cyanobacteria taxa when living in symbiotic associations than when free-living, even though many seem capable of both (Bergman *et al.*, 2007). Although symbiotic associations may not be obligate, the mutual benefits seem to favour it wherever nitrogen is limiting.

Symbiotic associations with cyanobacteria occur in many groups of plants, ranging from trees, ferns, legumes, and bryophytes such as moss (Bergman *et al.*, 2007; Bothe *et al.*, 2007). Cyanobacterial symbionts (*cyanobionts*) may be found in specialized plant structures such as root nodules, that have evolved to host them, or may be epiphytic on the surface of plants, or even intracellular in the most intimate associations (Solheim and Zielke, 2002; Bothe *et al.*, 2007). Regardless of the accommodation arrangements, some mechanism usually exists (or is presumed) for the transfer of photosynthates to the cyanobiont, and fixed nitrogen to the host

(Böhme, 1998; Solheim and Zielke, 2002). Various chemical signals appear to be involved in communication between the partners of these relationships. Plant hormones may attract motile cyanobacteria propagules (hormogonia) and induce changes in cyanobacteria morphology (Böhme, 1998; Turetsky, 2003; Bergman *et al.*, 2007; Bothe *et al.*, 2007). Host plants may be able to regulate N-fixation rates by chemical signals to their cyanobionts, causing physiological changes or even by regulating their abundance (Solheim and Zielke, 2002; Bergman *et al.*, 2007). Antimicrobial properties of moss tissues and high phenol concentrations may make bryophytes easily able to discourage cyanobacterial colonization, although the prevalence of associations in mature boreal forest stands otherwise lacking in cyanobacterial symbioses suggests that they can also be amicable hosts when the need arises (Turetsky, 2003; DeLuca *et al.*, 2007).

Pleurozium schreberi (Brid.) Mitt. is the most common and abundant species of feather moss in the boreal forest, present in 90% of the range, and covering at least half of the boreal forest floor in most locations (see Benscoter and Vitt, 2007). It is also host to heterocystous nitrogen-fixing cyanobacteria that may be found on the surface, or under leaves of the shoots (DeLuca *et al.*, 2002). These cyanobionts include members of several genera, most commonly *Nostoc*, *Stigonema*, and *Calothrix* (DeLuca *et al.*, 2002; Houle *et al.*, 2006; DeLuca *et al.*, 2007). These cyanobionts have also been found in association with several common boreal forest feather moss species, including *P. schreberi*, *Hylocomium splendens*, and *Ptillium crista-castrensis* (DeLuca *et al.*, 2002; Houle *et al.*, 2006; Lagerström *et al.*, 2007; Zackrisson *et al.*, 2009). Despite widespread distributions, cyanobacterial diversity appears to show a high degree of host-specificity, at least at the genetic level (Ininbergs *et al.*, 2011).

N-fixation rates by these cyanobacteria rival other inputs of nitrogen, challenging us to re-examine nitrogen-limitation within boreal forest ecosystems (DeLuca *et al.*, 2002). Nevertheless, N-fixation rates are also highly variable, and may be controlled by both environmental and biotic factors. The relative importance of biotic and environmental controls remains an unresolved question in this system, as with general research on the importance of biodiversity for ecosystem function.

N-fixation rates may be correlated to cyanobacteria abundance (DeLuca *et al.*, 2007), and therefore controls might act indirectly by changing cyanobacteria populations, or directly by modifying N-fixation activity of static populations. Collembola have been observed grazing on mats of nitrogen-fixing cyanobacteria in arctic environments, which suggests the possibility of top-down regulation of

N-fixation, by controlling cyanobacteria populations as well as herbivory-induced N-fixation (Birkemoe and Liengen, 2000). Temperature, light, moisture, and nutrient availability are the most important environmental controls on N-fixation rates (Turetsky, 2003; Lindo and Gonzalez, 2010; Sorensen and Michelsen, 2011; Gundale *et al.*, 2012a,b). The frequency and timing of moisture supply can also have large effects on N-fixation rates, in addition to the total amount (Jackson *et al.*, 2010; Gundale *et al.*, 2012b). What remains unclear, however, is whether these environmental conditions regulate N-fixation rates directly, or by changing the abundance of symbiotic cyanobacteria.

Different genera of cyanobacteria appear to fix nitrogen at different rates, depending on temperature (Gentili *et al.*, 2005). For the same protein content, *Calothrix* cells had the highest rates of N-fixation at 30 °C, while *Nostoc* cells fixed the most nitrogen at 13 °C (Gentili *et al.*, 2005). Cyanobacteria taxa associated with boreal forest therefore has the potential to exhibit the kind of response diversity and redundancy that theory predicts should act as a buffer to reduce variability in ecosystem-level nitrogen-fixation under changing environmental conditions.

Given the energy requirements of N-fixation, the process may not be worthwhile when nitrogen is not limiting. Studies of boreal forest stands of different ages have found that N-fixation rates are low or non-existent when nitrogen is abundant following fires, but increases as stands mature and nitrogen becomes more limiting (Zackrisson *et al.*, 2004; DeLuca *et al.*, 2008). This negative relationship between available N and N-fixation has led to a view of negative feedbacks between the two inputs, described as a "nitrostat", regulating total N inputs (DeLuca *et al.*, 2008; Menge and Hedin, 2009). Reciprocal transplant experiments in the field suggest this difference is related to changes in cyanobacteria abundance induced by changes in available nitrogen (DeLuca *et al.*, 2007). Menge and Hedin (2009), however, found a positive association between available nitrogen and N-fixation rates, mediated by bryophyte abundance at highly fertile sites.

1.5 The boreal forest bryosphere: a natural model system to study ecosystem responses to climate change and habitat fragmentation

Moss contributes to ecosystem-level productivity, nutrient cycling, and also creates structural habitat for a highly diverse multitrophic food web, including N-fixing cyanobacteria, other bacteria, fungi, microarthropods and many other invertebrates

(Lindo and Gonzalez, 2010). Mosses are grazed by very few animals, possibly due to high concentrations of phenols and other unpalatable compounds (Turetsky, 2003; Lindo and Gonzalez, 2010). Moss tends to form tightly packed mats on forest floors, forming a transition zone between above-ground terrestrial and atmospheric processes, and below-ground processes characteristic of soils. This boundary layer of moss, together with inhabiting biota, has been termed "the bryosphere", to recognize its unique contribution to ecosystem processes, and potential as a natural model system (Lindo and Gonzalez, 2010). The bryosphere is well-suited to studying the relationships between biodiversity and ecosystem processes, at physical scales small enough to make experimental manipulation feasible.

Boreal forest moss is a major player in stand-level carbon and nitrogen cycling, which can affect ecosystem properties of the forest as a whole, with potential for climate feedbacks across an extensive circumpolar range. Climate change is expected to cause increases in temperature and changes in precipitation patterns throughout the region, with potential impacts on N-fixation and microarthropod communities. Fragmentation is also an important driver of biodiversity change at large scales, while the brysophere is a natural model system that has previously been used to demonstrate non-random biodiversity loss caused by fragmentation. The boreal forest bryosphere is therefore a highly tractable model microecosystem for the study of interactive effects of climate change and habitat fragmentation on biodiversity and ecosystem processes. A potential diversity of nitrogen-fixing cyanobacteria, and a highly diverse decomposer food web, offer several biotic communities and relevant processes to examine, with relevance to the boreal forest, and other systems facing climate change in fragmented landscapes.

The boreal forest bryosphere combines several attributes of a tractable experimental system, with unique features relevant to questions concerning the relationship between biodiversity and ecosystem function. A highly diverse detritus-based food web within the bryosphere is well-suited to studying environmental change and dispersal in competitive and multitrophic communities (Gonzalez *et al.*, 1998; Srivastava *et al.*, 2004; Lindo and Gonzalez, 2010). Positive interactions, such as facultative symbioses between moss and cyanobacteria, can buffer stressful environmental conditions, and represent an understudied aspect of biodiversity-ecosystem function relationships (Bertness and Callaway, 1994; Mulder *et al.*, 2001). All this biota is also linked to nitrogen cycling, with further implications for productivity and decomposition within the boreal forest as a whole. Many classic experiments on ecosystem function have focused on plant productivity or

biomass (Loreau *et al.*, 2001), whereas this system also includes N-fixation as a dynamic ecosystem process, which is affected by a range of biotic interactions between mosses and multiple cyanobacteria taxa. The relative importance of biotic interactions and environmental conditions also remains untested with respect to N-fixation, productivity, and decomposition in the boreal forest bryosphere, despite the implications for carbon cycling and global climate feedbacks.

1.6 Thesis Overview

In order to explore the environmental and ecological controls over processes important for nitrogen cycling in boreal forest moss, I carried out a field experiment that combined habitat fragmentation and simulated climate change, while measuring several ecological state variables and ecosystem processes. The next chapter describes the overall design of the field experiment and implications for statistical analysis. Subsequent chapters present analyses of different subsets of data collected from the experiment, exploring the effects on: (1) cyanobacteria and nitrogen fixation, (2) moss production and decomposition under environmental change, (3) N-fixation and moss growth, including the importance of available nitrogen, and (4) microarthropod community structure within the moss, and a test of hypothetical top-down control on cyanobacteria. General conclusions are summarized in the final chapter.

References

Bascompte J, Solé RV (1996) Habitat fragmentation and extinction thresholds in spatially explicit models. Journal of Animal Ecology 65(4):465–473

Bellard C, Bertelsmeier C, Leadley P, Thuiller W, Courchamp F (2012) Impacts of climate change on the future of biodiversity. Ecology Letters pp no–no, doi:10.1111/j.1461-0248.2011.01736.x

Benscoter BW, Vitt DH (2007) Evaluating feathermoss growth: a challenge to traditional methods and implications for the boreal carbon budget. Journal of Ecology 95(1):151–158, doi:10.1111/j.1365-2745.2006.01180.x

Bergman B, Rai A, Rasmussen U (2007) Cyanobacterial associations. In: Elmerich C, Newton WE (eds.) Nitrogen Fixation: Origins, Applications, and Research

Progress, vol. 5, Springer Netherlands, Dordrecht, The Netherlands, pp 257–301

Bernier PY, Desjardins RL, Karimi-Zindashty Y, Worth D, Beaudoin A, Luo Y, Wang S (2011) Boreal lichen woodlands: A possible negative feedback to climate change in eastern North America. Agricultural and Forest Meteorology 151(4):521–528, doi:10.1016/j.agrformet.2010.12.013

Bertness MD, Callaway R (1994) Positive interactions in communities. Trends in Ecology & Evolution 9(5):191–193

Birkemoe T, Liengen T (2000) Does collembolan grazing influence nitrogen fixation by cyanobacteria in the high Arctic? Polar Biology 23(8):589–592, doi:10.1007/s003000000133

Böhme H (1998) Regulation of nitrogen fixation in heterocyst-forming cyanobacteria. Trends in Plant Science 3(9):346–351

Bonan GB, Van Cleve K (1992) Soil temperature nitrogen mineralization and carbon source-sink relationships in boreal forests. Canadian Journal of Forest Research 22(5):629–639

Bothe H, Ferguson SJ, Newton WE (2007) Biology of the Nitrogen Cycle. Elsevier, Amsterdam, The Netherlands

Botkin DB, Saxe H, Araujo MB, Betts R, Bradshaw RHW, Cedhagen T, Chesson P, Dawson TP, Etterson JR, Faith DP, Ferrier S, Guisan A, Hansen AS, Hilbert DW, Loehle C, Margules C, New M, Sobel MJ, Stockwell DRB (2007) Forecasting the effects of global warming on biodiversity. Bioscience 57(3):227–236, doi:10.1641/b570306

Brooker RW, Travis JMJ, Clark EJ, Dytham C (2007) Modelling species' range shifts in a changing climate: The impacts of biotic interactions, dispersal distance and the rate of climate change. Journal of Theoretical Biology 245(1):59–65

Burrows MT, Schoeman DS, Buckley LB, Moore P, Poloczanska ES, Brander KM, Brown C, Bruno JF, Duarte CM, Halpern BS, Holding J, Kappel CV, Kiessling W, O'Connor MI, Pandolfi JM, Parmesan C, Schwing FB, Sydeman WJ, Richardson AJ (2011) The pace of shifting climate in marine and

terrestrial ecosystems. Science 334(6056):652–655, doi:10.1126/science.1210288

Cardinale BJ, Nelson K, Palmer MA (2000) Linking species diversity to the functioning of ecosystems: on the importance of environmental context. Oikos 91:175–183

Cardinale BJ, Palmer MA, Collins SL (2002) Species diversity enhances ecosystem functioning through interspecific facilitation. Nature 415(6870):426–429

Cardinale BJ, Srivastava DS, Emmett Duffy J, Wright JP, Downing AL, Sankaran M, Jouseau C (2006) Effects of biodiversity on the functioning of trophic groups and ecosystems. Nature 443(7114):989–992

Cardinale BJ, Matulich KL, Hooper DU, Byrnes JE, Duffy E, Gamfeldt L, Balvanera P, O'Connor MI, Gonzalez A (2011) The functional role of producer diversity in ecosystems. Am J Bot 98(3):572–592, doi:10.3732/ajb.1000364

Cardinale BJ, Duffy JE, Gonzalez A, Hooper DU, Perrings C, Venail P, Narwani A, Mace GM, Tilman D, Wardle DA, Kinzig AP, Daily GC, Loreau M, Grace JB, Larigauderie A, Srivastava DS, Naeem S (2012) Biodiversity loss and its impact on humanity. Nature 486(7401):59–67, doi:10.1038/nature11148

Chapin FS, Zavaleta ES, Eviner VT, Naylor RL, Vitousek PM, Reynolds HL, Hooper DU, Lavorel S, Sala OE, Hobbie SE, Mack MC, Diaz S (2000) Consequences of changing biodiversity. Nature 405(6783):234–242

Chapin I F S, Oechel WC, Van Cleve K, Lawrence W (1987) The role of mosses in the phosphorus cycling of an Alaskan USA black spruce forest. Oecologia 74(2):310–315

Chapin I F Stuart, Walker BH, Hobbs RJ, Hooper DU, Lawton JH, Sala OE, Tilman D (1997) Biotic control over the functioning of ecosystems. Science 277(5325):500–504, doi:10.1126/science.277.5325.500

Chen IC, Hill JK, Ohlemüller R, Roy DB, Thomas CD (2011) Rapid range shifts of species associated with high levels of climate warming. Science 333(6045):1024–1026, doi:10.1126/Science.1206432

Chesson P (2000) Mechanisms of maintenance of species diversity. Annual Review of Ecology and Systematics 31:343–366

Cottingham KL, Brown BL, Lennon JT (2001) Biodiversity may regulate the temporal variability of ecological systems. Ecology Letters 4(1):72–85

Cumming GS (2007) Global biodiversity scenarios and landscape ecology. Landscape Ecology 22(5):671–685, doi:10.1007/s10980-006-9057-3

Davidson EA, Janssens IA (2006) Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440(7081):165–173, doi:10.1038/Nature04514

Davies KF, Melbourne BA, Margules CR (2001) Effects of within- and between-patch processes on community dynamics in a fragmentation experiment. Ecology 82(7):1830–1846

Davis AJ, Jenkinson LS, Lawton JH, Shorrocks B, Wood S (1998a) Making mistakes when predicting shifts in species range in response to global warming. Nature 391(6669):783–786

Davis AJ, Lawton JH, Shorrocks B, Jenkinson LS (1998b) Individualistic species responses invalidate simple physiological models of community dynamics under global environmental change. Journal of Animal Ecology 67(4):600–612

Davis MB, Shaw RG (2001) Range shifts and adaptive responses to Quaternary climate change. Science 292(5517):673–679

DeLuca T, Zackrisson O, Gentili F, Sellstedt A, Nilsson MC (2007) Ecosystem controls on nitrogen fixation in boreal feather moss communities. Oecologia 152(1):121–130, doi:10.1007/s00442-006-0626-6

DeLuca TH, Zackrisson O, Nilsson MC, Sellstedt A (2002) Quantifying nitrogen-fixation in feather moss carpets of boreal forests. Nature 419(6910):917–920, doi:10.1038/nature01051

DeLuca TH, Zackrisson O, Gundale MJ, Nilsson MC (2008) Ecosystem feedbacks and nitrogen fixation in boreal forests. Science 320(5880):1181, doi:10.1126/science.1154836

Descamps-Julien B, Gonzalez A (2005) Stable coexistence in a fluctuating environment: an experimental demonstration. Ecology 86(10):2815–2824

Doak DF, Bigger D, Harding EK, Marvier MA, O'Malley RE, Thomson D (1998) The statistical inevitability of stability-diversity relationships in community ecology. American Naturalist 151(3):264–276

Dobson A, Lodge D, Alder J, Cumming GS, Keymer J, McGlade J, Mooney H, Rusak JA, Sala O, Wolters V, Wall D, Winfree R, Xenopoulos MA (2006) Habitat loss, trophic collapse, and the decline of ecosystem services. Ecology 87(8):1915–1924

Doerr VAJ, Barrett T, Doerr ED (2011) Connectivity, dispersal behaviour and conservation under climate change: a response to Hodgson et al. Journal of Applied Ecology 48(1):143–147, doi:10.1111/j.1365-2664.2010.01899.x

Ehrlich PR, Ehrlich AH (1981) Extinction: The causes and consequences of the disappearance of species. Random House, New York, New York, USA

Elmqvist T, Folke C, Nystrom M, Peterson G, Bengtsson J, Walker B, Norberg J (2003) Response diversity, ecosystem change, and resilience. Frontiers in Ecology and the Environment 1(9):488–494

Eugster W, Rouse WR, Pielke Sr RA, McFadden JP, Baldocchi DD, Kittel TGF, Chapin FS, Liston GE, Vidale PL, Vaganov E, Chambers S (2000) Land-atmosphere energy exchange in Arctic tundra and boreal forest: available data and feedbacks to climate. Global Change Biology 6(S1):84–115, doi:10.1046/j.1365-2486.2000.06015.x

Fahrig L (2003) Effects of habitat fragmentation on biodiversity. Annual Review of Ecology Evolution and Systematics 34:487–515, doi:10.1146/annurev.ecolsys.34.011802.132419

Fukami T, Morin PJ (2003) Productivity-biodiversity relationships depend on the history of community assembly. Nature 424(6947):423–426

Gamfeldt L, Hillebrand H, Jonsson PR (2008) Multiple functions increase the importance of biodiversity for overall ecosystem functioning. Ecology 89(5):1223–1231, doi:10.1890/06-2091.1

Gaston KJ (2000) Global patterns in biodiversity. Nature 405(6783):220–227

Gentili F, Nilsson MC, Zackrisson O, DeLuca TH, Sellstedt A (2005) Physiological and molecular diversity of feather moss associative N2-fixing cyanobacteria. Journal of Experimental Botany 56(422):3121–3127, doi:10.1093/jxb/eri309

Gilbert F, Gonzalez A, Evans-Freke I (1998) Corridors maintain species richness in the fragmented landscapes of a microecosystem. Proceedings of the Royal Society B: Biological Sciences 265(1396):577–582

Gilman SE, Urban MC, Tewksbury J, Gilchrist GW, Holt RD (2010) A framework for community interactions under climate change. Trends in Ecology & Evolution 25(6):325–331, doi:10.1016/J.Tree.2010.03.002

Gonzalez A, Holt RD (2002) The inflationary effects of environmental fluctuations in source-sink systems. Proceedings of the National Academy of Sciences of the United States of America 99(23):14,872–14,877

Gonzalez A, Loreau M (2009) The causes and consequences of compensatory dynamics in ecological communities. Annual Review of Ecology, Evolution, and Systematics 40(1):393–414, doi:10.1146/annurev.ecolsys.39.110707.173349

Gonzalez A, Lawton JH, Gilbert FS, Blackburn TM, Evans-Freke I (1998) Metapopulation dynamics, abundance, and distribution in a microecosystem. Science 281(5385):2045–2047

Gonzalez A, Mouquet N, Loreau M (2009) Biodiversity as spatial insurance: the effects of habitat fragmentation and dispersal on ecosystem functioning. In: Naeem S, Bunker DE, Hector A, Loreau M, Perrings C (eds.) Biodiversity, Ecosystem Functioning and Ecosystem Services, Oxford University Press

Grant EHC, Nichols JD, Lowe WH, Fagan WF (2010) Use of multiple dispersal pathways facilitates amphibian persistence in stream networks. Proceedings of the National Academy of Sciences of the United States of America 107(15):6936–6940, doi:10.1073/Pnas.1000266107

Gravel D, Mouquet N, Loreau M, Guichard F (2010) Patch Dynamics, Persistence, and Species Coexistence in Metaecosystems. American Naturalist 176(3):289–302, doi:10.1086/655426

Gross K, Cardinale BJ (2005) The functional consequences of random vs. ordered species extinctions. Ecology Letters 8(4):409–418

Gundale MJ, Nilsson M, Bansal S, JÃd'derlund A (2012a) The interactive effects of temperature and light on biological nitrogen fixation in boreal forests. New Phytologist 194(2):453–463, doi:10.1111/j.1469-8137.2012.04071.x

Gundale MJ, Wardle DA, Nilsson MC (2012b) The effect of altered macroclimate on N-fixation by boreal feather mosses. Biology Letters 8(5):805–808, doi:10.1098/rsbl.2012.0429

Hansen J, Sato M, Ruedy R (2012) Perception of climate change. Proceedings of the National Academy of Sciences 109(37):E2415–E2423, doi:10.1073/pnas.1205276109

Hanski I (1999) Metapopulation ecology. Oxford series in ecology and evolution, Oxford University Press, Oxford, UK

Hanski I (2005) Landscape fragmentation, biodiversity loss and the societal response – The longterm consequences of our use of natural resources may be surprising and unpleasant. Embo Reports 6(5):388–392, doi:10.1038/sj.embor.7400398

Hanski I, Gilpin M (1991) Metapopulation Dynamics – Brief-History and Conceptual Domain. Biological Journal of the Linnean Society 42(1-2):3–16

Hector A, Bagchi R (2007) Biodiversity and ecosystem multifunctionality. Nature 448(7150):188–190, doi:10.1038/nature05947

Hector A, Schmid B, Beierkuhnlein C, Caldeira MC, Diemer M, Dimitrakopoulos PG, Finn JA, Freitas H, Giller PS, Good J, Harris R, Hogberg P, Huss-Danell K, Joshi J, Jumpponen A, Korner C, Leadley PW, Loreau M, Minns A, Mulder CPH, O'Donovan G, Otway SJ, Pereira JS, Prinz A, Read DJ, Scherer-Lorenzen M, Schulze ED, Siamantziouras ASD, Spehn EM, Terry AC, Troumbis AY, Woodward FI, Yachi S, Lawton JH (1999) Plant diversity and productivity experiments in European grasslands. Science 286(5442):1123–1127

Heywood VH (1995) Global Biodiversity Assessment. Cambridge University Press, Cambridge, UK

Hodgson JA, Thomas CD, Wintle BA, Moilanen A (2009) Climate change, connectivity and conservation decision making: back to basics. Journal of Applied Ecology 46(5):964–969, doi:10.1111/J.1365-2664.2009.01695.X

Holling CS (1973) Resilience and stability of ecological systems. Annual Review of Ecology and Systematics 4(1):1–23, doi:10.1146/annurev.es.04.110173.000245

Holt RD, Barfield M, Gonzalez A (2003) Impacts of environmental variability in open populations and communities: "inflation" in sink environments. Theoretical Population Biology 64(3):315–330

Holyoak M, Leibold MA, Holt RD (2005) Metacommunities : spatial dynamics and ecological communities. University of Chicago Press, Chicago, Il.

Hooper DU, Vitousek PM (1997) The effects of plant composition and diversity on ecosystem processes. Science 277(5330):1302–1305, doi:10.1126/science.277.5330.1302

Hooper DU, Chapin I E S, Ewel JJ, Hector A, Inchausti P, Lavorel S, Lawton JH, Lodge DM, Loreau M, Naeem S, Schmid B, Setala H, Symstad AJ, Vandermeer J, Wardle DA (2005) Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. Ecological Monographs 75(1):3–35

Houlahan JE, Currie DJ, Cottenie K, Cumming GS, Ernest SKM, Findlay CS, Fuhlendorf SD, Gaedke U, Legendre P, Magnuson JJ, McArdle BH, Muldavin EH, Noble D, Russell R, Stevens RD, Willis TJ, Woiwod IP, Wondzell SM (2007) Compensatory dynamics are rare in natural ecological communities. Proceedings of the National Academy of Sciences of the United States of America 104(9):3273–3277, doi:10.1073/pnas.0603798104

Houle D, Gauthier SB, Paquet S, Planas D, Warren A (2006) Identification of two genera of N-2-fixing cyanobacteria growing on three feather moss species in boreal forests of Quebec, Canada. Canadian Journal of Botany-Revue Canadienne De Botanique 84(6):1025–1029, doi:10.1139/B06-059

Hughes JB, Roughgarden J (2000) Species diversity and biomass stability. American Naturalist 155(5):618–627

Hungate BA, Dukes JS, Shaw MR, Luo YQ, Field CB (2003) Nitrogen and climate change. Science 302(5650):1512–1513

Huston MA, Aarssen LW, Austin MP, Cade BS, Fridley JD, Garnier E, Grime JP, Hodgson J, Lauenroth WK, Thompson K, Vandermeer JH, Wardle DA, Hector A, Schmid B, Beierkuhnlein C, Caldeira MC, Diemer M, Dimitrakopoulos PG, Finn JA, Freitas H, Giller PS, Good J, Harris R, Hogberg P, Huss-Danell K, Joshi J, Jumpponen A, Korner C, Leadley PW, Loreau M, Minns A, Mulder CPH, O'Donovan G, Otway SJ, Pereira JS, Prinz A, Read DJ, Scherer-Lorenzen M, Schulze ED, Siamantziouras ASD, Spehn E, Terry AC, Troumbis AY, Woodward FI, Yachi S, Lawton JH (2000) No consistent effect of plant diversity on productivity. Science 289(5483):1255a, doi:10.1126/science.289.5483.1255a

Ininbergs K, Bay G, Rasmussen U, Wardle DA, Nilsson MC (2011) Composition and diversity of nifH genes of nitrogen-fixing cyanobacteria associated with boreal forest feather mosses. New Phytologist 192(2):507–517, doi:10.1111/j.1469-8137.2011.03809.x

IPCC (2002) Climate change and biodiversity. Technical Paper IPCC Technical Paper V, Intergovernmental Panel on Climate Change

Isbell F, Calcagno V, Hector A, Connolly J, Harpole WS, Reich PB, Scherer-Lorenzen M, Schmid B, Tilman D, van Ruijven J, Weigelt A, Wilsey BJ, Zavaleta ES, Loreau M (2011) High plant diversity is needed to maintain ecosystem services. Nature 477(7363):199–202, doi:10.1038/nature10282

Ives AR, Hughes JB (2002) General relationships between species diversity and stability in competitive systems. American Naturalist 159(4):388–395

Ives AR, Klug JL, Gross K (2000) Stability and species richness in complex communities. Ecology Letters 3(5):399–411

Jackson BG, Martin P, Nilsson MC, Wardle DA (2010) Response of feather moss associated N2 fixation and litter decomposition to variations in simulated rainfall intensity and frequency. Oikos 120(4):570–581, doi:10.1111/j.1600-0706.2010.18641.x

Janssens IA, Luyssaert S (2009) Carbon cycle: Nitrogen's carbon bonus. Nature Geosci 2(5):318–319, doi:10.1038/ngeo505

Kardol P, Reynolds WN, Norby RJ, Classen AT (2011) Climate change effects on soil microarthropod abundance and community structure. Applied Soil Ecology 47(1):37–44, doi:10.1016/J.Apsoil.2010.11.001

Lagerström A, Nilsson MC, Zackrisson O, Wardle DA (2007) Ecosystem input of nitrogen through biological fixation in feather mosses during ecosystem retrogression. Functional Ecology 21(6):1027–1033, doi:10.1111/j.1365-2435.2007.01331.x

Leibold MA, Holyoak M, Mouquet N, Amarasekare P, Chase JM, Hoopes MF, Holt RD, Shurin JB, Law R, Tilman D, Loreau M, Gonzalez A (2004) The metacommunity concept: a framework for multi-scale community ecology. Ecology Letters 7(7):601–613, doi:10.1111/j.1461-0248.2004.00608.x

Lindahl BO, Taylor AFS, Finlay RD (2002) Defining nutritional constraints on carbon cycling in boreal forests: towards a less 'phytocentric' perspective. Plant and Soil 242(1):123–135, doi:10.1023/A:1019650226585

Lindo Z, Gonzalez A (2010) The bryosphere: an integral and influential component of the Earth's biosphere. Ecosystems 13(4):612–627, doi:10.1007/s10021-010-9336-3

Logan T, Charron I, Chaumont D, Houle D (2011) Atlas de scénarios climatiques pour la forêt québécoise. Tech. Rep. ISBN papier : 976-2-923292-06-9 ISBN web : 978-2-923292-05-2, Ouranos et MRNF, URL: http://www.ouranos.ca/media/publication/162_AtlasForet2011.pdf

Loreau M (2000) Biodiversity and ecosystem functioning: recent theoretical advances. Oikos 91(1):3–17

Loreau M, Hector A (2001) Partitioning selection and complementarity in biodiversity experiments. Nature 412(6842):72–76

Loreau M, Holt RD (2004) Spatial flows and the regulation of ecosystems. American Naturalist 163(4):606–615

Loreau M, Naeem S, Inchausti P, Bengtsson J, Grime JP, Hector A, Hooper DU, Huston MA, Raffaelli D, Schmid B, Tilman D, Wardle DA (2001)

Biodiversity and ecosystem functioning: current knowledge and future challenges. Science 294(5543):804–808, doi:10.1126/science.1064088

Loreau M, Mouquet N, Gonzalez A (2003a) Biodiversity as spatial insurance in heterogeneous landscapes. Proceedings of the National Academy of Sciences of the United States of America 100(22):12,765 – 12,770, doi:10.1073/pnas.2235465100

Loreau M, Mouquet N, Holt RD (2003b) Meta-ecosystems: a theoretical framework for a spatial ecosystem ecology. Ecology Letters 6(8):673–679, doi:10.1046/j.1461-0248.2003.00483.x

MacArthur R (1955) Fluctuations of Animal Populations and a Measure of Community Stability. Ecology 36(3):533–536

MacArthur RH, Wilson EO (2001) The theory of island biogeography. Princeton landmarks in biology, Princeton University Press, Princeton, N.J. ; Oxford

Maestre FT, Quero JL, Gotelli NJ, Escudero A, Ochoa V, Delgado-Baquerizo M, García-Gómez M, Bowker MA, Soliveres S, Escolar C, García-Palacios P, Berdugo M, Valencia E, Gozalo B, Gallardo A, Aguilera L, Arredondo T, Blones J, Boeken B, Bran D, ConceiçÃčo AA, Cabrera O, Chaieb M, Derak M, Eldridge DJ, Espinosa CI, Florentino A, Gaitán J, Gatica MG, Ghiloufi W, Gómez-González S, Gutiérez JR, Hernández RM, Huang X, Huber-Sannwald E, Jankju M, Miriti M, Monerris J, Mau RL, Morici E, Naseri K, Ospina A, Polo V, Prina A, Pucheta E, Ramírez-Collantes DA, RomÃčo R, Tighe M, Torres-Díaz C, Val J, Veiga JP, Wang D, Zaady E (2012) Plant species richness and ecosystem multifunctionality in global drylands. Science 335(6065):214–218, doi:10.1126/science.1215442

Matthews DP, Gonzalez A (2007) The inflationary effects of environmental fluctuations ensure the persistence of sink metapopulations. Ecology 88(11):2848–2856

May RM (1974) Stability and complexity in model ecosystems, [2d edn. Princeton University Press, Princeton, N.J.

McCann KS (2000) The diversity-stability debate. Nature 405(6783):228–233

McInerny G, Travis JMJ, Dytham C (2007) Range shifting on a fragmented landscape. Ecological Informatics 2(1):1–8

McLaughlin JF, Hellmann JJ, Boggs CL, Ehrlich PR (2002) Climate change hastens population extinctions. Proceedings of the National Academy of Sciences of the United States of America 99(9):6070–6074, doi:10.1073/Pnas.052131199

Menge DNL, Hedin LO (2009) Nitrogen fixation in different biogeochemical niches along a 120 000-year chronosequence in New Zealand. Ecology 90(8):2190–2201, doi:10.1890/08-0877.1

Mikkelsen TN, Beier C, Jonasson S, Holmstrup M, Schmidt IK, Ambus P, Pilegaard K, Michelsen A, Albert K, Andresen LC, Arndal MF, Bruun N, Christensen S, Danbaek S, Gundersen P, Jorgensen P, Linden LG, Kongstad J, Maraldo K, Prieme A, Riis-Nielsen T, Ro-Poulsen H, Stevnbak K, Selsted MB, Sorensen P, Larsen KS, Carter MS, Ibrom A, Martinussen T, Miglietta F, Sverdrup H (2008) Experimental design of multifactor climate change experiments with elevated CO2, warming and drought: the CLIMAITE project. Functional Ecology 22(1):185–195, doi:10.1111/J.1365-2435.2007.01362.X

Millennium Ecosystem Assessment (2005) Ecosystems and human well-being: Biodiversity synthesis. Tech. rep., World Resources Institute, URL: http://www.maweb.org/

Mouquet N, Loreau M (2003) Community patterns in source-sink metacommunities. American Naturalist 162(5):544–557

Mulder CPH, Uliassi DD, Doak DF (2001) Physical stress and diversity-productivity relationships: The role of positive interactions. Proceedings of the National Academy of Sciences of the United States of America 98(12):6704–6708

Naeem S (1998) Species redundancy and ecosystem reliability. Conservation Biology 12(1):39–45, doi:10.1046/j.1523-1739.1998.96379.x

Naeem S, Li S (1997) Biodiversity enhances ecosystem reliability. Nature 390(6659):507–509

Naeem S, Thompson LJ, Lawler SP, Lawton JH, Woodfin RM (1994) Declining biodiversity can alter the performance of ecosystems. Nature 368(6473):734–737

van Nes EH, Scheffer M (2004) Large species shifts triggered by small forces. American Naturalist 164(2):255–266

Norberg J, Swaney DP, Dushoff J, Lin J, Casagrandi R, Levin SA (2001) Phenotypic diversity and ecosystem functioning in changing environments: A theoretical framework. Proceedings of the National Academy of Sciences of the United States of America 98(20):11,376–11,381, doi:10.1073/pnas.171315998

Norberg J, Urban MC, Vellend M, Klausmeier CA, Loeuille N (2012) Eco-evolutionary responses of biodiversity to climate change. Nature Clim Change advance online publication, doi:10.1038/nclimate1588

Norby RJ, Warren JM, Iversen CM, Medlyn BE, McMurtrie RE (2010) CO2 enhancement of forest productivity constrained by limited nitrogen availability. Proceedings of the National Academy of Sciences of the United States of America doi:10.1073/pnas.1006463107

O'Connor NE, Crowe TP (2005) Biodiversity loss and ecosystem functioning: Distinguishing between number and identity of species. Ecology 86(7):1783–1796

O'Donnell JA, Turetsky MR, Harden JW, Manies KL, Pruett LE, Shetler G, Neff JC (2009) Interactive Effects of Fire, Soil Climate, and Moss on CO2 Fluxes in Black Spruce Ecosystems of Interior Alaska. Ecosystems 12(1):57–72, doi:10.1007/s10021-008-9206-4

Opdam P, Wascher D (2004) Climate change meets habitat fragmentation: linking landscape and biogeographical scale levels in research and conservation. Biological Conservation 117(3):285–297, doi:10.1016/j.biocon.2003.11.008

Parmesan C (2006) Ecological and evolutionary responses to recent climate change. Annual Review of Ecology Evolution and Systematics 37:637–669, doi:10.1146/annurev.ecolsys.37.091305.110100

Parmesan C, Yohe G (2003) A globally coherent fingerprint of climate change impacts across natural systems. Nature 421(6918):37–42, doi:10.1038/nature01286

Pearson RG, Dawson TP (2003) Predicting the impacts of climate change on the distribution of species: are bioclimate envelope models useful? Global Ecology and Biogeography 12(5):361–371

Pearson RG, Dawson TP (2005) Long-distance plant dispersal and habitat fragmentation: identifying conservation targets for spatial landscape planning under climate change. Biological Conservation 123(3):389–401, doi:10.1016/j.biocon.2004.12.006

Peng C, Apps MJ (1999) Modelling the response of net primary productivity (NPP) of boreal forest ecosystems to changes in climate and fire disturbance regimes. Ecological Modelling 122(3):175–193

Petchey OL (2000) Species diversity, species extinction, and ecosystem function. American Naturalist 155(5):696–702

Peterson G, Allen CR, Holling CS (1998) Ecological resilience, biodiversity, and scale. Ecosystems 1(1):6–18

Raffaelli D (2004) How extinction patterns affect ecosystems. Science 306(5699):1141–1142, doi:10.1126/science.1106365

Rastetter EE, Gough L, Hartley AE, Herbert DA, Nadelhoffer KJ, Williams M (1999) A revised assessment of species redundancy and ecosystem reliability. Conservation Biology 13(2):440–445

Reich PB, Tilman D, Isbell F, Mueller K, Hobbie SE, Flynn DFB, Eisenhauer N (2012) Impacts of biodiversity loss escalate through time as redundancy fades. Science 336(6081):589–592, doi:10.1126/science.1217909

Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier RY (1979) Generic assignments, strain histories and properties of pure cultures of cyanobacteria. Journal of General Microbiology 111:1–61

Rockstrom J, Steffen W, Noone K, Persson A, Chapin FS, Lambin EF, Lenton TM, Scheffer M, Folke C, Schellnhuber HJ, Nykvist B, de Wit CA, Hughes T, van der Leeuw S, Rodhe H, Sorlin S, Snyder PK, Costanza R, Svedin U, Falkenmark M, Karlberg L, Corell RW, Fabry VJ, Hansen J, Walker B, Liverman D, Richardson K, Crutzen P, Foley JA (2009) A safe operating space for humanity. Nature 461(7263):472–475

Rosenzweig ML (1996) Species Diversity In Space and Time, 2nd edn. Cambridge University Press, Cambridge, UK

Sala OE, Chapin FS, Armesto JJ, Berlow E, Bloomfield J, Dirzo R, Huber-Sanwald E, Huenneke LF, Jackson RB, Kinzig A, Leemans R, Lodge DM, Mooney HA, Oesterheld M, Poff NL, Sykes MT, Walker BH, Walker M, Wall DH (2000) Global biodiversity scenarios for the year 2100. Science 287(5459):1770–1774, doi:10.1126/science.287.5459.1770

Scheffer M, Carpenter S, Foley JA, Folke C, Walker B (2001) Catastrophic shifts in ecosystems. Nature 413(6856):591–596

Scheffer M, Bascompte J, Brock WA, Brovkin V, Carpenter SR, Dakos V, Held H, van Nes EH, Rietkerk M, Sugihara G (2009) Early-warning signals for critical transitions. Nature 461(7260):53–59

Soja AJ, Tchebakova NM, French NHF, Flannigan MD, Shugart HH, Stocks BJ, Sukhinin AI, Parfenova EI, Chapin FS, Stackhouse PW (2007) Climate-induced boreal forest change: Predictions versus current observations. Global and Planetary Change 56(3-4):274–296, doi:10.1016/J.Glopacha.2006.07.028

Solan M, Cardinale BJ, Downing AL, Engelhardt KAM, Ruesink JL, Srivastava DS (2004) Extinction and ecosystem function in the marine benthos. Science 306:1177–1180

Solheim B, Zielke M (2002) Associations between cyanobacteria and mosses. In: Rai A, Bergman B, Rasmussen U (eds.) Cyanobacteria in Symbiosis, Kluwer Academic Publishers, Netherlands, pp 137–152

Sorensen PL, Michelsen A (2011) Long-term warming and litter addition affects nitrogen fixation in a subarctic heath. Global Change Biology 17(1):528–537, doi:10.1111/j.1365-2486.2010.02234.x

Srivastava DS, Kolasa J, Bengtsson J, Gonzalez A, Lawler SP, Miller TE, Munguia P, Romanuk T, Schneider DC, Trzcinski MK (2004) Are natural microcosms useful model systems for ecology? Trends in Ecology & Evolution 19(7):379–384

Staddon P, Lindo Z, Crittenden PD, Gilbert F, Gonzalez A (2010) Connectivity, non-random extinction and ecosystem function in experimental metacommunities.

Ecology Letters 13(5):543–552, doi:10.1111/J.1461-0248.2010.01450.X

Taggart RE, Cross AT (2009) Global greenhouse to icehouse and back again: the origin and future of the boreal forest biome. Global and Planetary Change 65(3-4):115–121, doi:10.1016/j.gloplacha.2008.10.014

Thomas CD, Cameron A, Green RE, Bakkenes M, Beaumont LJ, Collingham YC, Erasmus BFN, de Siqueira MF, Grainger A, Hannah L, Hughes L, Huntley B, van Jaarsveld AS, Midgley GF, Miles L, Ortega-Huerta MA, Peterson AT, Phillips OL, Williams SE (2004) Extinction risk from climate change. Nature 427(6970):145–148, doi:10.1038/nature02121

Tilman D (1996) Biodiversity: population versus ecosystem stability. Ecology 77(2):350–363

Tilman D, Knops J, Wedin D, Reich P, Ritchie M, Siemann E (1997) The influence of functional diversity and composition on ecosystem processes. Science 277(5330):1300–1302

Tilman D, Lehman CL, Bristow CE (1998) Diversity-stability relationships: statistical inevitability or ecological consequence? American Naturalist 151(3):277–282

Tilman D, Reich PB, Knops J, Wedin D, Mielke T, Lehman C (2001) Diversity and productivity in a long-term grassland experiment. Science 294(5543):843–845

Travis JMJ (2003) Climate change and habitat destruction: a deadly anthropogenic cocktail. Proceedings of the Royal Society B: Biological Sciences 270(1514):467–473, doi:10.1098/rspb.2002.2246

Turetsky MR (2003) The role of bryophytes in carbon and nitrogen cycling. The Bryologist 106(3):395–409, doi:10.1639/05

Vellend M (2010) Conceptual Synthesis in Community Ecology. Quarterly Review of Biology 85(2):183–206

Villalpando SN, Williams RS, Norby RJ (2009) Elevated air temperature alters an old-field insect community in a multifactor climate change experiment. Global Change Biology 15(4):930–942, doi:10.1111/j.1365-2486.2008.01721.x

Vinebrooke RD, Cottingham KL, Norberg J, Scheffer M, Dodson SI, Maberly SC, Sommer U (2004) Impacts of multiple stressors on biodiversity and ecosystem functioning: the role of species co-tolerance. Oikos 104(3):451–457

Walther GR, Post E, Convey P, Menzel A, Parmesan C, Beebee TJC, Fromentin JM, Hoegh-Guldberg O, Bairlein F (2002) Ecological responses to recent climate change. Nature 416(6879):389–395, doi:10.1038/416389a

Watkinson AR, Gill JA (2002) Climate change and dispersal. In: Bullock JM, Kenward RE, Hails RS (eds.) Dispersal Ecology, Blackwell Science, Malden, MA, pp 410–428

Wigley TML, Smith RL, Santer BD (1998) Anthropogenic Influence on the Autocorrelation Structure of Hemispheric-Mean Temperatures. Science 282(5394):1676–1679, doi:10.1126/science.282.5394.1676

With KA, King AW (1999) Extinction thresholds for species in fractal landscapes. Conservation Biology 13(2):314–326

Yachi S, Loreau M (1999) Biodiversity and ecosystem productivity in a fluctuating environment: the insurance hypothesis. Proceedings of the National Academy of Sciences of the United States of America 96(4):1463–1468, doi:10.1073/pnas.96.4.1463

Zackrisson O, DeLuca TH, Nilsson MC, Sellstedt A, Berglund LM (2004) Nitrogen fixation increases with successional age in boreal forests. Ecology 85(12):3327–3334, doi:10.1890/04-0461

Zackrisson O, DeLuca T, Gentili F, Sellstedt A, Jäderlund A (2009) Nitrogen fixation in mixed Hylocomium splendens moss communities. Oecologia doi:10.1007/s00442-009-1299-8

Zavaleta ES, Pasari JR, Hulvey KB, Tilman GD (2010) Sustaining multiple ecosystem functions in grassland communities requires higher biodiversity. Proceedings of the National Academy of Sciences 107(4):1443–1446, doi:10.1073/pnas.0906829107

CHAPTER 2

SEC-C: The Schefferville Experiment on Climate Change (& Fragmentation)

The details of the experiment, its design and treatments are presented here in full, along with implications for statistical analyses. Some information in this chapter will be repeated in later chapters, where relevant and necessary for each to stand alone as a self-contained manuscript.

The field experiment described here was intended to combine habitat fragmentation and climate change in a factorial manner, permitting the assessment of effects of each in isolation, and in combination, thus providing a method to measure possible non-additive effects. We anticipated a range of extinction scenarios that would provide insight into how ecosystem properties change under community disassembly, particularly under ordered extinctions observed in previous fragmentation experiments (Gonzalez and Chaneton, 2002; Wright *et al.*, 2007). Although fragmentation and climate change are both drivers of environmental change, we had difficulty coming up with an acronym that captured all treatments, and preferred a simple one that happened to highlight the climate change aspects.

All raw data collected from the experiment, as well as scripts used to process and analyze them in `R v2.12` (R Development Core Team, 2010) are available online at:

`http://www.github.com/jawhiteley/SECC.R.JAW/`

Data are saved as `.csv` files (comma-separated values, readable by a wide range of computer software), and metadata is included in plain text files. The organization

of the files, and instructions for working with the project in R, are described in a README file in the main project directory. The entire directory is managed and stored as a git repository (http://git-scm.com/), meaning that a full version history of the files is available, and it can easily be copied or *cloned*. Note that the use of git software is not required to access the files: they can simply be copied as a directory of files (or downloaded from the URL given above). The data and files are made available under the Gnu General Public License (GPL) version 3 or later (included with the files). You are welcome and encouraged to use the material for your own purposes. I only ask that appropriate attribution and credit are given, and let me know if any of it was useful to you. When in doubt, you may cite this thesis, or relevant publications.

2.1 Study Site

The field experiment was set up just outside of Schefferville, Québec, Canada (54°48′N 66°50′W) (Figure 2.1). The experiment was spread out over eight blocks within an area of boreal forest approx. 100×200 m, centred near 54°47′44″N 66°47′20″W (642150 mE, 6074288 mN UTM grid 19 U) , roughly 1 km southeast of the airport at the edge of the town (Figures 2.2 and 2.3). This site is also briefly described by Lindo *et al.* (2012).

The Schefferville area was chosen for the field experiment partly due to its location in a transition zone near the northern limits of the boreal forest in eastern North America (Figure 2.1). The McGill Subarctic Research Station is also located in Schefferville, which makes for a convenient and well-equipped base of operations. Schefferville is south of the southern limit of continuous permafrost and includes areas of tundra at high elevations, with areas of boreal forest mixed with lichen-dominated open areas at lower elevations, as well as wetlands and small lakes. The topography is dominated by alternating ridges and valleys oriented in an approximately northwest to southeast direction (Fitzjarrald and Moore, 1994). Soils are typically well-drained and oligotrophic (Moore, 1980).

This area experiences continental weather typical of the region, with average air temperatures historically ranging from −24 °C in the winter to 16 °C in the summer (Lechowicz and Adams, 1978; Environment Canada, 2012). Air temperatures only remain above 0 °C from about May to September (Lechowicz and Adams, 1978). An average of 408 mm of rain falls on Schefferville each year, primarily during the growing season, although at least some of the 440 cm of annual

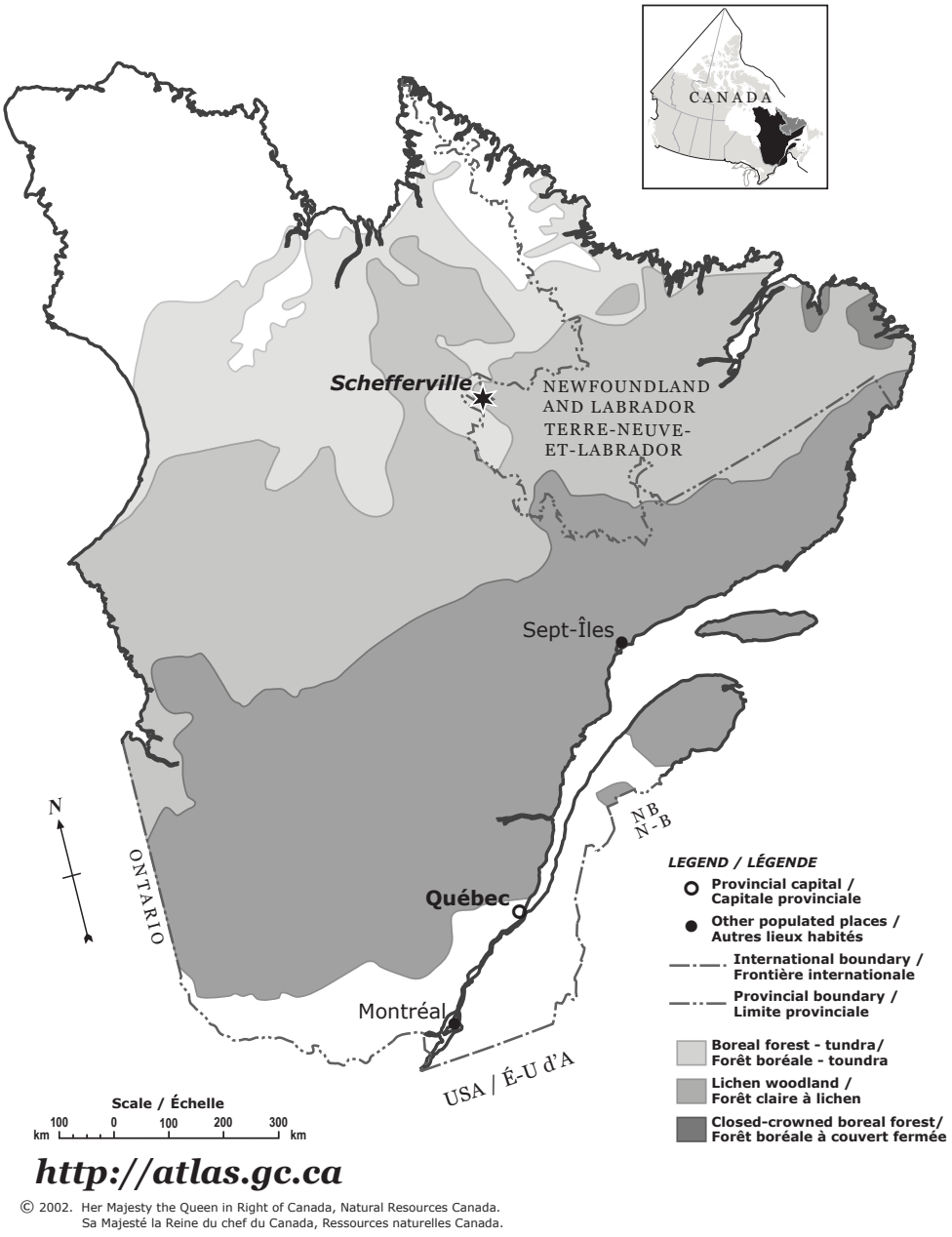

Figure 2.1

Map of Québec, Canada, showing location of Schefferville (indicated by the star), near the northern limits of boreal lichen woodland. The site itself was within spruce-moss forest with a sparse canopy (see text). Modified from http://atlas.gc.ca/ *, and Payette et al., 2001.*

Figure 2.2 *Location of study area (white rectangle), and McGill Subarctic Research Station (indicated by McGill crest northwest of the airport, west of the runway).*
Image from Google Earth, ©2012 Digital Globe, ©2012 Google. Used in accordance with Google Earth Content Rules & Guidelines for academic publication.

Figure 2.3 *Locations of eight (8) replicate blocks throughout the study area, outlined as a white rectangle approximately 100 × 200 m in size (2 ha).*
Image from Google Earth, ©2012 Digital Globe, ©2012 Google. Used in accordance with Google Earth Content Rules & Guidelines for academic publication.

snowfall has been observed at any month of the year (Environment Canada, 2012).

The study site is dominated by a sparse canopy of scattered spruce trees, including both *Picea mariana* (Mill.) and *P. glauca* (Moench) Voss. (Moore, 1980). The understory includes dwarf birch (*Betula glandulosa* Michx.) and Labrador tea (*Ledum groenlandicum* Oeder.), while the ground is covered by a continuous carpet of feather moss, most of which is *Pleurozium schreberi* (Brid.) Mitt., with occasional patches or individual shoots of *Hylocomium splendens* (Hedw.) or *Ptillum crista-castrensis* (Hedw.) (Moore, 1980). Although we have not been able to confirm the amount of time since the last fire at the site, we believe it to be at least 100 years, possibly as much as 200 years, based on the size of the largest trees, and local knowledge (T. Moore, pers. comm.). This would make it a relatively old stand, in mid- to late-succession (Zackrisson *et al.*, 2004).

2.2 Experimental design

Figure 2.4
Field experiment site, showing full and partial chambers, and experimental moss landscapes.

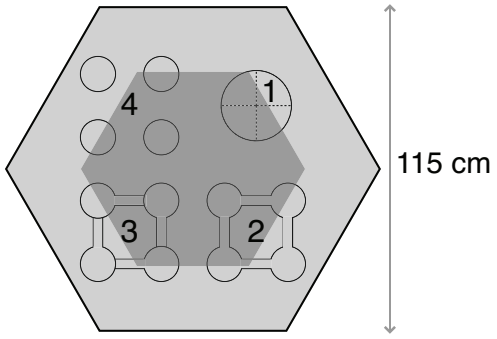

Figure 2.5
Diagram representing layout of fragmentation treatments inside chambers used to simulate climate change, to scale. Fragmentation treatments, in clockwise order beginning with top-right: (1) Contiguous, (2) Corridors, (3) Pseudo-Corridors, (4) Isolated. The inner shaded hexagon shows the approximate area open to vertical precipitation; the actual 'rain shadow' around the outer margins varied across chambers, depending on topography, prevailing wind, and other physical conditions.

The experiment consisted of experimental bryophyte patches and associated biota, subjected to climate change and habitat fragmentation treatments, which were

combined in a nested factorial design, to test for main effects and interactions. A four-patch metacommunity of each level of fragmentation (contiguous, corridors, pseudo-corridors, and isolated) was included in each chamber treatment (ambient, partial and full chambers), for a factorial nested design (Figures 2.4, 2.5). Fragmented metacommunities were separated by 10 cm within chambers, and chamber treatments were at least 1 m apart. All chamber treatments and contained landscapes of metacommunities were oriented along a North-South axis, such that one wall of the hexagonal chambers faced North and the opposing wall faced South. This was to control for a possible warming gradient observed in ITEX chambers (Marion *et al.*, 1997).

The full combination of treatments were replicated to provide three destructive sample events over the duration of the experiment, at eight locations (blocks) throughout the study area (Figure 2.3). We set up the experiment over the summer of 2007, and collected samples destructively at three different dates from all 8 blocks: August 2008 (12 months), June 2009 (22 months), and August 2009 (24 months). In this region, August is late summer, but June is often still spring: Some patches still had snow on them during this period, and were sampled immediately following snowmelt.

2.3 Habitat fragmentation treatments

Experimental metacommunities of feather mosses and associated fauna were constructed by cutting out patches from the moss carpet on the forest floor, in one of four arrangements: a single large *contiguous* patch; an equivalent area divided into four patches, each connected by two *corridors*; four patches of the same size, each connected to one *pseudo-corridor*; and four *isolated* patches (Figure 2.5). Contiguous patches were contained within a continuous area 25 cm in diameter (491 cm^2), while each of the four patches in the fragmented treatments were 12.5 cm in diameter (122.7 $cm^2 \times 4 = 491$ cm^2 total metacommunity area).

Patches were isolated by cutting patches of moss of appropriate size from the moss carpet, and placing them into plastic pots, which were replaced in the same location in the moss carpet, to try to maintain normal hydrological characteristics, while placing a dispersal barrier around the sides of the patch. The pots were 9 cm deep, and moss added was no deeper than 8 cm, leaving the surface of the bryosphere about 1 cm below the tops of the pots at the start of the experiment. The pots had holes in the bottoms to allow water drainage, and may have allowed faunal

dispersal of unknown rates between the experimental patches and underlying soil.

Corridors were created by cutting and replacing a rectangle of moss 3×10 cm, lined with 6 mil polyethylene film along the sides, but open along the bottoms to avoid disrupting water flow. Pseudo-corridors allowed a control for the extra habitat area provided by corridors, but with the same degree of isolation as the unconnected fragments: each patch and connected pseudo-corridor were isolated from the others in the same community.

Previous studies have detected fragmentation effects in microarthropod communities using similar patch arrangements and sizes, but using moss on an underlying rock substrate (Gilbert *et al.*, 1998; Gonzalez *et al.*, 1998; Gonzalez and Chaneton, 2002). Boreal forest feather mosses, however, grow on a soil and humus substrate on the forest floor rather than solid rock, which poses additional challenges to isolating patches without altering moisture characteristics independently of the isolation treatments.

Precautions were taken not to fully enclose moss patches in impermeable plastic, in an attempt to maintain similar moisture dynamics as ambient moss. Unfortunately, the many gaps also allowed dispersal of some biota between experimental metacommunities and the underlying substrate. Therefore, *fragmentation* treatments are also sometimes described as *isolation* treatments, to avoid implying that the experimental metacommunities are completely isolated from the surrounding matrix, while still capturing the different limitations on dispersal into, and out of, experimental patches. From this perspective, isolated patches remain the most isolated, while corridor and pseudo-corridor treatments are more open, due to gaps in the walls of the containing pots where corridors are connected. The contiguous treatments remain the most highly connected, albeit primarily to each other, rather than the surrounding matrix, due to fewer holes in the pots in general.

2.4 Simulated climate change treatments

We simulated climate change conditions with open-top chambers based on the design for those used by ITEX in tundra systems (Marion *et al.*, 1997). Such chambers have been used for many years to passively warm plant and soil communities in field experiments on ecological effects of climate change (Elmendorf *et al.*, 2012). Passive greenhouse apparatus have been used on antarctic soil communities, where experimenters noted the artefacts caused by a closed

Figure 2.6 *Top view of an open top chamber (OTC) treatment used to simulate climate change. A 'rain shadow' of drought conditions is visible as lighter-coloured moss around the periphery of the chamber.*

Figure 2.7 *Photograph of ambient treatment, with moss landscapes composed of one 4-patch network of each level of fragmentation. Temperature and relative humidity dataloggers (HOBO pro v2) are also visible, attached to wooden steaks and covered by a solar shade constructed from half of a plastic pot. Dataloggers were deployed in this intensive layout during the first year of the experiment, to establish any small-scale differences within chamber treatments (see Figure 2.6).*

chamber on precipitation and snow cover (Kennedy, 1995). Open-top chambers have become more popular since, because they allow a compromise between precipitation throughput, air exchange, and warming (Marion *et al.*, 1997; Shen and Harte, 2000). Passive systems have the advantage of being relatively inexpensive, and logistically simpler to apply in remote locations, but they suffer from the lack of direct control over the degree, timing, and other characteristics of the warming effect (Shen and Harte, 2000; Elmendorf *et al.*, 2012). They may also affect other variables in interactive ways, such as soil moisture, and should therefore be used with caution and appropriate monitoring to characterize the actual effects resulting from the treatments (Carlyle *et al.*, 2011).

The chambers used in this experiment were hexagonal, with walls at a 60° angle, measuring 115 cm between walls at the base and 40 cm tall (Figures 2.6, 2.4). The opening between walls at the top of the chamber was 69 cm across. Panels were constructed of Sun-Lite fibreglass (by Solar Components Corporation), supported by aluminium angles between panels, and wooden support strips along the top. All components were fastened with UV-resistant plastic cable ties ("zip ties").

The experiment included *ambient* landscapes without chambers, full *chambers* as described above, and *partial chambers* with walls only along the top-half of the frames, allowing air flow at the surface, and reducing the precipitation shadow effect (Figure 2.4). Partial chambers were generally intermediate between full chambers and ambient conditions, in both warming and drought effects (not shown). Due to time constraints, some data was not collected from all patches in the partial chamber treatments (notably fauna data, and cyanobacteria density). For reasons of simplicity and consistency, partial chambers were excluded from most analyses even when data was available.

Although the open-top chambers allow precipitation to fall through, we found that the sloped walls effectively prevented precipitation from reaching the outer edges of the chambers. The chambers therefore also include a precipitation gradient affecting moisture levels within individual patches. *Inner* patches of each metacommunity, closest to the chamber center, receive ambient levels of precipitation; *outer* patches in the periphery of chambers, farthest from the center, receive minimal levels; and *intermediate* patches in between receive intermediate levels. Outer chamber patches were effectively subjected to a two-year drought.

During sampling in June 2009, 22 months into the experiment, we did observe some patches in depressions that flooded during snowmelt. However, in chambers on elevated moss patches, snow fall accumulated in the centre of chambers, but

rarely reached the outer edge, even when melting. Therefore, we describe moisture conditions in outer patches as a prolonged drought, interrupted by transient flooding in some replicates. This is consistent with some climate change scenarios predicting more frequent extreme events, including longer periods of drought in some areas (Grant *et al.*, 2006; Lindner *et al.*, 2010; Heyder *et al.*, 2011).

This combination of chamber-level warming and internal moisture gradient allows us to separate the warming and precipitation aspects of climate conditions on our study system, based on patch location (inner, outer, or intermediate). If patch location is not a significant factor in an analysis of variance, we would conclude that any chamber-level effects were primarily due to warming. If patch position is significant, conditional on an interaction between patch position and chamber treatment, we can compare *inner chamber* patches to *ambient* patches to assess the effect of warming alone, and compare *outer chamber* patches to *inner chamber* patches to measure the additional effect of drought.

Chamber effects

Simulated climate treatments often have unintended effects, and may not match projected climate change conditions (Kennedy, 1995; Marion *et al.*, 1997; Shen and Harte, 2000; Dabros *et al.*, 2010; Moise and Henry, 2010; Carlyle *et al.*, 2011). We measured the effects of our chambers on temperature and relative humidity with automatic data loggers (HOBO Pro v2, by Onset Computer Corporation). Dataloggers were placed with sensors 2 cm below the upper surface of the bryosphere, behind a small plastic sunshade to prevent direct solar warming of the main unit. The dataloggers recorded temperature and relative humidity every half-hour, year-round.

During the first year, we deployed five dataloggers in a single chamber of each type (ambient, partial, full), to determine the degree of spatial differences in warming effects: one in the centre, and one at each of the northern, southern, eastern, and western edges of the experimental patches within the chambers (Figure 2.7). During the second winter, we deployed five dataloggers per chamber at a different location, with one in the centre of each chamber and the other four in the outer patch of each fragmentation treatment. For the second summer and the following year, we deployed a single datalogger in the centre of each chamber type, at five blocks throughout the experiment, to measure average warming effects across multiple chambers.

Table 2.1

Summary of Temperature (°C) readings 2 cm below the moss surface. "Summer" is all readings from June to September in a given year: this is the most variable time period in the data. "Winter" includes all readings from December to April of the following year: this period is generally the most stable in readings, buffered by snow cover.
Values from 2007-08 – 2009-06 are an average of five dataloggers in a single treatment replicate. Values from 2009-06 – 2010-07 are an average of dataloggers in the centre of chamber treatments at five different blocks.

				mean Temperature °C		ΔT °C (Chamber - Ambient)	
Start	End	#	Season	Ambient	Chamber	Mean	Min. – Max.
2007-08-19	2008-08-02	1	All	2.13	2.18	0.06	-12.90 – 20.68
2007-08-19	2008-08-02	1	Summer	9.65	10.35	0.69	-12.90 – 16.21
2007-08-19	2008-08-02	1	Winter	-1.93	-2.46	-0.53	-3.41 – 4.33
2008-08-04	2009-06-14	1	All	0.60	-0.09	-0.95	-19.96 – 16.46
2008-08-04	2009-06-14	1	Summer	7.53	8.07	0.65	-3.69 – 16.46
2008-08-04	2009-06-14	1	Winter	-1.86	-3.30	-1.92	-19.96 – 1.02
2009-06-15	2009-08-10	5	All	11.91	11.72	-0.19	-21.01 – 21.42
2009-06-15	2009-08-10	5	Summer	11.91	11.72	-0.19	-21.01 – 21.42
2009-08-19	2010-07-08	5	All	0.85	1.00	0.24	-13.10 – 22.61
2009-08-19	2010-07-08	5	Summer	6.65	7.32	0.52	-13.10 – 22.61
2009-08-19	2010-07-08	5	Winter	-1.36	-1.35	0.19	-2.80 – 6.95

Table 2.2

Difference between Chamber and Ambient Temperature (°C) readings, for daily mean, maximum, and minimum.

		Δ Daily	Δ Daily	Δ Daily
Start	End	Mean ±sd	Max. ±sd	Min. ±sd
2007-08-19	2008-08-02	0.06 ±0.33	0.67 ±0.54	-0.07 ±0.36
2008-08-04	2009-06-14	-0.95 ±0.49	-0.17 ±0.27	-1.29 ±0.68
2009-06-15	2009-08-10	-0.20 ±0.95	0.48 ±1.96	-0.21 ±0.37
2009-08-19	2010-07-08	0.25 ±0.48	0.44 ±0.66	0.27 ±0.64

The difference in temperature between chambers and ambient treatments appeared to be highly variable over seasons, and within days (Table 2.1, 2.2). Chambers were 0.5–0.6 °C warmer than ambient conditions in the summer, with the exception of summer 2009 (average of 5 chambers). Winters, on the other hand, were cooler within chambers, and much less variable. The winter of 2008–2009 was particularly cooler within the chamber being monitored, although data from five replicate chambers (from 2009-06-05 on) suggest fairly similar average temperatures in ambient and chambers during the winter. Chambers also had the strongest effect on the daily maximum temperatures (Table 2.2), with a possible

Table 2.3
Summary of % Relative Humidity (RH) readings 2 cm below the moss surface.

				mean % RH		Δ % RH (Chamber - Ambient)	
Start	End	#	Season	Ambient	Chamber	Mean	Min. – Max.
2007-08-19	2008-08-02	1	All	79.52	79.66	0.14	-74.14 – 71.49
2007-08-19	2008-08-02	1	Summer	86.35	82.16	-4.19	-74.14 – 71.49
2007-08-19	2008-08-02	1	Winter	84.96	87.30	2.34	-8.33 – 48.03
2008-08-04	2009-06-14	1	All	78.22	85.06	5.25	-64.77 – 63.58
2008-08-04	2009-06-14	1	Summer	79.28	87.27	3.54	-46.64 – 63.58
2008-08-04	2009-06-14	1	Winter	85.85	87.63	1.56	-52.07 – 58.76
2009-06-15	2009-08-10	5	All	93.88	96.62	2.74	-34.76 – 75.34
2009-06-15	2009-08-10	5	Summer	93.88	96.62	2.74	-34.76 – 75.34
2009-08-19	2010-07-08	5	All	82.06	80.89	-1.41	-68.53 – 99.00
2009-08-19	2010-07-08	5	Summer	88.55	85.68	-4.38	-68.53 – 74.25
2009-08-19	2010-07-08	5	Winter	85.62	83.58	-1.57	-50.01 – 51.57

Table 2.4
Difference between Chamber and Ambient % Relative Humidity readings, for daily mean, maximum, and minimum.

Start	End	Δ Daily Mean ±sd	Δ Daily Max. ±sd	Δ Daily Min. ±sd
2007-08-19	2008-08-02	0.13 ±5.58	0.46 ±5.71	5.71 ±4.73
2008-08-04	2009-06-14	5.28 ±9.77	5.56 ±9.54	9.54 ±10.05
2009-06-15	2009-08-10	2.70 ±4.53	1.16 ±3.94	3.94 ±7.78
2009-08-19	2010-07-08	-1.41 ±6.64	-1.57 ±6.66	6.66 ±6.73

decrease in daily minima.

Differences in relative humidity were also highly variable, differing by over 70 percentage points in some cases (Table 2.3). Chambers appeared to have marginally lower relative humidity during the first summer, but had marginally higher humidity until the end of the sampling period of the experiment; Chambers monitored after sampling suggested slightly dryer conditions in the chamber centres, but this effect is small relative to overall high relative humidity in both ambient and chamber treatments.

We did not find any systematic gradients in temperature or relative humidity across the areas of the chamber covered by dataloggers (unlike Marion *et al.*, 1997), although we did not monitor the outermost margins (Figure 2.7). Relative humidity readings in upper moss layers are also not necessarily indicative of total moisture availability within the substrate, which was detectably lower in outer chamber

patches (Figure 2.8).

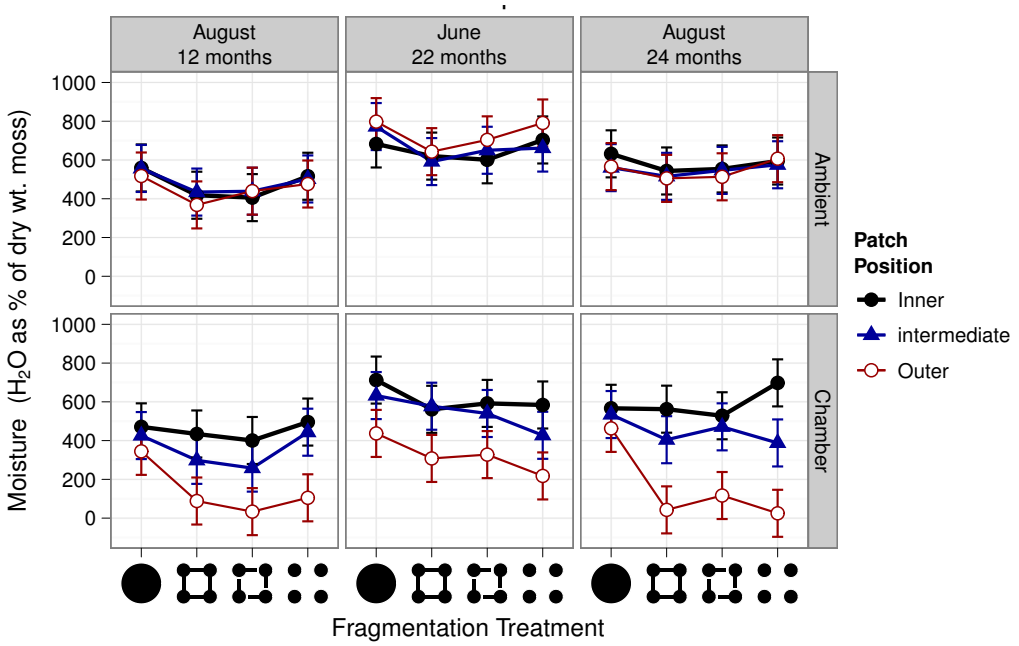

Figure 2.8

Water contents of moss patches at time of collection, as a percentage of moss dry weight, by fragmentation treatment and patch position, across chamber treatments and time from start of the experiment. Error bars represent 95% comparison intervals (Tukey's HSD Minimum Significant Ranges, based on a nested ANOVA and corrected for multiple comparisons across all plotted means; see chapter 3).

The moisture contents of the moss patches varied seasonally, and was strongly affected by patch position within chambers (Figure 2.8). Patches near the outer periphery of chambers were indeed drier than other patches on average, except for those in the contiguous fragmentation treatments. The physical connectivity in these treatments may have allowed moisture 'wicking' by capillary action from adjacent wet moss, thus maintaining similar levels of moisture in these outer patches, despite receiving less direct precipitation. Nevertheless, field observations suggest that such drought alleviation was only effective in deeper moss layers of these patches, leaving a very dry surface canopy, despite similar overall levels of moisture contents relative to other patches in the experiment. This sharp vertical moisture gradient may have implications for different processes that occur at different depths within the bryosphere.

Simulated climate change in the field

Consistent with many passive-warming open-top chambers (OTCs), ours warmed the moss most at daily maxima, during the summer (Kennedy, 1995; Shen and Harte, 2000; Dabros *et al.*, 2010). However, our chambers did not achieve temperature differences as high as other studies: Marion *et al.* (1997) measured warming of 1.2–1.8 °C at arctic tundra sites, while Dabros *et al.* (2010) used chambers in boreal forests of northwestern Québec to warm soils by 2–3 °C.

Our chambers were also smaller than those used in other studies, which may also explain the lower degree of warming observed (Marion *et al.*, 1997). We did not observe the same level of soil drying, or early snowmelt, as Dabros *et al.* (2010), who attributed early snowmelt to lower springtime temperatures due to early exposure to colder air. We observed similar cooling during snowmelt periods, as conditions in both chambers and ambient treatments were very stable while under winter snow cover. Low winter soil temperatures and even permafrost formation has also been observed in Schefferville, due to reduced snow and a resulting increase in exposure to colder air temperatures, particularly at higher elevations with high winds (Granberg, 1994).

Temperature changes inside open-top chambers can result from several factors, including the intended passive solar heating, but also changes in air turbulence at the boundary layer and energy exchange between chamber walls and vegetation (Drake *et al.*, 1989; Leadley and Drake, 1993). While some of these artefacts are also the result of CO_2 enrichment equipment, the physical presence of walls invariably alters air turbulence and mixing, which helps retain heat, but may also affect atmospheric mixing. Such changes in air turbulence may affect vegetative growth (Drake *et al.*, 1989), and perhaps gas exchange. Therefore, chamber effects include changes to energy exchange, as well as air circulation and precipitation caused by the physical structure of the chambers.

This experiment did include partial chambers to test for such artefacts. The partial chambers appeared to warm the moss somewhat less than full chambers in the summer (not shown), and allowed full precipitation to outer patches near their open periphery. The full data set for the experiment in the online repository does include data on N-fixation and moss growth in these partial chambers. Nevertheless, time constraints prevented a full sampling of these chambers and a decision was made to invest limited resources in the stronger contrast between ambient and full chambers at higher replication, than intensively sample these partial chambers. Later chapters also therefore focus on the more intensively sampled contrast

between ambient and altered conditions within OTCs, including warming and drought.

Most Global Circulation Models (GCMs) used to forecast global climate change predict greater warming in winter rather than summer, particularly at higher latitudes (Shen and Harte, 2000; Logan *et al.*, 2011), as well as increases of daily minima, leading to lower diurnal variation (Kennedy, 1995). Most of these temperature trends are quite the opposite of what our chambers, and most passive-warming methods are able to achieve (Kennedy, 1995; Marion *et al.*, 1997; Dabros *et al.*, 2010). Regional models predict greater precipitation, particularly in the form of snow in some areas of the boreal forest, such as Northern Quebec and Northern Europe (Jackson *et al.*, 2010; Logan *et al.*, 2011), but drier conditions in western Canada, Alaska, and southern Europe (Girardin *et al.*, 2004; Soja *et al.*, 2007; Lindner *et al.*, 2010; Sanderson *et al.*, 2011). Temperature-induced drought is a concern in some boreal regions, due to greater evapotranspiration, and increased risk of fire (Soja *et al.*, 2007). Nevertheless, winter cooling is a common occurrence in boreal regions during recent global warming, contrary to model forecasts (Cohen *et al.*, 2012).

Possible winter cooling may not be consistent with model forecasts, but is consistent with observed trends. Prolonged droughts may not be a concern in northern Quebec, but other areas of the boreal forest have already begun to show water limitation, and changes in the frequency of precipitation (Lindner *et al.*, 2010). Our climate change treatments are therefore relevant to some boreal forest regions, though not necessarily northern Quebec, given the large differences in regional climate trends and effects of global warming. More generally, these treatments represent a combination of warming and drought effects that offer a range of conditions in which to explore biotic and ecosystem-level responses to multiple environmental changes.

2.5 Implications for statistical analysis

Patch positions of differing drought conditions occur within each habitat fragmentation / isolation treatment, which were nested within simulated climate change treatments, replicated for collection at three time points during the experiment, across eight blocks. This could be described as a randomized complete block design, with elements of a 'split-plot' or hierarchical design. When analyzing data from such a design, it is necessary to account for the nested structure of the data

and the lack of independence within experimental units of different sizes (Sokal and Rohlf, 1981): although each chamber includes $4 \times 4 = 16$ patches, there is really only a single chamber. Failing to account for this would be to commit the error of pseudo-replication, artificially inflating sample sizes, degrees of freedom, and biasing hypothesis tests (Hurlbert, 1984).

The design of the SEC-C experiment therefore includes the following hierarchical structure, from largest to smallest experimental units:

Relative size	**Experimental unit**	Treatment levels
Largest	**Block**	8 locations within the study area (Figure 2.3)
•	**Time**	Date of sample collection: *12 months* August 2008 *22 months* June 2009 *24 months* August 2009
•	**Chamber**	Simulated climate change (section 2.4): *ambient*, *partial chambers*, (full) *chambers*
•	**Fragmentation (isolation)**	Connectivity or openness (section 2.2): *contiguous*, *corridors*, *pseudo-corridors*, *isolated*
Smallest	**Patch Position**	Position within chambers (section 2.4): *inner*, *intermediate*, *outer*

Each factor is nested within levels of the preceding factor in the list. I have taken this hierarchical structure into account where appropriate in statistical analyses of these data presented here.

Although samples were taken at different times throughout the two years of the experiment, it is important to note that for most data, this sampling was destructive: samples at different times were taken from different experimental units (chambers and all included treatments). Therefore, data from different time are independent, and a repeated-measures type analysis is not required.

The only exception to this is moss growth data, which was collected throughout the course of the experiment, always from patches collected at the end (the final time-point), and additional patches from replicates that were never collected (for a time point that was dropped due to time constraints, but left for follow-up sampling). Therefore, an additional "measurement time" factor should be included as the smallest experimental unit for moss growth data, to account for the lack of independence between sampling times (see chapter 4). Given that measurement periods were unequal, it is safer and simpler to aggregate growth estimates over each year of the experiment, to keep time periods equal (Zuur *et al.*, 2007).

Many variables were only measured at a subset of all treatment combinations. Typically, only combinations with adequate coverage were included in individual analyses, as presented in the following chapters.

References

Carlyle CN, Fraser LH, Turkington R (2011) Tracking soil temperature and moisture in a multi-factor climate experiment in temperate grassland: Do climate manipulation methods produce their intended effects? Ecosystems 14(3):489–502, doi:10.1007/S10021-011-9425-Y

Cohen JL, Furtado JC, Barlow MA, Alexeev VA, Cherry JE (2012) Arctic warming, increasing snow cover and widespread boreal winter cooling. Environmental Research Letters 7(1), doi:10.1088/1748-9326/7/1/014007

Dabros A, Fyles JW, Strachan IB (2010) Effects of open-top chambers on physical properties of air and soil at post-disturbance sites in northwestern Quebec. Plant and Soil 333(1-2):203–218, doi:10.1007/s11104-010-0336-z

Drake BG, Leadley PW, Arp WJ, Nassiry D, Curtis PS (1989) An open top chamber for field studies of elevated atmospheric CO2 concentration on saltmarsh vegetation. Functional Ecology 3(3):363–371, doi:10.2307/2389377

Elmendorf SC, Henry GHR, Hollister RD, Björk RG, Bjorkman AD, Callaghan TV, Collier LS, Cooper EJ, Cornelissen JHC, Day TA, Fosaa AM, Gould WA, Grétarsdóttir J, Harte J, Hermanutz L, Hik DS, Hofgaard A, Jarrad F, Jónsdóttir IS, Keuper F, Klanderud K, Klein JA, Koh S, Kudo G, Lang SI, Loewen V, May JL, Mercado J, Michelsen A, Molau U, Myers-Smith IH, Oberbauer SF, Pieper S, Post E, Rixen C, Robinson CH, Schmidt NM, Shaver GR, Stenström A, Tolvanen A, Totland c, Troxler T, Wahren CH, Webber PJ, Welker JM, Wookey PA (2012) Global assessment of experimental climate warming on tundra vegetation: heterogeneity over space and time. Ecology Letters 15(2):164–175, doi:10.1111/j.1461-0248.2011.01716.x

Environment Canada. Canadian Climate Normals or Averages 1971-2000, Schefferville, QC [online] (2012), URL: http://climate.weatheroffice.gc.ca/climate_normals/index_e.html [cited May 6, 2012]

Fitzjarrald DR, Moore KE (1994) Growing-season boundary-layer climate and surface exchanges in a sub-arctic lichen woodland. Journal of Geophysical Research-Atmospheres 99(D1):1899–1917, doi:10.1029/93jd01019

Gilbert F, Gonzalez A, Evans-Freke I (1998) Corridors maintain species richness in the fragmented landscapes of a microecosystem. Proceedings of the Royal Society B: Biological Sciences 265(1396):577–582

Girardin MP, Tardif J, Flannigan MD, Wotton BM, Bergeron Y (2004) Trends and periodicities in the Canadian Drought Code and their relationships with atmospheric circulation for the southern Canadian boreal forest. Canadian Journal of Forest Research 34(1):103–119, doi:10.1139/x03-195

Gonzalez A, Chaneton EJ (2002) Heterotroph species extinction, abundance and biomass dynamics in an experimentally fragmented microecosystem. Journal of Animal Ecology 71(4):594–602

Gonzalez A, Lawton JH, Gilbert FS, Blackburn TM, Evans-Freke I (1998) Metapopulation dynamics, abundance, and distribution in a microecosystem. Science 281(5385):2045–2047

Granberg HB (1994) Mapping heat-loss zones for permafrost prediction at the northern alpine limit of the boreal forest using high-resolution C-band SAR. Remote Sensing of Environment 50(3):280–286, doi:10.1016/0034-4257(94)90077-9

Grant RF, Black TA, Gaumont-Guay D, Kljun N, Barrc AG, Morgenstern K, Nesic Z (2006) Net ecosystem productivity of boreal aspen forests under drought and climate change: Mathematical modelling with Ecosys. Agricultural and Forest Meteorology 140(1-4):152–170, doi:10.1016/j.agrformet.2006.01.012

Heyder U, Schaphoff S, Gerten D, Lucht W (2011) Risk of severe climate change impact on the terrestrial biosphere. Environmental Research Letters 6(3), doi:03403610.1088/1748-9326/6/3/034036

Hurlbert SH (1984) Pseudoreplication and the Design of Ecological Field Experiments. Ecological Monographs 54(2):187–211

Jackson BG, Martin P, Nilsson MC, Wardle DA (2010) Response of feather moss associated N2 fixation and litter decomposition to variations in simulated

rainfall intensity and frequency. Oikos 120(4):570–581, doi:10.1111/j.1600-0706.2010.18641.x

Kennedy AD (1995) Temperature Effects of Passive Greenhouse Apparatus in High-Latitude Climate-Change Experiments. Functional Ecology 9(2):340–350

Leadley PW, Drake BG (1993) Open top chambers for exposing plant canopies to elevated CO2 concentration and for measuring net gas-exchange. Vegetatio 104:3–15, doi:10.1007/Bf00048141

Lechowicz MJ, Adams MS (1978) Diurnal and seasonal structure of the climate at Scheffer Ville, Quebec. Arctic and Alpine Research 10(1):95–104

Lindner M, Maroschek M, Netherer S, Kremer A, Barbati A, Garcia-Gonzalo J, Seidl R, Delzon S, Corona P, Kolstrom M, Lexer MJ, Marchetti M (2010) Climate change impacts, adaptive capacity, and vulnerability of European forest ecosystems. Forest Ecology and Management 259(4):698–709, doi:10.1016/j.foreco.2009.09.023

Lindo Z, Whiteley JA, Gonzalez A (2012) Traits explain community disassembly and trophic contraction following experimental environmental change. Global Change Biology 18(8):2448–2457, doi:10.1111/j.1365-2486.2012.02725.x

Logan T, Charron I, Chaumont D, Houle D (2011) Atlas de scénarios climatiques pour la forêt québécoise. Tech. Rep. ISBN papier : 976-2-923292-06-9 ISBN web : 978-2-923292-05-2, Ouranos et MRNF, URL: http://www.ouranos.ca/media/publication/162_AtlasForet2011.pdf

Marion GM, Henry GHR, Freckman DW, Johnstone J, Jones G, Jones MH, Levesque E, Molau U, Molgaard P, Parsons AN, Svoboda J, Virginia RA (1997) Open-top designs for manipulating field temperature in high-latitude ecosystems. Global Change Biology 3(s1):20–32, doi:10.1111/j.1365-2486.1997.gcb136.x

Moise ERD, Henry HAL (2010) Like moths to a street lamp: exaggerated animal densities in plot-level global change field experiments. Oikos 119(5):791–795, doi:10.1111/J.1600-0706.2009.18343.X

Moore TR (1980) The nutrient status of subarctic woodland soils. Arctic and Alpine Research 12(2):147–160

Payette S, Fortin MJ, Gamache I (2001) The subarctic forest-tundra: the structure of a biome in a changing climate. Bioscience 51(9):709–718, doi:10.1641/0006-3568(2001)051[0709:TSFTTS]2.0.CO;2

R Development Core Team (2010) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria, URL: http://www.R-project.org/, ISBN 3-900051-07-0

Sanderson MG, Hemming DL, Betts RA (2011) Regional temperature and precipitation changes under high-end (>= 4 degrees C) global warming. Philosophical Transactions of the Royal Society a-Mathematical Physical and Engineering Sciences 369(1934):85–98, doi:10.1098/rsta.2010.0283

Shen KP, Harte J (2000) Ecosystem climate manipulations. In: Sala OE, Jackson RB, Mooney HA, Howarth RW (eds.) Methods in Ecosystem Science, Springer, New York, pp 353–369

Soja AJ, Tchebakova NM, French NHF, Flannigan MD, Shugart HH, Stocks BJ, Sukhinin AI, Parfenova EI, Chapin FS, Stackhouse PW (2007) Climate-induced boreal forest change: Predictions versus current observations. Global and Planetary Change 56(3-4):274–296, doi:10.1016/J.Glopacha.2006.07.028

Sokal RR, Rohlf FJ (1981) Biometry: The Principles and Practice of Statistics in Biological Research, 3rd edn. W. H. Freeman and Company, New York

Wright DH, Gonzalez A, Coleman DC (2007) Changes in nestedness in experimental communities of soil fauna undergoing extinction. Pedobiologia 50(6):497–503

Zackrisson O, DeLuca TH, Nilsson MC, Sellstedt A, Berglund LM (2004) Nitrogen fixation increases with successional age in boreal forests. Ecology 85(12):3327–3334, doi:10.1890/04-0461

Zuur AF, Ieno EN, Smith GM (2007) Analysing Ecological Data. Statistics for Biology and Health, Springer, New York, URL: http://www.highstat.com/book1.htm

CHAPTER 3

Biotic nitrogen-fixation in the bryosphere inhibited more by drought than warming or habitat fragmentation

Keywords: climate-change, habitat fragmentation, nitrogen-fixation, cyanobacteria, *Pleurozium schreberi*, boreal forest

Status: submitted to Oecologia

Abstract

Climate change and habitat fragmentation are leading factors affecting global biodiversity. Previous studies have focused mostly on each of these drivers independently, with very few studies on their combined effects on biodiversity and ecosystem processes. The boreal forest is of particular interest to climate change research, due to its large circumpolar distribution. Carbon uptake in this ecosystem is nitrogen-limited, therefore factors affecting nutrient cycling in the boreal forest can have consequences for global climate. We used a two-year field experiment to investigate the response of biotic nitrogen-fixation by cyanobacteria associated with boreal forest bryophytes, in a factorial experiment combining simulated climate change with habitat fragmentation treatments. We simulated climate change conditions using open-top fibreglass greenhouse chambers in the field, which increased mean and maximum temperatures, and created a precipitation gradient from ambient levels in the centre, to extreme drought conditions at the periphery of the chamber. The dry patches near the chamber walls exhibited almost no N-fixation, despite having similar densities of cyanobacteria as other patches. We found no significant effect of fragmentation on cyanobacteria densities. Rates of N-fixation were best explained by a combination of several variables, and two-way interactions with the notable exception of interactions between fragmentation and climate treatments. These results suggest that cyanobacteria responded physiologically to drought by reducing N-fixation activity long before any changes in density. Ecosystem processes, such as N-fixation, can respond to environmental change much more rapidly than changes in the underlying community structure, which can delay insurance effects of biodiversity in such situations.

3.1 Introduction

Climate change encompasses changes in a range of climate variables, including temperature, precipitation, and atmospheric composition over large spatial and temporal scales. These environmental changes are expected to lead to a variety of changes in species and communities, including alterations to species distribution, phenology, and community composition (Parmesan and Yohe, 2003; Thomas *et al.*, 2004; Parmesan, 2006; Burrows *et al.*, 2011). Such changes in biotic communities are likely to have ecosystem consequences, with the potential for feedbacks to the climate system. What remains uncertain is how changes in climatic conditions will

interact with other drivers of biodiversity change. Interactions between climate change and habitat fragmentation are of particular concern, because these are two of the leading drivers of biodiversity change, and because of the potential for synergistic effects (Sala *et al.*, 2000; IPCC, 2002; Watkinson and Gill, 2002). Many species rely on dispersal to find suitable conditions during periods of environmental change, but habitat fragmentation impedes dispersal, leading to population declines at local and regional scales.

The boreal forest is one of the largest terrestrial biomes, covering more than 10% of the Earth's land surface (Taggart and Cross, 2009), and can therefore affect some aspects of global climate, including modifying planetary albedo, and greenhouse gas concentrations such as carbon dioxide (Bonan and Van Cleve, 1992; Mäkipää *et al.*, 1999). Boreal forests may act as a carbon source or sink, depending on the net balance between rates of production and decomposition at the ecosystem level (Markham, 2009). Productivity in boreal forests is typically nitrogen-limited (Lindahl *et al.*, 2002), which could prevent anticipated "fertilization" effects of rising CO_2 levels associated with climate change (Norby *et al.*, 2010). Understanding the ecosystem controls on nitrogen in the boreal forest is therefore crucial to predicting climate change impacts in the region, as well as understanding the potential feedbacks to the climate system, through changes in carbon uptake.

Bryophytes on the boreal forest floor play an important role in nutrient cycling within a forest stand. They act as a "nutrient sponge" by intercepting and taking up nearly all nutrient deposition to the forest floor (Chapin III *et al.*, 1987; Turetsky, 2003). Bryophytes are able to capture CO_2 emissions from underlying soils, and primary production of bryophytes can equal or even exceed that of overstory vegetation (Lindo and Gonzalez, 2010). Bryophytes also create unique habitats for a multitrophic, highly diverse community of associated organisms, ranging from microbiota to arthropod fauna. The bryosphere is therefore the combination of bryophytes and their associated fauna, which form an important boundary layer between soils and the atmosphere that integrates above- and below-ground processes Lindo:2010.Bryosphere.

The discovery of nitrogen-fixing cyanobacteria living in association with bryophytes revealed a significant biotic input of nitrogen to boreal forest ecosystems (DeLuca *et al.*, 2002). Rates of N-fixation measured in the field are often correlated with cyanobacteria abundance (DeLuca *et al.*, 2007; Lindo and Gonzalez, 2010), and increase with forest stand age, possibly as a consequence of reduced available nitrogen (DeLuca *et al.*, 2008). N-fixation rates by cyanobacteria vary by species

and temperature (Gentili *et al.*, 2005; Sorensen and Michelsen, 2011), water availability (Gundale *et al.*, 2009, 2012b), as well as light quantity and quality (Turetsky, 2003; Gundale *et al.*, 2012a). Recent lab experiments suggest that N-fixation rates are negatively affected by dry moss conditions, which can result from reduced frequency of precipitation, or temperature induced evapotranspiration (Gundale *et al.*, 2012b). N-fixation rates may often be temperature-limited in boreal forest mosses, with nitrogenase activity reaching optimal levels around 25 °C (Vitousek *et al.*, 2002; Houlton *et al.*, 2008; Gundale *et al.*, 2012a). Light intensity, however, interacts strongly with temperature, increasing N-fixation rates at low temperatures, but causing possible damage to cells at high temperatures (Gundale *et al.*, 2012a).

Nevertheless, there are few multifactor experiments that assess how biotic nitrogen-fixation by cyanobacteria may respond under a range of climate conditions (e.g., Gundale *et al.*, 2012a,b), including changes in temperature and precipitation over the long-term, and none that do so in a context of habitat fragmentation. Given the many environmental factors that are known to affect N-fixation rates, and the unknown factors affecting cyanobacteria abundance and distribution, it is important to conduct experiments in the field, under realistic conditions, while maintaining a bryosphere structure that is as undisturbed as possible.

We therefore measured bryosphere responses to simulated climate change conditions interacting with habitat fragmentation, in a field experiment in the boreal forest of northern Québec, Canada. A factorial design allowed us to measure independent and interactive effects of temperature, moisture, and fragmentation of the bryosphere habitat. We measured changes in cyanobacteria density and nitrogen-fixation associated with boreal forest moss over two years of experimental treatment.

We expected cyanobacteria to respond physiologically to changing environmental conditions, including temperature and moisture, at least over the short term. As stressful conditions such as drought persist over longer periods, we also expected to observe changes in species composition caused by declines in cyanobacteria abundance and changes in the distribution of species across patches of different levels of precipitation. Given that different species of cyanobacteria fix nitrogen at different rates, depending on temperature (Gentili *et al.*, 2005), we predicted that long-term warming would lead to changes in species composition within patches, with subsequent changes in overall levels of cyanobacterial N-fixation.

Little is known about the mechanisms responsible for maintaining local

cyanobacterial diversity but local extinction-colonization dynamics may be important. Heterocystous N-fixing cyanobacteria are non-motile when in symbiotic association with moss, but they are capable of dispersal via hormogonia, a motile reproductive phase of their life-cycle (Pawlowski and Bergman, 2007). Hormogonia disperse by means of propulsion through water films that surround moss surfaces, and exhibit chemotaxis towards bryophyte extracts. Some cyanobacteria may be capable of long-distance aerial dispersal in dry Antarctic habitats (Marshall and Chalmers, 1997), but this has not been observed in the genera found in the present study. We therefore predicted that moss habitat connectivity would allow cyanobacterial dispersal, through contiguous water films, and prevent long-term population declines in the presence of stressful fluctuations in environmental conditions (Leibold *et al.*, 2004). In the absence of community-level changes, only intraspecific compensation through physiological acclimation or local adaptation to changing climate are likely to sustain N-fixation. The potential for physiological or demographic responses to sustain N-fixation under expected climate change conditions is unknown. The results of this experiment provide an initial response to this question.

3.2 Methods

Study site

The field experiment was set up just outside of Schefferville, Québec, Canada (54°48′N 66°50′W). The experiment was spread out over an area of boreal forest approx. 100×200 m, located 1.6 km southeast of the McGill Subarctic Research Station near the edge of town, centred near 54°47′44″N 66°47′20″W.

Schefferville is south of the southern limit of continuous permafrost, but near the northern limit of the boreal forest in eastern North America, in a transition zone from woodland to tundra (Payette *et al.*, 2001). This area includes areas of tundra at high elevations, with areas of boreal forest mixed with lichen-dominated open areas at lower elevations, as well as wetlands and many small lakes. The topography is dominated by alternating ridges and valleys oriented in an approximately northwest to southeast direction (Fitzjarrald and Moore, 1994). Soils are typically well-drained, oligotrophic (Moore, 1980).

This area experiences continental weather typical of the region, with average air temperatures historically ranging from −24 °C in the winter to 16 °C in the summer (Lechowicz and Adams, 1978; Environment Canada, 2012). Air

temperatures only remain above 0 °C from about May to September (Lechowicz and Adams, 1978), resulting in a short growing season of about 100-120 days on average. An average of 408 mm of rain falls on Schefferville each year, primarily during the growing season, although at least some of 440 cm of annual snowfall has been observed at any month of the year (Environment Canada, 2012).

The study site is dominated by a sparse canopy of scattered spruce trees, including both *Picea mariana* (Mill.) and *P. glauca* (Moench) Voss. (Moore, 1980). The understory includes dwarf birch (*Betula glandulosa* Michx.) and Labrador tea (*Ledum groenlandicum* Oeder.), while the ground is covered by a continuous carpet of feather moss, most of which is *Pleurozium schreberi*(Brid.) Mitt., with occasional patches or individual shoots of *Hylocomium splendens* (Hedw.) or *Ptillum crista-castrensis* (Hedw.) (Moore, 1980)

Experimental design

The experiment included climate change and habitat fragmentation treatments, combined in a fully-factorial design, to test for the full range of main effects and interactions. Experimental meta-communities of bryophytes and associated fauna were constructed by cutting out patches from the moss carpet on the forest floor, in one of four fragmentation treatments: a single large contiguous patch; an equivalent area divided into four patches each connected by two corridors; four patches of the same size each connected to one pseudo-corridor; and four isolated patches (Figure 3.1). Large contiguous patches were 25 cm in diameter (491 cm^2), while each of the four patches in the fragmented treatments were 12.5 cm in diameter (122.7 $cm^2 \times 4 = 491$ cm^2 total metacommunity area).

Patches were isolated by cutting patches of moss of appropriate size from the moss carpet, and placing them into plastic flower pots, which were replaced into the moss carpet, to try to maintain ambient hydrological characteristics, while placing a dispersal barrier around the sides of the patch. The pots were 9 cm deep, and moss added was no deeper than 8 cm, leaving the surface of the bryosphere about 1 cm below the tops of the pots at the start of the experiment. The pots had holes in the bottoms to allow water drainage, and likely allowed faunal dispersal of unknown rates between the experimental patches and underlying soil. Corridors were created by cutting and replacing a rectangle of moss 3×10 cm, lined with 6 mil polyethylene film along the sides, but open along the bottoms. Pseudo-corridors allowed a control for the extra habitat area provided by corridors, but with the same

degree of isolation as the unconnected fragments (each patch & connected pseudo-corridor were isolated from the others in the same community).

All fragmentation levels were included within simulated climate change treatments (see below), which were replicated to provide three destructive sample events over the duration of the experiment, at eight locations (blocks) throughout the study area. We set up the experiment over the summer of 2007, and collected samples destructively at three different dates from all 8 blocks: August 2008 (12 months), June 2009 (22 months), and August 2009 (24 months).

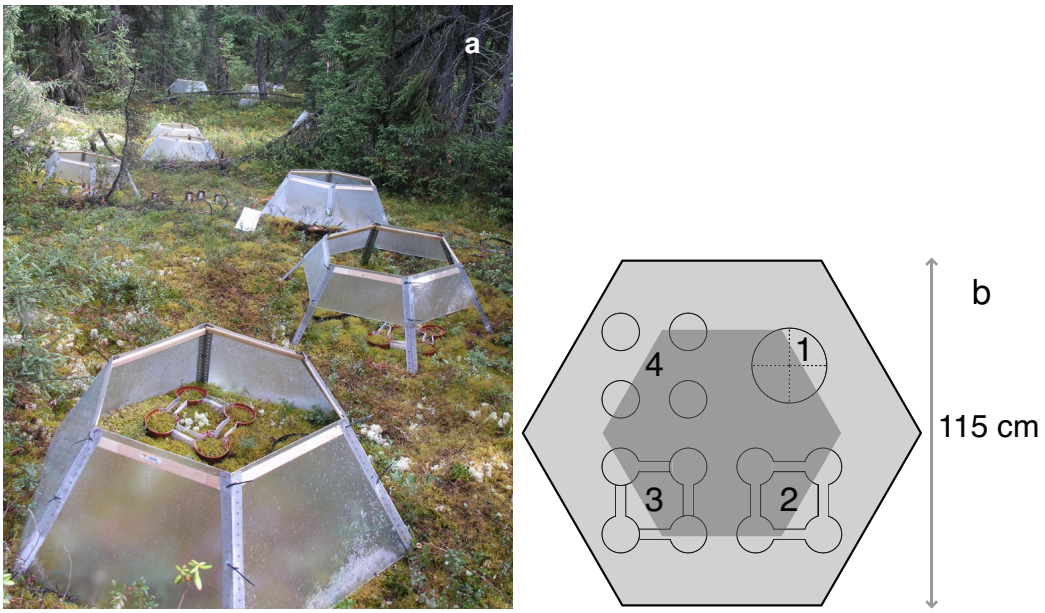

Figure 3.1
*(**a**) Photo of field experiment plots, showing full and partial chambers, and experimental moss landscapes.*
*(**b**) Diagram representing layout of fragmentation treatments inside chambers, to scale. Fragmentation treatments, in clockwise order beginning with top-right: (1) Contiguous, (2) Corridors, (3) Pseudo-Corridors, (4) Isolated. The inner shaded hexagon shows approximate area open to vertical precipitation; the actual 'rain shadow' varies across chambers, depending on local slope, aspect, prevailing wind, etc.*

Climate change treatments

We simulated climate change conditions with open-top chambers based on the design for those used by ITEX in tundra systems (Marion *et al.*, 1997). The chambers used in this experiment were hexagonal, with walls at a 60° angle, measuring 115 cm between walls at the base, 69 cm across at the top, and 40 cm tall. Panels were constructed of Sun-Lite fibreglass (by Solar Components Corporation),

supported by aluminium angles between panels, and wooden support strips along the top. All components were fastened with UV-resistant plastic cable ties ("zip ties").

The experiment included ambient landscapes without chambers, full chambers as described above, and partial chambers with walls only along the top-half of the frames, allowing air flow at the surface, and reducing the precipitation shadow effect (Figure 3.1). A four-patch meta-community of each level of the fragmentation treatment (contiguous, corridors, pseudo-corridors, and isolated) was included in each chamber treatment (ambient, partial and full chambers). Fragmentation meta-communities were separated by 10 cm within chambers, and chamber treatments (landscapes) were at least 1 m apart. All chamber treatments and contained landscapes of meta-communities were oriented along a north-south axis, such that one wall of the hexagonal chambers faced North and the opposing wall faced south. This was to control for a possible warming gradient observed in ITEX chambers (Marion *et al.*, 1997).

The effects of our chambers on temperature and relative humidity were measured by automatic data loggers (HOBO Pro v2, by Onset Computer Corporation). Dataloggers were placed with sensors 2 cm below the upper surface of the bryosphere, behind a small plastic sunshade to prevent direct solar warming of the main unit. The dataloggers recorded temperature and relative humidity every half-hour, year-round. During the first year, we placed five dataloggers in a single chamber of each type (ambient, partial, full), to determine the degree of spatial differences in warming effects: one in the centre, and one at each of the northern, southern, eastern, and western edges of the experimental patches within the chambers. During the second year, we deployed five dataloggers per chamber at a different location, with one in the centre of each chamber and the other four in the outer patch of each fragmentation treatment. For the remainder of the experiment, we deployed a single datalogger in the centre of each chamber type, at five blocks throughout the experiment, in the same landscapes that were sampled, to measure variance of warming effects across multiple chambers.

Although the open-top chambers allow precipitation to fall through, we found that the sloped walls effectively prevented precipitation from reaching the outer edges of the chambers. The chambers therefore also include a precipitation gradient affecting moisture levels within individual patches. Inner patches of each meta-community receive ambient levels of precipitation, outer patches receive minimal levels, and intermediate patches in between receive intermediate levels. Outer

patches were effectively treated with a two-year drought. During sampling in June 2009, 22 months into the experiment, we did observe some patches in depressions that flooded during snowmelt. However, in chambers on elevated moss patches, snow fall accumulated in the centre of chambers, but rarely reached the outer edge, even when melting. Therefore, we describe moisture conditions in outer patches as a prolonged drought, interrupted by transient flooding in some replicates. This is consistent with some climate change scenarios predicting more frequent extreme events, and longer droughts in some areas as a result of increases in global air temperature (Grant *et al.*, 2006; Lindner *et al.*, 2010; Heyder *et al.*, 2011).

This combination of chamber-level warming and internal moisture gradient allows us to separate the warming and precipitation aspects of climate conditions on our study system, based on patch location (inner, outer, or intermediate). If patch location were not a significant factor in an analysis of variance, we would conclude that any chamber-level effects were primarily due to warming, but if patch position were significant (leading to an interaction between patch position and chamber treatment), we would conclude that any chamber effects were a result of drying in the outer patches, rather than warming within the entire chamber.

N-fixation rates

We measured rates of biotic nitrogen fixation using an Acetylene Reduction Assay (ARA) (Schöllhorn and Burris, 1967; Hardy *et al.*, 1968; Mcnabb and Geist, 1979), which measures quantities of acetylene reduced to ethylene by nitrogenase enzymes in cyanobacteria heterocyst cells. Acetylene competes effectively with nitrogen gas for binding with nitrogenase in heterocysts (Schöllhorn and Burris, 1967), and previous studies have calibrated this method using parallel $^{15}N_2$ tracer experiments, and found a reduction ratio of 3:1 (3 mol acetylene reduced for every 1 mol nitrogen gas fixed by cyanobacteria) associated with *Pleurozium schreberi* in boreal forests DeLuca *et al.* (2002). This is consistent with theoretical predictions based on stoichiometry and lower H_2 formation in the presence of acetylene (Zehr and Montoya, 2007).

At the experimental site, we took 20 shoots of *Pleurozium schreberi* from each experimental patch and placed them into a 50 ml optically-clear polystyrene conical tube (Fisher Scientific), sealed with a rubber septum (Suba-Seal® 57 for 27 mm internal diameter opening). We then removed 10% of the headspace (5 ml), and replaced it with the same volume of acetylene gas (99.6% pure C_2H_2 from MEGS specialty gases, Montreal, Canada). We allowed the tubes to incubate for

24 hours, in the centre of their respective patches with the septum down. We then collected a 5 ml subsample of headspace gas into vacuum-sealed glass serum tubes with a rubber septum ("vacutainers") using blood collection needles. Gas samples were kept in the vacutainers during transport from the field site to the lab, and storage until processing with gas chromatography to quantify acetylene-derived ethylene produced by active nitrogenase in the moss system. Moss used for ARA measurements in the field were kept in their tubes, sealed with a plastic cap, and transported to the lab for quantification of cyanobacteria (see below).

A 1 ml gas sample was injected into a Shimadzu GC-2014 gas chromatograph with an injector temperature of 250 °C, FID at 250 °C and Carbosphere 80/100 column at 200 °C, using a flow rate of $30\,\mathrm{ml}\cdot\mathrm{min}^{-1}$ and Helium carrier gas. GCsolution software digitally integrated gas chromatography output. A calibration curve of known quantities of ethylene and acetylene gas was used to convert gas chromatography output to µmol gas per sample.

Cyanobacteria density

The moss samples used to measure N-fixation rates in the field were transported to the lab at McGill University, dried for 24 h at 30–40 °C in a Fisher Scientific drying oven. Preliminary samples indicated this drying had no observable effect on measured cyanobacteria abundance. The dry weight of each sample of 20 shoots was recorded to the nearest 0.1 mg. We selected two moss shoots of the 20 in each tube, each 6–7 cm long, to estimate cyanobacteria abundance by a novel use of sonication to dissociate cyanobacterial cells from the moss (see also Lindo and Whiteley, 2011). Both shoots were weighed together (dry weight), then placed in a 2 ml plastic centrifuge tube with 1 ml of deionized water, and sonicated by a Fisher Sonic Dismembrator 500 for 40 s at approx. 100 W (25% max. amplitude) with a horn frequency of 20 kHz. We agitated the tubes for 5 s on a vortex machine immediately prior to removing subsamples for counting. We then transferred two 10 µl subsamples to a hemacytometer to count cells under a compound microscope (Leica DM 2500) at 200x magnification, with fluorescence and a "Texas Red" filter to highlight cyanobacterial cells containing phycocyanin pigments. We counted cyanobacteria vegetative cells and heterocysts, identified according to Rippka *et al.* (1979).

We used a combination of sonication and mechanical agitation (vortex machine) to try to separate cyanobacteria cells and colonies from moss shoots and leaves. Long-term sonication is known to reduce cell division and disrupt other

cellular processes of *Microcystis* (Ahn *et al.*, 2003; Zhang *et al.*, 2006). Sonication is also used to remove contaminants (fungi, other bacteria, etc.) in the preparation of pure axenic cultures (Guillard, 2005). Therefore, we expected that sonication would also help break-down epiphytic attachments between cyanobacteria and moss leaves, as well as break apart large colonies, without affecting the phycocyanin pigments needed to identify and count cells. During trials, we observed that higher energies (Watts), or longer durations would physically rupture and damage cells, leading to reduced counts. Nevertheless, the settings reported here provided maximal extraction of cyanobacteria from moss shoots in sonication trials.

Statistical analysis

We performed all statistical analyses using `R` software (R Development Core Team, 2010), with the `reshape` (Wickham, 2007) and `plyr` (Wickham, 2011) packages for data processing. We used the `effects` package (Fox, 2003) to extract fitted values for a subset of model terms, and the `ggplot2` package (Wickham, 2009) to plot data and results.

We tested for effects of experimental treatments using a nested analysis of variance (ANOVA) separately on each response variable: cyanobacteria density and N-fixation rate. Sample date (time) was the largest experiment unit, with block, chamber, fragmentation, and finally patch position, nested hierarchically in descending order. Because each sample time includes independent samples, time is treated as a factor, rather than a repeated measure. We performed multiple comparisons of factor levels found to be significant in the ANOVA, using a method similar to Tukey's HSD (Sokal and Rohlf, 1981), calculating Minimum Significant Ranges using Mean Squared Error (MSE) from the appropriate nesting level in the ANOVA.

We tested for the relationship between N-fixation rate and cyanobacteria density using a multiple regression approach. We used the `glmulti` package (Calcagno and de Mazancourt, 2010) to perform multi-model selection and model-averaging. We used the package's genetic algorithm with default settings to search for 256 "best" models, as measured by the Akaike Information Criterion (AIC), including main effects and 2-way interactions as candidate model terms from available explanatory variables. We performed four replicate genetic algorithm searches, and combined them to assemble a confidence set of 256 of the best models found (Calcagno and de Mazancourt, 2010). Candidate models included **Cyanobacteria** cell density and **Moisture** contents of the substrate (both linear

and quadratic terms) as continuous variables, and experimental treatments **Block**, sample **Time** (12, 22, and 24 months after starting the experiment), **Chamber** (*Ambient* or *Full Chamber*), and patch **Position** within the chamber (*inner* or *outer*), as categorical factors (Table 3.4). Both cyanobacteria density and acetylene reduction rates were log-transformed to linearize the relationship and reduce the influence of infrequent large values.

We applied regression tree analysis (Zuur *et al.*, 2007), using the `rpart` package (Therneau *et al.*, 2010), to explore higher-order interactions, and identify the most influential variables on N-fixation rates. The data were not transformed for this analysis. Regression trees make fewer assumptions than classical regression modelling, employing a non-parametric iterative algorithm, which is complementary to the simultaneous parametric analysis of linear regression. The results helped guide linear regression analysis, suggesting which interactions might be most important, and potential non-linear effects.

3.3 Results

Chamber effects

Table 3.1

Summary of Temperature (°C) readings 2 cm below the moss surface.

			mean Temperature °C		ΔT °C (Chamber - Ambient)	
Start	End	Season	Ambient	Chamber	Mean	Min. – Max.
2007-08-19	2008-08-02	All	2.13	2.18	0.06	-12.90 – 20.68
2007-08-19	2008-08-02	Summer	9.65	10.35	0.69	-12.90 – 16.21
2007-08-19	2008-08-02	Winter	-1.93	-2.46	-0.53	-3.41 – 4.33
2008-08-04	2009-06-14	All	0.60	-0.09	-0.95	-19.96 – 16.46
2008-08-04	2009-06-14	Summer	7.53	8.07	0.65	-3.69 – 16.46
2008-08-04	2009-06-14	Winter	-1.86	-3.30	-1.92	-19.96 – 1.02
2009-06-15	2009-08-10	All	11.91	11.72	-0.19	-21.01 – 21.42
2009-06-15	2009-08-10	Summer	11.91	11.72	-0.19	-21.01 – 21.42
2009-08-19	2010-07-08	All	0.85	1.00	0.24	-13.10 – 22.61
2009-08-19	2010-07-08	Summer	6.65	7.32	0.52	-13.10 – 22.61
2009-08-19	2010-07-08	Winter	-1.36	-1.35	0.19	-2.80 – 6.95

Chambers showed variable degrees of warming, with most warming occurring during snow-free periods in the summer (Table 3.1), at daily maxima (Table 3.2). Nevertheless, an increase in daily minimum was also observed in some chambers during the experiment (Table 3.2).

Table 3.2
Difference between chamber and ambient temperature (°C) readings, for daily mean, maximum, and minimum.

Start	End	Δ Daily Mean ±sd	Δ Daily Max. ±sd	Δ Daily Min. ±sd
2007-08-19	2008-08-02	0.06 ±0.33	0.67 ±0.54	-0.07 ±0.36
2008-08-04	2009-06-14	-0.95 ±0.49	-0.17 ±0.27	-1.29 ±0.68
2009-06-15	2009-08-10	-0.20 ±0.95	0.48 ±1.96	-0.21 ±0.37
2009-08-19	2010-07-08	0.25 ±0.48	0.44 ±0.66	0.27 ±0.64

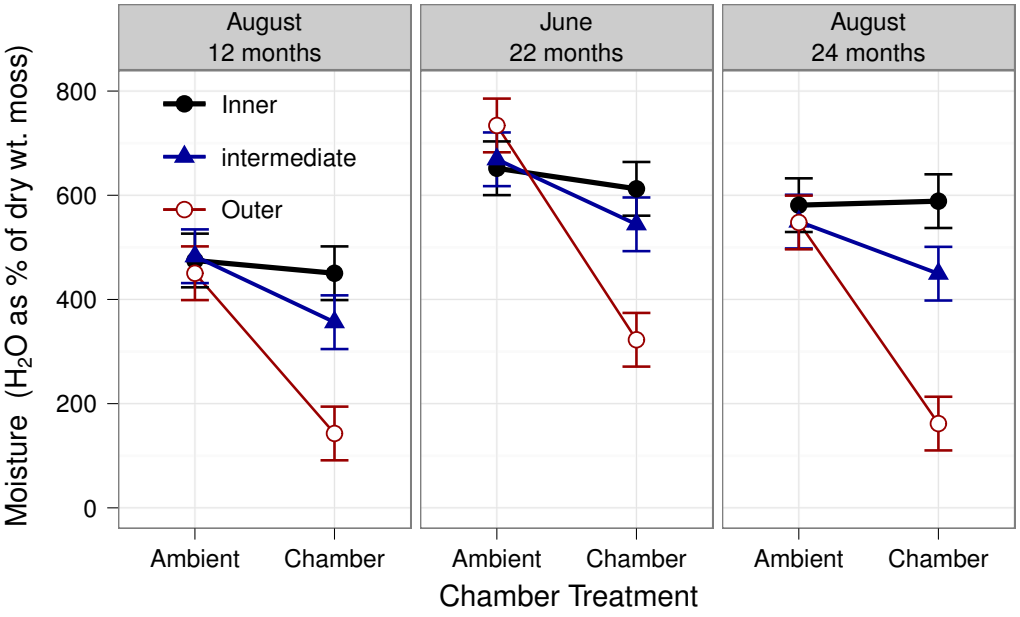

Figure 3.2
Water content of moss patches at time of collection, as a percentage of moss dry weight, at 12, 22, and 24 months into the experiment. Values are means, by chamber treatment and patch position (n = 32 for each point). Error bars represent 95% comparison intervals (Tukey's HSD Minimum Significant Ranges).

Nested analysis of variance of moss water contents revealed a significant interaction between chamber, fragmentation, and patch position, which also changed through time (Table 3.3). Patches in the outer corners of the chambers were consistently drier, except for those in the "contiguous" fragmentation treatment (Figure 3.8) Corridor treatments had significantly lower water contents than contiguous or isolated patches on average, which we attribute to the number of openings in the pots used to contain the patches. The magnitude of this difference across fragmentation treatments (100–200 percentage points) is also less than the gradient observed from outer to inner patches within chambers (200–300 percentage

points). Although patterns did depend on season, the magnitude did not change appreciably after the first 12 months. Dry conditions were interrupted only briefly during spring, 22 months into the experiment, when snowmelt led to observed flooding in some patches. This was definitely a wetter period overall, yet outer patches in the chambers still only contained about half as much water as all other patches (Figure 3.2, 22 months).

Nitrogen-Fixation and Cyanobacteria

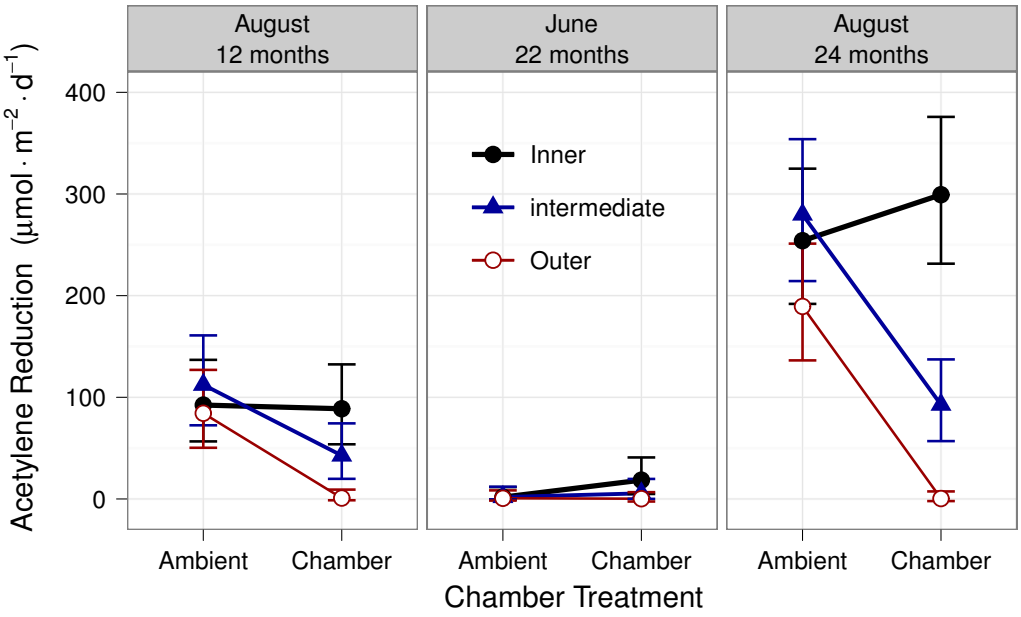

Figure 3.3
Rates of nitrogen-fixation in moss patches, measured in situ as rates of Ethylene produced by reduction of acetylene by nitrogenase enzymes, at 12, 22, and 24 months into the experiment. Values are means (n = 32 each), with error bars representing 95% comparison intervals (Tukey's HSD Minimum Significant Ranges).

Nested analysis of variance of nitrogen fixation rates revealed a significant interaction between chamber treatment, patch position, and time (Table 3.3). The significant interaction between chamber treatment and patch position ('dryness') was a result of lower rates of N-fixation observed in dry outer patches within the chambers, relative to wet inner chamber patches. Ambient patches showed no difference between patches of different positions. Measurements of Acetylene Reduction thus followed observed patterns in water contents very closely in late summer (Figure 3.3). Rates of nitrogen-fixation were lowest in outer chamber patches, which were also the driest patches in the experiment. All patches showed

Table 3.3

*F and P-values, with relevant degrees of freedom (df), for nested analysis of variance (ANOVA) of main response variables. Significant p-values (below α of 0.05) are highlighted in bold and indicated with asterisks: ** if $P < 0.01$, * if $P < 0.05$. Marginally significant P-values ($P < 0.01$) are indicated by •.*

	df		% Moisture			ARA (N-fixation)			Cyanobacteria cell density		
Term	*between*	*within*	*F*	*p*		*F*	*p*		*F*	*p*	
Time	2	14	11.0	**0.001**	**	14.4	**< 0.001**	**	0.7	0.517	
Chamber	1	21	42.1	**0.001**	**	16.9	**< 0.001**	**	3.7	0.067	•
Time × Chamber	2	21	0.2	0.807		8.9	**0.002**	**	1.2	0.326	
Fragmentation	3	126	13.3	**< 0.001**	**	1.8	0.148		1.1	0.358	
Time × Fragmentation	6	126	0.6	0.711		0.6	0.689		1.5	0.171	
Chamber × Fragmentation	3	126	2.1	0.104		0.8	0.500		0.5	0.703	
Time × Chamber × Fragmentation	6	126	0.9	0.513		0.4	0.907		0.4	0.881	
Position	2	336	113.6	**< 0.001**	**	70.4	**< 0.001**	**	2.4	0.127	
Time × Position	4	336	5.3	**< 0.001**	**	11.4	**< 0.001**	**	0.5	0.620	
Chamber × Position	2	336	126.7	**< 0.001**	**	44.2	**< 0.001**	**	0.1	0.767	
Fragmentation × Position	6	336	4.3	**< 0.001**	**	1.3	0.269		0.9	0.464	
Time × Chamber × Position	4	336	1.6	0.180		6.4	**< 0.001**	**	0.6	0.567	
Time × Fragmentation × Position	12	336	1.2	0.289		1.0	0.409		1.4	0.240	
Chamber × Fragmentation × Position	6	336	4.8	**< 0.001**	**	0.9	0.524		0.3	0.824	
Time × Chamber × Fragmentation × Position	12	336	2.2	**0.014**	*	0.7	0.736		1.8	0.098	•

extremely low rates of acetylene reduction in the spring, with the notable exception of those near the centre of the chambers (Figure 3.3, 22 months).

No significant differences in total cell density were found for any experimental treatment (Table 3.3). Despite weak trends suggesting slightly higher cell densities in chambers, the variation was much higher than observed differences (Figure 3.4). The results were similar for heterocyst cell density, ignoring vegetative cells that do not participate directly in nitrogen-fixation, and the number of heterocyst cells increased linearly with total cells per sample (Figure 3.15). Results for total cell density are presented, to permit easier comparison with results of other studies. The heterocystous cyanobacteria in our samples were dominated by *Stigonema* sp., with a minority of *Nostoc*, and a few unidentified single-cell species (see Figure 3.14).

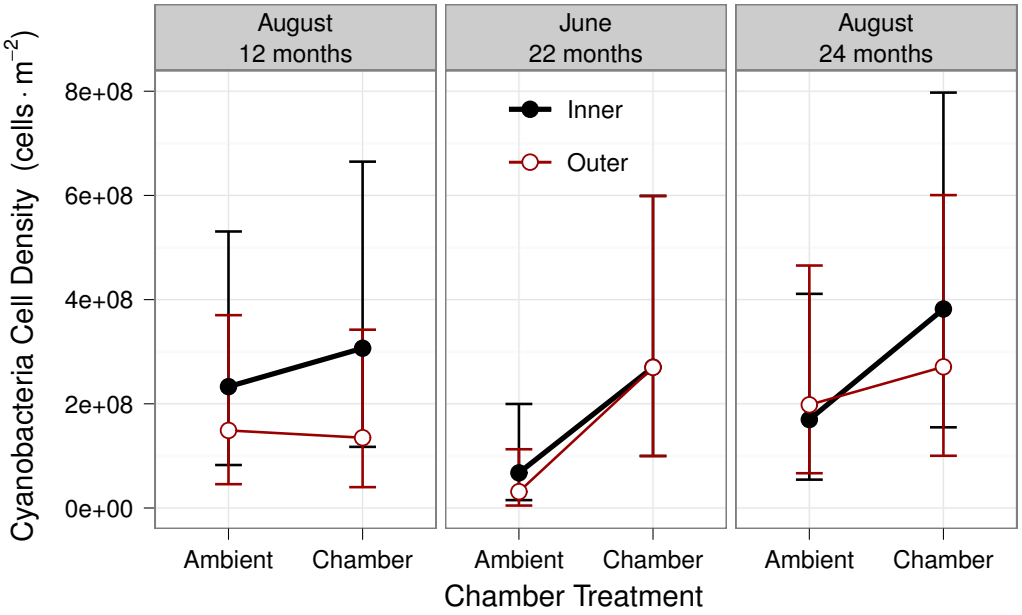

Figure 3.4
Density estimates of total cyanobacteria cells, at 12, 22, and 24 months into the experiment. Values are means (n = 32 each), with error bars representing 95% comparison intervals (Tukey's HSD Minimum Significant Ranges).

Cyanobacteria density alone is a poor predictor of observed N-fixation rates (Figures 3.5, 3.9). Although Cyanobacteria cell density does appear in the regression tree, time, moisture, fragmentation, and block all appear closer to the base of the tree, indicating that these variables account for more of the deviance, and may be more important than cell density in explaining short-term rates of N-fixation (Figure 3.9). Unsurprisingly, the two August samples (12, 24

months) group together, with the June sample (22 months) exhibiting overall lower rates of N-fixation. The lowest rates of N-fixation were found in dry patches (Moisture < 400–500 %), in contiguous or isolated patches (i.e. non-corridor fragmentation treatments). Note also that the wettest patches (Moisture > 600%) showed lower rates of N-fixation than dryer patches in other treatments (Figure 3.9). Regression trees of separate time points (not shown) showed a similar pattern where the highest rates of N-fixation occur in patches with intermediate moisture (300–600%), suggesting a non-linear, unimodal effect of moisture, interacting with other experimental treatments. The chamber treatment is notably absent from the regression tree, suggesting that warming had very little effect on N-fixation rates, relative to moisture, fragmentation, or time.

All main terms and several two-way interactions were found to be important in 256 of the best regression models, including interactions between cyanobacteria cell density and block, chamber, and moisture (Figure 3.5). The single best model with two-way interactions included the following terms (AIC=740, adjusted R^2 =0.74; $F_{62,321}$=18.3, P<<0.001; estimates and confidence intervals in supplementary materials):

$$
\begin{aligned}
&log_{10}(Acetylene\ Reduction) = \\
&\quad 1 + Time + Block + Chamber + Fragmentation + Position \\
&\quad + log_{10}(Cyanobacteria) + Moisture + Moisture^2 + Block \times Time \\
&\quad + Time \times Chamber + Time \times Position + Chamber \times Position \\
&\quad + Moisture \times log_{10}(Cyanobacteria) + Moisture^2 \times log_{10}(Cyanobacteria) \\
&\quad + Block \times log_{10}(Cyanobacteria) + Block \times Moisture + Block \times Moisture^2 \\
&\quad + Time \times Moisture + Chamber \times log_{10}(Cyanobacteria)
\end{aligned}
$$

Cyanobacteria cell density has a greater effect on N-fixation rates within chambers, both in inner and dry outer patches (Figure 3.12). The relationship between N-fixation rates and cyanobacteria cell density also depends somewhat on available moisture, with neutral or even negative relationships in very dry *and* very wet patches. The interaction between cyanobacteria, moisture, and blocks, are visualized in the supplementary materials.

Both regression trees (Figure 3.9) and regression models (Figure 3.11) suggest higher average rates of N-fixation in corridor and pseudo-corridor fragmentation treatments, independent of other experimental treatments. There is little support for interactions between fragmentation and climate variables of

temperature, as affected by chambers, and moisture (Figure 3.5).

Rates of N-fixation are positively related to cyanobacteria cell density, after removing effects of other variables, but the relationship is relatively weak, explaining only 4% of residual variation (Figure 3.6). Although cyanobacteria do play a role, environmental factors of moisture, fragmentation treatment, block location, and time, have a greater effect on observed rates of N-fixation.

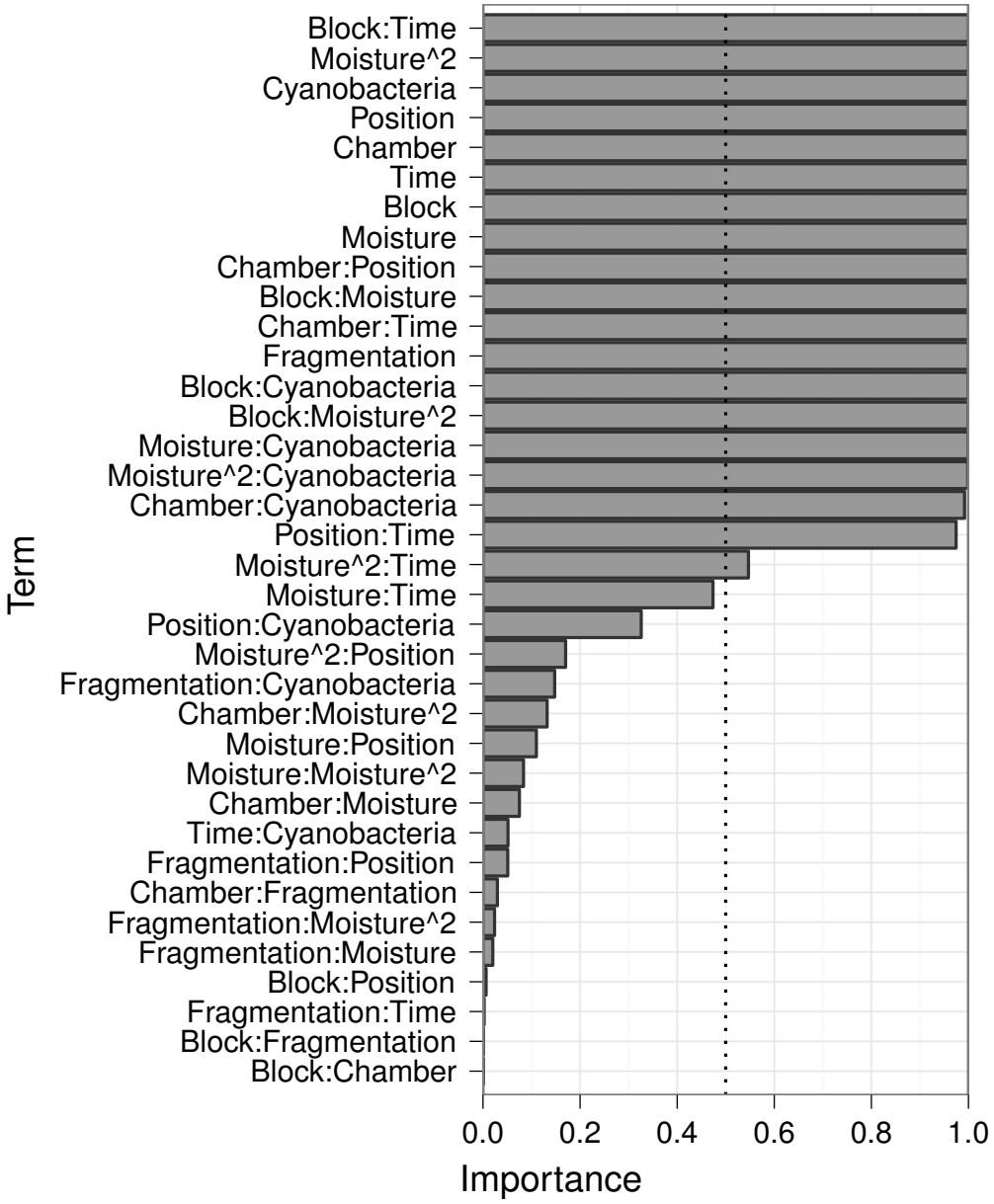

Figure 3.5

Model-averaged importance of terms used to explain acetylene reduction rates in linear models. Importance is calculated as the average term AIC weights across the 256 best models identified by 4 genetic algorithm searches of possible combinations of candidate model terms. The best model found included all terms above 50%, indicated by the dotted line (Moisture $\times$ Time interaction, and terms above).

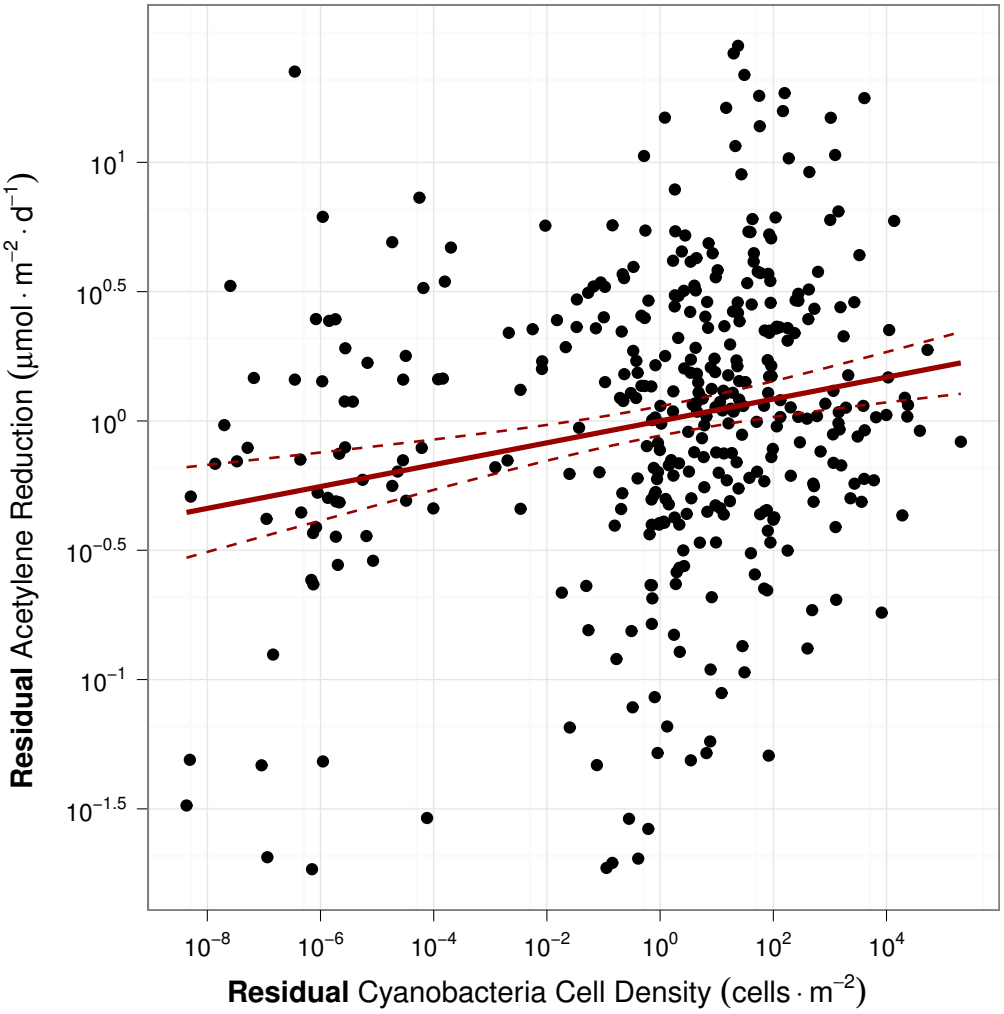

Figure 3.6

Partial linear regression of acetylene reduction rates as a function of cyanobacteria cell density, after removing effects of moisture contents and other experimental treatments. Lines indicate fitted values and point-wise 95% confidence intervals. Adjusted $r^2 = 0.04$ *(*$y = 0.042x$*;* $F_{1,382} = 17.62$*,* $P = 0.000034$*).*

3.4 Discussion

Nitrogen-fixation rates were no different between inner patches in chamber and ambient conditions. We therefore conclude that warming by chambers had no measurable effect on N-fixation rates. This may be explained by the relatively low and inconsistent warming effects of the passive chambers used in the experiment. The lowest rates of N-fixation, however, occurred in outer chamber patches, the driest patches in the experiment. Because the experiment did not include dry patches under ambient conditions, we can not rule out the possibility that warming and drought acted together to reduce N-fixation rates. Other experiments have demonstrated strong negative effects of reducing precipitation frequency on moisture contents and N-fixation rates (Gundale *et al.*, 2009; Jackson *et al.*, 2010; Gundale *et al.*, 2012b). Although N-fixation rates do vary across large temperature gradients, warming can also have indirect effects on N-fixation by increasing evapotranspiration and the associated negative effects of drought stress (Gundale *et al.*, 2012a,b). Further research on interactions between temperature and moisture on N-fixation rates would clarify the relative importance of these factors within the context of climate change conditions.

No statistical effect of fragmentation was detected on either N-fixation rates or cyanobacteria density when considered individually (Table 3.3), yet open corridor and pseudo-corridor treatments seem to exhibit rates of biotic N-fixation roughly twice as high as contiguous and fully isolated patches (Figures 3.9, 3.11). The reasons are somewhat unclear, and this pattern is inconsistent with expectations of changes in dispersal associated with the fragmentation treatments. It is possible that greater disturbance associated with setting up corridor treatments may have triggered a slight boost in N-fixation rates (T. DeLuca, pers. comm.), although a two-year boost seems unlikely. Fragmentation therefore appears to have subtle effects on nitrogen-fixing cyanobacteria, which were detectable in some analyses, but not others.

We also detected a statistically significant, but biologically weak difference in water contents among fragmentation treatments. Despite the ability of contiguous moss patches to mitigate some drying within chambers (Figure 3.8), N-fixation was low on average, across outer patches in all fragmentation treatments. We believe that contiguous moss patches are able to wick moisture from adjacent patches, thus maintaining similar overall moisture levels to other contiguous patches. This wicking effect may not occur near the moss surface, however, allowing dry

conditions to persist in roughly the first 3 cm below the tips of feather moss shoots (the bryosphere "canopy"), where the majority of N-fixing cyanobacteria occur (J. A. Whiteley, unpublished data).

We simulated climate change conditions in the field by modifying temperature and precipitation within open-top chambers. Maximum and average temperatures increased throughout the chambers. The greatest increases in temperature occurred in chambers with less canopy cover to the south of their position. Canopy shading would have reduced incident solar radiation, reducing the passive warming effect of a greenhouse chamber. On average, our measurements are similar to observed warming of soil temperatures by comparable chambers in arctic tundra (Marion *et al.*, 1997). We observed cooling more often than was observed in similar chambers used in tundra habitats, likely due to lower solar input under even a sparse boreal forest canopy. Nevertheless, wintertime cooling along with summer and autumn warming may be consistent with observed climate trends (Cohen *et al.*, 2012). A drought gradient within the chambers ranged from moisture conditions equivalent to ambient conditions in the centre, to extremely dry conditions in outer patches in the periphery of the chambers. The drought conditions could have been caused by decreased direct precipitation, blocked by the chamber walls, or by increased evapotranspiration as a result of energy absorption near chamber walls. In either case, drying occurred rapidly and persisted throughout the duration of the experiment.

Cyanobacteria densities were not significantly different across any experimental treatments, despite such a strong drought gradient. The drought-tolerance of symbiotic cyanobacteria is not well-described, though the species observed in this experiment appear able to survive nearly two years of drought. After 22 months, in early summer, we observed the highest densities of cyanobacteria in chambers, with no difference between dry outer and wet inner patches. Nevertheless, the highest rates of N-fixation were observed in the wet inner chamber patches. These samples were collected in spring, immediately following snowmelt — in some cases, a single day after being uncovered. Some of these experimental units were flooded by melting snow, providing a brief reprieve from long-term drought, which returned over the ensuing summer. The effect can be seen in Figure 3.2, where outer chamber patches, although dryer than other patches at the same point in time, are significantly wetter than either late-summer sampling periods before or after. It is noteworthy that, despite this short-term drought relief, only the inner chamber patches exhibited detectable rates of N-fixation, whereas the dry outer patches remained functionally

inactive. Either the drought relief did not last long enough for cyanobacteria to begin fixing nitrogen prior to sampling, or they had been so damaged by prolonged drought that they were no longer capable, despite continuing to appear in our density measurements.

An alternative explanation of our results might be that cyanobacteria are not responsible for the observed changes in N-fixation rates. Other N-fixing bacteria, less tolerant of drought stress, may account for the variation in ecosystem processes. Nevertheless, absence of proof does not constitute proof of absence. We observed marked variation in the relationship between cyanobacteria density and N-fixation rates (Figure 3.12), which suggests instead that cyanobacteria respond physiologically to environmental stress, long before mortality leads to demographic changes.

N-fixation in ambient treatments were comparable to those measured in similar ecosystems in Northern Sweden (DeLuca *et al.*, 2002, 2007; Zackrisson *et al.*, 2009): Assuming a ratio of 3 mol acetylene reduced : 1 mol N_2 fixed, and a 120-day growing season for Schefferville, an average rate of 100 $\mu mol \cdot m^{-2} \cdot d^{-1}$ acetylene reduced corresponds to 1.1 $kg\ N \cdot ha^{-1} \cdot yr^{-1}$ fixed; the highest rates of acetylene reduction in our experiment correspond to about 3 $kg\ N \cdot ha^{-1} \cdot yr^{-1}$. Note that these are unlikely to correspond to true annual rates, given the degree of temporal variability in N-fixation rates across seasons (DeLuca *et al.*, 2002), but are provided for comparison.

Our results suggest that precipitation changes associated with climate change will have larger impacts on nitrogen fixation than small changes in temperature. Although Gentili *et al.* (2005) found species-dependent temperature responses to N-fixation rates, they reported results only for the cyanobacteria genera *Calothrix* and *Nostoc*. A large majority of cyanobacteria in our samples were *Stigonema* sp. The sensitivity of this genus to temperature and moisture remains unknown. N-fixation rates in boreal forest moss have been shown to respond to drought and rates of experimental watering (Gundale *et al.*, 2009; Jackson *et al.*, 2010). Our results confirm the sensitivity of N-fixation rates to substrate moisture in situ, although our data also suggests an overall unimodal response of N-fixation to moisture, with rates declining above 600% moisture content (Figure 3.9). Our results also suggest that this response may be de-coupled from cyanobacteria density.

Previous studies of cyanobacteria-bryophyte associations have found a positive relationship between cyanobacteria density and N-fixation rates (DeLuca *et al.*, 2007; Lindo and Whiteley, 2011). We also found a significantly positive

but comparatively weak relationship between cyanobacteria density and rates of N-fixation, after accounting for experimental treatments. It is clear that patches with moderate densities of cyanobacteria may not be fixing any nitrogen at measurable rates when under drought stress. Cyanobacteria in this system likely respond to drought stress by entering a state of metabolic dormancy, persisting but not actively fixing nitrogen. For example, *Nostoc commune* is known to be able to survive desiccation for several years, undergoing several structural, physiological, and biochemical changes during drying and rewetting (Scherer and Potts, 1989). What is somewhat surprising is that prolonged drought has long-term effects on N-fixation rates, preventing recovery during intermittent wet periods despite the continued presence of apparently intact (fluorescent) cyanobacteria colonies.

Dessication and rewetting also affects moss physiology, with nutrients and metabolites often released during rewetting (Bewley, 1995; Turetsky, 2003). Drought may affect cyanobacteria directly, but may also cause longer-term indirect effects in N-fixation by causing stress to the host mosses. Reduced photosynthesis, loss of nutrients, and even structural changes to moss tissues can all have negative consequences for cyanobionts.

Our results demonstrate that the links between cyanobacteria populations and biotic N-fixation at the ecosystem level are more complex than perhaps previously thought. Although cyanobacteria density alone was a poor predictor of N-fixation rates, it is clear that this relationship is contingent on several environmental factors, including moisture, temperature, available nitrogen, and other unknown factors, which may account for the variation across blocks (spatial location) in our results.

A given number of cyanobacteria heterocyst cells of the same species will likely fix nitrogen at rates that vary over time (DeLuca *et al.*, 2002), depending on environmental conditions such as temperature (Gentili *et al.*, 2005), moisture (Gundale *et al.*, 2009), available nitrogen (DeLuca *et al.*, 2008), soil age (Menge and Hedin, 2009), and perhaps other factors such as availability of light or other nutrients. A precise estimate of the effect of cyanobacteria density on N-fixation rates is only truly possible by controlling for all other conditions, which is exceedingly difficult to do in the field. This may explain why the strength of the relationship between cyanobacteria density and N-fixation rates varies markedly across different studies. A stronger relationship may be more likely in cases where other factors are less variable, or better controlled.

The rapid response of N-fixation in contrast to little or no change in cyanobacteria populations also challenges our understanding of the relationship

between biodiversity and ecosystem function. Theoretical descriptions of this relationship assume that ecosystem-level process rates, usually productivity, are directly proportional to population sizes of species contributing to that process (Yachi and Loreau, 1999). Many theoretical studies assume that population dynamics occur relatively quickly in response to environmental conditions, with process rates calculated after such changes. However, we observed large changes in an ecosystem process, even a complete cessation of nitrogen-fixation, with no observed changes in the abundance of relevant species. This suggests that ecosystems can respond very rapidly in functional terms, due to physiological responses by dominant species, before population dynamics allow species better suited to new conditions to increase in abundance and compensate for new environmental conditions (Gonzalez and Loreau, 2009). As a result, the insurance effect of biodiversity (Loreau *et al.*, 2003) may occur only after an initial decline in ecosystem-level processes.

A major concern of climate change impacts is the potential for non-additive effects with other factors affecting biodiversity, particularly habitat loss and fragmentation (Sala *et al.*, 2000). We found no evidence of interaction between simulated climate change conditions and habitat fragmentation treatments. Habitat fragmentation is expected to amplify species extinction caused by climate change, by preventing dispersal needed for species to track preferred environmental conditions (Watkinson and Gill, 2002). Our experimental design did not provide opportunities for dispersal away from temperature increases, although the precipitation gradient did span the full range of connectivity treatments in the experiment. Cyanobacteria are only capable of dispersal during early stages of their life cycle, as immature hormogonia (Bergman *et al.*, 2007; Pawlowski and Bergman, 2007), and the environmental triggers of motile stages are not well understood, apart from unknown compounds produced by some plant hosts (Bergman *et al.*, 2007). It is unlikely that hormogonia would be capable of motility in a dessicated environment, and cyanobacteria may cope with drought by reducing metabolic activity rather than emigrating from unfavourable patches. For cyanobacteria, even cases of high physical connectivity of moss habitat may have low functional connectivity (Doerr *et al.*, 2011) if the habitat is too dry for propagules to traverse.

Moisture contents is an important factor in the ecology of bryophyte-associated nitrogen-fixing cyanobacteria in the boreal forest. Whether a result of warming, precipitation quantity or frequency, or a combination of factors,

prolonged periods of drought may have long-term impacts on N-fixation and nitrogen dynamics in boreal forests.

Conclusion

We observed almost total suppression of biotic nitrogen-fixation by cyanobacteria associated with boreal forest bryophytes in response to induced drought in a field experiment. Drying had a stronger effect on realized rates of N-fixation than temperature, independent of cyanobacterial cell density, which did not differ between treatments. Our results suggest a rapid and sustained physiological response by cyanobacteria to environmental change in the absence of measurable changes in density. N-fixation by cyanobacteria is ultimately the outcome of cyanobacteria density in the context of local environmental conditions. This highlights the fact that ecosystem processes may respond to environmental change much faster than the abundance of species responsible. Therefore, long-term monitoring of ecosystem processes, relevant biodiversity, and the links between them, will be needed to accurately understand and predict consequences of ongoing environmental change.

Acknowledgements

This project was supported by a Post-Graduate Scholarship from the Natural Sciences and Engineering Research Council of Canada (NSERC) to JAW, with a Natural Sciences and Engineering Research Council discovery grant and Canada Research Chair funding to AG. Z. Lindo helped with field work, and provided friendly review during manuscript preparation. O. Choulik provided logistical support in Schefferville. M. Mehta helped with cyanobacteria data collection. J. Connolly provided generally helpful advice on mixed modelling approaches.

References

Ahn CY, Park MH, Joung SH, Kim HS, Jang KY, Oh HM (2003) Growth inhibition of cyanobacteria by ultrasonic radiation: laboratory and enclosure studies. Environ Sci Technol 37(13):3031–3037, doi:10.1021/es034048z

Bergman B, Rai A, Rasmussen U (2007) Cyanobacterial associations. In: Elmerich C, Newton WE (eds.) Associative and Endophytic Nitrogen-fixing Bacteria

and Cyanobacterial Associations, Nitrogen Fixation: Origins, Applications, and Research Progress, vol. 5, Springer Netherlands, Dordrecht, The Netherlands, pp 257–301

Bewley JD (1995) Physiological aspects of desiccation tolerance - A retrospect. Int J Plant Sci 156(4):393–403, doi:10.2307/2475057

Bonan GB, Van Cleve K (1992) Soil temperature nitrogen mineralization and carbon source-sink relationships in boreal forests. Can J For Res 22(5):629–639, doi:10.1139/x92-084

Burrows MT, Schoeman DS, Buckley LB, Moore P, Poloczanska ES, Brander KM, Brown C, Bruno JF, Duarte CM, Halpern BS, Holding J, Kappel CV, Kiessling W, O'Connor MI, Pandolfi JM, Parmesan C, Schwing FB, Sydeman WJ, Richardson AJ (2011) The pace of shifting climate in marine and terrestrial ecosystems. Science 334(6056):652–655, doi:10.1126/science.1210288

Calcagno V, de Mazancourt C (2010) glmulti: an R package for easy automated model selection with (generalized) linear models. J Stat Softw 34(12):1–29

Chapin III FS, Oechel WC, Van Cleve K, Lawrence W (1987) The role of mosses in the phosphorus cycling of an Alaskan USA black spruce forest. Oecologia 74(2):310–315, doi:10.1007/BF00379375

Cohen JL, Furtado JC, Barlow MA, Alexeev VA, Cherry JE (2012) Arctic warming, increasing snow cover and widespread boreal winter cooling. Environ Res Lett 7(1), doi:10.1088/1748-9326/7/1/014007

DeLuca T, Zackrisson O, Gentili F, Sellstedt A, Nilsson MC (2007) Ecosystem controls on nitrogen fixation in boreal feather moss communities. Oecologia 152(1):121–130, doi:10.1007/s00442-006-0626-6

DeLuca TH, Zackrisson O, Nilsson MC, Sellstedt A (2002) Quantifying nitrogen-fixation in feather moss carpets of boreal forests. Nature 419(6910):917–920, doi:10.1038/nature01051

DeLuca TH, Zackrisson O, Gundale MJ, Nilsson MC (2008) Ecosystem feedbacks and nitrogen fixation in boreal forests. Science 320(5880):1181, doi:10.1126/science.1154836

Doerr VAJ, Barrett T, Doerr ED (2011) Connectivity, dispersal behaviour and conservation under climate change: a response to Hodgson et al. J Appl Ecol 48(1):143–147, doi:10.1111/j.1365-2664.2010.01899.x

Environment Canada. Canadian Climate Normals or Averages 1971-2000, Schefferville, QC [online] (2012), URL: http://climate.weatheroffice.gc.ca/climate_normals/index_e.html [cited May 6, 2012]

Fitzjarrald DR, Moore KE (1994) Growing-season boundary-layer climate and surface exchanges in a sub-arctic lichen woodland. J Geophys Res-Atmos 99(D1):1899–1917, doi:10.1029/93jd01019

Fox J (2003) Effect displays in R for generalised linear models. J Stat Softw 8(15):1–27

Gentili F, Nilsson MC, Zackrisson O, DeLuca TH, Sellstedt A (2005) Physiological and molecular diversity of feather moss associative N2-fixing cyanobacteria. J Exp Bot 56(422):3121–3127, doi:10.1093/jxb/eri309

Gonzalez A, Loreau M (2009) The causes and consequences of compensatory dynamics in ecological communities. Annu Rev Ecol Evol Syst 40(1):393–414, doi:10.1146/annurev.ecolsys.39.110707.173349

Grant RF, Black TA, Gaumont-Guay D, Kljun N, Barr AG, Morgenstern K, Nesic Z (2006) Net ecosystem productivity of boreal aspen forests under drought and climate change: Mathematical modelling with Ecosys. Agric For Meteorol 140(1-4):152–170, doi:10.1016/j.agrformet.2006.01.012

Guillard RRL (2005) Purification methods for microalgae. In: Andersen RA (ed.) Algal culturing techniques, Elsevier/Academic Press, Burlington, Mass.

Gundale MJ, Gustafsson H, Nilsson MC (2009) The sensitivity of nitrogen fixation by a feathermoss-cyanobacteria association to litter and moisture variability in young and old boreal forests. Can J For Res 39(12):2542–2549, doi:10.1139/X09-160

Gundale MJ, Nilsson M, Bansal S, Jäderlund A (2012a) The interactive effects of temperature and light on biological nitrogen fixation in boreal forests. New Phytol 194(2):453–463, doi:10.1111/j.1469-8137.2012.04071.x

Gundale MJ, Wardle DA, Nilsson MC (2012b) The effect of altered macroclimate on N-fixation by boreal feather mosses. Biol Lett 8(5):805–808, doi:10.1098/rsbl.2012.0429

Hardy RWF, Holsten RD, Jackson EK, Burns RC (1968) Acetylene-ethylene assay for N2 fixation – laboratory and field evaluation. Plant Physiol 43(8):1185–1207

Heyder U, Schaphoff S, Gerten D, Lucht W (2011) Risk of severe climate change impact on the terrestrial biosphere. Environ Res Lett 6(3), doi:10.1088/1748-9326/6/3/034036

Houlton BZ, Wang YP, Vitousek PM, Field CB (2008) A unifying framework for dinitrogen fixation in the terrestrial biosphere. Nature 454(7202):327–U34, doi:10.1038/nature07028

IPCC (2002) Climate change and biodiversity. Technical Paper IPCC Technical Paper V, Intergovernmental Panel on Climate Change

Jackson BG, Martin P, Nilsson MC, Wardle DA (2010) Response of feather moss associated N2 fixation and litter decomposition to variations in simulated rainfall intensity and frequency. Oikos 120(4):570–581, doi:10.1111/j.1600-0706.2010.18641.x

Lechowicz MJ, Adams MS (1978) Diurnal and seasonal structure of the climate at Scheffer Ville, Quebec. Arct Alp Res 10(1):95–104

Leibold MA, Holyoak M, Mouquet N, Amarasekare P, Chase JM, Hoopes MF, Holt RD, Shurin JB, Law R, Tilman D, Loreau M, Gonzalez A (2004) The metacommunity concept: a framework for multi-scale community ecology. Ecol Lett 7(7):601–613, doi:10.1111/j.1461-0248.2004.00608.x

Lindahl BO, Taylor AFS, Finlay RD (2002) Defining nutritional constraints on carbon cycling in boreal forests: towards a less 'phytocentric' perspective. Plant Soil 242(1):123–135, doi:10.1023/A:1019650226585

Lindner M, Maroschek M, Netherer S, Kremer A, Barbati A, Garcia-Gonzalo J, Seidl R, Delzon S, Corona P, Kolstrom M, Lexer MJ, Marchetti M (2010) Climate change impacts, adaptive capacity, and vulnerability of European forest ecosystems. For Ecol Manag 259(4):698–709, doi:10.1016/j.foreco.2009.09.023

Lindo Z, Gonzalez A (2010) The bryosphere: an integral and influential component of the Earth's biosphere. Ecosystems 13(4):612–627, doi:10.1007/s10021-010-9336-3

Lindo Z, Whiteley JA (2011) Old trees contribute bio-available nitrogen through canopy bryophytes. Plant Soil 342(1-2):1–8, doi:10.1007/s11104-010-0678-6

Loreau M, Mouquet N, Gonzalez A (2003) Biodiversity as spatial insurance in heterogeneous landscapes. Proc Natl Acad Sci USA 100(22):12,765 – 12,770, doi:10.1073/pnas.2235465100

Mäkipää R, Karjalainen T, Pussinen A, Kellomäki S (1999) Effects of climate change and nitrogen deposition on the carbon sequestration of a forest ecosystem in the boreal zone. Can J For Res 29(10):1490–1501, doi:10.1139/x99-123

Marion GM, Henry GHR, Freckman DW, Johnstone J, Jones G, Jones MH, Levesque E, Molau U, Molgaard P, Parsons AN, Svoboda J, Virginia RA (1997) Open-top designs for manipulating field temperature in high-latitude ecosystems. Glob Chang Biol 3(s1):20–32, doi:10.1111/j.1365-2486.1997.gcb136.x

Markham JH (2009) Variation in moss-associated nitrogen fixation in boreal forest stands. Oecologia 161(2):353–359, doi:10.1007/S00442-009-1391-0

Marshall WA, Chalmers MO (1997) Airborne dispersal of antarctic terrestrial algae and cyanobacteria. Ecography 20(6):585–594, doi:10.1111/j.1600-0587.1997.tb00427.x

Mcnabb DH, Geist JM (1979) Acetylene-reduction assay of symbiotic N2 fixation under field conditions. Ecology 60(5):1070–1072, doi:10.2307/1936873

Menge DNL, Hedin LO (2009) Nitrogen fixation in different biogeochemical niches along a 120 000-year chronosequence in New Zealand. Ecology 90(8):2190–2201, doi:10.1890/08-0877.1

Moore TR (1980) The nutrient status of subarctic woodland soils. Arct Alp Res 12(2):147–160

Norby RJ, Warren JM, Iversen CM, Medlyn BE, McMurtrie RE (2010) CO2 enhancement of forest productivity constrained by limited nitrogen availability. Proc Natl Acad Sci USA doi:10.1073/pnas.1006463107

Parmesan C (2006) Ecological and evolutionary responses to recent climate change. Annu Rev Ecol Evol Syst 37:637–669, doi:10.1146/annurev.ecolsys.37.091305.110100

Parmesan C, Yohe G (2003) A globally coherent fingerprint of climate change impacts across natural systems. Nature 421(6918):37–42, doi:10.1038/nature01286

Pawlowski K, Bergman B (2007) Plant symbioses with frankia and cyanobacteria. In: Bothe H, Ferguson SJ, Newton WE (eds.) Biology of the nitrogen cycle, Elsevier, Boston, pp 165–178

Payette S, Fortin MJ, Gamache I (2001) The subarctic forest-tundra: the structure of a biome in a changing climate. Bioscience 51(9):709–718, doi:10.1641/0006-3568(2001)051[0709:TSFTTS]2.0.CO;2

R Development Core Team (2010) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria, URL: http://www.R-project.org/, ISBN 3-900051-07-0

Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier RY (1979) Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J Gen Microbiol 111(Mar):1–61

Sala OE, Chapin FS, Armesto JJ, Berlow E, Bloomfield J, Dirzo R, Huber-Sanwald E, Huenneke LF, Jackson RB, Kinzig A, Leemans R, Lodge DM, Mooney HA, Oesterheld M, Poff NL, Sykes MT, Walker BH, Walker M, Wall DH (2000) Global biodiversity scenarios for the year 2100. Science 287(5459):1770–1774, doi:10.1126/science.287.5459.1770

Scherer S, Potts M (1989) Novel water stress protein from a desiccation-tolerant cyanobacterium. Purification and partial characterization. J Biol Chem 264(21):12,546–12,553

Schöllhorn R, Burris RH (1967) Acetylene as a competitive inhibitor of N2 fixation. Proc Natl Acad Sci USA 58(1):213–216, doi:10.1073/pnas.58.1.213

Sokal RR, Rohlf FJ (1981) Biometry: The Principles and Practice of Statistics in Biological Research, 3rd edn. W. H. Freeman and Company, New York

Sorensen PL, Michelsen A (2011) Long-term warming and litter addition affects nitrogen fixation in a subarctic heath. Glob Chang Biol 17(1):528–537, doi:10.1111/j.1365-2486.2010.02234.x

Taggart RE, Cross AT (2009) Global greenhouse to icehouse and back again: the origin and future of the boreal forest biome. Glob Planet Chang 65(3-4):115–121, doi:10.1016/j.gloplacha.2008.10.014

Therneau TM, Atkinson B, R port by Brian Ripley (2010) rpart: Recursive Partitioning. URL: http://CRAN.R-project.org/package=rpart, R package version 3.1-48

Thomas CD, Cameron A, Green RE, Bakkenes M, Beaumont LJ, Collingham YC, Erasmus BFN, de Siqueira MF, Grainger A, Hannah L, Hughes L, Huntley B, van Jaarsveld AS, Midgley GF, Miles L, Ortega-Huerta MA, Peterson AT, Phillips OL, Williams SE (2004) Extinction risk from climate change. Nature 427(6970):145–148, doi:10.1038/nature02121

Turetsky MR (2003) The role of bryophytes in carbon and nitrogen cycling. Bryologist 106(3):395–409, doi:10.1639/05

Vitousek PM, Cassman K, Cleveland C, Crews T, Field CB, Grimm NB, Howarth RW, Marino R, Martinelli L, Rastetter EB, Sprent JI (2002) Towards an ecological understanding of biological nitrogen fixation. Biogeochemistry 57(1):1–45, doi:10.1023/A:1015798428743

Watkinson AR, Gill JA (2002) Climate change and dispersal. In: Bullock JM, Kenward RE, Hails RS (eds.) Dispersal Ecology, Blackwell Science, Malden, MA, pp 410–428

Wickham H (2007) Reshaping data with the reshape package. J Stat Softw 21(12)

Wickham H (2009) ggplot2: elegant graphics for data analysis. Springer New York, h.wickham@gmail.com, doi:10.1007/978-0-387-98141-3, URL: http://had.co.nz/ggplot2/book

Wickham H (2011) plyr: Tools for splitting, applying and combining data. h.wickham@gmail.com, URL: http://CRAN.R-project.org/package=plyr, R package version 1.4

Yachi S, Loreau M (1999) Biodiversity and ecosystem productivity in a fluctuating environment: the insurance hypothesis. Proc Natl Acad Sci USA 96(4):1463–1468, doi:10.1073/pnas.96.4.1463

Zackrisson O, DeLuca T, Gentili F, Sellstedt A, Jäderlund A (2009) Nitrogen fixation in mixed *Hylocomium splendens* moss communities. Oecologia doi:10.1007/s00442-009-1299-8

Zehr JP, Montoya J (2007) Measuring N2 fixation in the field. In: Bothe H, Ferguson SJ, Newton WE (eds.) Biology of the nitrogen cycle, Elsevier, Boston, pp 193–205

Zhang G, Zhang P, Liu H, Wang B (2006) Ultrasonic damages on cyanobacterial photosynthesis. Ultrasonics Sonochemistry 13(6):501–505, doi:10.1016/j.ultsonch.2005.11.001

Zuur AF, Ieno EN, Smith GM (2007) Analysing Ecological Data. Statistics for Biology and Health, Springer, New York, URL: http://www.highstat.com/book1.htm

3.5 Supplementary Tables

Table 3.4

Terms included in candidate linear regression models.

abbrev.	term	type	values / units
ARA	Acetylene reduction (N-fixation) rate	*response*	$\log_{10}(\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{d}^{-1})$
Cells	Cyanobacteria cell density	continuous	$\log_{10}(\text{cells/m}^2)$
Moisture	Moisture contents of moss substrate	continuous	proportion of substrate dry weight
Moisture^2	Quadratic term	continuous	proportion . . . ^{2}
Block	Experimental Blocks (locations)	categorical	1–8
Chamber	Open-Top Chamber type	categorical	*Ambient* or *Full Chamber*
Fragmentation	Fragmentation Treatment	categorical	*Continuous*, *corridors*, *pseudo-corridors*, or *isolated*
Position	Patch Position within chamber	categorical	*inner* or *outer*
Time	Time of sample collection during experiment	categorical	12, 22, 24 months since start of expt.

3.6 Supplementary Figures

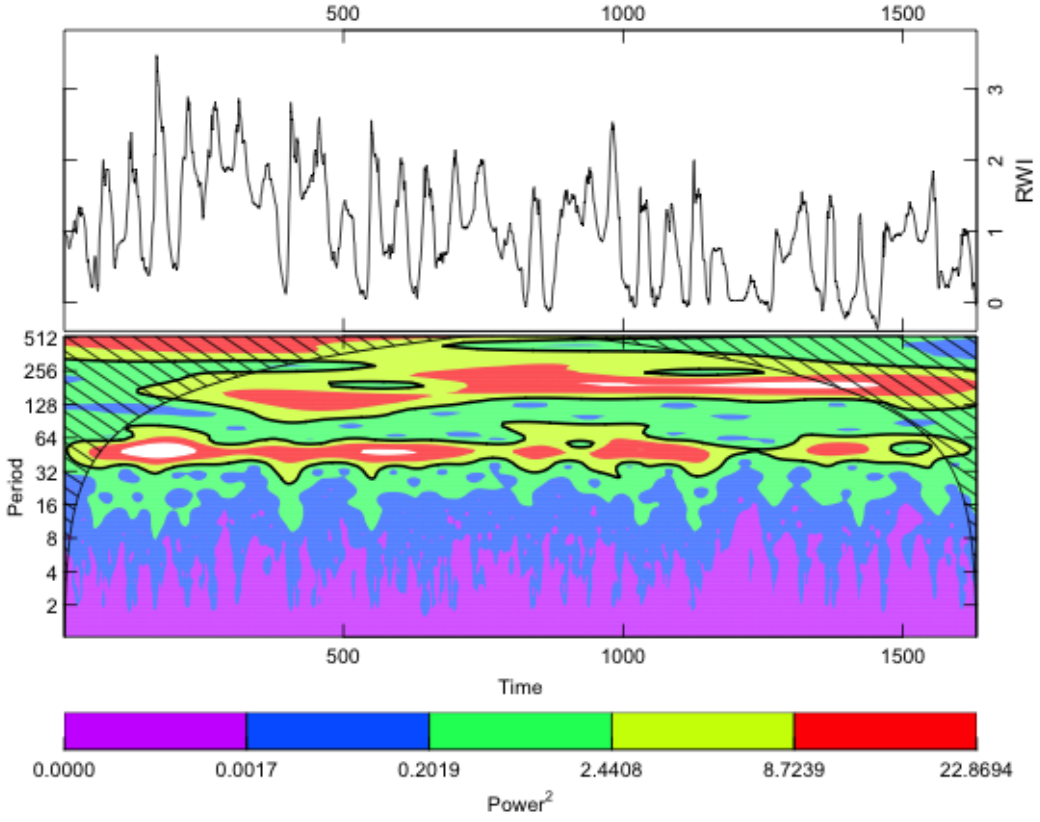

Figure 3.7

Wavelet analysis of effects of Chamber on temperature. Top panel shows a time series of the temperature difference between Chambers and Ambient plots (ΔT = Chamber - Ambient $^{\circ}C$). Temperature readings were collected every 30 minutes. Bottom panel shows power of fitting a "Mexican hat" wavelet; this highlights strong daily cycles (daily peak surrounded by nighttime minima), as well as 5-day cycles that may be related to dominant weather patterns (red band between 128 & 256 observations). Diagonal hatching indicates areas where the number of observations is insufficient for reliable estimates.

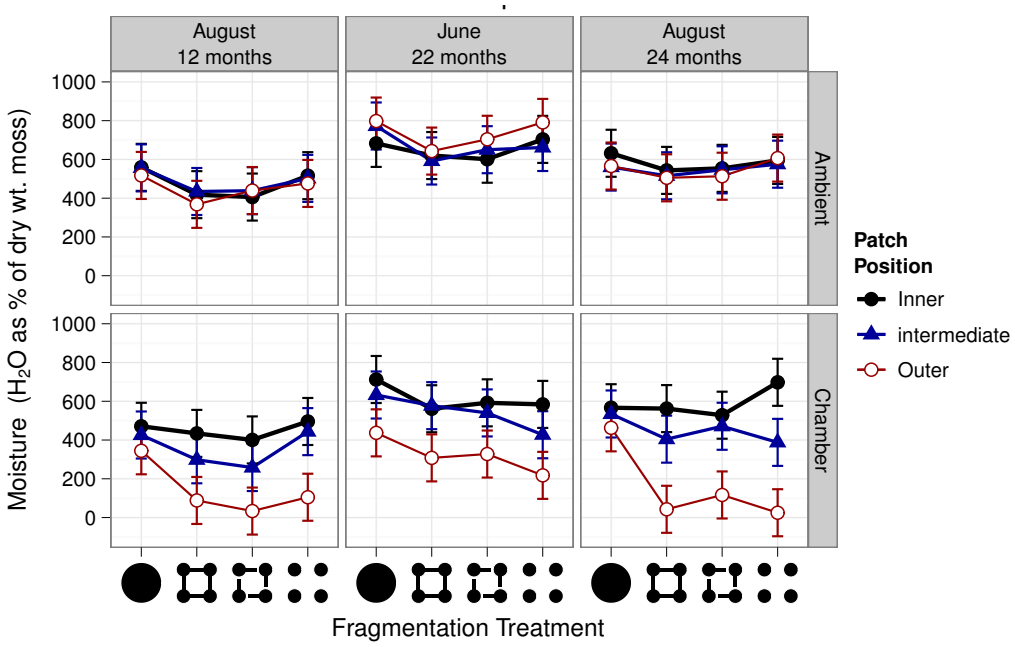

Figure 3.8

Water contents of moss patches at time of collection, as a percentage of moss dry weight, by fragmentation treatment and patch position, across chamber treatments and time from start of the experiment. Error bars represent 95% comparison intervals (Tukey's HSD Minimum Significant Ranges).

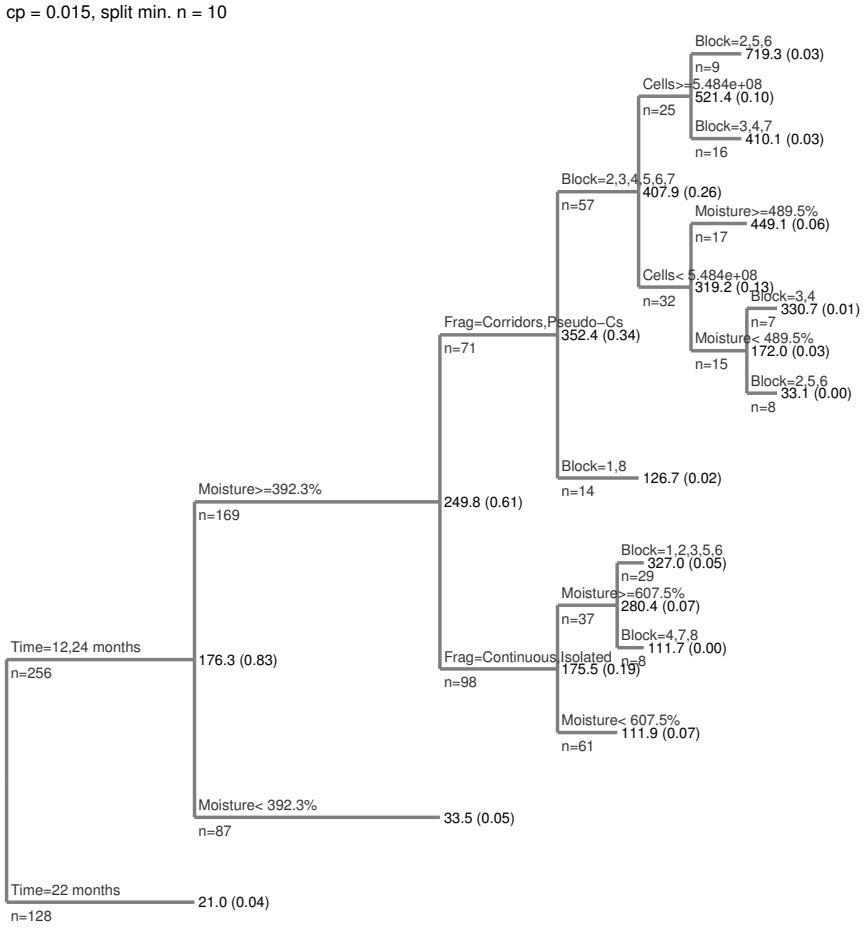

Figure 3.9

Regression Tree of acetylene reduction rates ($\mu mol \cdot m^{-2} \cdot d^{-1}$). Splitting criteria are indicated above each branch, with number of samples in each resulting split below. Mean values of each group and residual deviance in parentheses, are indicated at the tip of each branch (before any further splits).

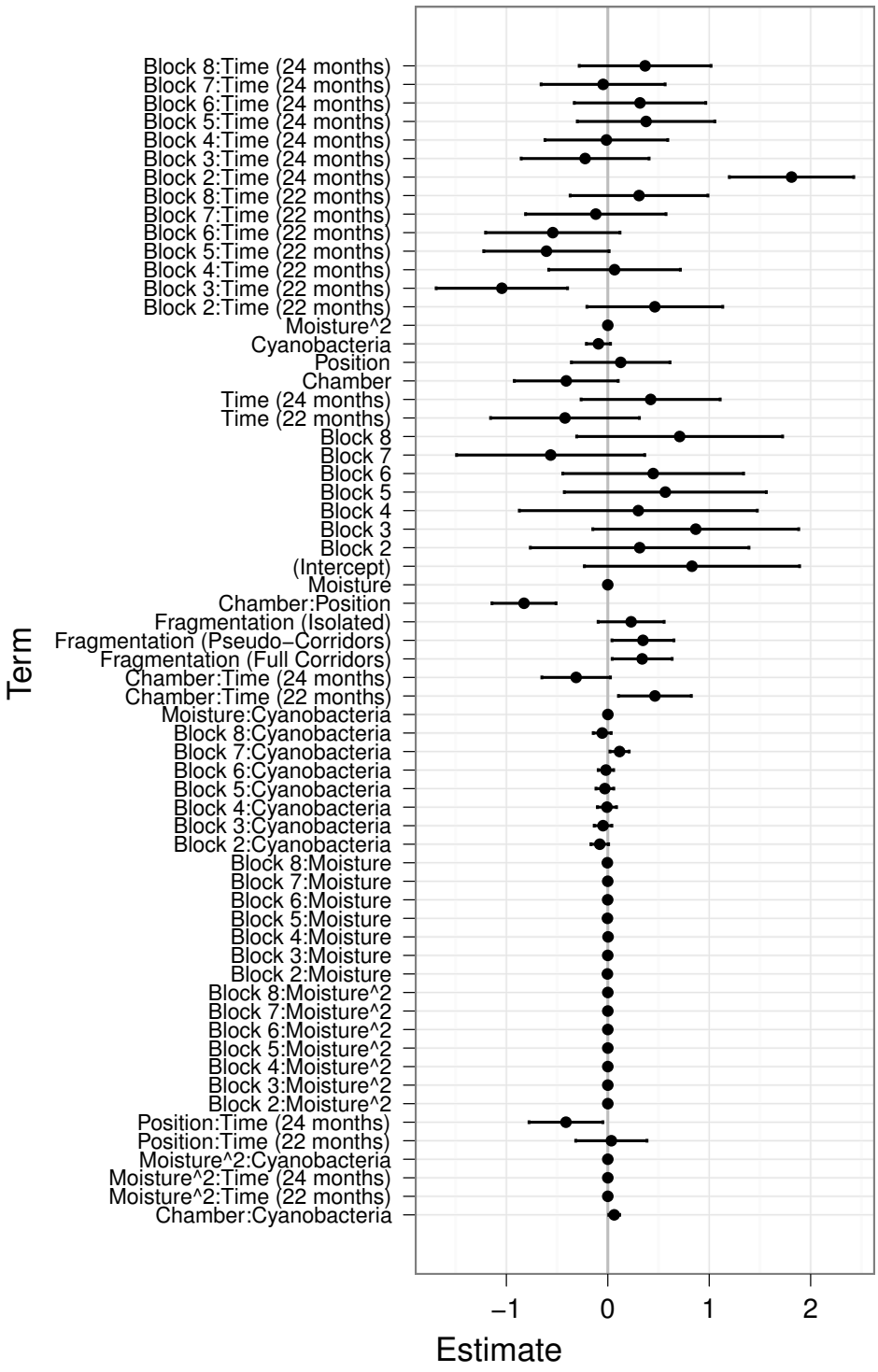

Figure 3.10

Estimates of linear model coefficient terms, averaged over the 256 best models found. Black circles indicate point estimates of coefficient values, and lines indicate 95% confidence intervals. Coefficients are included in the figure for terms with an 'importance' above 80%, calculated as the average term AIC weights across the best models identified (see Fig. 5 in the manuscript).

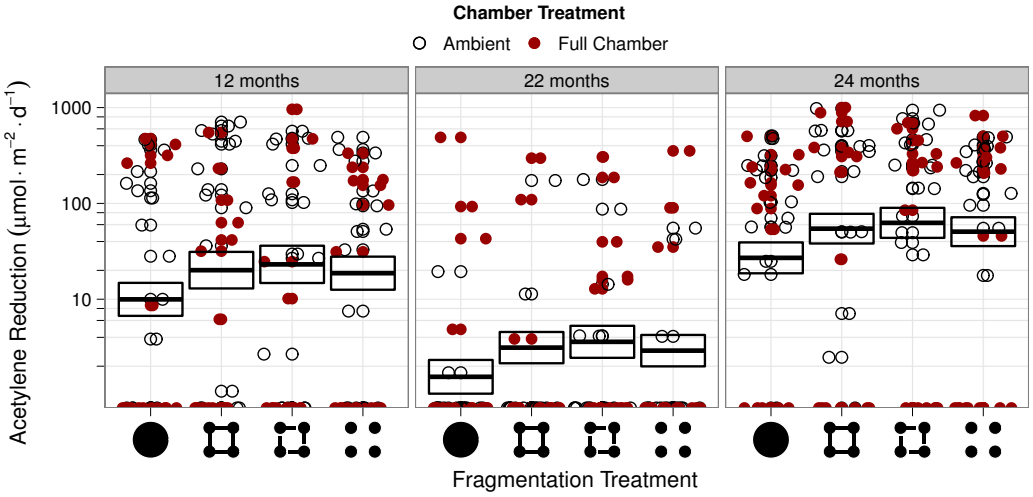

Figure 3.11

Acetylene reduction rates as a function of Fragmentation treatments, at each sampling time. Points have been jittered to reveal many overlapping 0-values for acetylene reduction rates. Boxes indicate fitted values (middle horizontal line) and 95% confidence intervals.

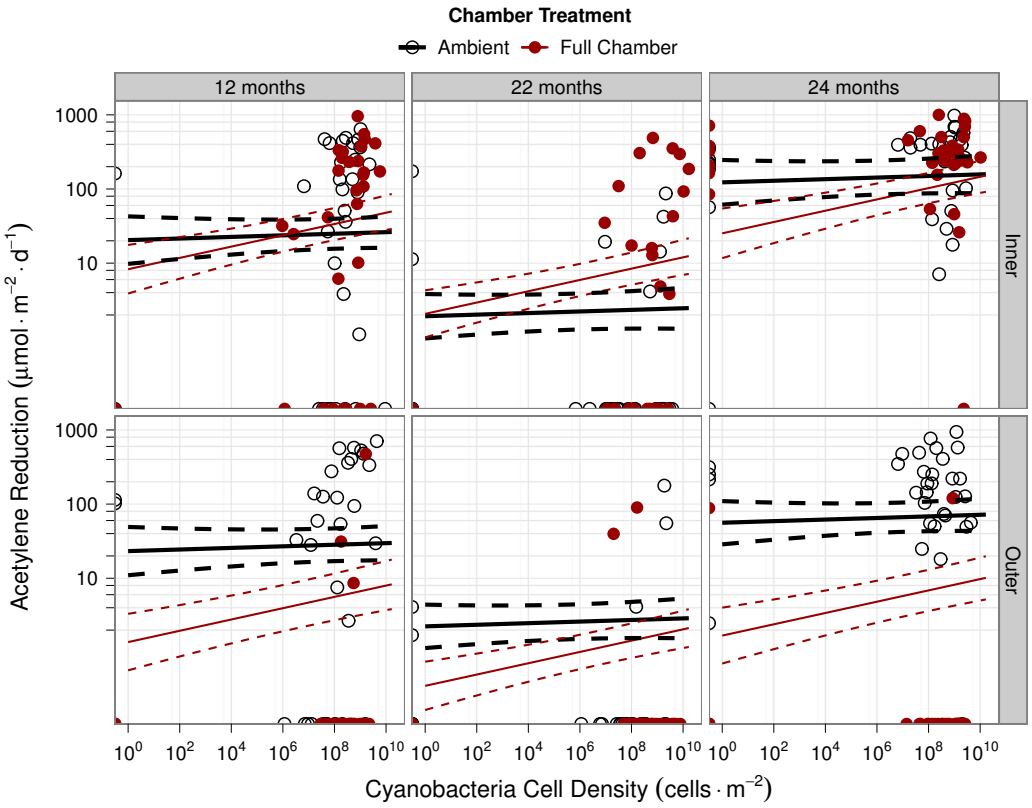

Figure 3.12

Acetylene reduction rates as a function of total cyanobacteria cell density, across time, chamber and position treatments. Solid lines show fitted values, with dashed lines showing point-wise 95% confidence intervals. Adjusted $R^2 = 0.74$ ($F_{62,321} = 18.31$, $p << 0.001$)

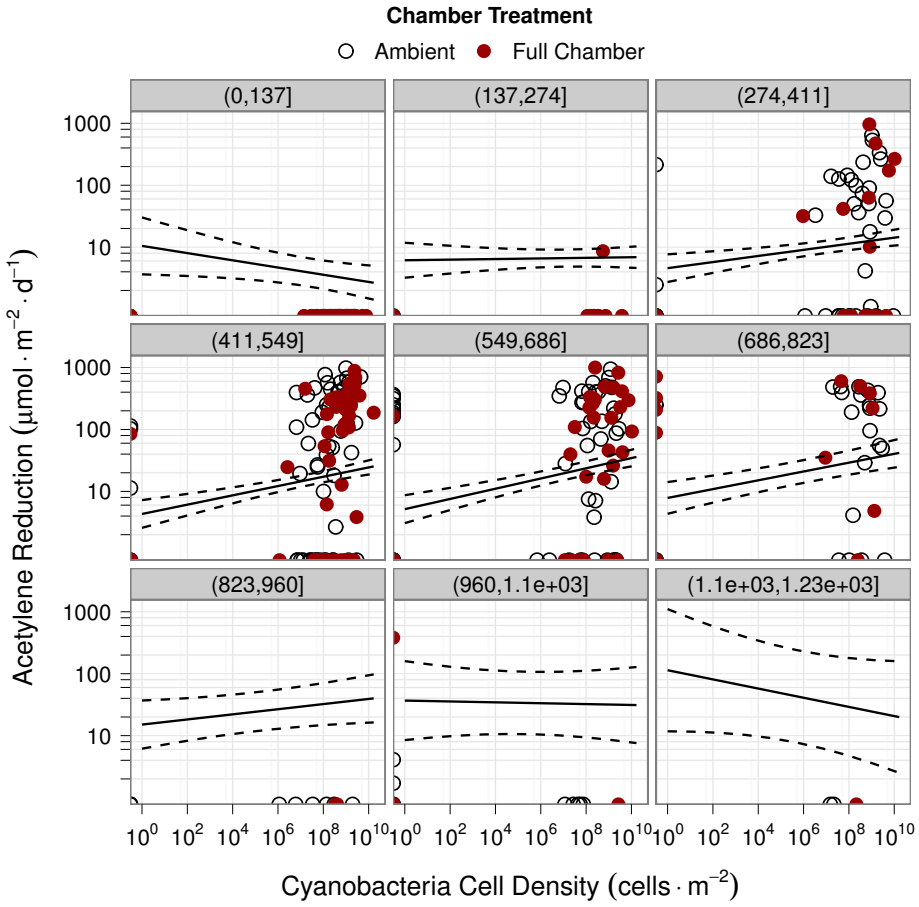

Figure 3.13

Acetylene reduction rates as a function of cyanobacteria cell density, across ranges of moisture contents (weight of water as a % of dry weight of moss substrate), divided into 9 roughly equal ranges. Numbers in grey boxes above each panel indicates the range of values for moisture contents represented in that panel. Low moisture values are in the upper-left panel, proceeding from left-to-right toward high moisture in the bottom-right panel. While higher rates of N-fixation are associated with higher densities of cyanobacteria at intermediate moisture values, this relationship becomes much weaker, and perhaps even negative, under extremely dry (< 275% moisture) or extremely wet (> 825% moisture) conditions.

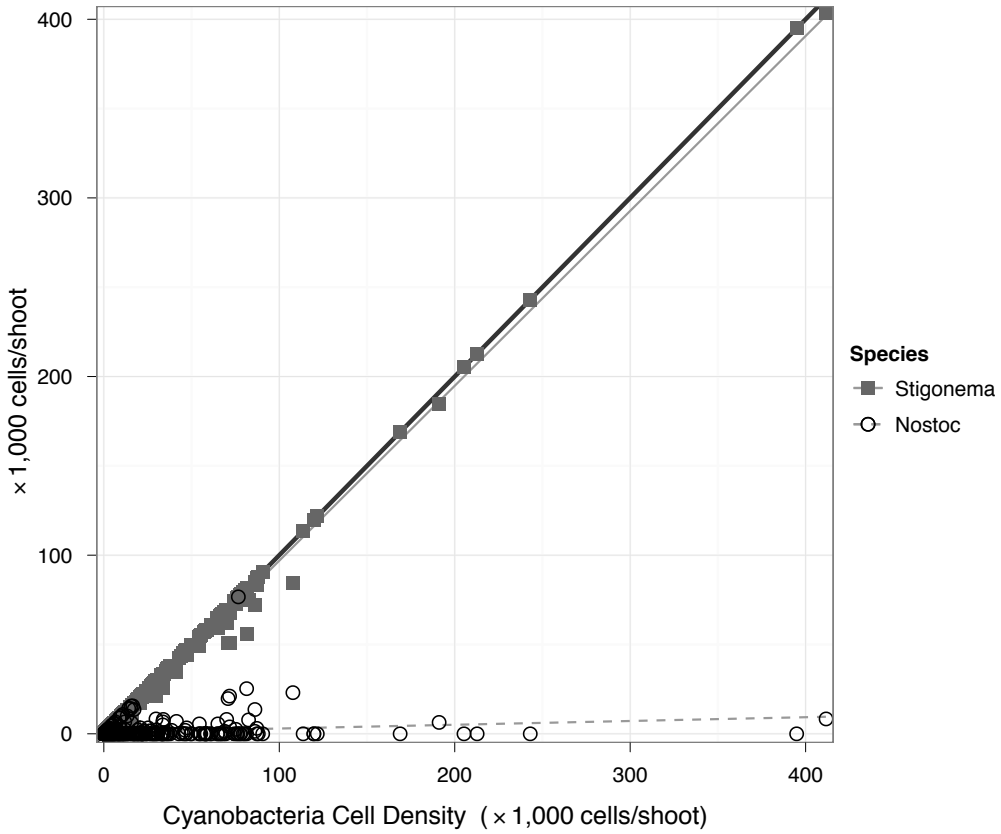

Figure 3.14
Species composition of cyanobacteria counts. Lines represent fitted GAMs (Additive Models). Most samples were dominated by Stigonema, with a minority of Nostoc cells.

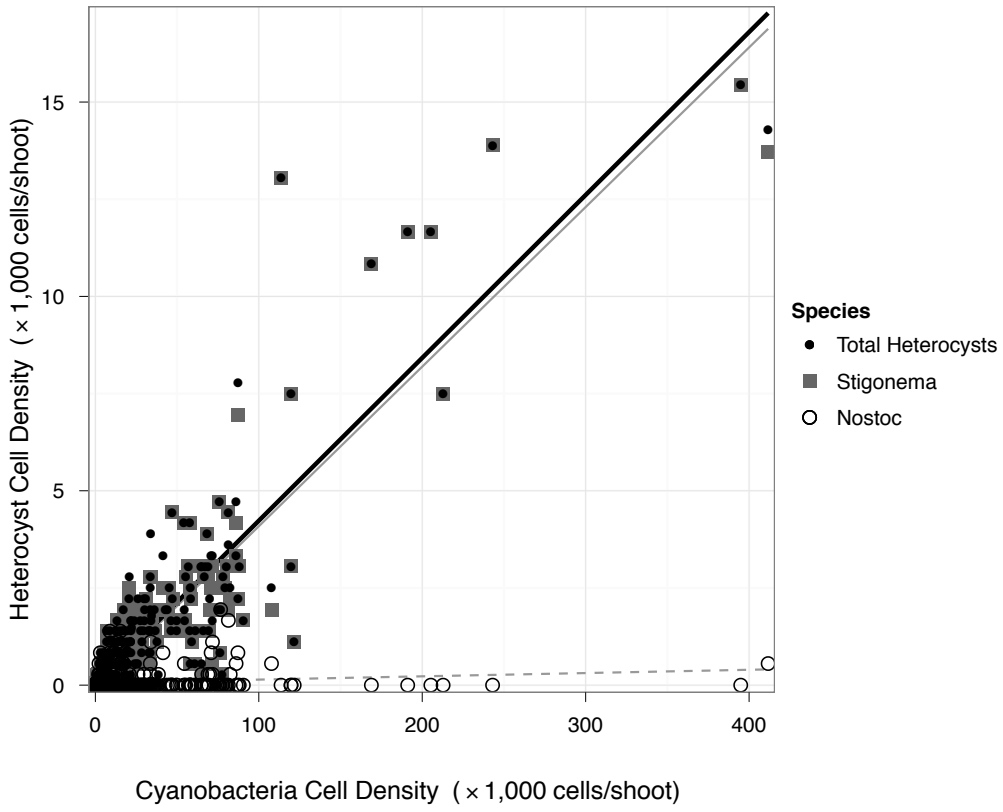

Figure 3.15

Number of heterocyst cells, as a function of total cells in each sample. Lines represent fitted GAMs (Additive Models), and suggest an average of 4-5% heterocysts. Samples were dominated by Stigonema spp., which also account for the majority of heterocysts in the samples.

Connecting statement

In chapter 3, I described experimental effects of combined habitat fragmentation and simulated climate change (warming and drought) on cyanobacteria and nitrogen-fixation across two years of a field experiment. The observed importance of drought also has implications for other ecosystem processes in the bryosphere, particularly productivity and decomposition. The relative rates of these processes under climate change conditions will ultimately determine whether boreal forests will continue to accumulate carbon in soil organic matter, or become a net emitter of carbon to the atmosphere. In the following chapter, I compare rates of moss growth, productivity, and decomposition within experimental patches, across all treatment combination. Although these measurements do not represent the full range of carbon cycling within a forest stand, the balance between productivity and decomposition within the moss layer will determine the supply of a significant portion of organic matter to boreal forest soils.

CHAPTER 4

Dry feather moss decomposition exceeds biomass production in a climate change experiment

Keywords: *Pleurozium schreberi*, net primary productivity, decomposition, boreal forest, bryosphere

Abstract

Boreal forest moss contributes to Net Ecosystem Production, and nutrient cycling, yet is often not considered in boreal forest carbon source/sink dynamics. The balance between Net Primary Production and decomposition will determine whether carbon will continue to accumulate in, or instead be released from boreal forest soils under climate change conditions. We used a two-year field experiment in northern Quebec, Canada to measure the interaction between moss connectivity, simulated climate warming, and drought, and resulting changes in biomass production and decomposition of *Pleurozium schreberi*, the dominant feather moss in black spruce stands. We found no overall effect of habitat connectivity, nor warming on either net primary production or decomposition. Drought was associated with severe reductions in moss productivity, which appeared to become more severe in the second year of the experiment (-13.3 $\pm$30.4 $g \cdot m^{-2} \cdot yr^{-1}$ 95% confidence interval NPP in dry patches). Drought also reduced decomposition from about 11% to 4.5% mass loss per year. The overall effect of drought was to cause *P. schreberi* to switch from net production of $100\,g \cdot m^{-2} \cdot yr^{-1}$ to a net loss of $40\,g \cdot m^{-2} \cdot yr^{-1}$ biomass. Drought may therefore lead to a significant reduction in litter inputs to soil, and less accumulation of carbon in boreal forest soils. These results suggest that moisture supply to boreal forest moss may have a more profound impact on carbon cycling within the moss layer than small increases in temperature predicted by global and regional climate models. Our results also serve as a reminder of the value of experimental approaches to understanding cumulative effects of multiple drivers of change at the ecosystem level. Bryophytes are likely to continue to be important for boreal forest ecosystem processes and nutrient cycling, and should therefore be incorporated into future research and modelling efforts to predict boreal forest responses to climate change conditions.

4.1 Introduction

Primary production typically exceeds rates of decomposition in boreal forest soils, leading to an accumulation of detritus and carbon compounds in the soil (Davidson and Janssens, 2006; Luyssaert *et al.*, 2008).The boreal forest now contains the largest pool of soil carbon in the world (Bonan and Van Cleve, 1992; Davidson and Janssens, 2006; Luyssaert *et al.*, 2008), within an area that covers 10% of the Earth's land surface (Taggart and Cross, 2009). Climate change may affect both decomposition and primary production throughout the boreal

forest, with the potential for feedback to the climate system, given the biome's extensive circumpolar distribution and considerable carbon stores. The balance between production and decomposition will determine whether the boreal forest will continue to act as a carbon sink, or become a carbon source, with potential positive feedback effects on global climate warming (Bonan and Van Cleve, 1992; Davidson and Janssens, 2006).

Climate change is expected to increase temperature, change precipitation patterns, and increase the frequency of extreme weather events, such as drought, throughout the boreal forest (IPCC, 2002; Girardin *et al.*, 2004; Soja *et al.*, 2007). There is concern that increasing temperature in the boreal forest may increase decomposition rates in the soil, leading to increased emissions of CO_2 (Davidson and Janssens, 2006). Higher concentrations of CO_2 in the atmosphere may not necessarily lead to a corresponding increase in primary production, which can be limited by other factors such as moisture or nitrogen availability (Janssens and Luyssaert, 2009). Predicting the net effect of climate change, including warming and drought, on boreal forest ecosystems remains a significant challenge.

Bryophytes are not always considered in carbon budgets and Net Ecosystem Production in boreal forests (Bond-Lamberty *et al.*, 2004), despite the fact that they can contribute as much as half of total Net Primary Production (NPP) in some stands, and they play an important role in nutrient cycling and soil processes (Turetsky, 2003; Lindo and Gonzalez, 2010). The moss layer forms an important transition zone at the interface between soils (the pedosphere) and the atmosphere, which can be called "the bryosphere" (*sensu* Lindo and Gonzalez, 2010). The bryosphere not only incorporates both above-ground and below-ground processes, it is also spatially-bounded within relatively small spatial scales, making it amenable to experimental manipulation and thus well-suited to be a natural model system.

Mosses are efficient scavengers of available nutrients, particularly nitrogen (Turetsky, 2003), which is often a limiting factor in primary production in boreal forests (DeLuca *et al.*, 2002; Menge *et al.*, 2008). Boreal forest bryophytes often form associations with nitrogen-fixing cyanobacteria, and therefore control nitrogen input to forest stands, particularly in later stages of succession when there are fewer available nutrients (Zackrisson *et al.*, 2004; DeLuca *et al.*, 2007). They can also control the temperature and moisture microclimate around them and of the top soil layer, affecting decomposition rates within the bryosphere (Turetsky, 2003; Jackson *et al.*, 2010).

Moss productivity can be affected by temperature, limiting nutrients (such as

water, nitrogen or phosphorous), light, and species composition (Turetsky, 2003). Moisture availability, variability, and timing are also known to affect feather moss production in the boreal forest (Frolking, 1997). We therefore predicted that drought would severely limit primary productivity of feather moss in field conditions.

Decomposition within the bryosphere is also affected by temperature, moisture, as well as pH, the composition of the litter, and soil biota, such as microbes, fungi, and detritovores (Bonan and Van Cleve, 1992; Turetsky, 2003; Lindo and Gonzalez, 2010; Jackson *et al.*, 2010). Moss litter is often slow to decompose, due to recalcitrant compounds that inhibit decomposition, and low nitrogen concentrations (Turetsky, 2003). Low temperatures can also reduce overall decomposition rates in the boreal forest (Davidson and Janssens, 2006), as well as dry conditions (O'Donnell *et al.*, 2009). The low thermal conductance of mosses can reduce temperatures within the moss layer, as well as evapotranspiration from moisture stored among tightly-packed shoots (Turetsky, 2003; Heijmans *et al.*, 2004). Excessively wet conditions in moss layers can also inhibit aerobic decomposition (Turetsky, 2003), although decomposition in upland feather mosses is likely much more moisture limited than in *Sphagnum* bogs (Bartsch and Moore, 1985; Moore and Basiliko, 2006). Mosses can therefore control microclimate conditions such as temperature and moisture than can directly affect decomposition rates. Understanding the net effects of both warming and drought on the bryosphere is an important part of predicting how carbon cycling will be affected by climate change in the boreal forest.

Habitat fragmentation is known to cause biodiversity loss in bryosphere fauna, with consequences for ecosystem processes (Lindo and Gonzalez, 2010; Staddon *et al.*, 2010). There is also great potential for the loss of habitat connectivity to interact with climate change conditions, leading to non-additive impacts on biodiversity and ecosystem function (Watkinson and Gill, 2002). While climate change will have direct effects on vegetation communities in the boreal forest, fragmentation can cause indirect, long-term impacts by impeding dispersal of biota, and preventing species from colonizing habitats to which they are pre-adapted. Because much of the decomposition and nutrient cycling in the bryosphere is carried out by biotic activity in the context of environmental conditions, we hypothesized that habitat connectivity may interact with climate change effects in the bryosphere, with isolated patches being more strongly negatively affected, with lower resilience to changing environmental conditions at the ecosystem level.

We set up a field experiment near the northern limit of the boreal forest in

eastern North America, to measure the possible interactive effects between habitat fragmentation and climate change on net primary production and decomposition of the boreal forest feather moss *Pleurozium schreberi* (Brid.) Mitt. *P. schreberi* is the most common species of feather moss in the boreal forest, forming a continuous carpet over the soil in areas associated with black spruce (*Picea mariana* (Mill.)). The focus of this study is the balance between biomass production and decomposition within the bryosphere itself, rather than carbon cycling across the entire boreal forest. We tested the following main hypotheses: (i) moss productivity exceeds decomposition under ambient conditions; (ii) warming increases decomposition rates faster than primary production; (iii) drought has a less severe impact on decomposition than productivity, leading to a net loss of biomass under most climate change conditions; (iv) habitat fragmentation interacts with climate change treatments, such that negative effects of drought are more severe in more isolated patches.

4.2 Methods

Study site

The experiment was conducted in a boreal forest stand approximately 100×200 m, located 1.6 km southeast of the McGill Subarctic Research Station near the edge of the town of Schefferville, Québec, Canada, 54°47′44″N 66°47′20″W. The study site is dominated by the feather moss *Pleurozium schreberi* .

The experiment was started, and baseline measurements collected, during the summer of 2007. Subsequent data and samples were collected after 12 months (August 2008), 22 months (June 2009), and 24 months (August 2009). Full details on the study site and experimental design are presented in chapter 2.

Climate change treatments

We simulated climate change conditions with open-top chambers based on the design for those used by ITEX in tundra systems (Marion *et al.*, 1997). The chambers used in this experiment were hexagonal, with Sun-Lite fibreglass walls at a 60° angle, measuring 115 cm between walls at the base, 69 cm across at the top, and 40 cm tall (full details in section 2.4).

Landscapes composed of one of each type of fragmentation treatment were randomly assigned to be covered by a full chamber, as described above, or an

ambient control, with no chamber over top. All chamber treatments and landscapes were oriented along a North-South axis, to ensure consistent exposure to incoming solar radiation.

These chambers warmed the moss layer an average of about 0.5 °C in the summers, and mild cooling in the winters ranging from -1.9 to +0.2 °C (see chapter 2 for full details). The walls of the open-top chambers also created a 'rain shadow' around the exterior of the chambers, such that the outer patches of each meta-community received very little precipitation, resulting in a prolonged drought, whereas inner patches received ambient levels of precipitation.

We measured the moisture contents of each patch by weighing each patch before and after drying (72 hours in a Tullgren funnel used to extract microarthropods for another analysis). The weight of the water removed by drying was divided by the dry weight of the moss substrate, resulting in the amount of moisture as a percentage of the substrate dry weight. In many cases, the patches contained more water than moss by weight, therefore many values are greater than 100%.

Experimental Design

The experiment included a fully factorial combination of chamber and fragmentation treatments, which allows testing of independent, as well as interactive effects of each treatment. Experimental meta-communities of four patches were constructed by cutting sections of moss out from the forest floor, and isolating them in plastic flower pots.

Each meta-community of four patches was arranged in one of four fragmentation configurations: a single large *contiguous* patch, an equivalent area divided into four patches each connected by two *corridors*, the same arrangement but with corridors only joined to a single patch (*pseudo-corridors* to control for the extra habitat associated with the corridor treatment), and four *isolated* patches (Figure 4.1). Large patches were 25 cm in diameter (491 cm^2), while each of the four patches in the other treatments were 12.5 cm in diameter (122.7 $cm^2 \times 4 = 491$ cm^2 total metacommunity area).

Patches were isolated from the surrounding matrix by placing moss into plastic pots in the same location as the source moss on the forest floor. The pots were 9 cm deep, and moss added was no deeper than 8 cm, leaving the surface of the bryosphere about 1 cm below the tops of the pots at the start of the experiment.

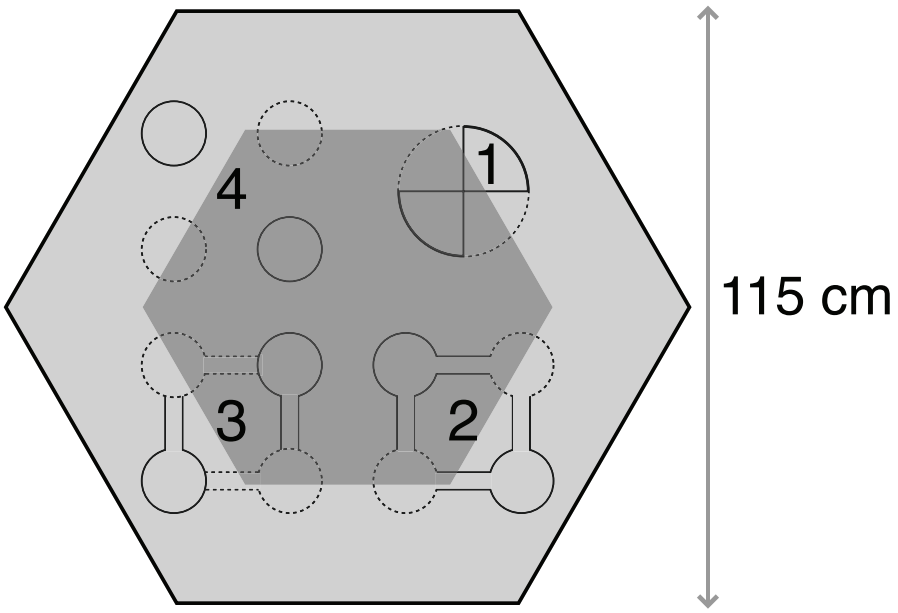

Figure 4.1
Diagram representing layout of fragmentation treatments inside chambers, to scale. Habitat fragmentation treatments, beginning with top-right: (1) Contiguous, (2) Corridors, (3) Pseudo-corridors, (4) Isolated. The inner shaded hexagon shows approximate area open to vertical precipitation; the actual 'rain shadow', shown in light grey, varies across chambers, depending on local slope, aspect, prevailing wind, and other small-scale differences in physical conditions.

Corridors were created by cutting and replacing a rectangle of moss 3×10 cm, lined with 6 mil polyethylene film along the sides, but open along the bottoms.

Habitat fragmentation treatments were nested within simulated climate change treatments, across eight replicate locations (blocks) throughout the study area (section 2.1). The full suite of experimental treatments thus includes (in decreasing size and nesting order): Blocks (the level of replication, not normally included as a fixed factor in statistical analyses), chambers, fragmentation, and patch position. Growth measurements were conducted on the same units at different periods during the experiment, so these also include time (or year) as the smallest experimental "treatment."

Moss Growth and Productivity

Pleurozium schreberi grows in individual stems, tightly packed, with growth occurring primarily near the apex, sometimes referred to as the "bryosphere canopy" (Lindo and Gonzalez, 2010). We measured linear extension of individual moss stems by marking them with a polyester thread carefully tied near the tip

of the shoot (see Figure 4.2), and measuring the distance from the marker to the tip at successive times throughout the experiment (after Clymo, 1970). The distance from marker to stem tip was measured using a digital caliper to the nearest 0.01 mm. Growth measurements were collected from the same marked shoots at the start of the experiment (baseline), and again after 12 months, 22 months, and 24 months at the end of the experiment. Moss growth in between each measurement is therefore the difference in the length measurement from the static marker to the tip, between each time point. Moss growth measurements are also therefore repeated measurements and not independent between time periods.

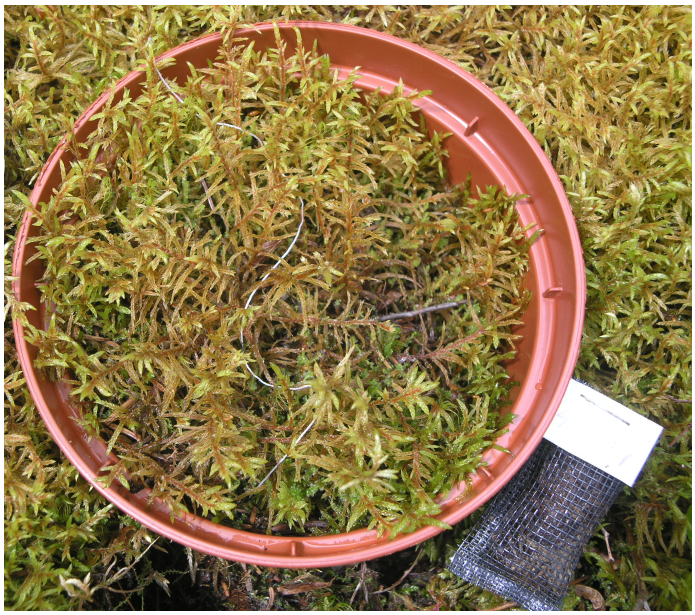

Figure 4.2
Experimental moss patch (fully isolated), showing white polyester thread marker for moss growth measurement, and moss litter bag for measuring decomposition (bottom right).

Moss growth was measured on one representative moss stem in each inner and outer patch. Additional replicate patches of each treatment combination were measured in the second year of the experiment, leading to double the sample size (in terms of patches, not moss stems per patch) during this period. Some data points were missing due to broken stems between measurements.

Although *Pleurozium schreberi* grows primarily at the apex of a single stem, lateral growth of branches at regular intervals also occurs (Benscoter and Vitt, 2007). However, lateral branch growth stops at a maximum length along the majority of the stem. Lateral growth therefore only occurs near the apex of the stem, in a region where lateral branches have not yet reached maximum length. Benscoter

and Vitt (2007) proposed a model of growth for *Pleurozium schreberi* that accounted for both linear stem extension, and lateral extension of the top-most branches, for the purpose of more accurate productivity estimates (Figure 4.3). They argue that productivity is underestimated by only using "bulk density" estimates of biomass to convert linear stem extensions to biomass production.

The model proposed by Benscoter and Vitt (2007) may be accurate, but it is rather complex and cumbersome, requiring calibration by measuring the length and mass of individual lateral branches of *P. schreberi* moss stems. We make the simplifying assumption that, although both the apical and lateral extension occur near the top of each moss stem, the net effect is an overall linear extension of approximately the same morphology. We therefore assume that biomass added in the growing region is equivalent to the amount that would be added by a linear addition of a given section farther down the shoot, where the lateral branches have all reached maximum length.

We measured the dry weight (in mg) of 1 cm sections of moss from patches throughout the experiment, to estimate bulk biomass for linear moss growth of a given unit length. Not surprisingly, the first cm of moss from the tip down was generally lower in mass than lower sections, so the average dry weight of the second and third cm were used to convert linear moss growth to biomass production. Three *P. schreberi* stems from each patch were divided into 1 cm sections, starting from the growing tip. We measured bulk biomass for three stems from inner and outer patches, contiguous and isolated fragmentation treatments, in ambient plots and chambers, from four blocks throughout the experiment. For patches where bulk estimates were not available, estimates from the three nearest neighbours within 10 m were averaged together.

To scale up to production estimates of $g \cdot m^{-2} \cdot yr^{-1}$, productivity values of a single shoot over a year of the experiment were multiplied by the fraction of the total patch dry weight represented by a single shoot (rather than an estimate of shoot density, which can be highly variable), converted to grams, and then multiplied by the number of patches (491 cm^2) in 1 m^2. Yearly estimates of biomass production per shoot were obtained by combining moss growth measurements from individual measurement periods as necessary, and converting total growth to biomass production, as above. The first measurement period was a single year (12 months), while the second and third measurements (22 and 24 months, respectively) were added together to estimate growth for the same shoots over the second year of the experiment.

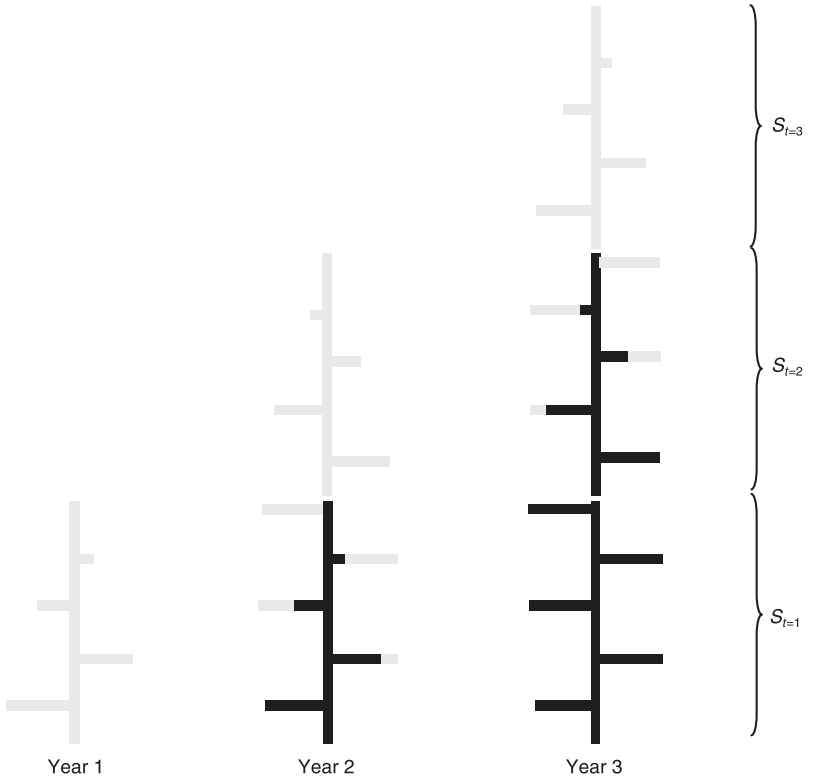

Figure 4.3
Conceptual diagram of a model of Pleurozium schreberi growth (from Benscoter and Vitt, 2007). Current year's growth is in grey, previous years' growth is in black. Rather than measuring individual branch lengths and extensions, we assume that a unit of extension at the tip is approximately equivalent to a linear-only extension of moss biomass with lateral branches at maximum length. Therefore, we used bulk estimates of moss biomass for 1 cm sections of moss stems, from the second and third cm sections below the growing tip.

Moss Decomposition

We measured decomposition of *Pleurozium schreberi* litter using litter bags (Figure 4.2). The litter bags were constructed from fibreglass screen door mesh (mesh size approximately 1.5 mm, cut into 5 × 5 cm squares, folded in half, and filled with approximately 0.4 g of moss litter (0.3–0.5 g). We recorded the weights of assembled litter bags prior to filling them with moss: this weight was subtracted from all subsequent weights, to consider only the weight of the contained litter. Once filled with moss litter, the edges of the litter bags were sealed using a heat sealer to melt the fibreglass mesh of the enclosing layers together. Litter bags were dried at 100 °C for 24 hours (also to kill the moss litter and prevent it from growing once deployed) and cooled to room temperature before recording initial dry weights.

We moistened dry litter bags by immersing in distilled water for 1 s prior to

transport to the field, to prevent undue loss of litter mass during handling (see Moore and Basiliko, 2006). At the field site, litter bags were inserted into experimental patches by cutting a 5 cm long opening into the moss with a knife, and placing the litter bags just above the bottom of the patches (approximately 6–7 cm below the moss surface).

We deployed the litter bags mid-way through the experiment, at 12 months (August 2008), and recovered them after 24 months (August 2009), allowing for decomposition over the final year of the experiment. Once recovered, litter bags were moistened by immersing in distilled water for 1 s to reduce mass lost during handling and transport back to the lab at McGill University in Montreal, Canada. Upon arrival, litter bags were immediately dried at 60 °C for 24 hours prior to processing. Litter bags were picked over to remove any remaining extraneous soil particles, rinsed, and dried again at 60 °C for 24 hours. We allowed the litter bags to cool to room temperature before recording final dry weights. Per cent mass loss over 1 yr was calculated as $100 \times (Dry\ weight_{initial} - Dry\ weight_{final}) / Dry\ weight_{initial}$. A set of 40 control litter bags were handled identically to those used in the experiment, but without being deployed in the moss for a year. They were brought back to the lab immediately and used to account for mass lost during handling (Moore and Basiliko, 2006).

Statistical analysis

We performed all statistical analyses using `R` software (R Development Core Team, 2010). All scripts and data are available online at: `http://github.com/jawhiteley/SECC.R.JAW`

We tested for the effect of experimental treatments on response variables using multiple regression modelling (Zuur *et al.*, 2007, 2009). Due to missing data and the nested nature of treatments, we fit a hierarchical regression model using the `nlme` package (Pinheiro *et al.*, 2011; Zuur *et al.*, 2007, 2009). The model fit is analogous to a nested analysis of variance (ANOVA), except that a maximum likelihood function is used to estimate coefficients, rather than the least squares of classic ANOVA.

We fit models for the response variables moss growth (linear), moss biomass production (per shoot), decomposition (% mass loss yr^{-1}), and net moss production (production - decomposition, in $g \cdot m^{-2} \cdot yr$). Explanatory variables for models fit at a single time point included: chamber, fragmentation, and patch position. These categorical factors were nested within each other, with experimental block

as the largest unit (blocks were the level of replication, so were not included as an explanatory variable). A separate model was fit for each measurement period of moss growth. A repeated measures analysis was not applied across all measurement periods, given that measurements occurred at unequal intervals.

Production estimates were aggregated to the two years of the experiment, to create intervals of equal length. Replicate patches added in the second year of the experiment were not included in this analysis, because they have no data available from the first year. The model fit to moss production included a year treatment (2 levels), as the smallest nested factor, to represent the repeated measurements on the same unit in both years. This is analogous to a "split-plot in time", and accounts for the lack of independence between observations from the same experimental units at different times (Sokal and Rohlf, 1981). Missing data prevented model convergence for the full range of interaction terms: three-way interaction between fragmentation, position, and year was omitted from the model for productivity over both years, as well as the dependent higher-order interaction among all four factors (chamber, fragmentation, position, and year).

We used sequential F-tests to calculate approximate *P*-values for the significance of model terms, which were confirmed with backward model-selection using likelihood ratio tests on AIC values (Zuur *et al.*, 2009). We used Tukey's method of Honestly Significant Differences (Tukey's HSD), implemented in the `multcomp` package (Hothorn *et al.*, 2008), to test for differences between treatment levels, where main factors or interaction terms were found to be significant in the fitted model (Sokal and Rohlf, 1981). We used the `effects` package (Fox, 2003) to extract fitted values for model terms significant in full models, and the `ggplot2` package (Wickham, 2009) to plot data and results. The `effects` package plots 95% confidence intervals around fitted values, which are useful for visually indicating differences that are likely to be statistically significant, but the results of Tukey's HSD are more appropriate for testing specific hypotheses about differences between treatment levels.

Decomposition estimates were only available for the second, final, year of the experiment, but there were no missing values, therefore we applied a simple nested analysis of variance (ANOVA), without time as an explanatory variable (chapter 3). Because decomposition data was per cent mass loss, the values were transformed using $arcsin(\sqrt{x})$. Fitted values and confidence limits were back-transformed for plotting on a linear scale. Minimum significant ranges, analogous to Tukey's HSD, were calculated for terms found to be significant in the nested ANOVA. The same

nested analysis of variances was used on moisture contents of moss patches (see chapter 3).

4.3 Results

Tests of significance of fitted model terms are presented as ANOVA-style tables in the supplementary materials, including degrees of freedom.Fragmentation had no statistically significant effect on any response variable presented here, including interaction terms. The most common significant interaction term was between chamber and position treatments, with dry outer chamber patches being significantly different from other patches.

Outer chamber patches were significantly drier than other patches, throughout the experiment (Figure 4.4). Most patches were slightly wetter during June (22 months), immediately following snow melt. There was a significant interaction between Time, Chamber, Fragmentation, and Patch Position for moisture contents: Contiguous patches in outer chambers held as much moisture as inner patches, whereas all other outer chamber patches were significantly drier (see supplementary Figure 4.9).

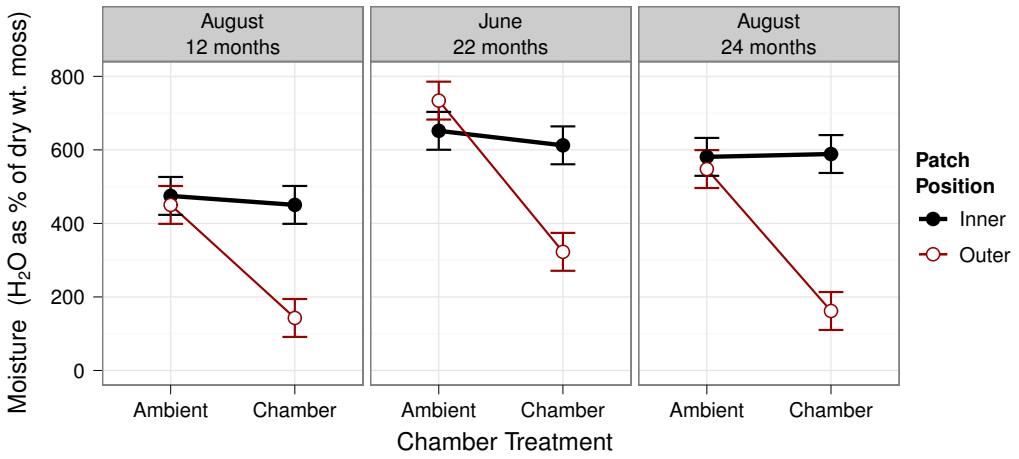

Figure 4.4

Water content of moss patches at time of collection, as a percentage of moss dry weight, at 12, 22, and 24 months into the experiment. Values are means, by chamber treatment and patch position (n = 32 for each point). Error bars represent 95% comparison intervals (Tukey's HSD Minimum Significant Ranges).

Moss growth and production

Moss growth was significantly lower in the dry, outer chamber patches, during all measurement periods (Figure 4.5). Moss growth was similar between the winter (12–22 months) and snow-free summer (22–24 months) of the second year of the experiment, with roughly 4 mm of growth during both times of the year in all but the dry outer chamber patches. Figure 4.5 suggests more growth in ambient and warm inner chamber patches during the second year overall, while warm and dry outer chamber patches declined to 0 on average.

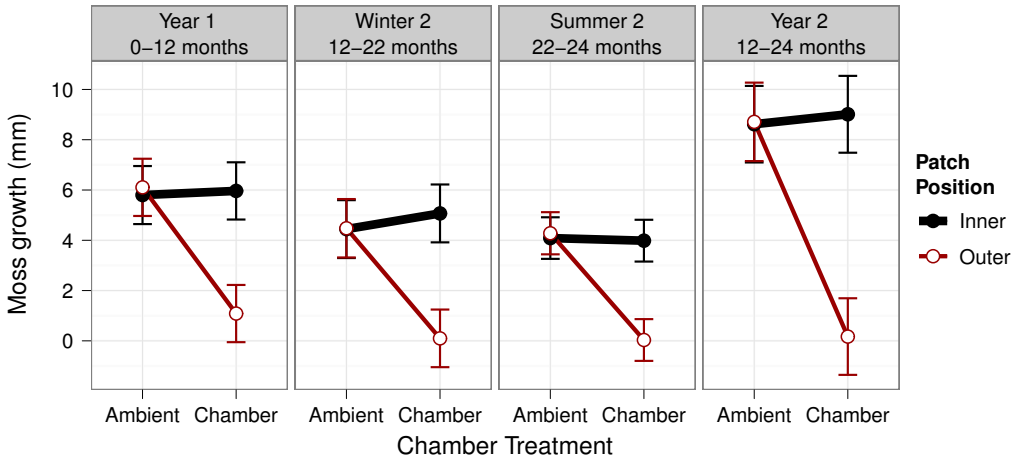

Figure 4.5
Fitted values and 95% confidence intervals for estimates of moss growth, by chamber treatment and patch position, for each measurement period (month "0" is August 2007). The final panel on the right shows growth over the entire second year of the experiment, aggregating values from the second and third panels from the left. The analysis was conducted separately for each period, therefore fitted values and confidence intervals are only comparable within each measurement period. *Tukey's HSD tests* within each panel *agree with the confidence interval overlaps shown in the figure (i.e., if the error bars in the figure* do not *overlap, the Tukey's HSD tests would have a P-value < 0.05). Growth rates appear to be similar between the winter (12–22 months) and snow-free summer (22–24 months) of the second year of the experiment, suggesting that roughly 4 mm of growth occurred during both times of the year, in all but the dry outer chamber patches.*

The analysis of moss biomass production between years confirms that moss productivity was significantly higher in the second year for all treatments, with the exception of dry outer chamber patches (Figure 4.6). Moss productivity in dry outer patches was not significantly different between years, but remained significantly lower than all other patches in both years. Moss stems in ambient patches added an average of 2 mg of biomass during the first year, but just over 3 mg in the second. Stems in dry outer chamber patches added an average amount of biomass

no different than 0 in both years. Some appear to have lost biomass (production < 0), particularly in the second year of the experiment. The difference in biomass production between dry outer patches and other patches in the experiment therefore increased in the second year of experimental treatments.

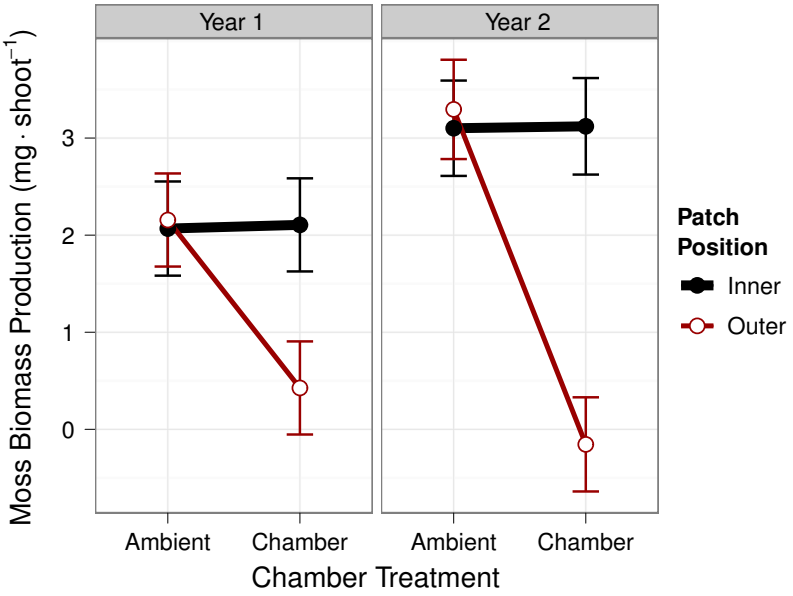

Figure 4.6

Fitted values and 95% confidence intervals for estimates of moss biomass production, by chamber treatment and patch position, for the first and second years of the experiment. Overall productivity is significantly higher in the second year of the experiment, except in the dry outer chamber patches, which are not significantly different between years (Tukey's HSD P=0.252). Tukey's HSD tests agree with the confidence interval overlaps shown in the figure (i.e., if the error bars in the figure do not *overlap, the Tukey's HSD tests would have a P-value < 0.05).*

Moss decomposition

Decomposition rates were significantly lower in dry outer chamber patches, which lost an average of approximately 5% of dry weight, a decline of 5–9 percentage points relative to other patches (Figure 4.7).

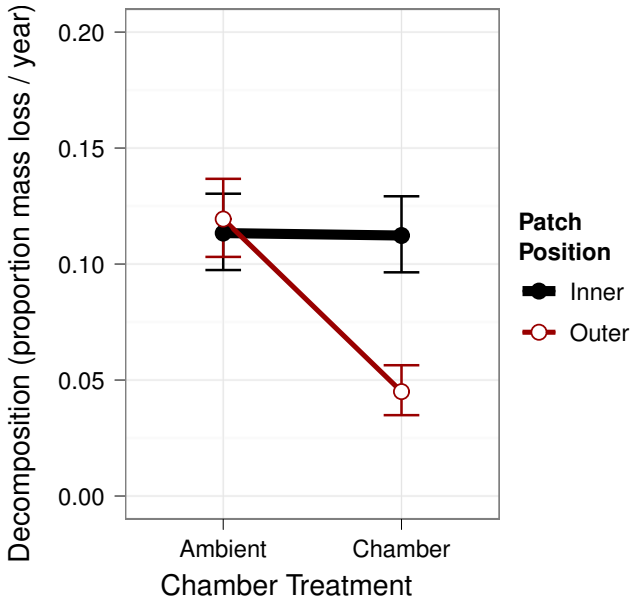

Figure 4.7
Fitted values and 95% comparison intervals (Tukey's HSD Minimum Significant Ranges) for estimates of Pleurozium schreberi litter decomposition, by chamber treatment and patch position, during the second year of the experiment. Overall decomposition rates are equal, apart from dry outer chamber patches, which is significantly lower than other treatments. Tukey's HSD tests agree with the confidence interval overlaps shown in the figure (i.e., if the error bars in the figure do not *overlap, the Tukey's HSD tests has a P-value < 0.05).*

Net moss biomass production

Net biomass production (moss productivity - decomposition) was around 100 $\pm$approx. 35 $g \cdot m^{-2} \cdot yr^{-1}$ in most patches, apart from the dry outer chamber patches, where net production was negative (-40.03 $\pm$33.54 $g \cdot m^{-2} \cdot yr^{-1}$). Nevertheless, the linear hypothesis test of the mean net production of outer chamber patches compared with a null value of 0 was inconclusive (Tukey's HSD P= 0.076).

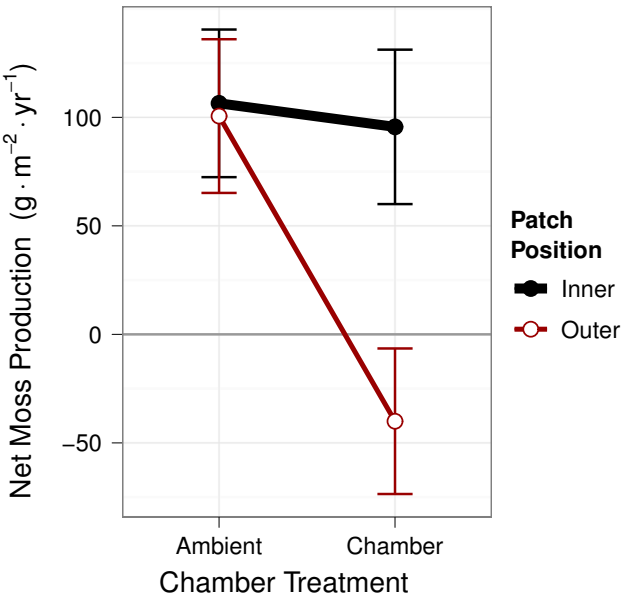

Figure 4.8
Fitted values and 95% confidence intervals for estimates of net biomass production (moss productivity - decomposition), by chamber treatment and patch position, across two years of the experiment. Mean net productivity was significantly lower in dry outer chamber patches, which is negative on average, but not significantly different from 0 according to a Tukey's HSD linear hypothesis test compared to a null value of 0 (P= 0.076). Tukey's HSD tests otherwise agree with the confidence interval overlaps shown in the figure (i.e., if the error bars in the figure do not *overlap, the Tukey's HSD tests has a P-value < 0.05).*

4.4 Discussion

Habitat connectivity

Our fragmentation treatments had no statistically significant effects on moss productivity or decomposition, refuting our last hypothesis. Nevertheless, fragmentation effects and higher-order interactions were approaching statistical significance for decomposition rates, and net biomass production (see supplementary tables), suggesting that a lack of power may have prevented the detection of subtle fragmentation effects in the experiment. The lack of a detectable effect should not be taken to mean that small-scale connectivity is unimportant in the bryosphere. The more open treatments in this experiment (corridors, and contiguous patches) were able to alleviate negative impacts of drought on microarthropods in this experiment (chapter 6). Furthermore, contiguous patches of moss in outer chambers contained similar total amounts of moisture as inner patches, which may be due to moisture moving through the moss by capillary action from adjacent patches.

Nevertheless, connectivity may not have had a detectable effect on productivity or decomposition for a number of possible reasons. One explanation may be that the wicking effect of contiguous patches only maintains moisture levels below the surface, leaving the surface layer, where productivity occurs, equally dry and inactive as other dry outer chamber patches. In addition, the biota responsible for most decomposition and nutrient cycling were not studied in this experiment (microbes and fungi in particular), and the dispersal of these species may not be affected by the fragmentation treatments applied in this experiment. The effects of fragmentation may also be delayed over time, and the indirect impacts on ecosystem processes such as decomposition and productivity may require more than two years to manifest in the experimental system. Longer-term studies may help to further disentangle the direct and indirect effects on ecosystem function in the bryosphere, as well as the relative importance of environmental and biotic controls.

Warming

Warming also had no detectable effect on growth, productivity, or decomposition of *Pleurozium schreberi* in this experiment. We expected higher rates under warmer conditions in all cases, particularly given observed and modelled increases in decomposition with temperature in the colder northern limits of the boreal forest (Bonan and Van Cleve, 1992). Nevertheless, the realized sensitivity of decomposition to temperature is complex and subject to many factors, but mostly the chemical composition of decomposing tissues, and other environmental conditions such as moisture (Davidson and Janssens, 2006).

Bryophyte litter is recalcitrant and decomposes more slowly than litter from many vascular plants (Turetsky, 2003; Davidson and Janssens, 2006; Lindo and Gonzalez, 2010). It is therefore possible that the decomposition of *P. schreberi* litter may not be as sensitive to temperature as other species, which would have important implications for soil carbon cycling under climate change conditions (Davidson and Janssens, 2006).

Mosses can prevent heat transfer from the air to lower soil layers (Turetsky, 2003), which may explain why decomposition was not affected by a measured increase in temperature 2 cm below the moss surface in this experiment (section 2.4). If most decomposition occurs at greater depths below the moss surface, the warming effect may not have translated at a sufficient magnitude to lead to changes in decomposition.

Drought

Drought within the outer chamber patches had significant and marked effects on both Net Primary Productivity and decomposition of *P. schreberi*. Biomass production was severely reduced in drought patches, with the difference increasing in the second year of drought, suggesting a non-linear response to drought: the magnitude of the effect may increase as drought duration increases. Moss growth and production was effectively zero on average for the second year of the experiment. Decomposition of *P. schreberi* was also lower in the dry outer chamber patches, but still above zero during the second year of the experiment. As predicted, the negative impacts of drought were more severe on productivity than decomposition, tipping the balance from net biomass accumulation to net biomass loss of *P. schreberi*.

Bryophytes are adapted to be somewhat desiccation-tolerant, being poikilohydric, though not as much as lichens or other taxa (Turetsky, 2003). Bryophytes can withstand periods of desiccation and quickly recover when re-hydrated, although this is often associated with a pulse of nutrients in runoff, as soluble carbon and nitrogen compounds are lost from moss tissues during the initial flush of moisture (Turetsky, 2003; Lindo and Gonzalez, 2010). Some patches in this experiment did experience periodic flooding during snowmelt in the spring, but this depended on the topography surrounding each chamber. Those on higher ground were much less likely to flood than those in depressions. Nevertheless, this periodic re-wetting during a long-term drought may have compounded drought stress by also leaching nutrients from the moss tissues, which may account for severe reductions in moss productivity, even negative growth in some patches (not including mass lost to decomposition).

The bryosphere typically encompasses a moisture gradient from surface layers that experience frequent drying, to deeper layers that remain wetter for longer periods (Lindo and Gonzalez, 2010). If decomposition occurs primarily in the below-ground area of the bryosphere, the wetter conditions may inhibit some aerobic decomposition (Turetsky, 2003), although the moisture levels in feather mosses are often low enough to be limiting for the decomposer community (Jackson *et al.*, 2010). Long-term drought, on the other hand, likely has strong negative effects on biota responsible for decomposition within the bryosphere (Jackson *et al.*, 2010; Lindo and Gonzalez, 2010). Therefore, the overall effect of long-term drought in this experiment remains negative, although there appear to be drought-tolerant taxa capable of carrying on with decomposition under prolonged drought

conditions, even when moss production itself has completely stopped.

Jackson *et al.* (2010) observed decomposition rates around 10 – 20 % in moss patches receiving infrequent or low watering in a greenhouse, but up to 60 % mass loss with heavy, frequent watering. Our observations are consistent with low or infrequent watering in ambient patches, and even lower in the driest patches. This suggests that an increase in the amount or frequency of precipitation events in this area could increase decomposition rates much more than moderate increases in winter temperatures. Such conditions are what are forecast by regional climate models for Northern Québec: increases in precipitation rather than warmer winter temperatures (Logan *et al.*, 2011).

Habitat connectivity was able to alleviate apparent drought severity, as measured by total moisture contents of the moss patches. This was likely due to moisture movement by capillary action to the dry outer patches (see supplementary materials). It is perhaps not surprising that this moisture wicking was insufficient to maintain productivity even in contiguous patches, because all growth in *P. schreberi* occurs in the top few centimeters, which remained dry throughout the experiment. Nevertheless, we might have expected such moisture wicking to keep decomposition rates in outer contiguous chamber patches similar to inner chamber or ambient patches, but this did not appear to be the case. This may partly explain why decomposition remained positive in outer chamber patches, however, given that varying levels of habitat connectivity may have alleviated drought severity in the deeper layers of the moss.

The vertical moisture gradient common to the bryosphere further suggests that drought effects may increase with drought duration, as water evaporates over time, and dry conditions move deeper over the course of a protracted drought period. Moss growth, in the upper layer, is immediately affected by drought, but decomposition and other nutrient cycling processes may take longer to respond, delaying feedbacks and indirect effects on moss growth and nutrient cycling throughout the boreal forest.

Implications for carbon cycling

We did not convert our estimates of Net Primary Production or decomposition to g of carbon, as we lacked a direct measurement of the carbon content of the moss in our experiment. This makes it difficult to compare our estimates with others in the literature, although is unlikely to have an effect on the relative rates observed within the experiment (unless new moss growth has a different proportion of Carbon

than senescent, decomposing moss tissues). Bond-Lamberty *et al.* (2004) assumed 45% carbon by mass for boreal forest feather mosses. If this assumption holds for our experimental site, then Net Primary Production would be in the range of 69 $\pm$14 $gC \cdot m^{-2} \cdot yr^{-1}$ in ambient patches, and -6.0 $\pm$13.7 $gC \cdot m^{-2} \cdot yr^{-1}$ in outer chamber patches. Decomposition would be approximately in the range of 23 $\pm$6.6 $gC \cdot m^{-2} \cdot yr^{-1}$ in ambient patches, and 12.2 $\pm$6.6 $gC \cdot m^{-2} \cdot yr^{-1}$ in outer chamber patches.

Our estimates of Net Primary Production by *P. schreberi* are comparable to other measurements in boreal spruce-moss forests. Bond-Lamberty *et al.* (2004) measured rates of bryophyte production well-drained stands in the range of 8–143 $gC \cdot m^{-2} \cdot yr^{-1}$, with the highest levels at intermediate stand age. Our estimates of moss biomass production are comparable to those estimated in models by Bonan and Van Cleve (1992), although our estimates of decomposition were much lower, possibly due to the specific nature of *P. schreberi* litter used in this experiment. Bisbee *et al.* (2001) found higher rates of NPP in *Sphagnum* mosses, but the dominance of ground cover by feather mosses led to a greater contribution to total NPP at a site in central Saskatchewan: 24 $gC \cdot m^{-2} \cdot yr^{-1}$ by feather mosses, which is lower than the ambient patches at our site.

Because this experiment did not measure CO_2 fluxes, nor control for nitrogen inputs, these results are not comparable with the complex interactions of these two nutrients at the ecosystem level (see, for example Bonan and Van Cleve, 1992; Magnani *et al.*, 2007). The goal of this experiment was to assess the relative importance of environmental, and ultimately biotic controls over moss production and decomposition within the bryosphere. This captures a potentially important input of litter, and therefore organic carbon, to the soil humus layers, where the rates of decomposition will determine the ecosystem-level carbon cycling for a given boreal forest stand.

Therefore, these results should be interpreted in the context of long-term indirect effects of community-level impacts of climate warming, drought, and habitat fragmentation, rather than the complete set of perturbations expected to affect the boreal forest in the coming century: CO_2-enrichment, nitrogen deposition, habitat fragmentation, and climate change, including changes in temperature, precipitation, as well as the spatial and temporal variability of these processes (Wilmking *et al.*, 2005; Soja *et al.*, 2007; Jackson *et al.*, 2010; Lindner *et al.*, 2010). Predicting ecosystem-level change under the combined effects of all these pressures remains a daunting challenge. This experiment represents one piece of

the solution: to understand how processes within the bryosphere are affected by a combination of small-scale fragmentation and climate change, and how the biotic components of the bryosphere are involved in ecosystem-level changes. Improving our understanding of processes within the boreal forest moss layer will improve our ability to predict how larger processes across the boreal forest will respond, including the cycling of important nutrients, such as nitrogen and carbon. The extensive circumpolar distribution of this ecosystem, and the general similarities across much of this range, means that such changes can have impacts at the global scale, including atmospheric carbon budgets, and climate (Luyssaert *et al.*, 2008; Taggart and Cross, 2009).

Regional climate models predict an overall increase in winter precipitation in northern Quebec, along with increases in winter temperatures (Logan *et al.*, 2011). The Canadian Drought Index is already lower in Quebec than the rest of Canada, and regional models forecast slight increases by 2090, mostly in the south of the province. Nevertheless, climate change is expected to impact temperature and precipitation unevenly across the globe, and other regions in the boreal forest have had increases in drought events, and duration (Grant *et al.*, 2006; Soja *et al.*, 2007). The boreal forest in Northern Europe, however, may also experience more abundant and frequent precipitation (see Jackson *et al.*, 2010), although warming can still lead to temperature-induced droughts by increasing rates of evapotranspiration in between precipitation events (Heijmans *et al.*, 2004).

Our results, and those of other studies, suggest that both temperature and moisture levels in the moss layer are important determinants of Net Primary Production and decomposition within the bryosphere, with important consequences for nutrient cycling and carbon budgets of boreal forest stands around the world. Temperature interacts with precipitation amount and frequency to ultimately affect the vertical moisture profile within the bryosphere, and the balance between biomass production and decomposition.

Our results suggest that drought can shift the balance between carbon uptake and loss within boreal forest moss, leading to reduction in the supply of litter to underlying soil layers, further reducing the carbon-sequestration potential of boreal ecosystems. These results can not by themselves be used to predict overall effects of climate change on carbon budgets in the boreal forest, but they do provide an important piece of the puzzle. They also demonstrate the important contribution made by bryophytes to boreal forest dynamics: bryophytes contribute significantly to ecosystem productivity, nutrient cycling, and other indirect effects on vascular

plants (Turetsky, 2003; Lindo and Gonzalez, 2010). Mosses can even intercept and fix carbon emitted from soil underneath, creating potentially complex interactions between processes at different depths below-ground (Lindo and Gonzalez, 2010). Studies and models of carbon cycling in the boreal forest must therefore incorporate bryophytes in order to make accurate predictions of whole-ecosystem responses.

Wet moss grows more, but also decomposes faster, particularly in warmer conditions. The vertical distribution of moisture within the bryosphere could be an important factor determining the balance between productivity and decomposition, which occur at different depths. Furthermore the full range of cumulative effects of climate warming and precipitation have rarely been studied in controlled experiments well-suited to disentangling the full range of effects, including their interactions.

What remains uncertain is how the small-scale processes measured in this experiment scale up to the bryosphere across the circumpolar distribution of the boreal forest, including a range of latitudes, environmental conditions, and moss species. The importance of scale-dependent processes and variability in both space and time should not be underestimated (Pearson and Dawson, 2003; Wiedermann *et al.*, 2009)

Conclusion

Our controlled experiment found that drought drastically reduced production of *Pleurozium schreberi*, but had a less severe effect on decomposition, with the net effect being a switch in the relative magnitude of production and decomposition, and a net loss of moss biomass under drought conditions. Neither warming, nor fragmentation had detectable effects on either productivity or decomposition in the boreal forest moss in this experiment, either due to smaller effect sizes or indirect effects that required even more time to manifest at the ecosystem-level. Time lags and potentially interactive effects of multiple drivers of global change pose a significant challenge to predicting ecosystem change under future conditions. Controlled ecosystem-level field experiments such as this offer a unique opportunity to measure the full range of impacts and interactions, and separate them in order to elucidate the mechanisms underlying cumulative impacts. Future research on carbon cycling in the boreal forest must incorporate processes operating in the bryosphere, and account for spatial and temporal variability at appropriate scales.

Acknowledgements

We are extremely grateful to H. McIntosh for collecting data on bulk biomass for 1 cm sections of *Pleurozium schreberi* stems, without which the full analysis would not have been possible. We also thank Z. Lindo for fruitful discussions and comments during manuscript preparation.

References

Bartsch I, Moore TR (1985) A preliminary investigation of primary production and decomposition in 4 peatlands near Schefferville, Quebec. Canadian Journal of Botany 63(7):1241–1248

Benscoter BW, Vitt DH (2007) Evaluating feathermoss growth: a challenge to traditional methods and implications for the boreal carbon budget. Journal of Ecology 95(1):151–158, doi:10.1111/j.1365-2745.2006.01180.x

Bisbee KE, Gower ST, Norman JM, Nordheim EV (2001) Environmental controls on ground cover species composition and productivity in a boreal black spruce forest. Oecologia 129(2):261–270

Bonan GB, Van Cleve K (1992) Soil temperature nitrogen mineralization and carbon source-sink relationships in boreal forests. Canadian Journal of Forest Research 22(5):629–639, doi:10.1139/x92-084

Bond-Lamberty B, Wang C, Gower ST (2004) Net primary production and net ecosystem production of a boreal black spruce wildfire chronosequence. Global Change Biology 10(4):473–487, doi:10.1111/j.1529-8817.2003.0742.x

Clymo RS (1970) The growth of sphagnum: methods of measurement. The Journal of Ecology 58(1):13–49

Davidson EA, Janssens IA (2006) Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440(7081):165–173, doi:10.1038/Nature04514

DeLuca T, Zackrisson O, Gentili F, Sellstedt A, Nilsson MC (2007) Ecosystem controls on nitrogen fixation in boreal feather moss communities. Oecologia 152(1):121–130, doi:10.1007/s00442-006-0626-6

DeLuca TH, Zackrisson O, Nilsson MC, Sellstedt A (2002) Quantifying nitrogen-fixation in feather moss carpets of boreal forests. Nature 419(6910):917–920, doi:10.1038/nature01051

Fox J (2003) Effect displays in R for generalised linear models. Journal of Statistical Software 8(15):1–27

Frolking S (1997) Sensitivity of spruce/moss boreal forest net ecosystem productivity to seasonal anomalies in weather. Journal of Geophysical Research-Atmospheres 102(D24):29,053–29,064, doi:10.1029/96JD03707

Girardin MP, Tardif J, Flannigan MD, Wotton BM, Bergeron Y (2004) Trends and periodicities in the Canadian Drought Code and their relationships with atmospheric circulation for the southern Canadian boreal forest. Canadian Journal of Forest Research 34(1):103–119, doi:10.1139/x03-195

Grant RF, Black TA, Gaumont-Guay D, Kljun N, Barr AG, Morgenstern K, Nesic Z (2006) Net ecosystem productivity of boreal aspen forests under drought and climate change: Mathematical modelling with Ecosys. Agricultural and Forest Meteorology 140(1-4):152–170, doi:10.1016/j.agrformet.2006.01.012

Heijmans M, Arp WJ, Chapin FS (2004) Controls on moss evaporation in a boreal black spruce forest. Global Biogeochemical Cycles 18(2), doi:Gb200410.1029/2003gb002128

Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. Biometrical Journal 50(3):346–363

IPCC (2002) Climate change and biodiversity. Technical Paper IPCC Technical Paper V, Intergovernmental Panel on Climate Change

Jackson BG, Martin P, Nilsson MC, Wardle DA (2010) Response of feather moss associated N2 fixation and litter decomposition to variations in simulated rainfall intensity and frequency. Oikos 120(4):570–581, doi:10.1111/j.1600-0706.2010.18641.x

Janssens IA, Luyssaert S (2009) Carbon cycle: Nitrogen's carbon bonus. Nature Geosci 2(5):318–319, doi:10.1038/ngeo505

Lindner M, Maroschek M, Netherer S, Kremer A, Barbati A, Garcia-Gonzalo J, Seidl R, Delzon S, Corona P, Kolstrom M, Lexer MJ, Marchetti M (2010) Climate change impacts, adaptive capacity, and vulnerability of European forest ecosystems. Forest Ecology and Management 259(4):698–709, doi:10.1016/j.foreco.2009.09.023

Lindo Z, Gonzalez A (2010) The bryosphere: an integral and influential component of the Earth's biosphere. Ecosystems 13(4):612–627, doi:10.1007/s10021-010-9336-3

Logan T, Charron I, Chaumont D, Houle D (2011) Atlas de scénarios climatiques pour la forêt québécoise. Tech. Rep. ISBN papier : 976-2-923292-06-9 ISBN web : 978-2-923292-05-2, Ouranos et MRNF, URL: http://www.ouranos.ca/media/publication/162_AtlasForet2011.pdf

Luyssaert S, Schulze ED, Boerner A, Knohl A, Hessenmoeller D, Law BE, Ciais P, Grace J (2008) Old-growth forests as global carbon sinks. Nature 455(7210):213–215, doi:10.1038/nature07276

Magnani F, Mencuccini M, Borghetti M, Berbigier P, Berninger F, Delzon S, Grelle A, Hari P, Jarvis PG, Kolari P, Kowalski AS, Lankreijer H, Law BE, Lindroth A, Loustau D, Manca G, Moncrieff JB, Rayment M, Tedeschi V, Valentini R, Grace J (2007) The human footprint in the carbon cycle of temperate and boreal forests. Nature 447(7146):849–851, doi:10.1038/nature05847

Marion GM, Henry GHR, Freckman DW, Johnstone J, Jones G, Jones MH, Levesque E, Molau U, Molgaard P, Parsons AN, Svoboda J, Virginia RA (1997) Open-top designs for manipulating field temperature in high-latitude ecosystems. Global Change Biology 3(s1):20–32, doi:10.1111/j.1365-2486.1997.gcb136.x

Menge DNL, Levin SA, Hedin LO (2008) Evolutionary tradeoffs can select against nitrogen fixation and thereby maintain nitrogen limitation. Proceedings of the National Academy of Sciences of the United States of America 105(5):1573–1578, doi:10.1073/pnas.0711411105

Moore T, Basiliko N (2006) Decomposition in boreal peatlands boreal peatland ecosystems. In: Wieder RK, Vitt DH (eds.) Boreal Peatland Ecosystems, Ecological Studies, vol. 188, Springer-Verlag, Berlin, Germany, pp 125–143

O'Donnell JA, Turetsky MR, Harden JW, Manies KL, Pruett LE, Shetler G, Neff JC (2009) Interactive Effects of Fire, Soil Climate, and Moss on CO2 Fluxes in Black Spruce Ecosystems of Interior Alaska. Ecosystems 12(1):57–72, doi:10.1007/s10021-008-9206-4

Pearson RG, Dawson TP (2003) Predicting the impacts of climate change on the distribution of species: are bioclimate envelope models useful? Global Ecology and Biogeography 12(5):361–371

Pinheiro J, Bates D, DebRoy S, Sarkar D, R Development Core Team (2011) nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-98

R Development Core Team (2010) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria, URL: http://www.R-project.org/, ISBN 3-900051-07-0

Soja AJ, Tchebakova NM, French NHF, Flannigan MD, Shugart HH, Stocks BJ, Sukhinin AI, Parfenova EI, Chapin FS, Stackhouse PW (2007) Climate-induced boreal forest change: Predictions versus current observations. Global and Planetary Change 56(3-4):274–296, doi:10.1016/J.Glopacha.2006.07.028

Sokal RR, Rohlf FJ (1981) Biometry: The Principles and Practice of Statistics in Biological Research, 3rd edn. W. H. Freeman and Company, New York

Staddon P, Lindo Z, Crittenden PD, Gilbert F, Gonzalez A (2010) Connectivity, non-random extinction and ecosystem function in experimental metacommunities. Ecology Letters 13(5):543–552, doi:10.1111/J.1461-0248.2010.01450.X

Taggart RE, Cross AT (2009) Global greenhouse to icehouse and back again: the origin and future of the boreal forest biome. Global and Planetary Change 65(3-4):115–121, doi:10.1016/j.gloplacha.2008.10.014

Turetsky MR (2003) The role of bryophytes in carbon and nitrogen cycling. The Bryologist 106(3):395–409, doi:10.1639/05

Watkinson AR, Gill JA (2002) Climate change and dispersal. In: Bullock JM, Kenward RE, Hails RS (eds.) Dispersal Ecology, Blackwell Science, Malden, MA, pp 410–428

Wickham H (2009) ggplot2: elegant graphics for data analysis. Springer New York, h.wickham@gmail.com, doi:10.1007/978-0-387-98141-3, URL: http://had.co.nz/ggplot2/book

Wiedermann MM, Gunnarsson U, Nilsson MB, Nordin A, Ericson L (2009) Can small-scale experiments predict ecosystem responses? An example from peatlands. Oikos 118(3):449–456, doi:10.1111/J.1600-0706.2008.17129.X

Wilmking M, D'Arrigo R, Jacoby GC, Juday GP (2005) Increased temperature sensitivity and divergent growth trends in circumpolar boreal forests. Geophysical Research Letters 32(L15715), doi:10.1029/2005gl023331

Zackrisson O, DeLuca TH, Nilsson MC, Sellstedt A, Berglund LM (2004) Nitrogen fixation increases with successional age in boreal forests. Ecology 85(12):3327–3334, doi:10.1890/04-0461

Zuur AF, Ieno EN, Smith GM (2007) Analysing Ecological Data. Statistics for Biology and Health, Springer, New York, URL: http://www.highstat.com/book1.htm

Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM (2009) Mixed Effects Models and Extensions in Ecology with R. Springer, New York, URL: http://www.highstat.com/book2.htm

4.5 Supplementary Figures

Ambient patches, and inner chamber patches contained roughly the same amount of moisture, ranging from 400 – 800 % by weight (Figure 4.9). Outer chamber patches are significantly drier, containing less than 100% moisture, except for those in the contiguous treatment. This difference is likely a result of moisture wicking from adjacent patches, alleviating the drying effect caused by reduced direct precipitation. Nevertheless, we observed that most of this wicking occurs below the surface of the moss, while the upper regions where most growth occurs (approx. 3 cm) remains dry. The driest patches contained nearly no moisture at all, with mean values outer chamber patches being not significantly different from 0 (except for contiguous patches). Intermediate patches within the chamber (between the inner and outer patches) were not significantly drier than inner or ambient patches, but appear to be somewhat intermediate between inner and outer patches, particularly among isolated patches.

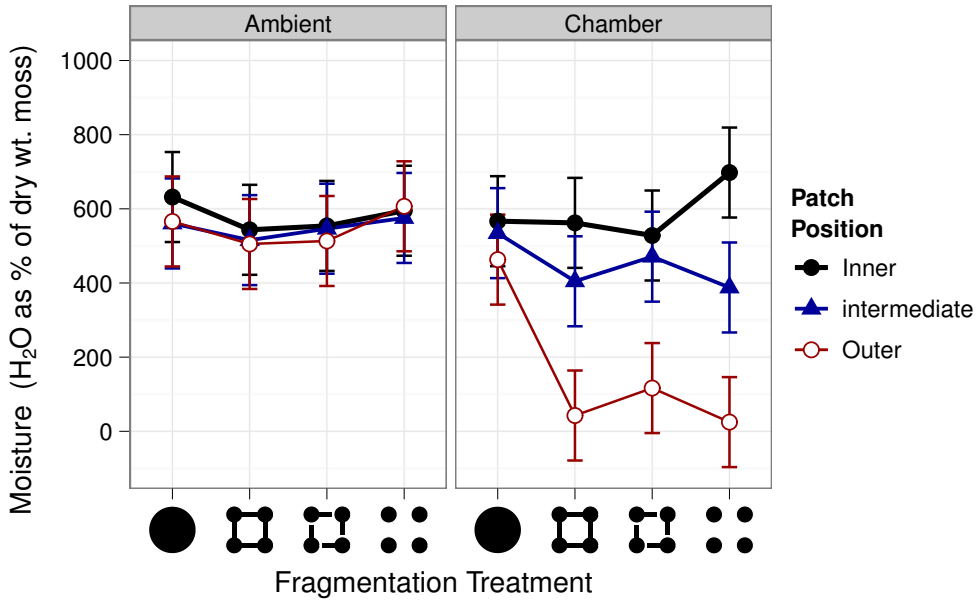

Figure 4.9
Water Contents of moss patches at time of collection (24 months; August 2009), as a percentage of moss dry weight. Values are means, by chamber, fragmentation treatment and patch position (n = 8 for each point). Error bars represent 95% comparison intervals (Tukey's HSD Minimum Significant Ranges).

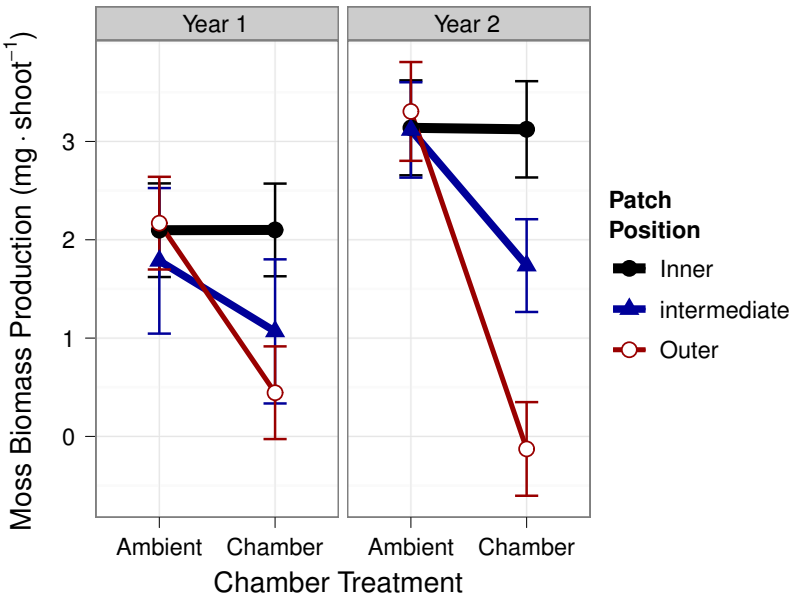

Figure 4.10
Fitted values and 95% confidence intervals for estimates of moss biomass production, by chamber treatment and patch position (including intermediate patches between inner and outer patches), across two years of the experiment. Tukey's HSD tests agree with the confidence interval overlaps shown in the figure (i.e., if the error bars in the figure do not *overlap, the Tukey's HSD tests has a P-value < 0.05). Although biomass production was significantly higher in the second year in ambient and inner chamber patches, there was no difference between years for outer or intermediate chamber patches. The net result is that intermediate chamber patches had intermediate rates of productivity in the first year, not significantly different from either inner or outer chamber patches (which are different from each other). In the second year, however, moss in intermediate chamber patches added significantly less biomass than the moss in inner or ambient patches, but significantly more than moss in the dry outer chamber patches.*

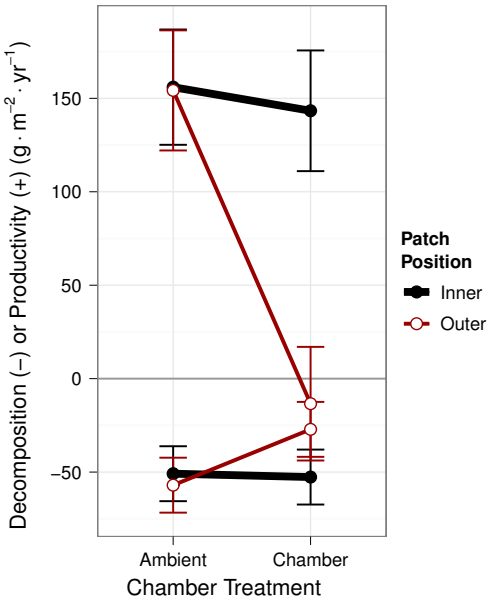

Figure 4.11

Fitted values and 95% confidence intervals for estimates of moss biomass production and decomposition, by chamber treatment and patch position during the second year of the experiment, in common units. Decomposition estimates were multiplied by -1 to present them as mass lost, while productivity is presented as biomass production. These values are presented for comparison with other values in the literature, although the main analysis was performed on the difference between productivity and decomposition in each patch (net biomass production).

4.6 ANOVA-type Tables

The following ANOVA-type tables present approximate *P*-values for terms in a fitted hierarchical model (using the `nlme` package in `R`). The *P*-values are based on sequential *F*-tests, while adding each term in the order listed. The output was produced using the `anova()` method for fitted `lme` model objects). Because of the differences between the hierarchical models used in this analysis and a standard ANOVA, these *P*-values are approximate and should be interpreted with caution, especially marginally significant values close to 0.05 (**?**). Nevertheless, they generally agree with the results of backward model-selection using likelihood ratio tests on nested models with different fixed effects (fit using Maximum Likelihood, rather than Restricted Maximum Likelihood). Notable exceptions are described in the table captions.

Model terms confirmed to be statistically significant are indicated by an asterisk * in the right-most column. Terms with marginal *P*-values near 0.05 in both the ANOVA-type tables here and backwards-model selection are marked with ·, and should be interpreted with caution.

The "Replicate" factor present in some models refers to additional replicates added at the level of Chambers, within each Block during the second year of the experiment. They are replicate patches, at all levels of other treatments, rather than additional sub-samples within the same experimental units. Given the planned nature of the experiment, and randomly-assigned treatments (and replicate designations), we expect no difference between replicates, but the term was included in the model to verify this.

Table 4.1
F-tests and approximate P-values of model terms added sequentially for linear moss growth during the first 12 months *of the experiment. Terms found to have significant effects are indicated in bold, and by a single asterisk * in the right-most column (based on approximate P-values much lower than 0.05, and confirmed by backwards model selection using likelihood ratio tests).*

	df^1	df_2	$F_{1,2}$	P	
(Intercept)	1	55	168.6	< 0.001	*
Chamber	1	7	22.1	0.002	*
Fragmentation	3	42	0.7	0.552	
Position	1	55	20.2	< 0.001	*
Chamber × Fragmentation	3	42	0.3	0.798	
Chamber × Position	1	55	25.5	< 0.001	*
Fragmentation × Position	3	55	0.4	0.772	
Chamber × Fragmentation × Position	3	55	0.4	0.754	

Table 4.2
F-tests and approximate P-values of model terms added sequentially for linear moss growth during months 13 – 22 *of the experiment (the second winter). Terms found to have significant effects are indicated in bold, and by a single asterisk * in the right-most column (based on approximate P-values much lower than 0.05, and confirmed by backwards model selection using likelihood ratio tests). The significant Replicate × Chamber × Position term is surprising, The importance of this term appears to be due to more growth in outer chamber patches, and slightly lower growth in all other patches, of the extra replicates: the overall effect being a smaller difference between treatment levels in the added replicates, relative to patches with repeated measurements. The general trends are the same, however.*

	df^1	df_2	$F_{1,2}$	P	
(Intercept)	1	103	62.2	< 0.001	*
Replicate	1	7	2.7	0.144	
Chamber	1	14	15.6	0.001	*
Fragmentation	3	84	0.8	0.514	
Position	1	103	36.6	< 0.001	*
Replicate × Chamber	1	14	0.2	0.657	
Replicate × Fragmentation	3	84	0.3	0.800	
Chamber × Fragmentation	3	84	0.7	0.553	
Replicate × Position	1	103	3.5	0.063	·
Chamber × Position	1	103	36.8	< 0.001	*
Fragmentation × Position	3	103	1.0	0.404	
Replicate × Chamber × Fragmentation	3	84	0.2	0.890	
Replicate × Chamber × Position	1	103	8.7	0.004	*
Replicate × Fragmentation × Position	3	103	0.3	0.841	
Chamber × Fragmentation × Position	3	103	1.9	0.128	
Replicate × Chamber × Fragmentation × Position	3	103	0.5	0.689	

Table 4.3

F-tests and approximate P-values of model terms added sequentially for linear moss growth during months 23 – 24 *of the experiment (the second summer). Terms found to have significant effects are indicated in bold, and by a single asterisk * in the right-most column (based on approximate P-values much lower than 0.05, and confirmed by backwards model selection using likelihood ratio tests). As expected, the Replicate × Chamber × Position interaction term is not significant.*

*Although the Fragmentation term appears to be significant (P=0.037), these are approximate P-values and should be interpreted with caution (**?**). Fragmentation was not statistically significant at any other time point, which suggests that fragmentation effects may take time (2 years or more) to begin to be detectable, although the evidence for this is currently weak. The possible Chamber × Fragmentation effect is driven by slightly higher rates of moss growth in contiguous patches overall, as well as ambient isolated patches.*

	df^1	df_2	$F_{1,2}$	P	
(Intercept)	1	107	87.9	< 0.001	*
Replicate	1	7	0.0	0.947	
Chamber	1	14	51.2	< 0.001	*
Fragmentation	3	84	3.0	0.037	·
Position	1	107	39.0	< 0.001	*
Replicate × Chamber	1	14	1.1	0.302	
Replicate × Fragmentation	3	84	0.5	0.718	
Chamber × Fragmentation	3	84	2.4	0.078	·
Replicate × Position	1	107	4.0	0.048	·
Chamber × Position	1	107	46.9	< 0.001	*
Fragmentation × Position	3	107	0.3	0.830	
Replicate × Chamber × Fragmentation	3	84	0.4	0.721	
Replicate × Chamber × Position	1	107	0.2	0.670	
Replicate × Fragmentation × Position	3	107	0.8	0.485	
Chamber × Fragmentation × Position	3	107	1.1	0.357	
Replicate × Chamber × Fragmentation × Position	3	107	0.9	0.456	

Table 4.4

F-tests and approximate P-values of model terms added sequentially for linear moss growth during months 13 – 24 *of the experiment (the second year). Terms found to have significant effects are indicated in bold, and by a single asterisk * in the right-most column (based on approximate P-values much lower than 0.05, and confirmed by backwards model selection using likelihood ratio tests). The Replicate × Chamber × Position term is approaching significance at the P= 0.05 threshold, and is driven by the pattern described in the model fit for months 13 – 22 (Table 4.2, above).*

	df^1	df_2	$F_{1,2}$	P	
(Intercept)	1	98	120.3	< 0.001	*
Replicate	1	7	1.7	0.237	
Chamber	1	14	52.2	< 0.001	*
Fragmentation	3	84	1.6	0.198	
Position	1	98	61.0	< 0.001	*
Replicate × Chamber	1	14	1.3	0.267	
Replicate × Fragmentation	3	84	0.7	0.562	
Chamber × Fragmentation	3	84	2.4	0.073	·
Replicate × Position	1	98	0.1	0.730	
Chamber × Position	1	98	62.4	< 0.001	*
Fragmentation × Position	3	98	0.6	0.629	
Replicate × Chamber × Fragmentation	3	84	0.3	0.814	
Replicate × Chamber × Position	1	98	3.3	0.074	·
Replicate × Fragmentation × Position	3	98	0.1	0.939	
Chamber × Fragmentation × Position	3	98	1.7	0.164	
Replicate × Chamber × Fragmentation × Position	3	98	0.7	0.530	

Table 4.5

F-tests and approximate P-values of model terms added sequentially for moss biomass production over both years *of the experiment. P-values are approximate, although none are ambiguous or near a threshold of 0.05. The same terms would be removed by backward-model selection using likelihood ratio tests. Terms determined to have significant effects are indicated in bold, and by a single asterisk * in the right-most column.*

	df^1	df_2	$F_{1,2}$	P	
(Intercept)	1	106	220.5	< 0.001	*
Chamber	1	7	43.3	< 0.001	*
Fragmentation	3	42	0.9	0.451	
Position	1	56	38.8	< 0.001	*
Year	1	106	23.3	< 0.001	*
Chamber × Fragmentation	3	42	0.7	0.556	
Chamber × Position	1	56	45.3	< 0.001	*
Chamber × Year	1	106	12.1	0.001	*
Fragmentation × Position	3	56	0.5	0.699	
Fragmentation × Year	3	106	0.5	0.681	
Position × Year	1	106	9.0	0.003	*
Chamber × Fragmentation × Position	3	56	0.6	0.608	
Chamber × Fragmentation × Year	3	106	0.2	0.870	
Chamber × Position × Year	1	106	11.2	0.001	*

Table 4.6

*Nested ANOVA of arcsin-square-root transformed decomposition, measured as the proportion of mass lost over the second year of the experiment. Significant p-values (below α of 0.05) are highlighted in bold and indicated with asterisks: *** if $P < 0.001$,** if $P < 0.01$, * if $P < 0.05$. Marginally significant P-values ($P < 0.01$) are indicated by •.*

	df	SS	Mean Square	F	P	
Residuals (Block)	7	0.28	0.04			
Chamber	1	0.16	0.16	8.55	**0.0222**	*
Residuals (Block/Chamber)	7	0.13	0.02			
Fragmentation	3	0.03	0.01	2.33	0.0883	•
Chamber × Fragmentation	3	0.01	0.00	0.62	0.6039	
Residuals (Block/Chamber/Fragmentation)	42	0.17	0.00			
Position	1	0.11	0.11	21.37	**< 0.0001**	***
Chamber × Position	1	0.15	0.15	28.72	**< 0.0001**	***
Fragmentation × Position	3	0.02	0.01	1.09	0.3593	
Chamber × Fragmentation × Position	3	0.01	0.00	0.42	0.7419	
Residuals (within)	56	0.29	0.01			

Table 4.7

F-tests and approximate P-values of model terms added sequentially for net moss biomass production *(Productivity - Decomposition), during the second year of the experiment. Terms found to have significant effects are indicated in bold, and by a single asterisk * in the right-most column (based on approximate P-values much lower than 0.05, and confirmed by backwards model selection using likelihood ratio tests). See text below for discussion of the significance of the three-way Chamber × Fragmentation × Position term.*

	df^1	df_2	$F_{1,2}$	P	
(Intercept)	1	43	30.9	< 0.001	*
Chamber	1	7	26.5	0.001	*
Fragmentation	3	42	0.5	0.693	
Position	1	43	24.7	< 0.001	*
Chamber × Fragmentation	3	42	1.2	0.309	
Chamber × Position	1	43	19.9	< 0.001	*
Fragmentation × Position	3	43	0.6	0.636	
Chamber × Fragmentation × Position	3	43	2.4	0.079	~

Although the *Chamber* × *Fragmentation* × *Position* treatment is borderline significant, a likelihood-ratio test comparing equivalent models with and without this term produced a P-value of 0.0497, which would be considered 'significant' at the 0.05 level, but is not as clear as the other terms. Allowing for heterogeneity between Fragmentation treatments results in a marginally better fit (as judged by the AIC, but not by BIC), and results in all Fragmentation terms being non-significant (e.g., *Chamber* × *Fragmentation* × *Position* Likelihood Ratio = 5.82, P= 0.1207). Doing so also makes extracting fitted values for marginal factors much less practical (and impossible via the `effects` package). We therefore used the model with results presented in Table 4.7 to generate graphical output, but conclude that there is insufficient evidence of a three-way interaction between Chamber, Fragmentation, and Position treatments.

Connecting statement

Previous chapters (3 and 4) presented effects of combined habitat fragmentation, warming and drought treatments on cyanobacteria densities, N-fixation, moss productivity, and decomposition. These processes are all related, with cyanobacteria fixing nitrogen, which supports moss growth, production, and decomposition. With moss growth as the focal endpoint, I test this set of hypothesized relationships among cyanobacteria, N-fixation, and moss growth in the following chapter. In chapter 5, I also include measurements of available nitrogen, to add to measurements of available moisture and the warming by experimental chambers, and compare the relative effects of these environmental conditions on each ecosystem process.

CHAPTER 5

Ecological controls on boreal forest moss growth and N-fixation in a simulated climate change experiment

Keywords: *Pleurozium schreberi*, biotic nitrogen fixation, heterocystous cyanobacteria, moss growth, boreal forest, bryosphere, drought

Abstract

Nitrogen-fixing cyanobacteria found in association with feather mosses provide an important source of nitrogen to boreal forest ecosystems, which are otherwise nitrogen-limited. As boreal forests in Canada are predicted to experience multiple interacting stressors, such as climate change and habitat fragmentation, a more holistic understanding of how multiple factors control nitrogen supply within these systems is imperative. We present results of a two-year field experiment that combined fragmentation and simulated climate change, using open top chambers to warm moss, and create a drought gradient. We measured and analyzed relationships between moisture, available nitrogen, cyanobacteria density, N-fixation rates, and growth rates of the dominant feather moss *Pleurozium schreberi*. We found that N-fixation rates were strongly regulated by moisture, perhaps nonlinearly, while cyanobacteria density had a weak positive effect. Available N had a subtle negative effect on N-fixation rates, after removing effects of other explanatory variables. We also found an overall positive effect of moisture on moss growth, as well as N-fixation rates, after accounting for the effect of moisture. Our results support "nitrostatic" negative feedbacks between available N and N-fixation rates in boreal forest moss, independent of cyanobacteria density on moss shoots. These results also highlight the important regulating role of moisture on ecosystem processes within the bryosphere, through direct effects, as well as potential long-term indirect effects through changes to nutrient dynamics and species interactions.

5.1 Introduction

Productivity in boreal forest systems is thought to be nitrogen-limited (Moore, 1980; DeLuca *et al.*, 2002; Menge *et al.*, 2008). Nitrogen-fixing heterocystous cyanobacteria living in association with boreal forest mosses provide an important input of nitrogen, particularly in late-succession stands (DeLuca *et al.*, 2002; Zackrisson *et al.*, 2004). Although recent work has demonstrated the importance of various environmental conditions in regulating biotic nitrogen fixation in this system, questions still remain concerning the full range of interactions, and the relationship between biotic N-fixation and moss productivity. This uncertainty limits our ability to make accurate predictions about how boreal systems will respond to projected changes in climate, such as temperature, precipitation, and their variation in space and time.

The boreal forest covers at least 10% of the earth's land surface and thus plays a role in regulating global climate (Eugster *et al.*, 2000; Taggart and Cross, 2009; Bernier *et al.*, 2011). Large pools of carbon currently stored in boreal forest soils may continue to accumulate atmospheric carbon, or release it, depending on the relative rates of primary production, respiration and decomposition (Davidson and Janssens, 2006; Luyssaert *et al.*, 2008; Wiedermann *et al.*, 2009). Despite increasing amounts of CO_2 in the atmosphere, carbon-uptake in boreal forests is often nitrogen-limited (Janssens and Luyssaert, 2009; Markham, 2009; Zackrisson *et al.*, 2009), while soil decomposition is limited more often by temperature or moisture availability (Davidson and Janssens, 2006; Jackson *et al.*, 2010). Whether boreal forests will continue to act as a carbon sink, or become a net emitter of carbon, will depend on several environmental factors and limiting nutrients, particularly nitrogen.

Ecosystem impacts of climate change may be further amplified by habitat fragmentation and isolation. Many organisms respond to changes in climate by relocating to track environmental conditions to which they are best adapted, if possible (Watkinson and Gill, 2002). This may be between different microclimates over short distances, such as different slopes, topography, or proximity to water bodies. Species can also shift distributions over much longer distances to track temperature and moisture gradients at continental scales (Parmesan *et al.*, 1999; Walther *et al.*, 2002; Taggart and Cross, 2009; Chen *et al.*, 2011). Finding suitable environmental conditions may be hampered in either case by a lack of connectivity between habitat patches, or habitats with different microclimate conditions, preventing necessary rates of dispersal. The combination of habitat fragmentation and climate change has raised major concerns over their potential synergy (Sala *et al.*, 2000; Opdam and Wascher, 2004; Ewers and Didham, 2006). Species able to survive climate change by dispersal, or those tolerant of habitat fragmentation under current conditions, may be unable to persist in the face of both drivers.

We used boreal forest moss as a model microecosystem to explore potential interactive effects of warming, drought, and habitat fragmentation. The moss layer, its biotic inhabitants, and combination of above and below ground processes makes for a tractable experimental system, and has been named "the bryosphere" (Lindo and Gonzalez, 2010). Moss habitats have been used as natural model systems to study effects of habitat fragmentation on native microfauna, often microarthropods (Lindo and Gonzalez, 2010). The cyanobacteria responsible for biotic N-fixation

in boreal feather mosses are often members of genera that form motile hormogonia that presumably disperse through water films within the bryosphere. We asked if they may also be affected by small scale fragmentation of moss patches, or whether extinctions in faunal food webs may have indirect effects on ecosystem processes such as N-fixation or moss growth, via trophic interactions or regulation of nutrient cycling (Birkemoe and Liengen, 2000; Lindo and Gonzalez, 2010). This paper will focus on the net responses of N-fixation by cyanobacteria associated with bryophytes, and the growth of the mosses themselves, in response to a range of experimentally manipulated and naturally varying environmental conditions.

The most common species of feather moss throughout the boreal forest is *Pleurozium schreberi* (Brid.) Mitt., which often hosts symbiotic nitrogen-fixing cyanobacteria (DeLuca *et al.*, 2002; Zackrisson *et al.*, 2004). These filamentous, heterocystous cyanobacteria can be found growing epiphytically on the surface of moss leaves, or in various crevices between moss leaves and shoots. They are often members of the genera *Nostoc*, *Stigonema*, or *Calothrix*, each of which fixes atmospheric nitrogen into ammonia at different rates depending on temperature (Gentili *et al.*, 2005), and perhaps other environmental factors.

These groups of cyanobacteria are frequently found in symbiotic association with bryophytes, fungi, and vascular plants, providing valuable fixed nitrogen that may be otherwise rare in the host's environment, in exchange for photosynthates (Vitousek *et al.*, 2002; Bothe *et al.*, 2007; Bergman *et al.*, 2007; Elmerich and Newton, 2007). Associations with feather mosses are somewhat less well understood, and the exchanges of such materials between host and cyanobiont have not been precisely measured, particularly in the case of *Pleurozium schreberi*.

The rates of N-fixation by cyanobacteria associated with *Pleurozium schreberi* are sizeable, contributing as much as half of all nitrogen inputs to mature boreal forest stands (DeLuca *et al.*, 2002). The fate of nitrogen fixed by cyanobacteria associated with *P. schreberi* is not generally known, however. It is often presumed to be transferred directly to the host (Bergman *et al.*, 2007), but if any were released directly to the environment, it would likely be immediately absorbed by moss anyway (Turetsky, 2003). In either case, the rates of N-fixation are sufficient to support primary production of both moss and trees under otherwise N-limited conditions, particularly in old-growth stands (DeLuca *et al.*, 2002; Zackrisson *et al.*, 2004; Luyssaert *et al.*, 2008; Lindo and Whiteley, 2011).

N-fixation rates in the bryosphere are affected by temperature (Gentili *et al.*, 2005; Markham, 2009) and moisture availability as well as variability (Gundale

et al., 2009; Jackson *et al.*, 2010; Gundale *et al.*, 2012). This combination of factors may account for observed short-term seasonal variation in N-fixation, with peaks occurring during summers (DeLuca *et al.*, 2002; Markham, 2009) when temperature, light, and moisture are favourable. Markham (2009) found N-fixation increasing monotonically with temperature during a single growing season, while experiments by Gentili *et al.* (2005) found different optimal temperatures for different cyanobacteria genera. This implies a potential for insurance effects of cyanobacteria diversity through functional compensation between cyanobiont taxa, which could buffer N-fixation rates in the face of temperature variations over longer timescales (Loreau *et al.*, 2003).

N-fixation rates by cyanobacteria may also be down-regulated by nitrogen available in the environment, within the moss layer, forming a "nitrostatic" ecosystem feedback between the two sources of bioavailable nitrogen (Menge and Hedin, 2009). When nitrogen is abundant, such as following a fire, N-fixation associated with mosses is lower, but tends to increase as available nitrogen declines and becomes more limiting with successional age (Zackrisson *et al.*, 2004; Lagerström *et al.*, 2007; DeLuca *et al.*, 2008). In cases of high nitrogen abundance, bryophytes would have no need for cyanobacterial symbionts and antimicrobial properties of bryophytes (Turetsky, 2003) may actively discourage the formation of symbioses until different chemical signals attract cyanobionts once nitrogen conditions become more limiting (DeLuca *et al.*, 2007). On the other hand, Menge and Hedin (2009) found a positive relationship between soil N availability and N-fixation, due primarily to increases in bryophyte biomass on more fertile sites. An observed negative relationship between N-fixation and abundant available N along chronosequences could be a product of successional age, rather than available nitrogen per se. Nutrient addition experiments, however, have showed significant declines in cyanobacteria abundance and N-fixation rates with N fertilization, demonstrating that the symbiotic interactions are highly sensitive to N availability (DeLuca *et al.*, 2007), but not to other additions of phosphorous or micronutrients (Markham, 2009).

Moisture, temperature, light, and nutrient availability (particularly nitrogen) are the most important environmental factors controlling rates of N-fixation by cyanobacteria associated with boreal forest moss (Gentili *et al.*, 2005; Bergman *et al.*, 2007), as well as moss growth (Turetsky, 2003; Lindo and Gonzalez, 2010). The relative importance of various factors controlling N-fixation and moss growth, however, remains an open question. Ultimately, the importance of a given factor

may be context-dependent, affected by whichever is most limiting at a given location or time (Frolking, 1997; Cleveland *et al.*, 1999; Gundale *et al.*, 2010), yet few studies have controlled, let alone measured several factors at a single site at the same time, to compare the relative magnitude of effects on N-fixation or moss growth.

We present results of a two-year field experiment designed to test the interactive effects of habitat fragmentation and simulated climate change conditions, on the moss and cyanobacteria association in the boreal forest. We simulated climate change using open-top chambers that passively warmed the air above the moss layer, and intercepted precipitation in the outer margins of the chambers. This experimental design allowed us to assess the relative importance of several potential ecological controls on cyanobacteria cell density, N-fixation rates, and linear growth rates of *Pleurozium schreberi*.

The effects of experimental treatments on most variables are presented in previous chapters. We now introduce measurements of total nitrogen (N) available within the moss layer, test for effects of experimental treatments, and then explore relationships between variables that might influence both N-fixation and moss growth in this system. Current knowledge of the system predicts a positive effect of moisture on all ecosystem properties. We would also predict total nitrogen to reduce cyanobacteria density, with indirect effects on N-fixation rates, while we might expect moss growth to be affected by the combination of nitrogen supplied from N-fixation by cyanobionts and what is directly available in the moss layer.

5.2 Methods

Study site

The field experiment was conducted in a boreal forest stand approximately 100 × 200 m, located 1.6 km southeast of the McGill Subarctic Research Station near the edge of the town of Schefferville, Québec, Canada, 54°47′44″N 66°47′20″W. The ground cover at the study site is dominated by the feather moss *Pleurozium schreberi*, under a sparse canopy of scatted spruce trees, including both *Picea mariana* (Mill.) and *P. glauca* (Moench) Voss. (Moore, 1980), and an understory of dwarf birch (*Betula glandulosa* Michx.) and Labrador tea (*Ledum groenlandicum* Oeder.). Full details on the study site and experimental design are presented in chapter 2.

We started the experiment during the summer of 2007. All data presented here was collected after two years of experimental treatments (August 2009), and includes measurements taken at the time of sample collection, such as N-fixation, cyanobacteria cell density, and moisture levels in the moss, as well as measurements integrated over the second year of the experiment, including moss growth and total available N within the moss layer.

Experimental design

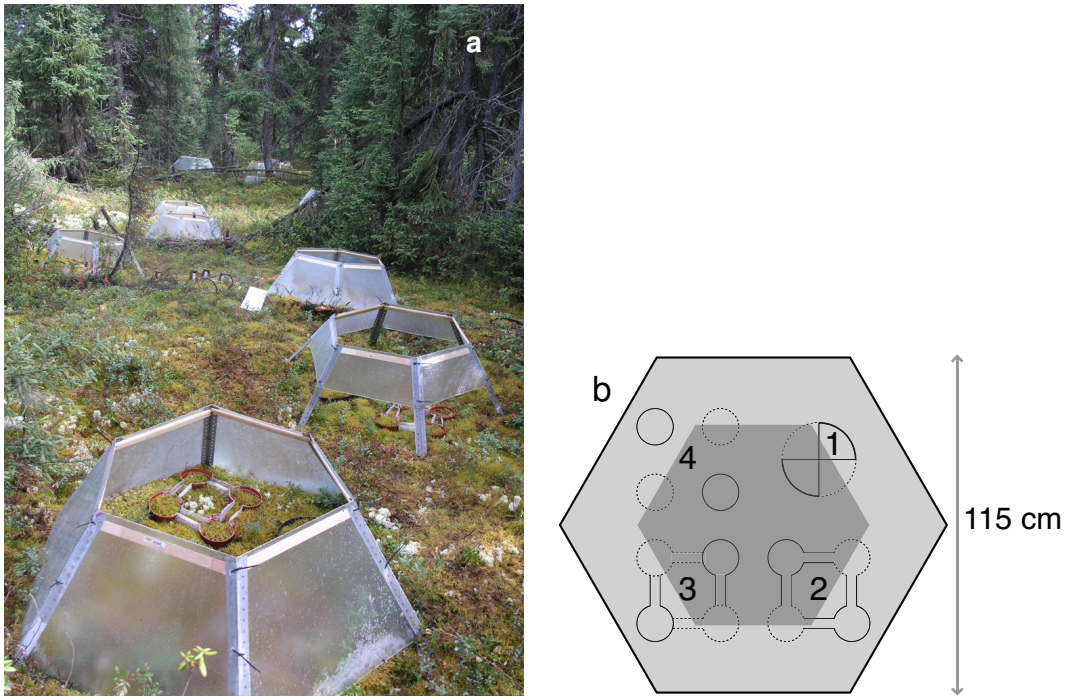

Figure 5.1 *Experimental design:*
*(**a**) View of one block of the field experiment, showing open-top chambers and ambient treatments. Partial chambers with the bottom half of the walls removed are also visible, but these were not included in the data presented here;*
*(**b**) Diagram representing layout of fragmentation treatments inside chambers, to scale. Habitat fragmentation treatments, beginning with top-right: (1) Contiguous, (2) Corridors, (3) Pseudo-corridors, (4) Isolated. The inner shaded hexagon shows approximate area open to vertical precipitation; the actual 'rain shadow', shown in light grey, varies across chambers, depending on local topography, prevailing wind, canopy cover, and other physical differences.*

The experiment included a factorial combination of climate change and habitat fragmentation treatments (Figure 5.1). Moss patches were isolated within plastic pots, 9 cm deep and 12.5 cm in diameter, in combinations of four patches, at four different levels of fragmentation from the surrounding matrix (and each

other). Fully *isolated* patches consisted of four patches in separate pots arranged in a square, separated by 10 cm from its nearest neighbour; a *corridor* treatment added 3 cm-wide corridors connecting each patch to both neighbours; a *pseudo-corridor* treatment consisted of the same layout as the corridor treatment, but each corridor was only connected to a single patch, to control for the added habitat area of the corridor treatments, with the same degree of connectivity as the isolated patches, and; *contiguous* patches included the same total area of moss in a single large patch 25 cm in diameter. These networks of patches were designed to create metacommunities, by allowing movement of fauna and propagules between patches, with varying levels of dispersal (see Leibold *et al.*, 2004).

Combinations of each of the four levels of habitat fragmentation were grouped together and randomly assigned to one of two simulated climate change treatments: an open top *chamber* (OTC), or an ambient control (Figure 5.1a).

This experimental layout was replicated at eight blocks throughout the study site, in areas dominated by *Pleurozium schreberi*, and open enough to allow space for the open top chambers to sit flush on the surface of the moss.

Climate change treatments

We simulated climate change conditions with open top chambers (OTCs), based on the hexagonal designs used by ITEX in tundra systems (Marion *et al.*, 1997). The OTCs used in this experiment were 115 cm wide at the base, 40 cm tall, with an opening 69 cm wide at the top. Chamber walls were constructed of Sun-Lite fiberglass (by Solar Components Corporation), supported by aluminium angles between panels, and wooden support strips along the top, fastened together with UV-resistant cable ties. Full details on chamber treatments and effects in this experiment are described in section 2.4.

Temperature and relative humidity were recorded in the center of each climate treatment with HOBO pro v2 dataloggers (Onset Computer Corporation) in five of the blocks. Temperature and relative humidity readings 2 cm below the moss surface were recorded every 30 minutes, year-round. Chambers were able to warm the moss surface by 0.5 °C during the summer, mostly during daily maxima (see section 2.4).

Although the open-top chambers allow precipitation to fall through, we found that the sloped walls effectively prevented precipitation from reaching the outer edges of the chambers, creating a precipitation gradient that affected moisture levels within individual patches. Inner patches of each metacommunity within chambers

received ambient levels of precipitation, outer patches received minimal levels, and intermediate patches in between received intermediate levels. We weighed each moss patch before and after drying to measure the moisture content held in the moss at the end of the second year of the experiment (see Results section for measured differences). Outer patches were therefore effectively treated with a two-year drought, interrupted briefly during spring when melting snow flooded some plots. This is consistent with some climate change scenarios predicting more frequent extreme events, and longer temperature-induced droughts in some areas (Grant *et al.*, 2006; Soja *et al.*, 2007; Lindner *et al.*, 2010).

Available nitrogen

We measured bioavailable nitrogen within the moss layer with Unibest PST-1 ion resin capsules (Unibest International, Bozeman, Montana, USA; unibestinc.com). These are spherical capsules 1.9 cm in diameter made of nylon mesh, containing 1 g of mixed anionic and cationic resin. They continuously adsorb elements from water in soil or other strata in direct contact with the capsules, in a manner analogous to absorption by living organisms (Skogley, 1992). They provide an integrated measure of available nutrients over the time they are in contact with the surrounding moss.

Ion resin capsules were installed within experimental patches by inserting a stainless steel blade roughly vertically into the moss to create an opening, then inserting the capsule to a depth of 6 cm below the moss surface, within 2–3 cm of the centre of the patch. The opening was then gently covered with moss.

At the end of the experiment, capsules were recovered from the moss and transported to the field station, where they were rinsed with distilled water and placed in a whirl-pak bag for shipping, with 1-2 ml of distilled water to keep them moist. Capsules were transported back to the lab at McGill University (Montreal, Canada) and stored at 6 °C for several months until processing.

Resin capsules were processed at the Soil Testing lab on McGill University's MacDonald Campus, to determine resin-sorbed nitrate (NO_3^-) and ammonium (NH_4^+). Each capsule was shaken on an orbital shaker for 20 minutes in three sequential 16 ml aliquots of 2 M KCl solution, which was decanted between aliquots, for a total of 48 ml of extract (after Johnson *et al.*, 2005; DeLuca *et al.*, 2007). The extracts were analyzed for ion concentrations on a Lachat flow injection analyzer. Concentrations in $mg \cdot l^{-1}$ were converted to $g \cdot m^{-2}$, based on the surface

area of the capsule, extractant volume (48 ml), and the number of days each capsule was left in the field experimental patches to incubate.

Capsules were installed in inner and outer patches in late July 2008, and collected in August 2009, resulting in an average incubation time of 395±6 days. Capsules were also installed into a randomly selected intermediate patch in early June 2009, and collected at the same time as the rest, incubating over 58±6 days exclusively during the summer. We found that most nitrogen adsorption occurs during the summer (see Supplementary Materials), and therefore could not standardize both measurements over a common time period. Only measurements from inner and outer patches are used in the analyses presented here.

Nitrogen-fixation

We measured rates of nitrogen-fixation using an Acetylene Reduction Assay (ARA), which measures quantities of acetylene reduced to ethylene by nitrogenase enzymes in cyanobacteria heterocyst cells (Schöllhorn and Burris, 1967; Hardy *et al.*, 1968; Mcnabb and Geist, 1979). In each experimental patch, we placed 20 shoots of *Pleurozium schreberi* into a 50 ml optically clear polystyrene tube, sealed with a rubber septum (see chapter 3 for full details). We then removed 10% of the headspace within the tubes (5 ml) and replaced it with 99.6% pure acetylene gas. We left the tubes in the experimental patch (septum down) overnight to incubate for 24 hours, before sampling 5 ml of gas into a vacutainer for transport back to the lab.

We analyzed 1 ml gas samples for acetylene and ethylene concentrations in a gas chromatograph (Shimadzu GC-2014) with an injector temperature of 250 °C, FID at 250 °C and Carbosphere 80/100 column at 200 °C, using a flow rate of $30\,\mathrm{ml}\cdot\mathrm{min}^{-1}$ and Helium carrier gas. GCsolution software digitally integrated gas chromatography output, and converted readings into concentrations based on a calibration curve created with known quantities of each gas. Further details of methods used to measure N-fixation and cyanobacteria density for this experiment are described in chapter 3.

Previous studies have calibrated the ARA method for *P. schreberi*, using parallel $^{15}N_2$ tracer experiments, and found a reduction ratio of 3:1 (3 mol acetylene reduced for every 1 mol nitrogen gas fixed by cyanobacteria) associated with *Pleurozium schreberi* in boreal forests DeLuca *et al.* (2002). We multiplied all measures of acetylene reduction rates by the expected nitrogen:acetylene ratio (1/3) to estimate N-fixation rates as $\mu\mathrm{mol\ N}\cdot\mathrm{m}^{-2}\cdot\mathrm{d}^{-1}$.

Cyanobacteria cell density

We selected two moss shoots of the 20 in each tube used for N-fixation measurements (see above), each 6–7 cm long, to estimate cyanobacteria abundance using a novel sonication technique to dissociate cyanobacterial cells from the moss (see also Lindo and Whiteley, 2011). Both shoots were placed in a 2 ml plastic centrifuge tube with 1 ml of deionized water, and sonicated for 40 s at approx. 100 W (25% max. amplitude) with a horn frequency of 20 kHz. We agitated the tubes for 5 s on a vortex machine immediately prior to taking subsamples for counting. We then transferred two 10 µl subsamples to a hemacytometer to count cells under a compound microscope at 200× magnification, with fluorescence and a "Texas Red" filter. We counted cyanobacteria vegetative cells and heterocysts, identified according to Rippka *et al.* (1979). Most cyanobacteria in our samples were identified as *Stigonema*, with a few *Nostoc*, many of which were in various stages of development (hormogonia, akinetes, and short young filaments).

Moss growth

We measured linear extension of individual moss stems by marking them with a polyester thread carefully tied near the tip of the shoot (see chapter 4), and measuring the distance from the marker to the tip with a digital caliper, at successive times throughout the experiment (after Clymo, 1970). Full details on moss growth measurements are presented in chapter 4. Measurements were taken at the beginning and the end of the second year of the experiment, providing a measure of linear growth over the entire second year. We measured moss growth on a single representative shoot in each patch included in the analysis. The marked shoot was also included in the sample of 20 shoots used to measure N-fixation rates (above), and as one of two shoots used to estimate cyanobacteria cell density, where possible (see above).

Hypotheses and statistical analysis

We tested a series of related hypotheses of relevant interactions among measured variables, and the relative importance of habitat fragmentation, temperature (chambers), available moisture, nitrogen, and cyanobacteria density on N-fixation rates, and moss growth. The effects of experimental treatments on most measured variables are presented previously (in chapters 3 and 4), except for available N in the moss layer. We began by testing the hypothesis that:

H1: *Available nitrogen (N) in the moss layer is affected by experimental warming, drought, fragmentation treatments, and their interaction.*

Cyanobacteria are principally responsible for N-fixation associated with boreal forest mosses. We therefore predicted that cyanobacteria density would respond to moisture and available nitrogen, driving resulting changes in N-fixation (DeLuca *et al.*, 2007; Gundale *et al.*, 2009):

H2: *Cyanobacteria cell density is positively related to available moisture;*

H3: *Cyanobacteria cell density is negatively related to available nitrogen;*

We predicted the following relationships and controls on N-fixation by cyanobacteria associated with *Pleurozium schreberi*:

H4: *N-fixation rates are positively related to cyanobacteria cell density;*

H5: *N-fixation rates are unimodally related to moisture content of the moss;*

H6: *N-fixation rates are positively related to temperature (warming by the presence of an open-top chamber);*

H7: *N-fixation rates are negatively related to total available N within the moss layer; and*

H8: *N-fixation rates are more negatively affected by drought in more isolated patches.*

We also predicted the following effects on *P. schreberi* growth:

H9: *Moss growth is positively related to moisture content of the moss;*

H10: *Moss growth is positively related to temperature (warming by the presence of an open-top chamber); and*

H11: *Moss growth is positively related to an interaction between N-fixation rates or Total N in the moss, assuming an overall negative relationship between N-fixation and Total N.*

H12: *Moss growth is more negatively affected by drought in more isolated patches.*

To test our first hypothesis (H1), we tested for the effects of chamber, fragmentation treatments, patch position, and all interactions, in a nested analysis of variance (ANOVA), including blocks as the largest experimental unit, followed by chamber and fragmentation treatments, with patches at the lowest level. When interactions terms were significant, we compared individual factor levels or interactions, using Minimum Significant Ranges, based on Tukey's HSD, to correct for multiple comparisons (Sokal and Rohlf, 1981).

Remaining hypotheses were tested within a multiple regression framework, by fitting linear models containing main effects and ecologically relevant interaction terms as predictors for the response variables of interest: N-fixation, and moss growth. We also used a multi-model approach to perform preliminary screening of model terms and two-way interactions that significantly improved model fit (Calcagno and de Mazancourt, 2010). Because we had more possible terms than could be tested using an exhaustive search in a reasonable amount of time, we employed a genetic algorithm to search possible combinations of candidate main terms and two-way interactions, keeping a confidence set of 256 of the best models identified over 6 replicate runs (Calcagno and de Mazancourt, 2010). The multi-model approach indicated which terms of interest were likely to explain a significant amount of variation in the data, using a measure of "importance" based on model weights of the confidence set of best models. We also used the confidence set to estimate coefficients and confidence intervals for each term, as an indication of effect sizes and uncertainty for all terms considered together. We chose a final combination of interaction terms of ecological interest, combined with those identified as highly "important" by multi-model selection, to fit a final model for each response variable, which we used to plot fitted values. Data exploration suggested that both N-fixation rates and moss growth variances were unequal across blocks, so final models were corrected for heterogeneity among blocks, which significantly improved the model fits, and reduced patterns in residuals.

N-fixation rates, cyanobacteria cell density, and total available N had many small values, with a few large ones, in a highly skewed distribution. We therefore log-transformed (with values of 0 remaining as 0) each of these variables to linearize relationships and keep residuals normally distributed. Moss growth rates could not be log-transformed due to several negative values (moss that actually shrank in dry patches - see below), however data exploration and model validation suggested such a transformation was not necessary.

Our model for N-fixation rates as a response variable included: blocks, chambers, and fragmentation as factors, as well as moisture content, cyanobacteria cell density, and available N as continuous variables. We also included a quadratic term for moisture to test for a possible unimodal relationship (see chapter 3). We excluded most interactions with Chambers, due to the absence of dry patches in the ambient treatments, which led to unrealistic fitted values in the ambient treatments and overall, and confounded the effects of moisture with the warming effect of the chambers. We also explicitly did not consider any interaction between the linear and quadratic moisture terms, which amounts to a cubic term, and was not theoretically justifiable or ecologically reasonable.

We tested for the "pure" effects of moisture, cyanobacteria density, and available N on N-fixation rates, by fitting partial regressions with N-fixation rates, after removing variation explained by all other terms in the model. This consists of re-fitting a mixed model without the explanatory variable of interest, and similar model with the variable of interest as the response instead; a linear model is then fit for the residuals of the first model on residuals of the second (Zuur *et al.*, 2007).

Our model for moss growth rates as a response variable included: blocks, chambers, and fragmentation as factors, as well as moisture content, N-fixation rates, and available N as continuous variables. We tested for the "pure" effects of moisture, N-fixation rates, and available N, by fitting partial regressions with moss growth rates as the response variable, after removing variation explained by all other terms in the model. Data exploration also suggested that moss growth rates, N-fixation rates, and moisture content were highly correlated. We therefore excluded N-fixation from the partial regression of moss growth on moisture content, but included moisture in the partial regression of moss growth on N-fixation. This allowed us to test how much variation is explained by moisture first, and how much remaining variation is explained by N-fixation rates. Both moisture and N-fixation were included in a partial regression on available N, to account for all variation explained by both.

We performed all calculations and statistical analyses in `R v2.12` (R Development Core Team, 2010), with the `nlme` package for mixed effects modelling (Pinheiro *et al.*, 2011), `glmulti` for multi-model selection and inference (Calcagno and de Mazancourt, 2010), `effects` for extracting partial effects of predictor variables on response variables from fitted models (Fox, 2003), and `ggplot2` to graph data and results (Wickham, 2009). All experimental data and analysis scripts are available online:

http://www.github.com/jawhiteley/SECC.R.JAW

Because of differences in data collection of different variables across experimental patches, and for reasons of simplicity, we restricted out analysis to data from *inner* and *outer* patches in ambient and chamber treatments, and all habitat fragmentation treatments, from all eight blocks. The data analyzed here thus includes: 8 blocks $\times$ 2 chamber treatments $\times$ 4 fragmentation treatments $\times$ 2 patches each (inner and outer) $=$ 128 data points for each variable. There were 10 moss growth measurements missing due to damaged or missing stems marked in the first measurement that could not be re-measured to calculate linear growth. We removed a single potential outlier for cyanobacteria density that was nearly twice as large as the next largest value ($> 5 \times 10^9$ cells $\cdot$ m^{-2}), and appeared to disproportionately influence model fits. We also removed a single measurement of moss growth above 30 mm $\cdot$ yr^{-1} as a potential outlier, and a single value of moisture content above 800% that may have been overly influential. There were several values as high as these in other data collected in this experiment, but with so few extreme values in the data analyzed here, they were potentially highly influential on linear model fits and were removed as a precaution against spurious results.

5.3 Results

Available water and nitrogen

Outer chamber patches were drier than all other patches, which were otherwise similar in moisture content (Figure 5.2a). Contiguous patches had total moisture levels similar to inner patches in the chambers (resulting in a significant three-way interaction between chambers, fragmentation, and patch position; Table 5.1). Based on field observations, we believe this difference is due to moss wicking moisture from adjacent patches, which occurred below the moss surface, creating a steep moisture gradient within outer contiguous patches in chamber. The upper few cm appeared just as dry as outer chamber patches in other fragmentation treatments (see chapter 3).

Preliminary exploration of data from ion resin capsules revealed that NO_3^- concentrations were low and relatively stable across all samples, while NH_4^+ was higher in concentration and more variable. We added both measurements together to estimate total nitrogen (N) available within the moss layer, which was analyzed for results presented here. Total available N differed overall by chamber and patch

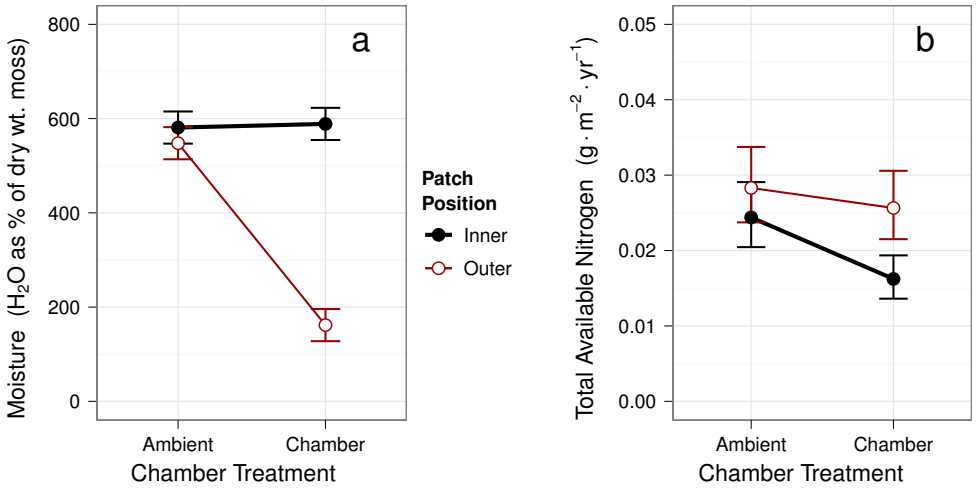

Figure 5.2

(a) Water content of moss patches at the end of the second year of the experiment, by chamber treatment and patch position.

(b) Total nitrogen available in the moss layer over the second year of the experiment, by chamber treatment and patch position.

Table 5.1

*F and P-values, with relevant degrees of freedom (df), for nested analysis of variance (ANOVA) of moss water content and total available N. Significant p-values (below α of 0.05) are highlighted in bold and indicated with asterisks: ** if $P < 0.01$, * if $P < 0.05$. Marginally significant P-values ($P < 0.01$) are indicated by •.*

	df		% Moisture			Total N		
Term	*between*	*within*	F	P		F	P	
Chamber	1	7	35.6	**< 0.001**	**	8.0	**0.025**	*
Fragmentation	3	42	13.9	**< 0.001**	**	1.10	0.367	
Chamber × Fragmentation	3	42	4.2	**0.011**	*	1.2	0.320	
Position	1	56	186.6	**< 0.001**	**	12.2	**0.001**	**
Chamber × Position	1	56	136.6	**< 0.001**	**	3.2	0.081	•
Fragmentation × Position	3	56	9.9	**< 0.001**	**	0.2	0.928	
Chamber × Fragmentation × Position	3	56	16.1	**< 0.001**	**	1.5	0.225	

position (see Table 5.1), but this appears to be driven largely by lower amounts in the inner chamber patches (Figure 5.2b).

Cyanobacteria

Based on multimodel selection results, we fitted a model including main effects of blocks, chamber, fragmentation, moisture, total available nitrogen, and a block × moisture interaction Figure 5.3. Cyanobacteria densities were not significantly

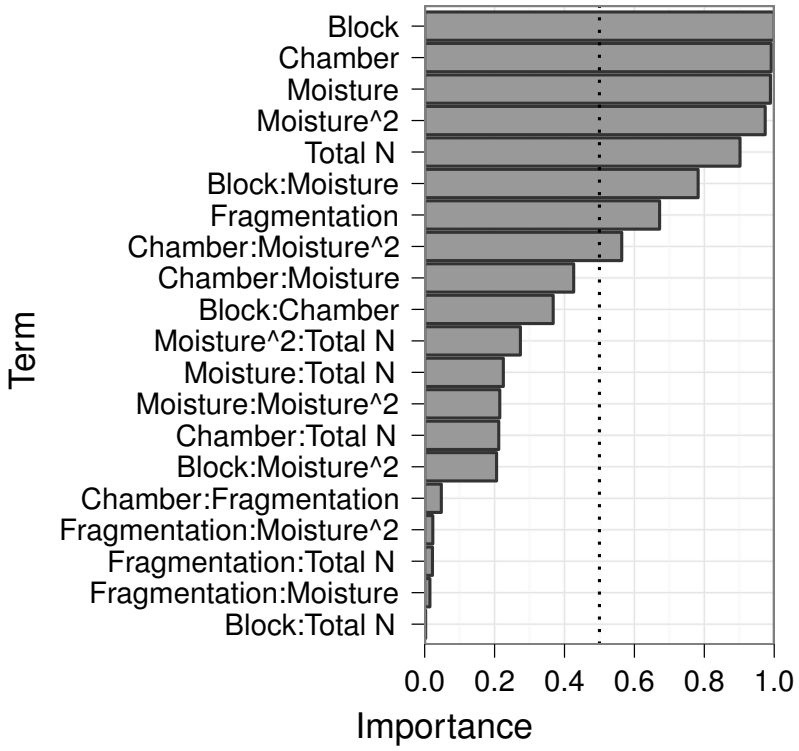

Figure 5.3

Model-averaged importance of terms used to explain cyanobacteria cell densities. Importance is calculated as the average term AIC weights over the 256 best models identified by 6 genetic algorithm searches of possible combinations of candidate model terms. The best model found included all terms above 50% (Chamber:Moisture^2 and up).

related to either moisture or total available N (Figure 5.4). There is a suggested unimodal relationship between cyanobacteria density and moisture, but there is too much uncertainty around the predicted values to be confident (Figure 5.4a). The "pure" effect of moisture on cyanobacteria density was also non-significant (Figure 5.5a). Total N had a marginally significant, but positive effect on cyanobacteria density, after removing effects of other variables (Figure 5.5b).

Nitrogen-fixation

A genetic algorithm search of combinations of candidate terms and two-way interactions revealed that most improved the model fit, and were present in a majority of the 256 best models found (Figure 5.6). The best model found by the search included nearly all 2-way interaction terms, with the exception of: block $\times$ total N, cyanobacteria cell density $\times$ moisture (and the quadratic term), and moisture^2 $\times$ total N. We decided not to consider interactions between chambers

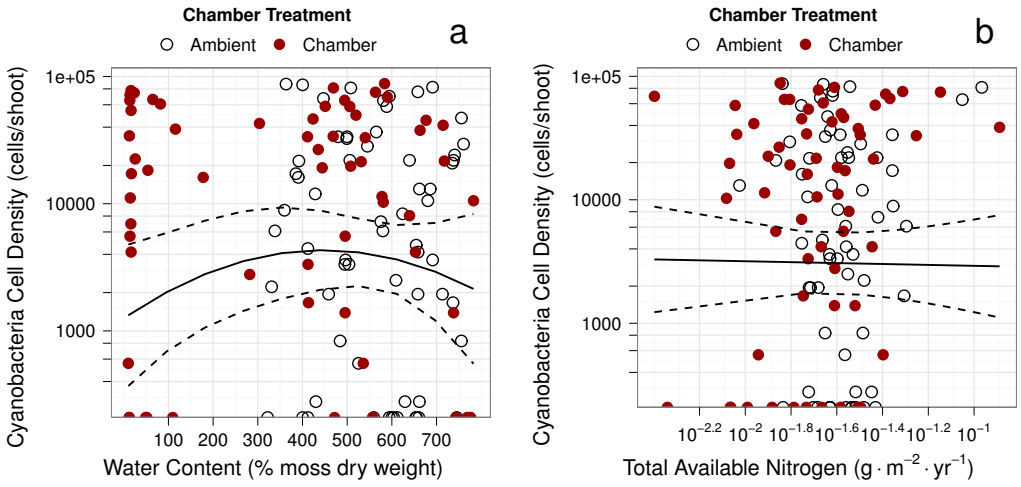

Figure 5.4

Cyanobacteria cell density as a function of (a) moisture content, and (b) total nitrogen (N) in the moss layer. Cyanobacteria densities were not significantly related to either variable, although there is a suggested unimodal relationship with moisture.

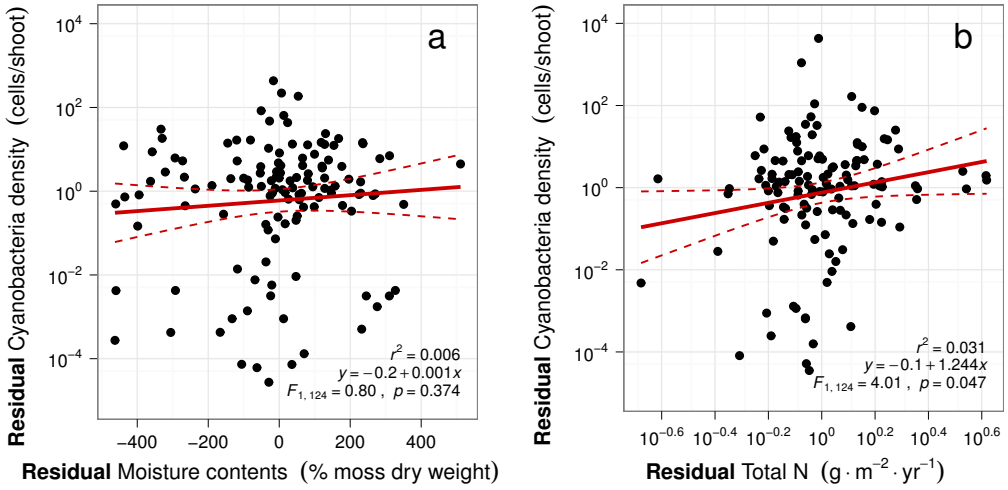

Figure 5.5

Partial regressions of cyanobacteria density as a function of (a) moisture content, and (b) total nitrogen (N) in the moss layer. Effects of blocks, chambers, fragmentation, block × moisture interactions, and each explanatory variable were accounted for and removed as part of the partial regressions. Cyanobacteria densities were not significantly affected by moisture, but there is a borderline significant positive effect of total nitrogen on cyanobacteria density, after removing other effects.

and continuous variables, due to the confounding absence of dry patches in ambient treatments (see Methods section), and we also excluded interactions between blocks and cyanobacteria density, and blocks with total N. We kept the block $\times$ moisture interaction terms, which significantly improved the model fit, but did not consider the others. The remaining interaction terms also suggested a three-way interaction between blocks, chambers, and fragmentation treatments, which also significantly improved model fit.

The final model we used to explore effects on N-fixation associated with *Pleurozium schreberi*, and for partial regressions, consisted of the following terms:

- Block
- Chamber
- Fragmentation (Isolation)
- Moisture
- Moisture^2
- Cyanobacteria cell density
- Total available N
- Block $\times$ Chamber
- Block $\times$ Fragmentation
- Chamber $\times$ Fragmentation
- Block $\times$ Moisture
- Block $\times$ Moisture^2
- Fragmentation $\times$ Moisture
- Fragmentation $\times$ Moisture^2
- Fragmentation $\times$ Cyanobacteria density
- Fragmentation $\times$ Total N
- Block $\times$ Chamber $\times$ Fragmentation

Although the model included many terms and interactions, we present only fitted values for the terms most relevant to the hypotheses of interest (see Methods section). Blocks were different on average, but these site-level differences are to be expected and not the focus of this research: they are accounted for in the model, allowing a clearer examination of other factors affecting N-fixation rates at the patch

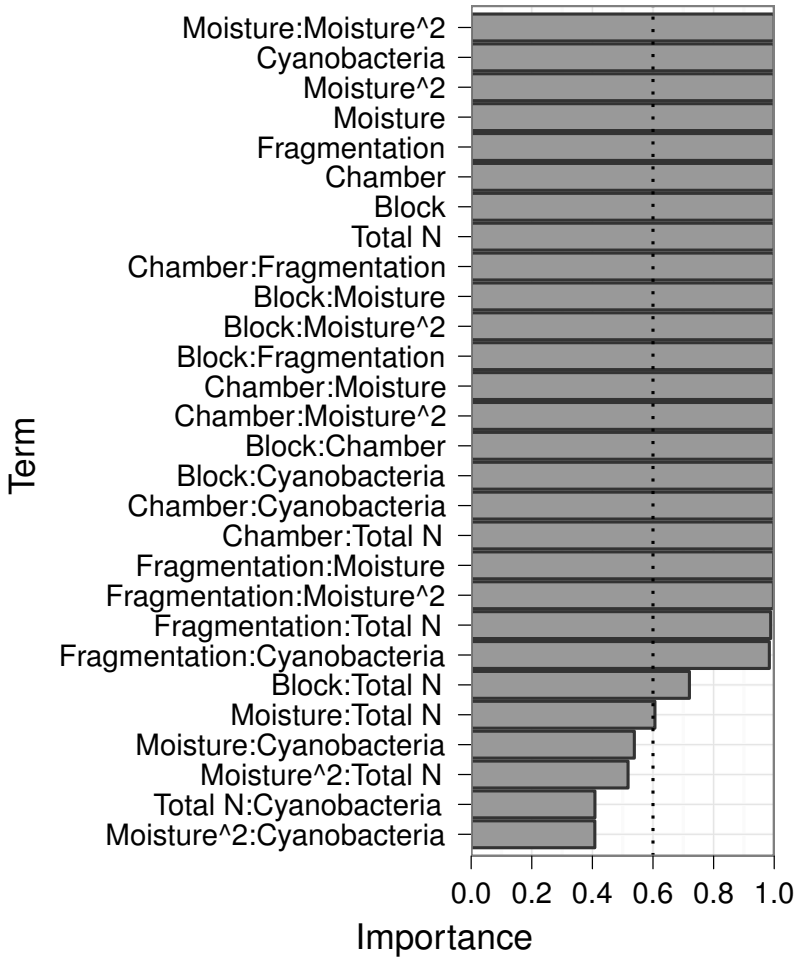

Figure 5.6

Model-averaged importance of terms used to explain N-fixation rates. Importance is calculated as the average term AIC weights over the 256 best models identified by 6 genetic algorithm searches of possible combinations of candidate model terms. The best model found included all terms above 60% (Moisture:Total N and up).

scale. We found no significant difference in N-fixation rates between chambers, although dry outer patches did reduce average N-fixation rates on average within chambers.

Moisture had a generally positive effect on N-fixation, with slight differences between fragmentation treatments (Figure 5.7a) There appears to be a threshold response to moisture, whereby N-fixation rates are reduced to 0 below about 300% moisture in the moss, but increases beyond this point (Figure 5.7a). Apart from interactions with cyanobacteria cell density and Total N, moisture was generally an important term in model fitting, and also explained nearly 65% of residual variation, after removing effects all other model terms (Figure 5.8a).

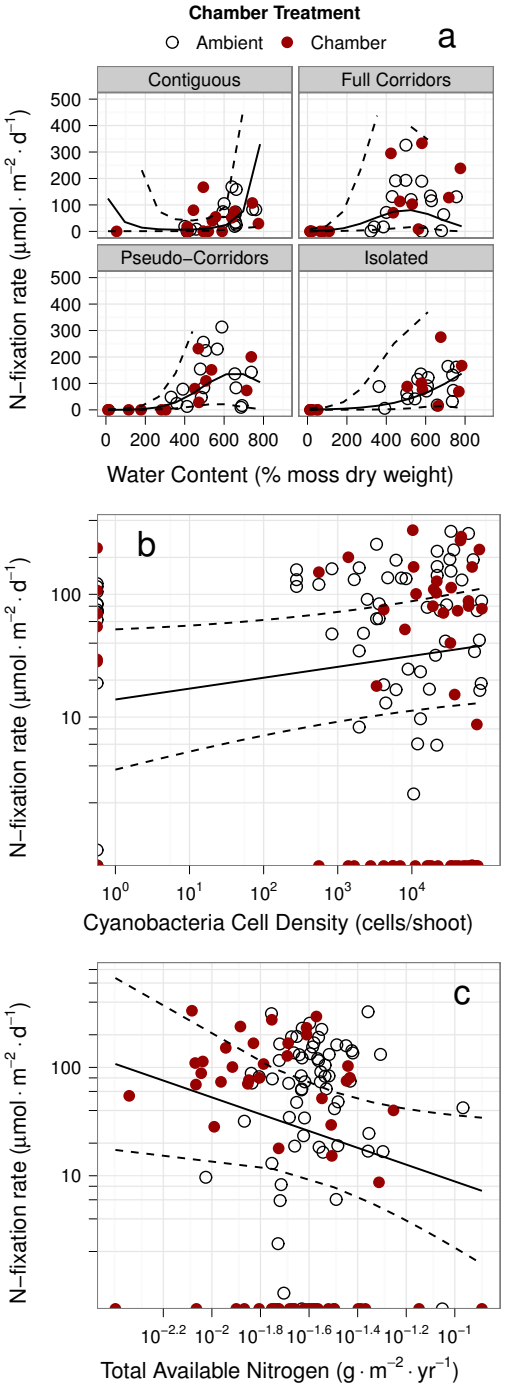

Figure 5.7

N-fixation rates as a function of (a) moisture content, by fragmentation treatment, (b) cyanobacteria cell density, and (c) total nitrogen (N) in the moss layer. The N-fixation axis was not log-transformed for response to moisture in order to better show the fit with small values of both variables, and the combination of linear and quadratic terms for moisture (on log-transformed N-fixation rates). N-fixation rates were lower on average in contiguous patches, while fitted values in pseudo- and full corridor patches suggest a possible unimodal response to moisture, with N-fixation rates no longer increasing at very high moisture levels.

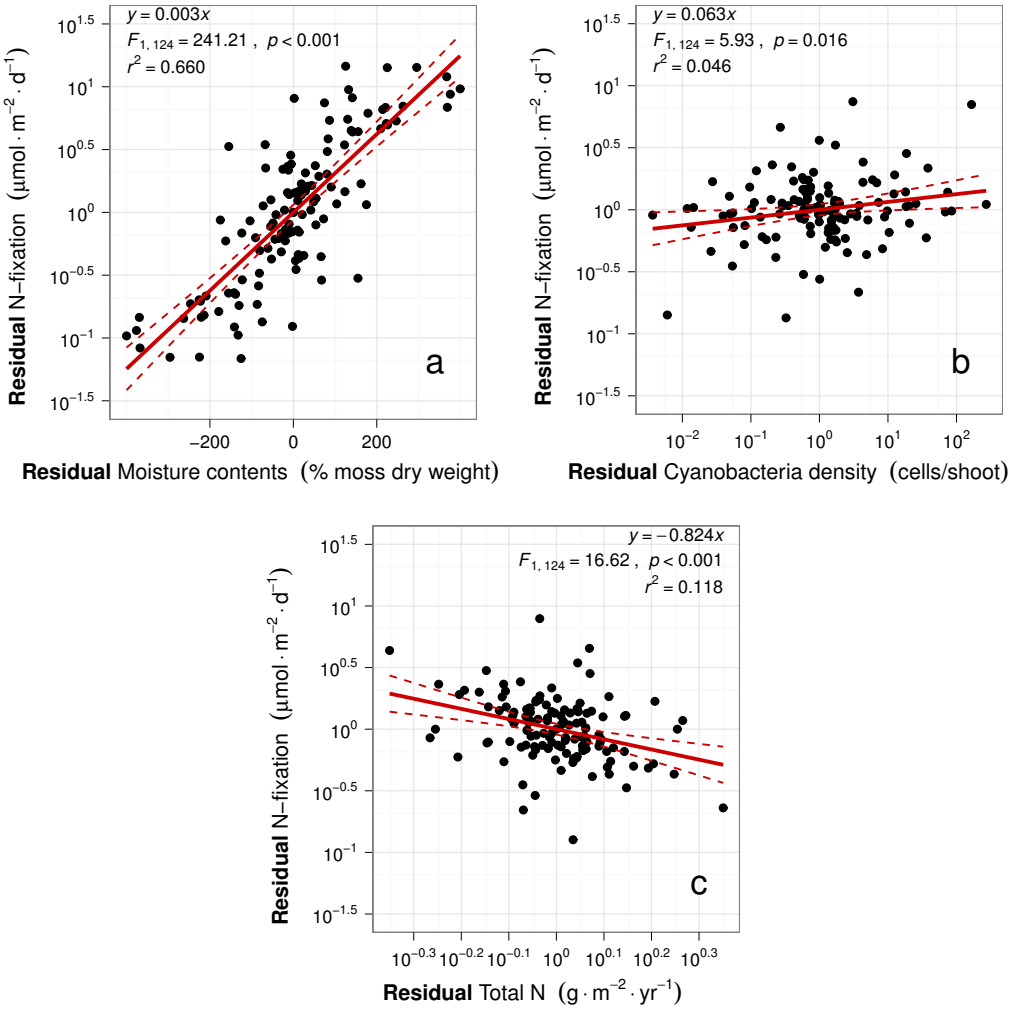

Figure 5.8

Partial regressions of N-fixation rates on (a) Moisture content of the moss, (b) cyanobacteria cell density, and (c) total N available in the moss layer, after accounting for other variables in the model (block, chamber, fragmentation, and important interactions - see text). A positive effect of moisture accounts for the largest proportion of the residual variance, followed by a negative effect of available N, and a small, but statistically significant positive effect of cyanobacteria density. The effect of moisture may be nonlinear (sigmoidal), suggesting a possible threshold response, and a maximal level beyond which moisture is no longer limiting.

Cyanobacteria density had an overall positive, but very weak effect on N-fixation rates (Figure 5.7b), accounting for only 6.5% of residual variation (Figure 5.8b). N-fixation rates were negatively affected by total available N (Figure 5.7c), and accounted for 10.8% of residual variation (Figure 5.8c).

Moss growth

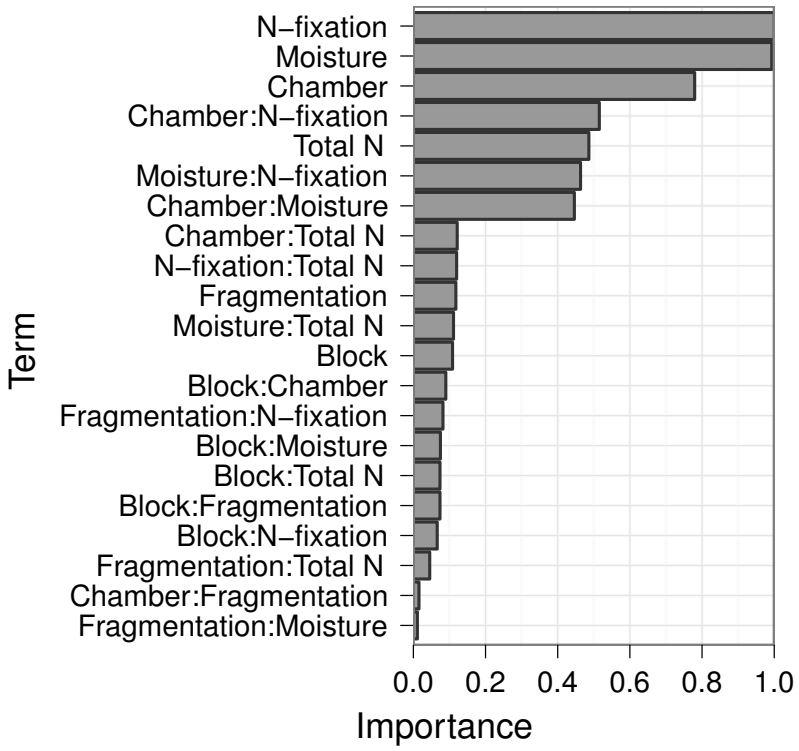

Figure 5.9
Model-averaged importance of terms used to explain Pleurozium schreberi growth rates. Importance is calculated as the average term AIC weights over the 256 best models identified by 6 genetic algorithm searches of possible combinations of candidate model terms. The best model found included only Moisture and N-fixation, which were highly correlated; removing N-fixation from consideration led to a best model that included Chamber, Moisture, and their interaction.

A genetic algorithm search suggested that very few terms improved model fit with respect to moss growth rates, unlike N-fixation rates. Moisture and N-fixation rates alone were deemed to be the only terms necessary in the "best" model found by the search algorithm, although Chamber, and interactions with moisture and N-fixation were also common in the best models found (Figure 5.9). Because N-fixation rates are highly influenced by moisture (see above), we also ran a search algorithm without N-fixation as a candidate term. This suggested a best

model that included chambers, moisture, and their interaction were also important. Nevertheless, we did not include interactions between chambers and moisture in the final model, due to a lack of dry patches in the ambient treatments, which resulted in unrealistic predicted values for such non-existent patches.

The final model we used to explore effects on growth rates of *Pleurozium schreberi*, and for partial regressions, consisted of the following terms:

- Block
- Chamber
- Fragmentation (Isolation)
- Moisture
- N-fixation rates
- Chamber × Moisture
- Chamber × N-fixation
- N-fixation × Moisture
- Chamber × Moisture × N-fixation

Both moisture and N-fixation appear to have overall positive effects on growth rates of the feather moss *Pleurozium schreberi* (Figure 5.11), at least within chambers (Figure 5.10). Total available N had no detectable effect on *P. schreberi* growth rates (Figures 5.10c, 5.11c).

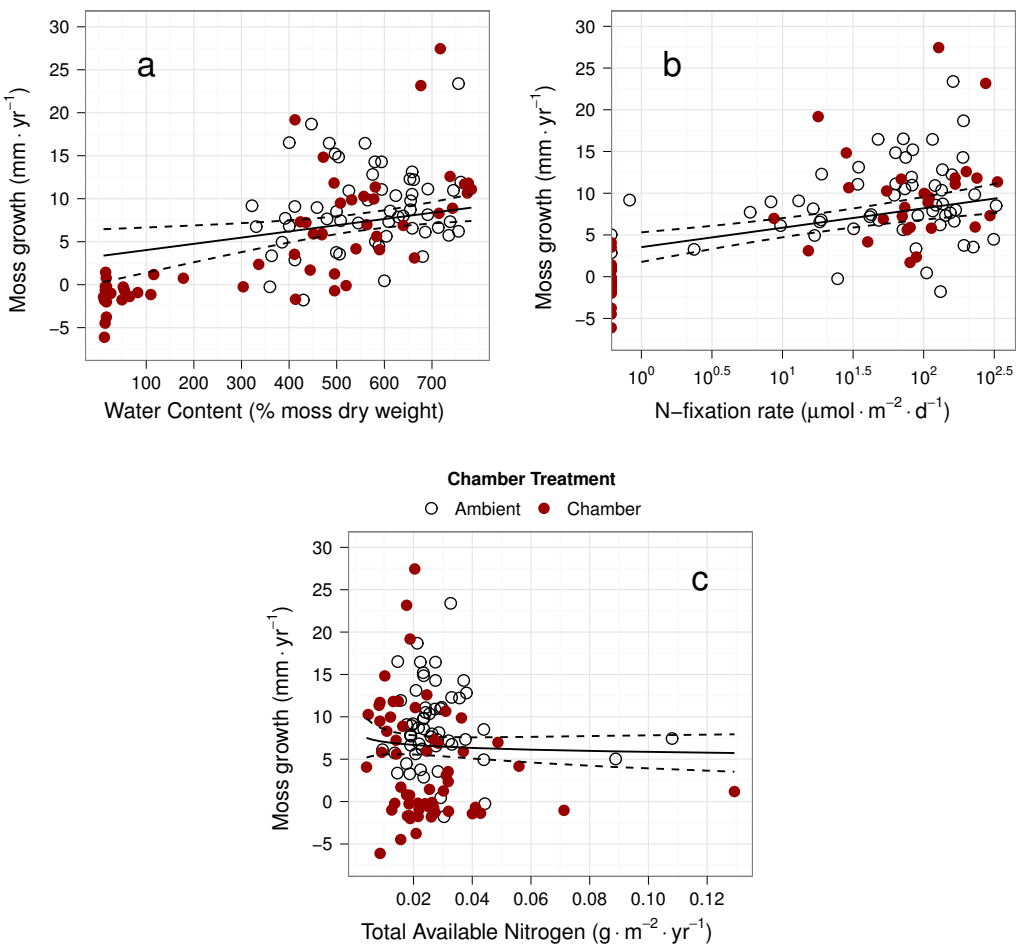

Figure 5.10

Predicted values of Pleurozium schreberi moss growth rates from our fitted model, as a function of (a) moisture content of moss, (b) N-fixation rates, and (c) total available N. As with N-fixation rates, the effect of moisture may be non-linear, with a threshold response near 300%, above which moss growth increases.

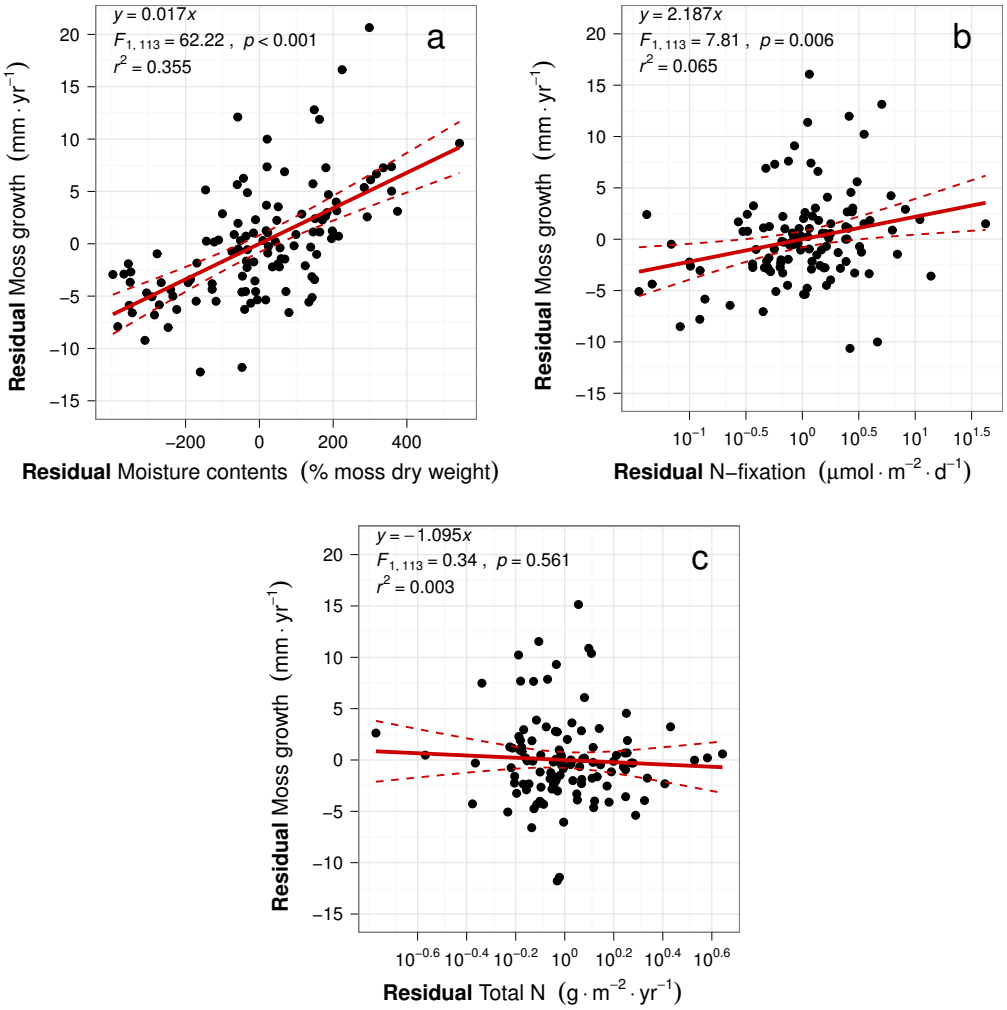

Figure 5.11

Partial regressions of moss growth rates on (a) Moisture content of the moss, (b) N-fixation rates, and (c) total N available in the moss layer, after accounting for other variables in the model (block, chamber, fragmentation, and important interactions - see text). The effect of N-fixation was not *included in the partial regression on moisture, given the established positive effect of moisture on N-fixation. The effect of moisture was included in other partial regressions. A positive effect of moisture accounts for the largest proportion of the residual variance. A positive effect of N-fixation remains significant (after removing the effect of moisture), while there is no statistically significant effect of total N on moss growth rates.*

5.4 Discussion

Our results were consistent with several of our hypotheses, and highlight the overall importance of moisture as a key environmental factor regulating biotic nitrogen-fixation and moss growth within the bryosphere. Non-linear threshold responses to moisture bear further exploration, and could affect predictions of biotic nitrogen-fixation under projected patterns of precipitation associated with climate change.

The test of our first hypothesis revealed unexpected patterns in available nitrogen across experimental treatments (H1). Total nitrogen available in the moss layer was lower across inner, compared to all outer patches, though perhaps only within chambers (Figure 5.2b). This pattern is very different than that observed in other measured variables in this experiment, which tended to be lower in the dry *outer* chamber patches (see chapters 3, 4, and 6).

Given that the ion resin capsules adsorb ions through direct contact with the water film in the surrounding substrate, we would have expected the dry patches to have adsorbed far less than others. This could be explained by dry moss releasing nutrients, including nitrogen during re-wetting (Bewley, 1995; Turetsky, 2003). Dry patches were occasionally flooded during spring snowmelt, and contiguous patches were able to wick moisture from adjacent wet patches, which may have caused releases of nitrogen from moss tissues. Nevertheless, dry outer chamber patches appear to have similar levels of available nitrogen as ambient patches, suggesting no effect of drought or moisture on total nitrogen available in the moss layer.

The difference in mean available nitrogen between all inner and outer patches, though statistically significant, amounted to approximately $10\,\mathrm{mg}\cdot\mathrm{m}^{-2}\cdot\mathrm{yr}^{-1}$, which is less than 10% of the range of observed values. Sloped chamber walls preventing direct precipitation should have also prevented nitrogen deposition; it is possible that they did, but that drought and re-wetting cycles released enough nitrogen from the moss in dry outer patches, but not in the inner chamber patches. Taking into account the overall negative effect of total N on rates of N-fixation (Figure 5.8c), the observed differences in mean total N between treatments may only translate to a 1% difference in N-fixation rates, and may therefore not be biologically significant. The overall variation in total N, however, was able to explain about 10% of the residual variation in N-fixation rates (Figure 5.8c), further supporting the hypothesis that N-fixation is down-regulated by available nitrogen (H7), though not as a result of changes in cyanobacteria density (H3, H4).

Contrary to our predictions (H2, H3), we found no effect of moisture

on cyanobacteria cell densities, and a possible positive effect of total available nitrogen, once effects of other variables had been removed (Figure 5.5). This differs from nitrostatic relationships observed in previous experiments (DeLuca *et al.*, 2007), although our result is not as strong as other positive relationships mediated by a fertilizing effect on overall bryophyte biomass (Menge and Hedin, 2009). The patterns in nitrogen-fixation observed in this experiment appear to be due to physiological responses of cyanobacteria, rather than changes in population abundance. These cyanobacteria communities are heavily dominated by *Stigonema*, suggesting that there was little turnover in composition over large ranges of total density (see also chapter 3).

N-fixation rates were positively, but weakly related to cyanobacteria cell density (Figure 5.7b), as predicted (H4), although the strength of relationship is lower than might have been expected from previous research using reciprocal transplant experiments between sites (DeLuca *et al.*, 2007). These results are consistent with the hypothesis that nitrogen fixation can respond physiologically at short time scales, without any demographic changes in cyanobacteria populations (discussed in chapter 3). Long-term N-fixation rates over an entire year may be more dependent on cyanobacteria abundance, which may change over several years, as reflected in forest stands of different ages (Zackrisson *et al.*, 2004; DeLuca *et al.*, 2007). N-fixation rates also change much more rapidly at shorter time scales within years or seasons (DeLuca *et al.*, 2002), irrespective of cyanobacteria density, in response to environmental factors such as temperature (Gentili *et al.*, 2005), moisture (Gundale *et al.*, 2009; Jackson *et al.*, 2010), and perhaps even available nitrogen.

Previous research has found negative relationships between available nitrogen and N-fixation rates across stands of different ages (Zackrisson *et al.*, 2004; DeLuca *et al.*, 2008), but also an overall positive effect of N-fertilization on N-fixation, mediated by increases in bryophyte abundance (Menge and Hedin, 2009). Transplanting moss patches between stands of different ages has suggested that, although moss may be capable of regulating cyanobiont density, other site-specific factors, including available nitrogen, may play a greater role in determining realized rates of N-fixation (DeLuca *et al.*, 2007). Our results confirm that available nitrogen can affect short-term N-fixation rates, after accounting for cyanobacteria density (Figure 5.7c).

This implies that nitrogen conditions within the moss layer may act both to down-regulate N-fixation at short time scales, and induce changes in cyanobacteria

densities over longer time scales. Effects of atmospheric nitrogen deposition on N-fixation rates and total ecosystem nitrogen supply may therefore depend as much on the variability of inputs, as well as the total amount. Consistent deposition of nitrogen may select against sustained N-fixation by cyanobionts, whereas irregular additions may be buffered by N-fixation during intervening periods, with cyanobionts being maintained in the system.

We found no significant evidence of an effect of passive warming by open top chambers on N-fixation rates (H6), although it is possible that N-fixation responds to temperature at even finer temporal scales than measured here (we only have direct temperature readings from 5 of the 8 blocks), or than caused by long-term warming. We did, however, find a strong effect of moisture on N-fixation rates (Figure 5.8), which increases monotonically, rather than the predicted unimodal relationship (H5). Nevertheless, some fitted values in our model do suggest that N-fixation rates may no longer increase at very high levels of moisture (Figure 5.7a)

Our data suggests a threshold response of both N-fixation and moss growth rates to moisture, whereby both are suppressed below about 300% moisture in the moss, but increases above this amount. Previous analyses have suggested a potential unimodal response to moisture, with N-fixation rates declining at very high moisture levels in excess of 800% (see chapter 3). The data presented here did not include such high moisture levels, which occurred primarily during the spring melt. High moisture in this experiment is therefore somewhat confounded with season, associated weather conditions, and other potential influences on N-fixation rates. Further research controlling for moisture availability and supply might clarify the sensitivity of N-fixation by cyanobacteria to predicted weather conditions, and variability of N-fixation rates in space and time (see Jackson *et al.*, 2010; Gundale *et al.*, 2012). If moisture effects are indeed nonlinear, with threshold effects on ecosystem processes, it would further complicate attempts to model and predict N-fixation under a suite of environmental conditions.

We also predicted that fragmentation would increase the severity of drought (H8). Our results instead suggest lower overall rates of N-fixation in contiguous patches, and a consistent effect of drought, with no detectable N-fixation below 300% moisture (Figure 5.7a). Lower N-fixation rates in contiguous patches may be an artefact of less disturbance when isolating the patches during set-up of the experiment; there is anecdotal evidence that such disturbance can trigger higher than ambient rates of N-fixation in *Pleurozium schreberi* (T. DeLuca, pers. comm.).

As predicted (H9), *Pleurozium schreberi* growth rates were positively affected

by moisture (Figure 5.11a), with negative growth rates observed in the driest patches (Figure 5.10a). We found no significant effect of passive warming by the chambers on moss growth rates (H10), nor of habitat fragmentation (H12), which had very low model-averaged weights in the multi-model analysis (Figure 5.9). The effect of moisture again appeared to display a threshold response: almost no growth occurred below 300% moisture, which only occurred within chambers (Figure 5.10a).

We also detected a significantly positive effect of N-fixation rates on moss growth, after removing the effect of moisture (Figure 5.8b), but no pure effect of available nitrogen, even after removing effects of other variables (Figure 5.11c). We also found little evidence for an interaction between N-fixation rates and available N, which is contrary to our predictions (H11). We had expected that moss growth rates would respond more to a combined effect, where high rates of N-fixation might compensate for low levels of available N, thus reducing nitrogen limitation on primary production. This seems particularly reasonable given that N-fixation is negatively related to available N (Figure 5.8c). The overall positive effect of N-fixation, and lack of a response to available N suggests that nitrogen may be limiting across the entire study area. Higher available nitrogen may still down-regulate N-fixation rates, but not enough to reduce the benefits to moss growth.

Moss photosynthesis may also feedback on N-fixation by providing more photosynthates to fuel N-fixation by cyanobacteria, or production of moss biomass as habitat for cyanobionts (Menge and Hedin, 2009). If growth rates were too high, however, cyanobacteria would need to continually re-colonize moss tissues closer to the moss canopy, in order to access light and photosynthates from active tissues. The potential for both negative and positive feedbacks between moss growth and N-fixation suggests that they may form a self-regulating system. There may be a balance of forces between light, nutrient, and moisture limitation that controls N-fixation rates directly (Turetsky, 2003; Gundale *et al.*, 2009; Lindo and Gonzalez, 2010), but may be modified by the physical conditions created by moss habitat as it grows vertically (see Lindo and Gonzalez, 2010). At larger site or landscape scales, however, the total area of moss cover, and dispersal of cyanobionts may play a greater role in determining stand-level N-fixation rates and nitrogen supply, which would appear as a positive feedback, limited only by environmental controls such as moisture, nutrients, and canopy cover (see Menge and Hedin, 2009).

These results underscore the importance of moisture to ecosystem processes within the bryosphere. Drought is a major limiting factor for many processes by reducing biological activity, the supply of nutrients other than water (e.g., N-

fixation by cyanobacteria), in addition to direct impacts on primary production. Drought may therefore also have long-term indirect effects on moss productivity, by limiting nutrient availability and increasing variability. Drought may also reduce decomposition rates, but less than productivity, with important implications for the carbon balance of boreal forest stands (see chapter 4). The importance of moisture is particularly relevant for boreal forest regions that are predicted to experience more frequent, longer droughts (Grant *et al.*, 2006; Soja *et al.*, 2007), or other changes in precipitation variability (Girardin *et al.*, 2004; Logan *et al.*, 2011). Even in areas where precipitation is predicted to increase, temperature increases may still lead to temperature-induced droughts by enhancing evapotranspiration (Soja *et al.*, 2007).

Conclusion

We were able to detect subtle positive effects of cyanobacteria density on N-fixation rates, and a negative effect of available nitrogen, supporting a nitrostatic understanding of N-fixation associated with boreal forest moss. We also found evidence for a direct benefit of cyanobacterial association for the host moss, in the form of increased growth rates. Overall, however, our results confirm the importance of moisture as a key environmental control on both N-fixation rates and *Pleurozium schreberi* growth in boreal forests. We also add that these effects may be nonlinear, with possible threshold and unimodal responses of N-fixation and moss growth across a wide range of moisture levels. Temperature-induced droughts may have direct negative effects on a variety of ecosystem processes within boreal forest moss, but the implications for nutrient cycling also suggest the potential for large-scale and long-term indirect effects. Models and predictions of climate change effects on boreal forests should therefore consider the overall effects of moisture supply, in addition to changes in temperature, particularly within the moss layers of the forest floor.

Acknowledgements

This project was supported by funding from the Canada Research Chair program, awarded to AG. JAW was supported by an NSERC PGS-D scholarship, and received travel funding from Northern Scientific Training Program of Indian and Northern Affairs Canada (now Aboriginal and Northern Development Canada).

We thank Hélène Lalande, laboratory coordinator at McGill University's Soil Testing lab, for advice in designing the extraction protocol for the ion resin capsules, and for

analyzing extractants for nitrogen compounds. Thanks to Zoë Lindo for help in the field, and many fruitful discussions about moss, cyanobacteria, nitrogen cycling, and soil ecology. Thanks to Mitran Mehta for help counting cyanobacteria, and Oksana Choulik for logistical support in Schefferville.

References

Bergman B, Rai A, Rasmussen U (2007) Cyanobacterial associations. In: Elmerich C, Newton WE (eds.) Nitrogen Fixation: Origins, Applications, and Research Progress, vol. 5, Springer Netherlands, Dordrecht, The Netherlands, pp 257–301

Bernier PY, Desjardins RL, Karimi-Zindashty Y, Worth D, Beaudoin A, Luo Y, Wang S (2011) Boreal lichen woodlands: A possible negative feedback to climate change in eastern North America. Agricultural and Forest Meteorology 151(4):521–528, doi:10.1016/j.agrformet.2010.12.013

Bewley JD (1995) Physiological aspects of desiccation tolerance - A retrospect. International Journal of Plant Sciences 156(4):393–403, doi:10.2307/2475057

Birkemoe T, Liengen T (2000) Does collembolan grazing influence nitrogen fixation by cyanobacteria in the high Arctic? Polar Biology 23(8):589–592, doi:10.1007/s003000000133

Bothe H, Ferguson SJ, Newton WE (2007) Biology of the Nitrogen Cycle. Elsevier, Amsterdam, The Netherlands

Calcagno V, de Mazancourt C (2010) glmulti: an R package for easy automated model selection with (generalized) linear models. Journal of Statistical Software 34(12):1–29

Chen IC, Hill JK, Ohlemüller R, Roy DB, Thomas CD (2011) Rapid range shifts of species associated with high levels of climate warming. Science 333(6045):1024–1026, doi:10.1126/Science.1206432

Cleveland CC, Townsend AR, Schimel DS, Fisher H, Howarth RW, Hedin LO, Perakis SS, Latty EF, Von Fischer JC, Elseroad A, Wasson MF (1999) Global patterns of terrestrial biological nitrogen (N-2) fixation in natural

ecosystems. Global Biogeochemical Cycles 13(2):623–645, doi:10.1029/1999GB900014

Clymo RS (1970) The growth of sphagnum: methods of measurement. The Journal of Ecology 58(1):13–49

Davidson EA, Janssens IA (2006) Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440(7081):165–173, doi:10.1038/Nature04514

DeLuca T, Zackrisson O, Gentili F, Sellstedt A, Nilsson MC (2007) Ecosystem controls on nitrogen fixation in boreal feather moss communities. Oecologia 152(1):121–130, doi:10.1007/s00442-006-0626-6

DeLuca TH, Zackrisson O, Nilsson MC, Sellstedt A (2002) Quantifying nitrogen-fixation in feather moss carpets of boreal forests. Nature 419(6910):917–920, doi:10.1038/nature01051

DeLuca TH, Zackrisson O, Gundale MJ, Nilsson MC (2008) Ecosystem feedbacks and nitrogen fixation in boreal forests. Science 320(5880):1181, doi:10.1126/science.1154836

Elmerich C, Newton WE (2007) Nitrogen Fixation: Origins, Applications, and Research Progress, Life Sciences, Agriculture, Plant Sciences and Forestry, vol. 5. Springer Netherlands, Dordrecht, The Netherlands, URL: http://www.springerlink.com/content/q66652/

Eugster W, Rouse WR, Pielke Sr RA, McFadden JP, Baldocchi DD, Kittel TGF, Chapin FS, Liston GE, Vidale PL, Vaganov E, Chambers S (2000) Land-atmosphere energy exchange in Arctic tundra and boreal forest: available data and feedbacks to climate. Global Change Biology 6(S1):84–115, doi:10.1046/j.1365-2486.2000.06015.x

Ewers RM, Didham RK (2006) Confounding factors in the detection of species responses to habitat fragmentation. Biological Reviews 81(1):117–142, doi:10.1017/s1464793105006949

Fox J (2003) Effect displays in R for generalised linear models. Journal of Statistical Software 8(15):1–27

Frolking S (1997) Sensitivity of spruce/moss boreal forest net ecosystem productivity to seasonal anomalies in weather. Journal of Geophysical Research-Atmospheres 102(D24):29,053–29,064, doi:10.1029/96JD03707

Gentili F, Nilsson MC, Zackrisson O, DeLuca TH, Sellstedt A (2005) Physiological and molecular diversity of feather moss associative N2-fixing cyanobacteria. Journal of Experimental Botany 56(422):3121–3127, doi:10.1093/jxb/eri309

Girardin MP, Tardif J, Flannigan MD, Wotton BM, Bergeron Y (2004) Trends and periodicities in the Canadian Drought Code and their relationships with atmospheric circulation for the southern Canadian boreal forest. Canadian Journal of Forest Research 34(1):103–119, doi:10.1139/x03-195

Grant RF, Black TA, Gaumont-Guay D, Kljun N, Barr AG, Morgenstern K, Nesic Z (2006) Net ecosystem productivity of boreal aspen forests under drought and climate change: Mathematical modelling with Ecosys. Agricultural and Forest Meteorology 140(1-4):152–170, doi:10.1016/j.agrformet.2006.01.012

Gundale MJ, Gustafsson H, Nilsson MC (2009) The sensitivity of nitrogen fixation by a feathermoss-cyanobacteria association to litter and moisture variability in young and old boreal forests. Canadian Journal of Forest Research 39(12):2542–2549, doi:10.1139/X09-160

Gundale MJ, Wardle DA, Nilsson MC (2010) Vascular plant removal effects on biological N fixation vary across a boreal forest island gradient. Ecology 91(6):1704–1714, doi:10.1890/09-0709.1

Gundale MJ, Wardle DA, Nilsson MC (2012) The effect of altered macroclimate on N-fixation by boreal feather mosses. Biology Letters 8(5):805–808, doi:10.1098/rsbl.2012.0429

Hardy RWF, Holsten RD, Jackson EK, Burns RC (1968) Acetylene-ethylene assay for N2 fixation – laboratory and field evaluation. Plant Physiology 43(8):1185–1207

Jackson BG, Martin P, Nilsson MC, Wardle DA (2010) Response of feather moss associated N2 fixation and litter decomposition to variations in simulated

rainfall intensity and frequency. Oikos 120(4):570–581, doi:10.1111/j.1600-0706.2010.18641.x

Janssens IA, Luyssaert S (2009) Carbon cycle: Nitrogen's carbon bonus. Nature Geosci 2(5):318–319, doi:10.1038/ngeo505

Johnson DW, Verburg PSJ, Arnone JA (2005) Soil extraction, ion exchange resin, and ion exchange membrane measures of soil mineral nitrogen during incubation of a tallgrass prairie soil. Soil Science Society of America Journal 69(1):260–265

Lagerström A, Nilsson MC, Zackrisson O, Wardle DA (2007) Ecosystem input of nitrogen through biological fixation in feather mosses during ecosystem retrogression. Functional Ecology 21(6):1027–1033, doi:10.1111/j.1365-2435.2007.01331.x

Leibold MA, Holyoak M, Mouquet N, Amarasekare P, Chase JM, Hoopes MF, Holt RD, Shurin JB, Law R, Tilman D, Loreau M, Gonzalez A (2004) The metacommunity concept: a framework for multi-scale community ecology. Ecology Letters 7(7):601–613, doi:10.1111/j.1461-0248.2004.00608.x

Lindner M, Maroschek M, Netherer S, Kremer A, Barbati A, Garcia-Gonzalo J, Seidl R, Delzon S, Corona P, Kolstrom M, Lexer MJ, Marchetti M (2010) Climate change impacts, adaptive capacity, and vulnerability of European forest ecosystems. Forest Ecology and Management 259(4):698–709, doi:10.1016/j.foreco.2009.09.023

Lindo Z, Gonzalez A (2010) The bryosphere: an integral and influential component of the Earth's biosphere. Ecosystems 13(4):612–627, doi:10.1007/s10021-010-9336-3

Lindo Z, Whiteley JA (2011) Old trees contribute bio-available nitrogen through canopy bryophytes. Plant and Soil 342(1-2):1–8, doi:10.1007/s11104-010-0678-6

Logan T, Charron I, Chaumont D, Houle D (2011) Atlas de sceÌĄnarios climatiques pour la foreÌĆt queÌĄbeÌĄcoise. Tech. Rep. ISBN papier : 976-2-923292-06-9 ISBN web : 978-2-923292-05-2, Ouranos et MRNF, URL: http://www.ouranos.ca/media/publication/162_AtlasForet2011.pdf

Loreau M, Mouquet N, Gonzalez A (2003) Biodiversity as spatial insurance in heterogeneous landscapes. Proceedings of the National Academy of Sciences of the United States of America 100(22):12,765 – 12,770, doi:10.1073/pnas.2235465100

Luyssaert S, Schulze ED, Boerner A, Knohl A, Hessenmoeller D, Law BE, Ciais P, Grace J (2008) Old-growth forests as global carbon sinks. Nature 455(7210):213–215, doi:10.1038/nature07276

Marion GM, Henry GHR, Freckman DW, Johnstone J, Jones G, Jones MH, Levesque E, Molau U, Molgaard P, Parsons AN, Svoboda J, Virginia RA (1997) Open-top designs for manipulating field temperature in high-latitude ecosystems. Global Change Biology 3(s1):20–32, doi:10.1111/j.1365-2486.1997.gcb136.x

Markham JH (2009) Variation in moss-associated nitrogen fixation in boreal forest stands. Oecologia 161(2):353–359, doi:10.1007/S00442-009-1391-0

Mcnabb DH, Geist JM (1979) Acetylene-reduction assay of symbiotic N2 fixation under field conditions. Ecology 60(5):1070–1072, doi:10.2307/1936873

Menge DNL, Hedin LO (2009) Nitrogen fixation in different biogeochemical niches along a 120 000-year chronosequence in New Zealand. Ecology 90(8):2190–2201, doi:10.1890/08-0877.1

Menge DNL, Levin SA, Hedin LO (2008) Evolutionary tradeoffs can select against nitrogen fixation and thereby maintain nitrogen limitation. Proceedings of the National Academy of Sciences of the United States of America 105(5):1573–1578, doi:10.1073/pnas.0711411105

Moore TR (1980) The nutrient status of subarctic woodland soils. Arctic and Alpine Research 12(2):147–160

Opdam P, Wascher D (2004) Climate change meets habitat fragmentation: linking landscape and biogeographical scale levels in research and conservation. Biological Conservation 117(3):285–297, doi:10.1016/j.biocon.2003.11.008

Parmesan C, Ryrholm N, Stefanescu C, Hill JK, Thomas CD, Descimon H, Huntley B, Kaila L, Kullberg J, Tammaru T, Tennent WJ, Thomas JA, Warren M

(1999) Poleward shifts in geographical ranges of butterfly species associated with regional warming. Nature 399(6736):579–583, doi:10.1038/21181

Pinheiro J, Bates D, DebRoy S, Sarkar D, R Development Core Team (2011) nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-98

R Development Core Team (2010) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria, URL: http://www.R-project.org/, ISBN 3-900051-07-0

Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier RY (1979) Generic assignments, strain histories and properties of pure cultures of cyanobacteria. Journal of General Microbiology 111(Mar):1–61

Sala OE, Chapin FS, Armesto JJ, Berlow E, Bloomfield J, Dirzo R, Huber-Sanwald E, Huenneke LF, Jackson RB, Kinzig A, Leemans R, Lodge DM, Mooney HA, Oesterheld M, Poff NL, Sykes MT, Walker BH, Walker M, Wall DH (2000) Global biodiversity scenarios for the year 2100. Science 287(5459):1770–1774, doi:10.1126/science.287.5459.1770

Schöllhorn R, Burris RH (1967) Acetylene as a competitive inhibitor of N2 fixation. Proceedings of the National Academy of Sciences of the United States of America 58(1):213–216, doi:10.1073/pnas.58.1.213

Skogley EO (1992) The universal bioavailability environment/soil test unibest. Communications in Soil Science and Plant Analysis 23(17):2225 – 2246, doi:10.1080/00103629209368736

Soja AJ, Tchebakova NM, French NHF, Flannigan MD, Shugart HH, Stocks BJ, Sukhinin AI, Parfenova EI, Chapin FS, Stackhouse PW (2007) Climate-induced boreal forest change: Predictions versus current observations. Global and Planetary Change 56(3-4):274–296, doi:10.1016/J.Glopacha.2006.07.028

Sokal RR, Rohlf FJ (1981) Biometry: The Principles and Practice of Statistics in Biological Research, 3rd edn. W. H. Freeman and Company, New York

Taggart RE, Cross AT (2009) Global greenhouse to icehouse and back again: the origin and future of the boreal forest biome. Global and Planetary Change 65(3-4):115–121, doi:10.1016/j.gloplacha.2008.10.014

Turetsky MR (2003) The role of bryophytes in carbon and nitrogen cycling. The Bryologist 106(3):395–409, doi:10.1639/05

Vitousek PM, Cassman K, Cleveland C, Crews T, Field CB, Grimm NB, Howarth RW, Marino R, Martinelli L, Rastetter EB, Sprent JI (2002) Towards an ecological understanding of biological nitrogen fixation. Biogeochemistry 57(1):1–45, doi:10.1023/A:1015798428743

Walther GR, Post E, Convey P, Menzel A, Parmesan C, Beebee TJC, Fromentin JM, Hoegh-Guldberg O, Bairlein F (2002) Ecological responses to recent climate change. Nature 416(6879):389–395, doi:10.1038/416389a

Watkinson AR, Gill JA (2002) Climate change and dispersal. In: Bullock JM, Kenward RE, Hails RS (eds.) Dispersal Ecology, Blackwell Science, Malden, MA, pp 410–428

Wickham H (2009) ggplot2: elegant graphics for data analysis. Springer New York, h.wickham@gmail.com, doi:10.1007/978-0-387-98141-3, URL: http://had.co.nz/ggplot2/book

Wiedermann MM, Gunnarsson U, Nilsson MB, Nordin A, Ericson L (2009) Can small-scale experiments predict ecosystem responses? An example from peatlands. Oikos 118(3):449–456, doi:10.1111/J.1600-0706.2008.17129.X

Zackrisson O, DeLuca TH, Nilsson MC, Sellstedt A, Berglund LM (2004) Nitrogen fixation increases with successional age in boreal forests. Ecology 85(12):3327–3334, doi:10.1890/04-0461

Zackrisson O, DeLuca T, Gentili F, Sellstedt A, Jäderlund A (2009) Nitrogen fixation in mixed Hylocomium splendens moss communities. Oecologia doi:10.1007/s00442-009-1299-8

Zuur AF, Ieno EN, Smith GM (2007) Analysing Ecological Data. Statistics for Biology and Health, Springer, New York, URL: http://www.highstat.com/book1.htm

5.5 Multi-model coefficient estimates

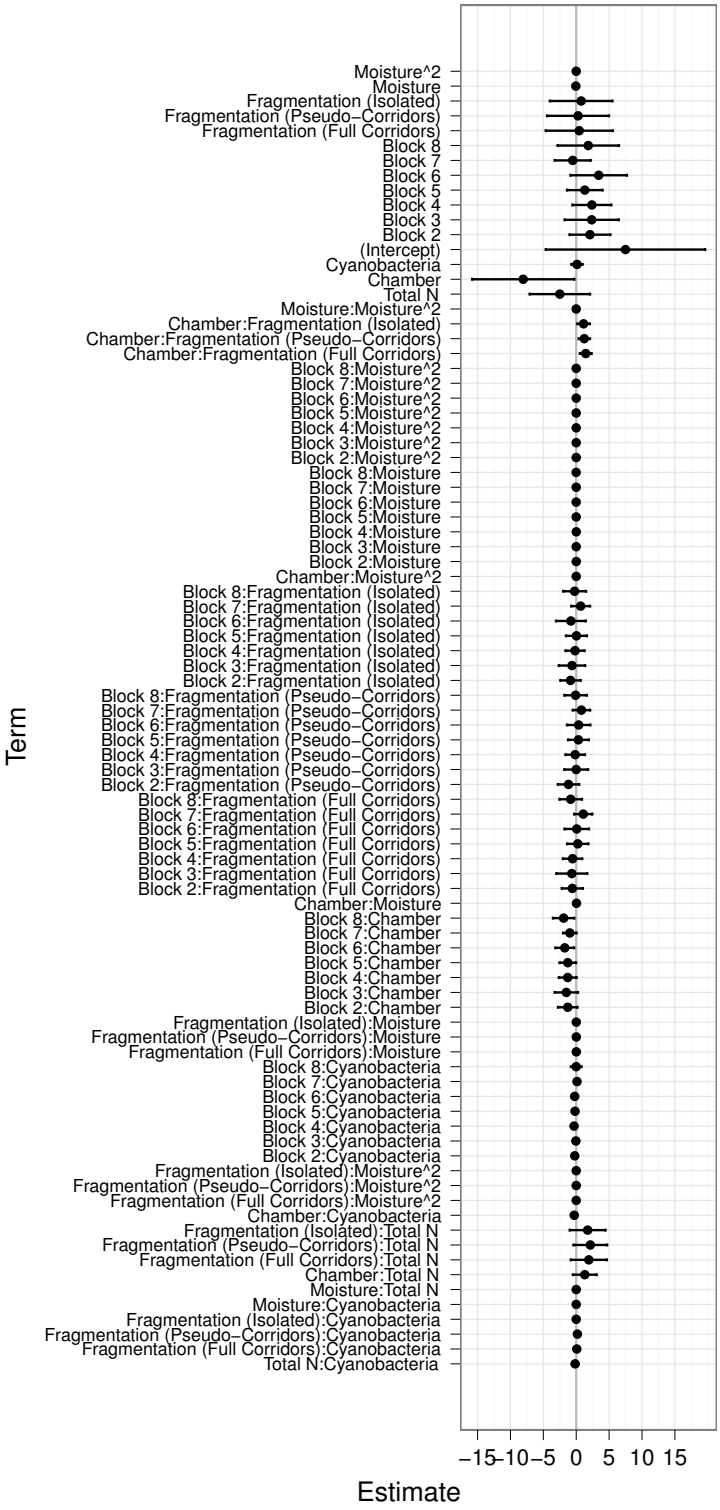

Figure 5.12
Estimates of coefficients and 95% confidence intervals for predictors of N-fixation rates, based on the 256 best models found using a genetic search algorithm of candidate model terms. Only coefficients with an "importance" of at least 50% are shown, which were included in the best model found by the search algorithm, and those included in the final model used for predictions and partial regressions.

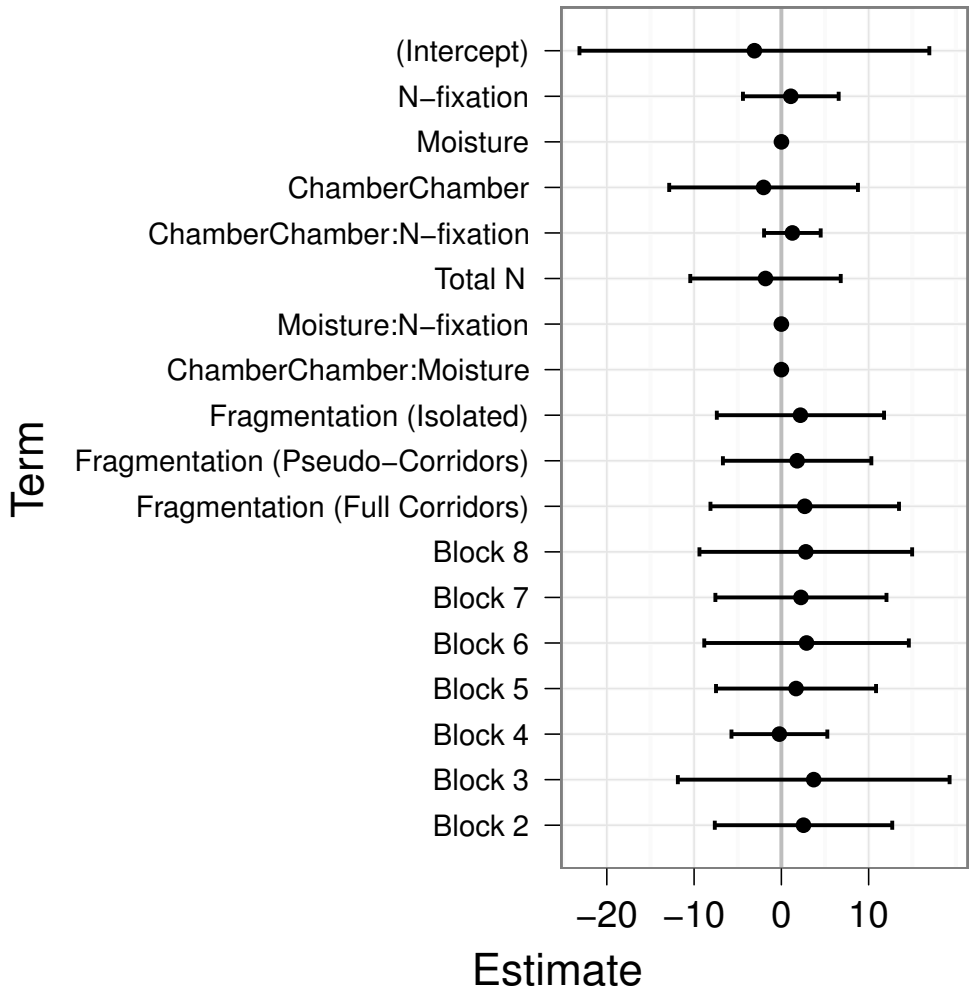

Figure 5.13
Estimates of coefficients and 95% confidence intervals for predictors of moss growth rates, based on the 256 best models found using a genetic search algorithm of candidate model terms. Only coefficients with an "importance" of at least 50% are shown, which were included in the best model found by the search algorithm, and those included in the final model used for predictions and partial regressions.

Connecting statement

Previous chapters have focused on the association between *Pleurozium schreberi* and N-fixing cyanobacteria (chapters 3 and 5), productivity and decomposition (chapters 4 and 5). In chapter 6, I explore effects of experimental treatments on microarthropod communities associated with *P. schreberi*, and test for evidence of possible top-down control of cyanobacteria densities by Collembola.

CHAPTER 6

Synergistic effects of habitat isolation and simulated climate change on a microarthropod community in boreal forest moss

Jonathan A. Whiteley[1], Zoë Lindo[2], and Andrew Gonzalez[1]

[1] Department of Biology, McGill University, 1205 Docteur Penfield, Montréal QC, Canada, H3A 1B1

[2] Department of Biology, Western University, London, Ontario, Canada, N6A 3K7

Keywords: bryosphere, climate change, habitat isolation, microarthropods, extinction

Abstract

The combined impacts of climate change and habitat fragmentation on biodiversity and community structure remain uncertain and difficult to predict. We applied passive warming, drought, and habitat isolation treatments in a two-year multifactorial field experiment to examine individual and interactive effects on a boreal forest microarthropod community associated with *Pleurozium schreberi*. We counted predatory prostigmatid and mesostigmatid mites, as well as Collembola, and tested for putative trophic interactions between predator, grazer guilds, and nitrogen-fixing cyanobacteria. We detected no effects of warming, or habitat isolation under ambient conditions. Microarthropod species richness and abundances declined in warm, dry patches, but was mitigated by increasing openness and connectivity to nearby wet patches. Community structure differed between isolated dry patches and other treatment combinations, primarily due to a subset of species able to persist in these patches. Species abundances were positively correlated overall, with the highest positive correlations between predator and grazer species in warm, dry, isolated patches. We found no evidence of trophic cascades, nor top-down regulation of nitrogen-fixing cyanobacteria densities. Environmental conditions therefore appear to exert stronger control over microarthropod community structure than biotic interactions, within the boreal forest bryosphere over a two-year period. Habitat connectivity did alleviate drought stress, suggesting interactive effects of climate change and habitat fragmentation, and underscores the importance of dispersal for mediating effects of environmental change, particularly over the long-term.

6.1 Introduction

Climate change and habitat fragmentation are two of the biggest drivers of global biodiversity loss (Sala *et al.*, 2000; Mantyka-Pringle *et al.*, 2012). Their combined impacts are of particular concern, because of their potential to cause synergistic extinctions, greater than would be predicted from each acting independently. Even species tolerant of a single stressor may be overcome by both. If species are correlated in their environmental tolerances, extinctions may be highly non-random, with important consequences for ecosystem processes. Furthermore, the lack of redundancy resulting from species co-tolerance could prevent functional compensation for extinctions by remaining species and profoundly limit the

resilience of ecosystems in the face of multiple stressors (Chapin *et al.*, 2000; Elmqvist *et al.*, 2003; Vinebrooke *et al.*, 2004).

Species exhibit a range of potential responses to climate change. They can: adapt to new conditions, disperse to track suitable environmental conditions, persist in a maladaptive state, go extinct, or not respond (Watkinson and Gill, 2002). Evolution to adapt to new conditions requires a large enough population with sufficient standing variation or mutation rates, and time to allow several generations of reproduction (Parmesan, 2006). Although evolutionary rescue is possible in theory (Gomulkiewicz and Holt, 1995), and laboratory conditions (Bell and Gonzalez, 2011), evidence suggests that the probability is still very low, except for large populations. Most species at risk are unlikely to have the opportunity to adapt to changing environmental conditions before going extinct at the current pace of climate change (Davis and Shaw, 2001; Burrows *et al.*, 2011).

Dispersal allows species to track the environmental conditions to which they are best adapted, even as those conditions move in space. This can occur at continental scales, as climate envelopes shift to higher latitudes and altitudes, but can also occur at smaller spatial scales, in the form of changes in microclimates (Watkinson and Gill, 2002). Large-scale shifts in species distributions have already been observed in responses to climate change (Walther *et al.*, 2002; Parmesan and Yohe, 2003; Parmesan, 2006; Chen *et al.*, 2011), and many more are expected (Thomas *et al.*, 2004). If a species' new optimal range is separated by their existing range by fragmented habitat, within an inhospitable matrix that is difficult to cross, dispersal is likely to be impeded, along with access to suitable habitat of sufficient quality and quantity. Habitat fragmentation and isolation are therefore seen as possible aggravating factors to climate change, potentially causing synergistic effects on biodiversity.

Despite theoretical work and simulation models (Travis, 2003; McInerny *et al.*, 2007), there are few experimental tests of the interactive effects of climate change and habitat isolation, particularly under field conditions. We present the results of a field experiment in the boreal forest of Northern Québec, Canada, which applied habitat isolation, mild warming, and prolonged drought treatments in a multifactorial design, to a community of bryophyte-associated microarthropods.

Passive warming has been observed to cause increases in microarthropod abundance (Kennedy, 1994; Coulson *et al.*, 1996; Mcgeoch *et al.*, 2006; Kardol *et al.*, 2011), except in combination with drought, which generally causes declines in both richness and abundance (Lindberg *et al.*, 2002; Kardol *et al.*, 2011).

Mcgeoch *et al.* (2006) found individualistic responses to drought, warming and shading in an Antarctic soil community, suggesting unpredictable patterns of extinction and community change under changes in climate over a single year. Chisholm *et al.* (2011) found fewer local and regional species richness and abundance in a greenhouse experiment, and that these patterns were affected by patch connectivity and spatial arrangement. This suggests that dispersal can significantly affect community response to environmental change, with the potential for synergistic effects between climate change, especially drought, and habitat connectivity.

Habitat fragmentation is known to cause species extinctions and reductions in density of microarthropods associated with bryophytes (Gilbert *et al.*, 1998; Gonzalez *et al.*, 1998; Gonzalez and Chaneton, 2002; Staddon *et al.*, 2010). Experiments have also consistently demonstrated that large-bodied, predatory microarthropods tend to be the most likely, and the earliest, bryofauna to disappear from isolated habitat patches (Gilbert *et al.*, 1998). Such species typically require more habitat area to meet foraging needs than other species, but are also motile enough to disperse across relatively long distances through inhospitable terrain.

There is general concern for the potential synergistic effects of habitat fragmentation and climate change, particularly if species responses are correlated across drivers (Vinebrooke *et al.*, 2004). This would lead to a greater number of extinctions under a combination of climate change and habitat isolation than would be expected from an additive combination of the effects of each driver measured individually. Furthermore, species extinctions may alter inter-specific interactions such as competition or predation, leading to potential indirect effects on community structure and function (Davis *et al.*, 1998; Brooker *et al.*, 2007). Although bryofauna are known to include a highly diverse and multitrophic community of species, specific interactions are often poorly understood (Lindo and Gonzalez, 2010). The relative importance of interspecific interactions and abiotic controls on species abundance remains a subject of debate (Houlahan *et al.*, 2007)

Although larger-bodied, predatory species are more negatively affected by habitat isolation, they may also be more drought-tolerant, depending on their level of sclerotization (Lindo *et al.*, 2012), and access to sufficient prey resources. On the other hand, smaller, less sclerotized taxa may be more tolerant to habitat isolation, due to smaller habitat requirements and shorter generation times, yet are also very intolerant of dessication. Species tolerant to one form of environmental stress or another are unlikely to be tolerant of multiple uncorrelated stresses, leading

to a suite of additional species lost when stressful conditions are experienced in combination (Brook *et al.*, 2008; Mantyka-Pringle *et al.*, 2012). Therefore, we predict that a combination of habitat isolation and climate stress would lead to non-additive species extinctions, particularly among large, predatory, well-sclerotized taxa.

6.2 Methods

Study site

The experiment was conducted in a boreal forest stand approximately 100×200 m, located 1.6 km southeast of the McGill Subarctic Research Station near the edge of the town of Schefferville, Québec, Canada, 54°47′44″N 66°47′20″W. Full details on the study site and experimental design are available in chapter 2.

Experimental Design

Climate and habitat isolation treatments were combined in a fully factorial nested design, to allow comparison of independent and interactive effects. Experimental meta-communities were constructed by cutting four patches out of the moss carpet on the forest floor. Each meta-community of four patches was arranged in one of three configurations: a single large contiguous patch, an equivalent area divided into four patches each connected by two corridors, and four isolated patches (Figure 6.1). Large patches were 25 cm in diameter (491 cm^2), while each of the four patches in the other treatments were 12.5 cm in diameter (122.7 $cm^2 \times 4 = 491$ cm^2 total metacommunity area).

Patches were isolated from the surrounding habitat matrix by placing moss into plastic pots in the same location as the source moss on the forest floor. The pots were 9 cm deep, and moss added was no deeper than 8 cm, leaving the surface of the bryosphere about 1 cm below the tops of the pots at the start of the experiment. Corridors were created by cutting and replacing a rectangle of moss 3×10 cm, lined with 6 mil polyethylene film along the sides, but open along the bottoms. Pseudo-corridors allowed a control for the extra habitat area provided by corridors, but with the same degree of isolation as the unconnected fragments (each patch and connected pseudo-corridor were isolated from the others in the same community).

The design of meta-communities was intended to disrupt faunal dispersal, without overly disrupting hydrologic characteristics of the bryosphere. However,

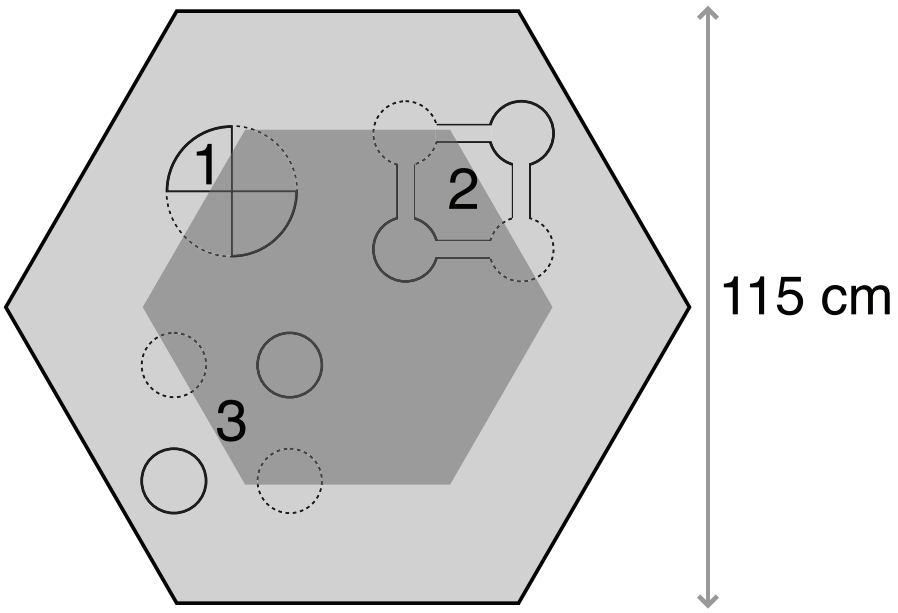

Figure 6.1
Diagram representing layout of fragmentation treatments inside chambers, to scale. Habitat isolation treatments, beginning with top-left: (1) Contiguous, (2) Corridors, (3) Isolated. The inner shaded hexagon shows approximate area open to vertical precipitation; the actual 'rain shadow' varies across chambers, depending on local slope, aspect, prevailing wind, and other small-scale differences in physical conditions.

drainage holes in the bottoms potentially did allow vertical faunal movement between patches and the underlying soil. Because corridors were also open along the bottom, the corridor and pseudo-corridor treatments are better interpreted as varying levels of "openness" to the surrounding habitat relative to the isolated patches, rather than closed systems of fragmented habitat. This type of isolation over a soil substrate is a notable difference between the type of patch isolation in this experiment, relative to similar field experiments performed on fragmented moss systems on a solid rock substrate (Gonzalez *et al.*, 1998; Gonzalez, 2000; Gonzalez and Chaneton, 2002).

Habitat isolation treatments were nested within simulated climate change treatments, across eight replicate locations (blocks) throughout the study area (see chapter 2). Due to time limitations, only data from a subset of four blocks was included in the analysis presented here.

We simulated climate change conditions with open-top chambers based on the design for those used by ITEX in tundra systems (Marion *et al.*, 1997). The chambers used in this experiment were hexagonal, with Sun-Lite fibreglass walls at a 60° angle, measuring 115 cm between walls at the base, 69 cm across at the top,

and 40 cm tall (full details in section 2.4).

Landscapes composed of one of each type of isolation treatment were randomly assigned to be covered by a full chamber, as described above, or an ambient control, with no chamber over top. All chamber treatments and landscapes were oriented along a North-South axis, to ensure consistent exposure to incoming solar radiation.

These chambers warmed the upper moss layer, 2 cm below the surface, an average of about 0.5 °C in the summers, and mild cooling in the winters ranging from -1.9 to +0.2 °C (see section 2.4 for full details). The walls of the open-top chambers also created a 'rain shadow' around the exterior of the chambers, such that the outer patches of each meta-community received very little precipitation, resulting in a prolonged drought, whereas inner patches received ambient levels of precipitation. Intermediate patches in between received intermediate levels of precipitation. Only inner and outer patches were included in the analysis presented here, due to time constraints.

Sample Collection & Identification

The entire microarthropod community was extracted from the bryosphere patches by heat extraction. Each patch was placed in a Tullgren funnel over vials of 70% ethanol for a period of 72 hours. Because heat extraction resulted in completely dry moss, the patches were weighed before and after extraction, to measure water content and total dry weight of the moss patch. The experiment was started in August 2007. Samples for the data presented here were collected in August 2009, after two continuous years of experimental treatments.

Enumerated microarthropods included Mesostigmata, predatory Prostigmata (members of the Bdellidae and Rhagidae families), and Collembola. Individuals were assigned to morphospecies (reliably identifiable operational taxonomic units) and identified to the lowest taxa possible, usually families, according to Krantz and Walter (2009). Immature individuals were grouped with the closest resembling adult taxa. Counts were also grouped into trophic levels: Predators include mesostigmatid mites, excluding fungivorous uropodid mites, as well as bdellid and rhagid prostigs; Grazers include uropodid mites and Collembola. We decided to focus on these groups, in part due to time constraints, and because we predicted the large-bodied predatory mites, and the unsclerotized Collembola with short generation times, would respond the most to temperature and drought (Lindberg *et al.*, 2002; Chisholm *et al.*, 2011; Kardol *et al.*, 2011) treatments.

Another taxa of interest in this system is nitrogen-fixing cyanobacteria, which are epiphytic on feather mosses, such as *Pleurozium schreberi* in the boreal forest. It is not known whether any bryofauna actively graze on these cyanobacteria, or exercise any kind of population regulation. Grazing effects by Collembola have been observed on cyanobacteria mats in the arctic (Birkemoe and Liengen, 2000), but those growing epiphytically on moss may enjoy a certain degree of protection from herbivory by growing under moss leaves, or in other parts of moss structure that are difficult to access by grazers (DeLuca *et al.*, 2002). We hypothesized a simple linear food chain with cyanobacteria as a primary producer, grazers that may feed on cyanobacteria (and other producers such as fungi or microbiota), and predatory mites that feed on these grazers and other species. The grazer community consisted of Collembola and fungivorous mesostigmatid mites, while the predator group included mesostigmatid mites, as well as bdellid and rhagid prostigs. Interactions between these faunal groups could have implications for nitrogen-fixation and nutrient cycling throughout the boreal forest, although there is no evidence for such interactions at present. No bryofauna or other species are known to graze on feather mosses in the boreal forest.

Statistical analyses

All statistical analyses were performed in the R statistical computing language (R Development Core Team, 2010), and all plots generated using the ggplot2 package (Wickham, 2009). We tested the response of single variables to experimental factors of habitat isolation, warming (chambers), and drought (the effect of *position* within *chambers*), within a nested analysis of variance (ANOVA) (Sokal and Rohlf, 1981; Crawley, 2007). Separate nested ANOVAs were applied to each response variable: observed species richness, community evenness, and density of both predators and grazers. Grazer densities were square-root transformed to satisfy certain assumptions of ANOVA: normal distribution of residuals and homogeneity of variances.

Where ANOVA revealed significant main or interaction effects, we performed multiple comparisons of factor levels, using a method similar to Tukey's HSD, calculating Minimum Significant Ranges (MSR) using Mean Squared Error (MSE) from the appropriate nesting level in the full ANOVA table (Sokal and Rohlf, 1981). These Minimum Significant Ranges are corrected for multiple comparisons relative to a confidence interval about each treatment mean. A graphical method was used to compare the density of each species in the most disturbed patches (*isolated outer*

chamber patches), relative to an appropriate control (e.g., *isolated inner chamber* patches, to identify difference in species-specific responses (Gonzalez *et al.*, 1998).

To measure the possible strength of trophic cascades, we also computed the spearman rank correlation coefficient for densities between putative trophic levels: predators, grazers, and cyanobacteria (producers). Cyanobacteria densities are reported in chapter 3. We predicted that drought stress combined with habitat isolation would reduce predator abundance faster than other levels, leading to a release of grazers, and a consequent reduction in cyanobacteria density. We therefore expected a positive correlation between predators and cyanobacteria, with both negatively correlated to grazers. There are certainly other taxa within each of these trophic levels other than what were enumerated in this study, particularly fungi, microbiota, nematodes, etc. Nevertheless, this is intended as an indirect way of inferring likely trophic relationships, within a system that is known to contain complex food webs, but for which many feeding relationships remain poorly studied (Lindo and Gonzalez, 2010).

Multivariate analyses of bryofauna communities were performed using the `vegan` package in `R v2.12` (Oksanen *et al.*, 2011). We explored multivariate relationships among samples using non-metric multidimensional scaling (nMDS). Species counts per gram dry weight of moss were log-transformed to reduce the influence of common, highly-abundant species (original values of 0 were kept as 0, rather than using a $log(x+1)$ transformation). We used the Bray-Curtis index of similarity (aka "percent similarity") on log-transformed densities in the nMDS analysis. The Bray-Curtis index is commonly-used and well-suited for ordination of ecological communities (Warwick and Clarke, 1991; Krebs, 1999; Whiteley and Bendell-Young, 2007; Borcard *et al.*, 2011).

Patterns of dissimilarity between communities in each combination of experimental factors were tested using Analysis of Similarity (ANOSIM; Clarke, 1993; Borcard *et al.*, 2011). The permutation-based ANOSIM approach is not able to test explicitly for interaction between main factors: it estimates the probability of observing a rank similarity within and between groups defined *a priori*, assuming a null hypothesis that all samples are from the same 'population' of sites (Warwick and Clarke, 1991; Clarke, 1993). We therefore employed a series of nested ANOSIM tests to assess whether differences between groups of one factor differed within levels of another factor (Whiteley and Bendell-Young, 2007). For example, we performed ANOSIM tests on each level of habitat isolation within each chamber treatment, to test for possible interactive effects of warming

and habitat isolation. The full suite of ANOSIM tests were performed on Bray-Curtis similarities of untransformed densities, log-transformed densities, as well as on Jaccard similarities of presence-absence data, to assess the sensitivity of the tests to differences in rare or common species. Untransformed data will place more weight on abundant species, whereas presence-absence data places equal weight on rare or abundant species.

6.3 Results

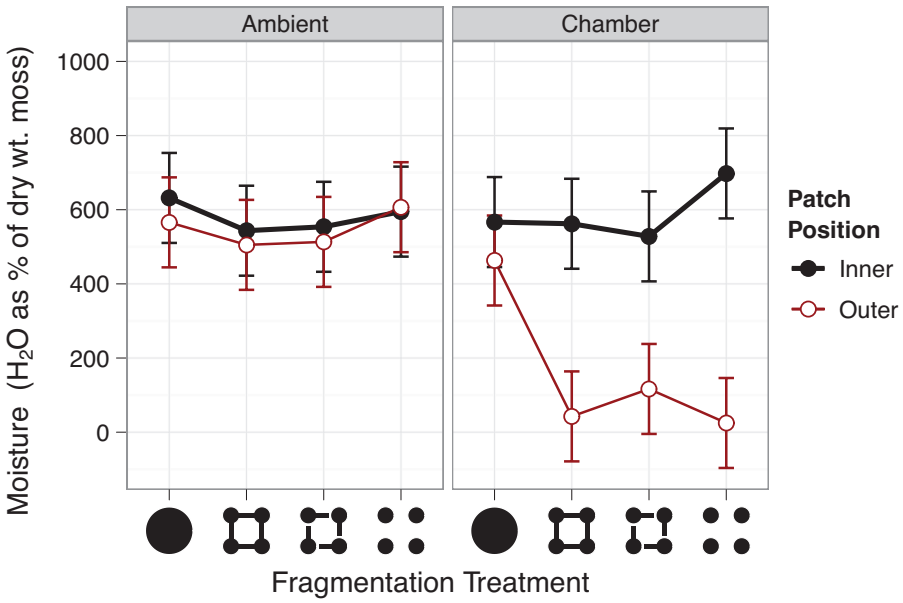

Figure 6.2
Average moisture contents of experimental patches, by habitat isolation and chamber treatments. Error bars represent 95% minimum significant differences, for multiple post hoc comparisons.

Outer chamber patches were significantly drier than inner chamber or ambient patches, with the exception of contiguous patches, which contained similar levels of moisture as inner or ambient patches (Figure 6.2). Analysis of variance revealed a statistically significant interaction between habitat isolation and position for all single variables of interest: Richness, Evenness, Predator density and Grazer density (Table 6.1). The three-way interaction between chamber, isolation, and position was never found to be significant at the 0.05 level, although the significance of multiple two-way interactions in most cases suggests that a lack of power may have prevented us from detecting the full three-way interaction effects.

Patches in the *outer chamber*, which experienced the most intense drought, also contained significantly fewer morphospecies (Figure 6.3), predators (Figure 6.4), and grazers (Figure 6.5). Results for evenness are not shown, but similar to those for morphospecies richness. More isolated patches also had fewer species than contiguous patches, but only in warm and dry *outer chamber* patches (Figure 6.3). Although this three-way interaction was not statistically significant according to the ANOVA, trends in the lower order terms are clearly driven by this underlying pattern, although large overall uncertainty at this scale may have obscured the signal.

There is a suggestion that isolation reduces predator density in the presence of drought (*outer chamber*) relative to *inner* and ambient patches (Figure 6.4). Habitat isolation is associated with fewer predators in chambers, relative to ambient conditions, driven largely by low predator densities in dry *outer chamber* patches, particularly in *isolated* and *corridor* patches. This further supports a three-way interaction despite a non-significant result for such a term in the ANOVA (Table 6.1). Although our results suggest higher predator density with increasing habitat isolation in ambient treatments, the overlap of comparison intervals means this may be a spurious pattern in our data.

Results for grazer density are roughly similar to those for predator density, with *outer chamber* patches in isolated or corridor treatments containing fewer than 5 individuals of any species per gram of moss by dry weight (Figure 6.5). Variability in grazer density was considerable, leading to large comparison intervals. Nevertheless, dry *outer isolated* patches contained fewer grazers than *inner* patches within chambers (Figure 6.5, right panel).

Drought in *isolated outer chamber* patches is associated in this experiment with greatly reduced densities of both predator and grazer species of microarthropods (Figure 6.6). A single species of *Entomobryidae* (a Collembola) was present in *outer* patches, but absent in *inner* patches, which is why it is the only species above the 1:1 correspondence line.

Predator and grazer species were generally positively correlated, most strongly within warmer and drier *outer chamber* patches. Grazers and cyanobacteria were also weakly positively correlated on average across most treatment combinations (Figure 6.7). Predators and cyanobacteria appear to be negatively correlated in some treatments, though perhaps uncorrelated overall.

Ordination of microarthropod communities revealed that most treatments were very similar to each other, except for warm, dry *outer chamber* patches in each

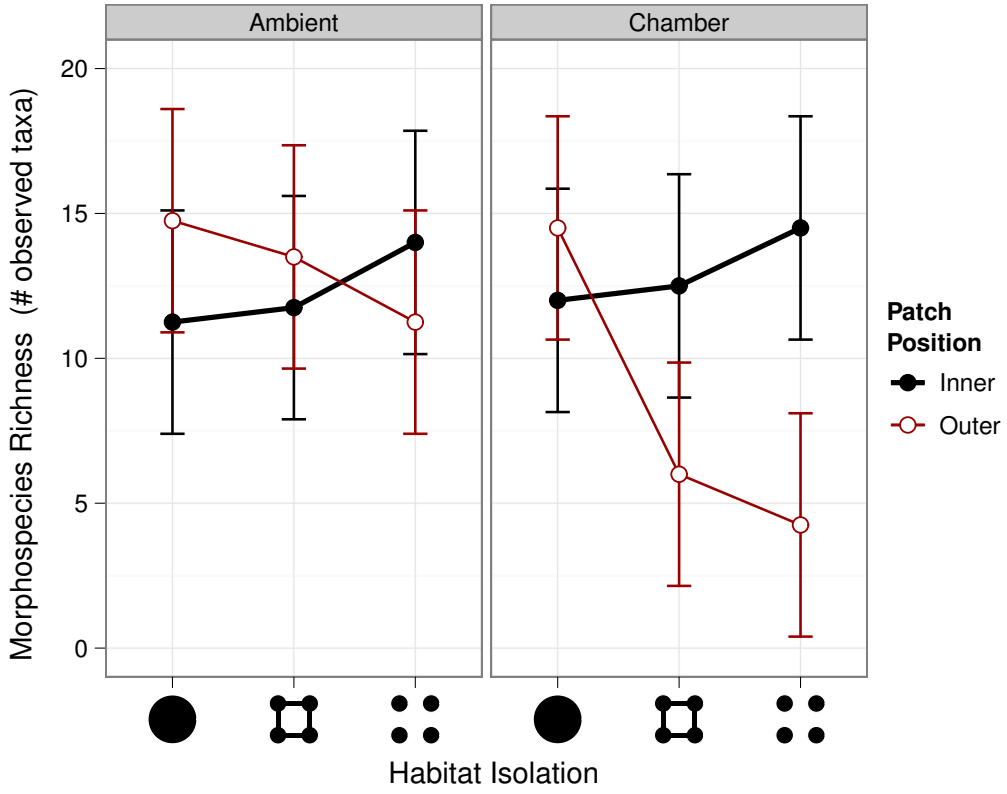

Figure 6.3

Microarthropod bryofauna morphospecies richness, of mesostigmatid mites and Collembola, by experimental treatments. Error bars represent 95% minimum significant differences, for multiple post hoc comparisons in each graph. There were significantly fewer taxa observed in dry outer chamber patches, with no difference detected among ambient or inner chamber treatments. Habitat isolation had no effect on taxonomic richness in inner patches, but had a negative effect on outer patches, with half the number of species in outer isolated *patches, than in* contiguous *or inner* isolated *patches within chambers. Although the three-way interaction between chamber, isolation, and position was not significant in the ANOVA model, it is likely that the differences detected among patch positions and isolation treatments are driven by differences between outer chamber patches and other experimental treatments. Similar patterns were observed for community evenness.*

isolation treatment (Figure 6.8). Outer contiguous patches in chambers appear to be as similar to inner patches in the same landscape as inner chamber patches across all isolation treatments. Increasing isolation, however, leads to increasing dissimilarity relative to *inner chamber* patches. These dry *outer chamber* patches in the more isolated treatments were characterized by a *Laelapidae* (sp.2) and *Entomobryidae* (sp.2). Three mesostigmatid mite species, and six Collembola species appeared to tolerate both drought and habitat isolation, with some less common Neelidae and Sminthuridae Collembola even present in the *outer isolated chamber* patches

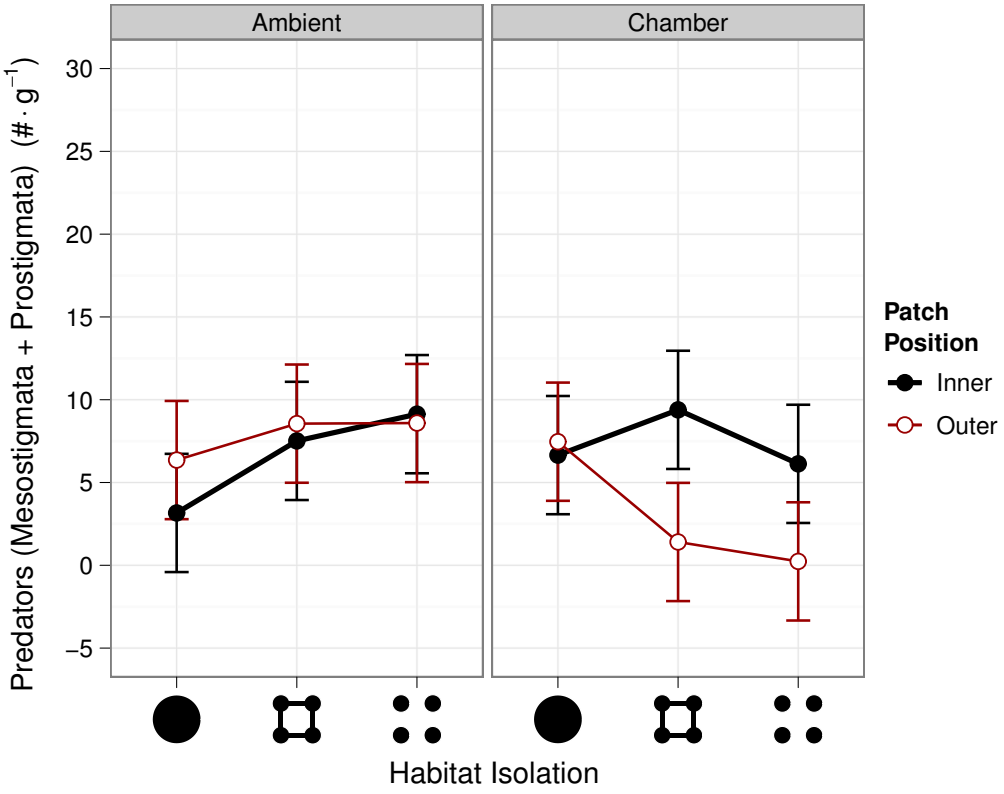

Figure 6.4

Bryofauna predator density by experimental treatment. "Predators" include predatory mesostigmatid mites and prostigmatid mites (members of the Bdellidae and Rhagidae groups). Error bars represent 95% minimum significant differences, for multiple post hoc comparisons in each graph. Compared with other treatments, roughly half the number of individuals per gram of moss were found in outer chamber patches. Although the nested ANOVA model did detect significant two-way interaction between habitat isolation and patch position, multiple comparisons did not clearly reveal which treatment levels might differ. Predator density appears to decrease with habitat isolation in outer or chamber patches. There is a suggestion that contiguous patches also had fewer predators than isolated patches in ambient treatments, but the only statistically significant difference is that there were half as many predators in isolated chamber patches (right panel).

(Figure 6.9). Some taxa, such as the *Zercon* sp. in our samples, were cosmopolitan throughout, although a few were unique to the warmer, drier conditions present in the *outer corridor chamber* patches, such as *Isotomidae* (sp. 4 & 5).

ANOSIM results confirm the patterns evident in the nMDS ordination: position treatments are significantly different within chambers, but not ambient treatments, whereas isolation was only significant among *outer chamber* patches (Table 6.2). The significance of an overall chamber effect is consistent with the differences between *outer* and *inner* patches within chambers, even though *inner*

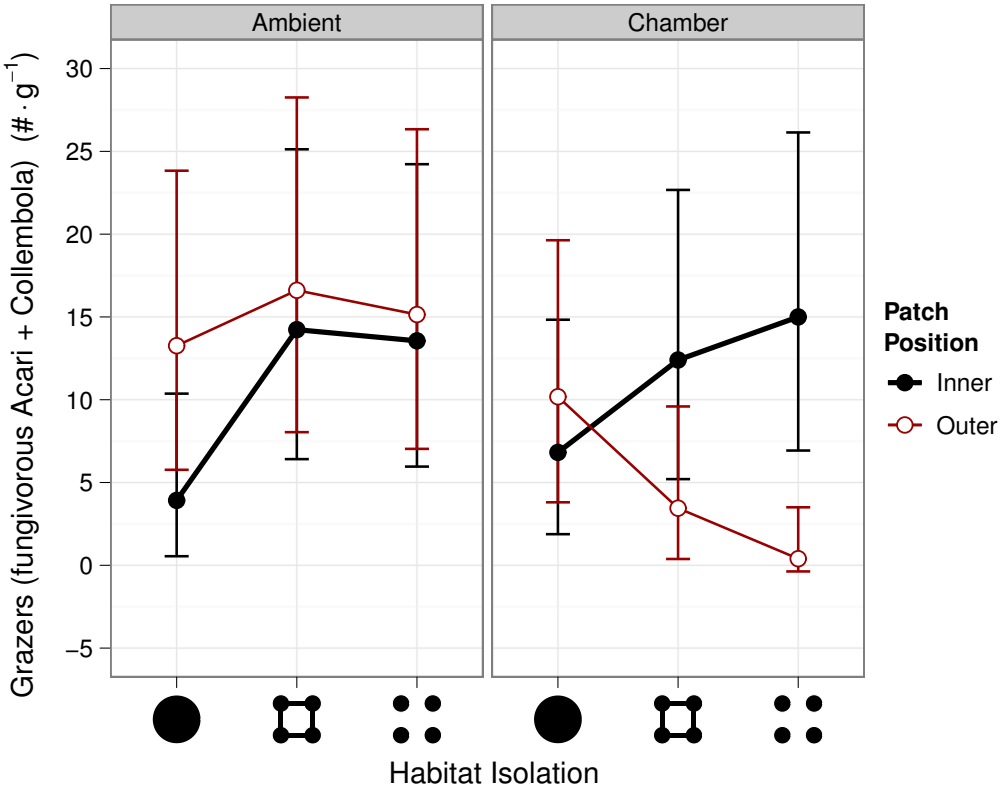

Figure 6.5

Bryofauna grazer density by experimental treatment. "Grazers" include fungivorous uropodine mites and Collembola. Error bars represent 95% minimum significant differences, for multiple post hoc comparisons in each graph. Compared with other treatments, less than half the number of individuals per gram of moss were found in outer chamber patches, particularly isolated and corridor treatments.

chamber patches are similar to ambient patches (see Figure 6.8).

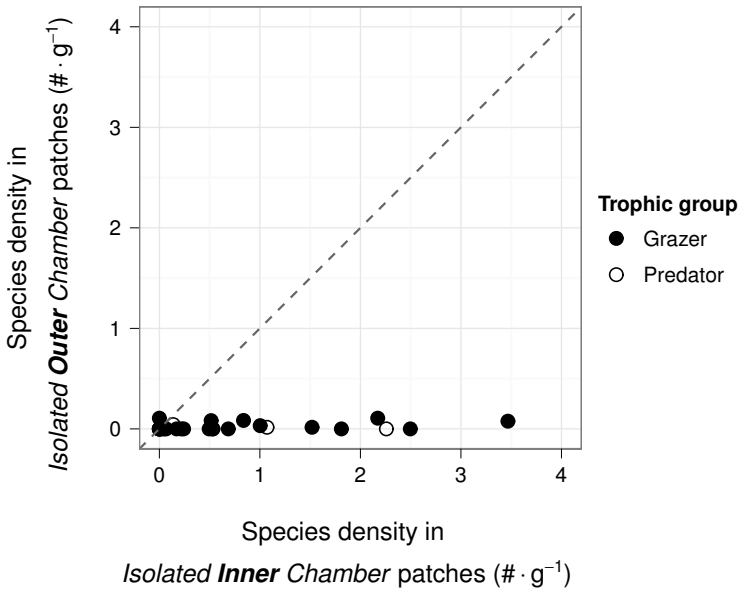

Figure 6.6

Densities of microarthropod morphospecies in isolated **outer** chamber *patches (warm and dry), relative to* isolated **inner** chamber *patches. The dashed line represents the 1:1 correspondence line: points above the line would be more abundant in warm, dry patches, while those below the line a more abundant in wetter inner patches. The only point above the line is a species of Entomobryidae that was absent in inner patches.*

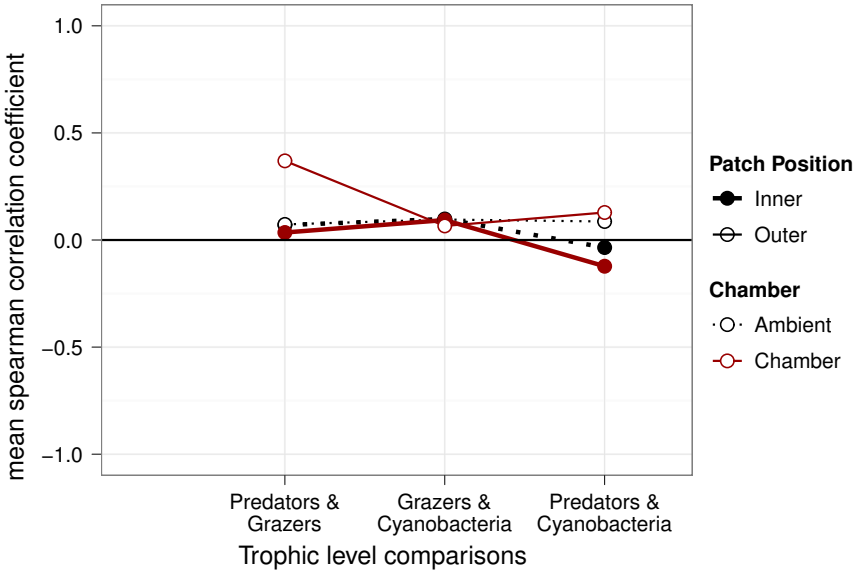

Figure 6.7

Average spearman rank correlation coefficients between species in each trophic group, across chamber and position treatments (isolation treatments were not compared, due to very small sample sizes: n = 4).

Table 6.1

*F and P-values, with relevant degrees of freedom (df), for nested analysis of variance (ANOVA) of main response variables. Significant P-values (below α of 0.05) are highlighted in bold and indicated with asterisks: ** if $P < 0.01$, * if $P < 0.05$. Marginally significant P-values ($0.05 < P < 0.1$) are indicated by •.*

	df		**Richness**			**Evenness**			**Predators**			**Grazers**		
Term	*between*	*within*	*F*	*P*		*F*	*P*		*F*	*P*		*F*	*P*	
Chamber	1	3	4.22	0.132		0.31	0.616		2.41	0.218		2.33	0.224	
Isolation	2	12	3.89	**0.050**	*	3.22	0.076	•	0.29	0.752		0.57	0.583	
Chamber × Isolation	2	12	2.66	0.111		0.36	0.702		6.17	**0.014**	*	2.03	0.174	
Position	1	18	5.50	**0.031**	*	9.71	**0.006**	**	4.07	0.059	•	1.78	0.199	
Chamber × Position	1	18	11.18	**0.004**	**	2.17	0.158		13.05	**0.002**	**	16.26	**0.001**	**
Isolation × Position	2	18	10.85	**0.001**	**	8.84	**0.002**	**	5.31	**0.015**	*	8.38	**0.003**	**
Chamber × Isolation × Position	2	18	1.90	0.178		1.71	0.209		1.54	0.241		1.65	0.219	

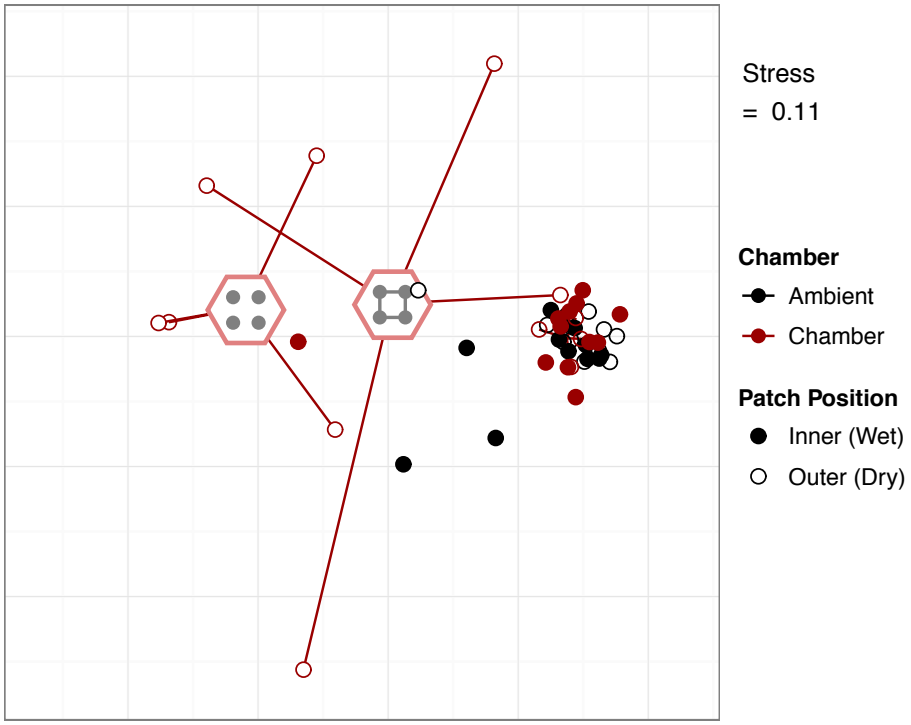

Figure 6.8
Non-metric Multidimensional Scaling (nMDS) ordination in 2-dimensions, for Bray-Curtis similarities of log-transformed microarthropod counts (#/g). Points for outer chamber *patches in* isolated *and* corridor *treatments have been joined by lines to their centroids, indicated by an icon for each treatment: other treatments are largely overlapping and clustered together. Stress = 0.11.*

Table 6.2

P-values for Analysis of Similarity (ANOSIM) among groups defined a priori, for a selection of distance metrics and transformations applied to the raw data. "Interaction terms" indicate where ANOSIM was performed on the last term, within the specified level of the first. e.g., "Outer Chamber $\times$ Isolation" indicates that ANOSIM was performed on Isolation treatments, among Outer Chamber patches.

Distance metric Transformation	Bray-Curtis -	Bray-Curtis $log(x+1)$	Jaccard presence-absence
Chamber	**0.028 ***	**0.012 ***	**0.014 ***
Position	0.189	0.154	0.167
Isolation	0.778	0.793	0.771
Chamber:*Ambient* $\times$ Position	0.553	0.573	0.571
Chamber:*Ambient* $\times$ Isolation	0.050 .	0.050 .	0.045 *
Chamber:*Chamber* $\times$ Position	**0.005 ** **	**0.008 ** **	**0.009 ** **
Chamber:*Chamber* $\times$ Isolation	0.107	0.093	0.102
Position:*Inner* $\times$ Isolation	0.272	0.264	0.262
Position:*Outer* $\times$ Isolation	0.285	0.273	0.310
Inner Chamber $\times$ Isolation	0.521	0.498	0.504
Outer Chamber $\times$ Isolation	**0.016 ***	**0.008 ** **	**0.015 ***

Figure 6.9

Occurrence of morphospecies within experimental treatment groups. Black cells indicated presence in at least one patch, while empty (white) cells indicate the taxon was not present in any patches in that treatment.

6.4 Discussion

These results show several interactive effects between climate and habitat isolation on the richness and abundance of bryofauna. While warming alone had no detectable effect on the bryofauna community, drought in *outer chamber* patches was associated with fewer species, especially in fully *isolated* patches. This suggests that habitat openness is able to mitigate species loss from severe drought stress, either by allowing dispersal of individuals into the patch, or by reducing the severity of the stress itself, as may be the case in contiguous patches (Gilbert *et al.*, 1998; Gonzalez *et al.*, 1998; Gonzalez and Chaneton, 2002). The overall effect demonstrates synergistic effects of drought and habitat isolation on microarthropod communities in the bryosphere.

The same pattern is evident for both predator and grazer densities within this system: both groups are similarly negatively affected by a combination of habitat isolation and drought, leading to a positive correlation in densities. The lack of strong negative correlations among any putative trophic levels considered in this experiment can be interpreted in two different ways. First, the trophic relationships between these groups may be weak, and these groups may not represent a strong trophic chain within the bryosphere. Second, the combined stress of drought and isolation may overwhelm any potential trophic cascades, such that mortality from environmental stress overwhelms those from consumption by higher trophic levels. These two interpretations are not mutually exclusive, although the second does not explain overall positive correlations within ambient or inner chamber patches. The observation of overall positive correlations in this system is consistent with larger meta-analyses that find generally positive, rather than negative correlations among most species, supporting the hypothesis that abiotic factors are more important for regulating populations than density-dependent factors such as competition or predation (Houlahan *et al.*, 2007).

The reduction in densities of predators and grazers explains the parallel reduction in richness observed in dry patches, as many species' densities are reduced to the point of extirpation. These losses also account for the observed differences in community composition. Despite broad similarities among communities in ambient patches and *inner chamber* patches, the *outer chamber* patches are more dissimilar from these large groups, with increasingly isolated patches showing greater dissimilarity to other patches, and more similarity to each other, as a result of common species persisting in the presence of both isolation and

drought.

Extinction did appear to be generally non-random, with the smaller, more abundant species being more likely to survive and be found in the warmer, drier, isolated patches (see Lindo *et al.*, 2012 for a full description of species traits associated with Oribatid mites in the experiment).

Contiguous patches seemed able to transfer moisture from adjacent wet patches by capillary action, thus maintaining similar overall levels of moisture as those patches receiving direct precipitation (personal observation). Although this 'wicking' maintained total moisture contents, we observed that it was only effective at alleviating drought stress in deeper moss layers, leaving the surface (moss canopy) still very dry (see chapter 4; Chisholm *et al.*, 2011). Nevertheless, from the perspective of microarthropods, the physical connectivity not only allows high dispersal of individuals between patches, but also of suitable moisture conditions.

Our results confirm the greater sensitivity of moss microarthropods to drought than warming (Hodkinson *et al.*, 1998; Kardol *et al.*, 2011). While neither isolation, nor warming alone elicited detectable changes in the bryofauna community, isolation does appear to be important in mediating the effects of drought. Fully isolated patches were isolated not only from nearby moss in terms of moisture, but also in terms of the ability of bryofauna to disperse laterally (vertical movement was not completely prevented in this experimental set-up). Outer chamber patches connected by corridors were just as dry as isolated patches (Figure 6.2), yet exhibited less biodiversity loss, and similar densities of bryofauna as inner patches. This demonstrates the synergistic effect of habitat isolation and climate change: more species are able to tolerate a single stress, than both in combination.

Environmental conditions, particularly moisture, appear to have been more important for regulating population densities in the bryosphere than trophic interactions, over the two years of this study. Although microarthropods may be adapted to frequent seasonal variations in moisture and temperature (Kardol *et al.*, 2011), it appears that many species share similar tolerances to drought stress, and are negatively affected as a whole community, rather than displaying potential for compensation. Over the longer-term, it is possible that interspecific interactions will become more important, leading to more indirect effects (Bender *et al.*, 1984; Menge, 1995). An 8-year experiment in boreal forest microarthropod communities found reduced abundances, but also marked differences in community structure: although most species declined in abundance, some increased and were more more common in drought treatments (Lindberg *et al.*, 2002). Compensatory

dynamics may require more time both for population growth rates to compensate for differential mortality rates, and for long-distance dispersal to supply phenotypes better-adapted to new prevailing environmental conditions. Compensation may also be more likely for smaller changes in environmental conditions, but ultimately depends on the distribution of traits and tolerances within a community (Norberg *et al.*, 2001; Gonzalez and Loreau, 2009; Kardol *et al.*, 2011; Lindo *et al.*, 2012).

These results demonstrate the importance of habitat connectivity for mitigating negative impacts of environmental change. This experiment was not designed along large-scale gradients, such as those at the continental scale that are expected to shift with projected climate change. Nevertheless, species can respond to climate change by relocating over relatively short distances, if they can find suitable microclimates that match their environmental tolerances (Watkinson and Gill, 2002). This experiment further supports the use of habitat corridors at landscape scales to facilitate dispersal and improve habitat quality and quantity to prevent species losses in the face of environmental change (Gilbert *et al.*, 1998; Rantalainen *et al.*, 2005; Williams *et al.*, 2005; Vos *et al.*, 2008; Samways *et al.*, 2010).

Long-term droughts, over areas much larger than in this experiment, may become more common in areas of the boreal forest under future climate change (Grant *et al.*, 2006; Soja *et al.*, 2007). Without adequate refugia, microarthropod densities may be negatively affected overall, with implications for soil processes such as decomposition (Seastedt, 1984; Rantalainen *et al.*, 2008). Such intense and long-term environmental change may be more likely to lead to wholesale community reorganization and sudden state shifts (Scheffer *et al.*, 2001; van Nes and Scheffer, 2004), than slow turnover or compensatory dynamics, particularly if there are no complementary species tolerant of drought conditions.

Conclusion

We combined habitat isolation with climate warming and drought in a multifactorial field experiment near the northern limit of the boreal forest. As predicted, moss-associated microarthropods were negatively affected by drought and warming in combination, which was mitigated by habitat openness. This confirms the synergistic effects of habitat isolation and environmental stress associated with climate change conditions. Maintaining habitat connectivity is therefore essential to maintaining biodiversity in the face of environmental change.

References

Bell G, Gonzalez A (2011) Adaptation and Evolutionary Rescue in Metapopulations Experiencing Environmental Deterioration. Science 332(6035):1327–1330, doi:10.1126/science.1203105

Bender EA, Case TJ, Gilpin ME (1984) Perturbation Experiments in Community Ecology: Theory and Practice. Ecology 65(1):1–13

Birkemoe T, Liengen T (2000) Does collembolan grazing influence nitrogen fixation by cyanobacteria in the high Arctic? Polar Biology 23(8):589–592, doi:10.1007/s003000000133

Borcard D, Gillet F, Legendre P (2011) Numerical Ecology with R. Use R!, Springer, New York, USA, URL: http://www.springer.com/statistics/life+sciences%2C+medicine+%26+health/book/978-1-4419-7975-9

Brook BW, Sodhi NS, Bradshaw CJA (2008) Synergies among extinction drivers under global change. Trends in Ecology & Evolution 23(8):453–460, doi:10.1016/j.tree.2008.03.011

Brooker RW, Travis JMJ, Clark EJ, Dytham C (2007) Modelling species' range shifts in a changing climate: The impacts of biotic interactions, dispersal distance and the rate of climate change. Journal of Theoretical Biology 245(1):59–65

Burrows MT, Schoeman DS, Buckley LB, Moore P, Poloczanska ES, Brander KM, Brown C, Bruno JF, Duarte CM, Halpern BS, Holding J, Kappel CV, Kiessling W, O'Connor MI, Pandolfi JM, Parmesan C, Schwing FB, Sydeman WJ, Richardson AJ (2011) The pace of shifting climate in marine and terrestrial ecosystems. Science 334(6056):652–655, doi:10.1126/science.1210288

Chapin FS, Zavaleta ES, Eviner VT, Naylor RL, Vitousek PM, Reynolds HL, Hooper DU, Lavorel S, Sala OE, Hobbie SE, Mack MC, Diaz S (2000) Consequences of changing biodiversity. Nature 405(6783):234–242

Chen IC, Hill JK, Ohlemüller R, Roy DB, Thomas CD (2011) Rapid range shifts of species associated with high levels of climate warming. Science 333(6045):1024–1026, doi:10.1126/Science.1206432

Chisholm C, Lindo Z, Gonzalez A (2011) Metacommunity diversity depends on connectivity and patch arrangement in heterogeneous habitat networks. Ecography 34(3):415–424, doi:10.1111/j.1600-0587.2010.06588.x

Clarke KR (1993) Non-parametric multivariate analysis of changes in community structure. Australian Journal of Ecology 18:117–143

Coulson SJ, Hodkinson ID, Webb NR, Block W, Bale JS, Strathdee AT, Worland MR, Wooley C (1996) Effects of experimental temperature elevation on high-arctic soil microarthropod populations. Polar Biology 16(2):147–153, doi:10.1007/bf02390435

Crawley MJ (2007) The R Book. John Wiley & Sons, Ltd., West Sussex, UK

Davis AJ, Jenkinson LS, Lawton JH, Shorrocks B, Wood S (1998) Making mistakes when predicting shifts in species range in response to global warming. Nature 391(6669):783–786

Davis MB, Shaw RG (2001) Range shifts and adaptive responses to Quaternary climate change. Science 292(5517):673–679

DeLuca TH, Zackrisson O, Nilsson MC, Sellstedt A (2002) Quantifying nitrogen-fixation in feather moss carpets of boreal forests. Nature 419(6910):917–920, doi:10.1038/nature01051

Elmqvist T, Folke C, Nystrom M, Peterson G, Bengtsson J, Walker B, Norberg J (2003) Response diversity, ecosystem change, and resilience. Frontiers in Ecology and the Environment 1(9):488–494

Gilbert F, Gonzalez A, Evans-Freke I (1998) Corridors maintain species richness in the fragmented landscapes of a microecosystem. Proceedings of the Royal Society B: Biological Sciences 265(1396):577–582

Gomulkiewicz R, Holt RD (1995) When Does Evolution by Natural-Selection Prevent Extinction. Evolution 49(1):201–207

Gonzalez A (2000) Community relaxation in fragmented landscapes: the relation between species richness, area and age. Ecology Letters 3(5):441–448

Gonzalez A, Chaneton EJ (2002) Heterotroph species extinction, abundance and biomass dynamics in an experimentally fragmented microecosystem. Journal of Animal Ecology 71(4):594–602

Gonzalez A, Loreau M (2009) The causes and consequences of compensatory dynamics in ecological communities. Annual Review of Ecology, Evolution, and Systematics 40(1):393–414, doi:10.1146/annurev.ecolsys.39.110707.173349

Gonzalez A, Lawton JH, Gilbert FS, Blackburn TM, Evans-Freke I (1998) Metapopulation dynamics, abundance, and distribution in a microecosystem. Science 281(5385):2045–2047

Grant RF, Black TA, Gaumont-Guay D, Kljun N, Barr AG, Morgenstern K, Nesic Z (2006) Net ecosystem productivity of boreal aspen forests under drought and climate change: Mathematical modelling with Ecosys. Agricultural and Forest Meteorology 140(1-4):152–170, doi:10.1016/j.agrformet.2006.01.012

Hodkinson ID, Webb NR, Bale JS, Block W, Coulson SJ, Strathdee AT (1998) Global change and Arctic ecosystems: Conclusions and predictions from experiments with terrestrial invertebrates on spitsbergen. Arctic and Alpine Research 30(3):306–313

Houlahan JE, Currie DJ, Cottenie K, Cumming GS, Ernest SKM, Findlay CS, Fuhlendorf SD, Gaedke U, Legendre P, Magnuson JJ, McArdle BH, Muldavin EH, Noble D, Russell R, Stevens RD, Willis TJ, Woiwod IP, Wondzell SM (2007) Compensatory dynamics are rare in natural ecological communities. Proceedings of the National Academy of Sciences of the United States of America 104(9):3273–3277, doi:10.1073/pnas.0603798104

Kardol P, Reynolds WN, Norby RJ, Classen AT (2011) Climate change effects on soil microarthropod abundance and community structure. Applied Soil Ecology 47(1):37–44, doi:10.1016/J.Apsoil.2010.11.001

Kennedy AD (1994) Simulated Climate-Change – a Field Manipulation Study of Polar Microarthropod Community Response to Global Warming. Ecography 17(2):131–140

Krantz GW, Walter DE (2009) A Manual of Acarology, 3rd edn. Texas Tech University Press, URL: http://books.google.ca/books?id=x00gAQAAMAAJ

Krebs CJ (1999) Ecological Methodology, 2nd edn. Benjamin/Cummings, Menlo Park, California

Lindberg N, Engtsson JB, Persson T (2002) Effects of experimental irrigation and drought on the composition and diversity of soil fauna in a coniferous stand. Journal of Applied Ecology 39(6):924–936

Lindo Z, Gonzalez A (2010) The bryosphere: an integral and influential component of the Earth's biosphere. Ecosystems 13(4):612–627, doi:10.1007/s10021-010-9336-3

Lindo Z, Whiteley J, Gonzalez A (2012) Traits explain community disassembly and trophic contraction following experimental environmental change. Global Change Biology 18(8):2448–2457, doi:10.1111/j.1365-2486.2012.02725.x

Mantyka-Pringle CS, Martin TG, Rhodes JR (2012) Interactions between climate and habitat loss effects on biodiversity: a systematic review and meta-analysis. Global Change Biology 18(4):1239–1252, doi:10.1111/j.1365-2486.2011.02593.x

Marion GM, Henry GHR, Freckman DW, Johnstone J, Jones G, Jones MH, Levesque E, Molau U, Molgaard P, Parsons AN, Svoboda J, Virginia RA (1997) Open-top designs for manipulating field temperature in high-latitude ecosystems. Global Change Biology 3(s1):20–32, doi:10.1111/j.1365-2486.1997.gcb136.x

Mcgeoch MA, Le Roux PC, Hugo EA, Chown SL (2006) Species and community responses to short-term climate manipulation: Microarthropods in the sub-Antarctic. Austral Ecology 31(6):719–731

McInerny G, Travis JMJ, Dytham C (2007) Range shifting on a fragmented landscape. Ecological Informatics 2(1):1–8

Menge BA (1995) Indirect Effects in Marine Rocky Intertidal Interaction Webs: Patterns and Importance. Ecological Monographs 65(1):21–74

van Nes EH, Scheffer M (2004) Large species shifts triggered by small forces. American Naturalist 164(2):255–266

Norberg J, Swaney DP, Dushoff J, Lin J, Casagrandi R, Levin SA (2001) Phenotypic diversity and ecosystem functioning in changing environments: A theoretical framework. Proceedings of the National Academy of Sciences of the United States of America 98(20):11,376–11,381, doi:10.1073/pnas.171315998

Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H (2011) vegan: Community Ecology Package. URL: http://CRAN.R-project.org/package=vegan, r package version 2.0-0

Parmesan C (2006) Ecological and evolutionary responses to recent climate change. Annual Review of Ecology Evolution and Systematics 37:637–669, doi:10.1146/annurev.ecolsys.37.091305.110100

Parmesan C, Yohe G (2003) A globally coherent fingerprint of climate change impacts across natural systems. Nature 421(6918):37–42, doi:10.1038/nature01286

R Development Core Team (2010) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria, URL: http://www.R-project.org/, ISBN 3-900051-07-0

Rantalainen ML, Fritze H, Haimi J, Pennanen T, Setala H (2005) Species richness and food web structure of soil decomposer community as affected by the size of habitat fragment and habitat corridors. Global Change Biology 11(10):1614–1627, doi:10.1111/j.1365-2486.2005.000999.x

Rantalainen ML, Haimi J, Fritze H, Pennanen T, Setala H (2008) Soil decomposer community as a model system in studying the effects of habitat fragmentation and habitat corridors. Soil Biology & Biochemistry 40(4):853–863, doi:10.1016/j.soilbio.2007.11.008

Sala OE, Chapin FS, Armesto JJ, Berlow E, Bloomfield J, Dirzo R, Huber-Sanwald E, Huenneke LF, Jackson RB, Kinzig A, Leemans R, Lodge DM, Mooney HA, Oesterheld M, Poff NL, Sykes MT, Walker BH, Walker M, Wall DH (2000) Global biodiversity scenarios for the year 2100. Science 287(5459):1770–1774, doi:10.1126/science.287.5459.1770

Samways MJ, Bazelet CS, Pryke JS (2010) Provision of ecosystem services by large scale corridors and ecological networks. Biodiversity and Conservation 19(10):2949–2962, doi:10.1007/S10531-009-9715-2

Scheffer M, Carpenter S, Foley JA, Folke C, Walker B (2001) Catastrophic shifts in ecosystems. Nature 413(6856):591–596

Seastedt TR (1984) The Role of Microarthropods in Decomposition and Mineralization Processes. Annual Review of Entomology 29:25–46

Soja AJ, Tchebakova NM, French NHF, Flannigan MD, Shugart HH, Stocks BJ, Sukhinin AI, Parfenova EI, Chapin FS, Stackhouse PW (2007) Climate-induced boreal forest change: Predictions versus current observations. Global and Planetary Change 56(3-4):274–296, doi:10.1016/J.Glopacha.2006.07.028

Sokal RR, Rohlf FJ (1981) Biometry: The Principles and Practice of Statistics in Biological Research, 3rd edn. W. H. Freeman and Company, New York

Staddon P, Lindo Z, Crittenden PD, Gilbert F, Gonzalez A (2010) Connectivity, non-random extinction and ecosystem function in experimental metacommunities. Ecology Letters 13(5):543–552, doi:10.1111/J.1461-0248.2010.01450.X

Thomas CD, Cameron A, Green RE, Bakkenes M, Beaumont LJ, Collingham YC, Erasmus BFN, de Siqueira MF, Grainger A, Hannah L, Hughes L, Huntley B, van Jaarsveld AS, Midgley GF, Miles L, Ortega-Huerta MA, Peterson AT, Phillips OL, Williams SE (2004) Extinction risk from climate change. Nature 427(6970):145–148, doi:10.1038/nature02121

Travis JMJ (2003) Climate change and habitat destruction: a deadly anthropogenic cocktail. Proceedings of the Royal Society B: Biological Sciences 270(1514):467–473, doi:10.1098/rspb.2002.2246

Vinebrooke RD, Cottingham KL, Norberg J, Scheffer M, Dodson SI, Maberly SC, Sommer U (2004) Impacts of multiple stressors on biodiversity and ecosystem functioning: the role of species co-tolerance. Oikos 104(3):451–457

Vos CC, Berry P, Opdam P, Baveco H, Nijhof B, O'Hanley J, Bell C, Kuipers H (2008) Adapting landscapes to climate change: examples of climate-proof ecosystem networks and priority adaptation zones. Journal of Applied Ecology 45(6):1722–1731, doi:10.1111/j.1365-2664.2008.01569.x

Walther GR, Post E, Convey P, Menzel A, Parmesan C, Beebee TJC, Fromentin JM, Hoegh-Guldberg O, Bairlein F (2002) Ecological responses to recent climate change. Nature 416(6879):389–395, doi:10.1038/416389a

Warwick RM, Clarke KR (1991) A comparison of some methods for analyzing changes in benthic community structure. Journal of the Marine Biological Association of the United Kingdom 71(1):225–244

Watkinson AR, Gill JA (2002) Climate change and dispersal. In: Bullock JM, Kenward RE, Hails RS (eds.) Dispersal Ecology, Blackwell Science, Malden, MA, pp 410–428

Whiteley J, Bendell-Young L (2007) Ecological implications of intertidal mariculture: observed differences in bivalve community structure between farm and reference sites. Journal of Applied Ecology 44(3):495–505

Wickham H (2009) ggplot2: elegant graphics for data analysis. Springer New York, h.wickham@gmail.com, doi:10.1007/978-0-387-98141-3, URL: http://had.co.nz/ggplot2/book

Williams P, Hannah L, Andelman S, Midgley G, Araujo M, Hughes G, Manne L, Martinez-Meyer E, Pearson R (2005) Planning for climate change: Identifying minimum-dispersal corridors for the Cape proteaceae. Conservation Biology 19(4):1063–1074, doi:10.1111/j.1523-1739.2005.00080.x

CHAPTER 7

Summary and Conclusion

Understanding and predicting how ecosystems respond to changing environments is a fundamental challenge in ecology, particularly during the current age of global change. Climate change and habitat fragmentation are expected to be the two biggest drivers of biodiversity change, particularly in combination (Sala *et al.*, 2000; Mantyka-Pringle *et al.*, 2012). Current theories predict non-random biodiversity loss, decline in ecosystem processes, and an increase in their variability as a result of environmental change coupled with habitat loss and fragmentation (Loreau *et al.*, 2003). Nevertheless, the relative importance of biodiversity and environmental controls on ecosystem processes remains an open empirical question (Loreau, 2000; Cardinale *et al.*, 2011). Experimental tests of existing theory are required to make robust predictions about the ecosystem effects of environmental changes acting alone or in synergy.

The SEC-C field experiment is the first attempt to assess changes in ecosystem structure and function under experimentally manipulated fragmentation and simulated climate change conditions (chapter 2). The factorial combination of treatments allowed the effect of each factor to be assessed independently, as well as their potential interactions. The experiment uses boreal forest moss (bryosphere) as a natural model system to study the effects of experimental treatments on cyanobacteria and microarthropod communities, and ecosystem rates of nitrogen-fixation, moss growth, and decomposition.

I found that nitrogen-fixation rates were more influenced by environmental conditions, primarily moisture and available nitrogen, than by variation in cyanobacteria abundance (chapters 3 and 5). Experimental warming had no detectable effects on N-fixation rates. Variation in cyanobacteria abundance could not be explained by any of the experimental treatments, suggesting a physiological

response to drought, rather than demographic responses to environmental stress or isolation, was primarily responsible for variation in N-fixation rates. Cyanobacteria composition did not vary, and was consistently dominated by *Stigonema* spp. Habitat isolation also appeared to have little direct effect on cyanobacteria communities or N-fixation in this experiment, other than possible increases in N-fixation as a result of disturbance associated with applying the treatments. The treatments seemed unable to influence cyanobacteria dispersal, or dispersal is not a limiting factor for this community.

These results challenge the paradigm that biodiversity provides insurance against environmental change, via compensation by functionally redundant taxa. Although I did observe both *Nostoc* and *Stigonema* spp. in our samples, neither seemed tolerant of drought, nor significantly affected by the warming treatments. This is consistent with an alternative view that most species in a community are correlated in their environmental tolerances (Houlahan *et al.*, 2007). Although diversity may provide insurance against some types of environmental change, at particular scales, the potential for compensation and resilience ultimately depends on the correlation of traits in response to environmental variables within a particular ecosystem (Webb *et al.*, 2010; Lindo *et al.*, 2012).

Moss growth rates also declined heavily under drought conditions, more so during the second year of the experiment (chapter 4). Moss growth was unrelated to available nitrogen, but positively related to N-fixation, suggesting overall nitrogen-limitation, and a continued benefit of nitrogen-fixation by symbiotic cyanobacteria (chapter 5).

Moss productivity and decomposition rates were also more strongly affected by drought than warming or fragmentation (chapter 4). Although both processes declined with drought, production was more negatively affected. The net effect of drought suggests that boreal forest moss can switch from a net uptake of carbon to a net loss of carbon, leading to reduced inputs of organic matter to soils. This does not account for all carbon in boreal forest ecosystems, but does justify concerns about carbon cycling under climate change conditions. Taking bryosphere processes into account will improve ecosystem-level understanding of carbon cycling, and allow for better predictions of potential feedbacks between climate change and the boreal forest.

Habitat isolation predictably reduced microarthropod abundance and richness, but only under drought conditions (chapter 6). There was a non-additive effect arising from the interaction between drought and habitat isolation. Certain

taxa appeared more tolerant of the combination of isolation and drought, depending on certain traits (explored in Lindo *et al.*, 2012). Larger-bodied, higher trophic taxa would be expected to be negatively affected by isolation more than observed in this experiment. Their mobility likely means that they view the landscapes at a larger scale than other microfauna, treating experimental patches as a part of a larger connected system (Ritchie, 1998). The result remains a non-additive change in community structure under the combination of drought and habitat isolation. Although community composition appeared to be resistant to drought, or habitat isolation separately, most groups were unable to tolerate both simultaneously. Connectivity appears to be able to mitigate drought effects, allowing declining populations to be maintained in the face of extreme, long-term environmental stress. More mobile, larger taxa, may still visit drought patches, provided adequate access (connectivity) is available.

Overall, the results of this experiment highlight the overriding importance of moisture in the bryosphere. Although temperature *per se* is certainly important at seasonal scales, small degrees of warming had little direct effect on most measured ecosystem processes. The magnitude of drought differences in the experiment may be larger than the magnitude of warming, which may account for the lack of observed temperature effects. Temperature may also have indirect effects, by altering rates of evapotranspiration, with the potential to induce drought conditions interactively with precipitation rates (Soja *et al.*, 2007). Nevertheless, moisture remains a fundamental determinant of N-fixation rates, moss growth and decomposition.

These results also support a view that ecosystem functions, such as N-fixation and moss growth, are more strongly regulated by environmental conditions, than species diversity in the bryosphere. Moisture and available nitrogen had stronger effects on N-fixation than cyanobacteria abundance. I observed very few cases where community structure explained variance in ecosystem processes, more than environmental drivers. Biodiversity certainly mediates changes in ecosystem processes, but these may be more often driven by large environmental changes. Species richness may only be important in relatively stable environments, or over long-term scales, while integrating over multiple environmental fluctuations.

Scale is fundamental in ecology, and this experiment is no exception. I followed treatments for two full years, which is longer than most lab experiments, without confounding space for time. Nevertheless, this may not be "long-term" as far as these natural communities are concerned. We still know so little about the

ecology of cyanobacteria and microarthropods, or processes that reliably determine relative abundances or patterns of spatial distributions. This presents a serious challenge to the use of the bryosphere as a natural model system in subarctic and polar regions (Lindo and Gonzalez, 2010). More research is needed on the ecology bryofauna and flora, and in particular how their interactions affect the ecosystem processes influenced by bryophytes.

7.1 Scaling up

The results of this experiment could be scaled up in two possible ways. The first involves extrapolating ecosystem processes of the bryosphere at the scale of the experiment to larger areas of boreal forest moss. The second would be scaling up by analogy, and extrapolating ecological processes within the experiment to larger-scale systems, such as the boreal forest itself.

The first type of extrapolation assumes that processes operating at the experimental scale apply equally at the landscape scale, within the same system. This assumes that scale-dependent processes are less important than certain ecological relationships, processes, or environmental controls, which may sometimes be the case (Wiedermann *et al.*, 2009). Dispersal is inherently scale-dependent, so conclusions about habitat fragmentation at the scale of this experiment may not be applicable to the same organisms at larger spatial scales. On the other hand, the role of environmental controls on processes such as N-fixation, moss growth, and decomposition, might be safely scaled up to larger areas of the boreal forest, depending on scales of variation in both environmental conditions, and the relevant biota (Benedetti-Cecchi, 2005). Further study on how such processes vary across scales would help to confirm the validity of this approach.

Using this approach, the results suggest that patterns of drought and precipitation will be extremely important in determining N-fixation rates, and therefore long-term supplies of nitrogen to some parts of the boreal forest. Knowing spatial and temporal patterns of precipitation, and drought, in addition to nitrogen deposition, will have to be taken into account to determine large-scale nitrogen cycling, and nutrient supplies that limit productivity.

The second approach to scaling up treats the moss layer much like a "miniature forest", with moss stems analogous to trees, and bryofauna as analogous to larger fauna inhabiting the forest. This approach is often taken when interpreting

results of similar experiments using the bryosphere as a model microecosystem, and asserting that the results are relevant to larger-scale multitrophic systems (Srivastava *et al.*, 2004; Lindo and Gonzalez, 2010). Studies using natural model systems more often seek general patterns in ecology, in addition to testing predictions for specific systems. Generality often depends on comparing processes at appropriate scales, however. Given the lack of information about actual dispersal rates or distances for most of the bryofauna and cyanobacteria, it is difficult to assess the generality of the observations and conclusions from such research, including the SEC-C experiment.

From this perspective, communities that appear tolerant of one stress, such as fragmentation or climate change, may be largely unable to cope with both. This insight has troubling implications for management, because it means we cannot necessarily predict impacts of environmental changes in an additive fashion: we must study multiple drivers in combination, in order to gain a more holistic understanding of the ecosystem consequences.

7.2 Future directions

The data set collected from the SEC-C experiment contains several highly-replicated ecosystem variables. Unexplored avenues of analysis remain. Structural equation modelling would be well-suited to analyze the complex network of hypothesized relationships among measured variables, for those with the time and experience. Although the data for microarthropods associated with the experiment is not as extensive as other variables, the combination of spatial, environmental, and community data presents opportunities for a range of analyses in numerical ecology (Legendre and Legendre, 1998; Borcard *et al.*, 2011), in addition to those presented here (chapter 6).

Furthermore, an unsampled replicate of all treatments at each block was left at the site at the end of sampling for this project, to allow treatment effects to continue over a longer time period (beyond the scope of my doctoral program). This presents an opportunity to follow-up and measure longer-term effects. There is also the possibility to examine system recovery from stress, by removing certain treatments and continuing to monitor N-fixation, moss growth rates, or microarthropod communities.

Given that we found a significant effect of available nitrogen, and growing concerns about N-deposition in the boreal forest (Mäkipää *et al.*, 1999; Sala *et al.*, 2000), further investigation of the sensitivity of N-fixation to variation in available

nitrogen would be enlightening. Is there a range of nitrogen availability over which biotic N-fixation is regulated with or without changes in cyanobacteria density? There are many more potential follow-up experiments that would provide valuable insight into the regulation of N-fixation by cyanobacteria associated with boreal forest mosses. Manipulations of nitrogen availability, and carbon dioxide enrichment provide additional factors that could interact with warming.

The SEC-C experiment has shown that bryosphere microecosystems can be amenable to large-scale field experiments, which can be used to test hypotheses, as well as answer pressing questions about ecosystem responses to environmental change. As our ecological understanding of nitrogen and carbon cycling, and the biota in the bryosphere improves, new opportunities are created for asking general ecological questions and testing a broader range of theory (Lindo and Gonzalez, 2010).

References

Benedetti-Cecchi L (2005) Unanticipated impacts of spatial variance of biodiversity on plant productivity. Ecology Letters 8(8):791–799

Borcard D, Gillet F, Legendre P (2011) Numerical Ecology with R. Use R!, Springer, New York, USA, URL: http://www.springer.com/statistics/life+sciences%2C+medicine+%26+health/book/978-1-4419-7975-9

Cardinale BJ, Matulich KL, Hooper DU, Byrnes JE, Duffy E, Gamfeldt L, Balvanera P, O'Connor MI, Gonzalez A (2011) The functional role of producer diversity in ecosystems. Am J Bot 98(3):572–592, doi:10.3732/ajb.1000364

Houlahan JE, Currie DJ, Cottenie K, Cumming GS, Ernest SKM, Findlay CS, Fuhlendorf SD, Gaedke U, Legendre P, Magnuson JJ, McArdle BH, Muldavin EH, Noble D, Russell R, Stevens RD, Willis TJ, Woiwod IP, Wondzell SM (2007) Compensatory dynamics are rare in natural ecological communities. Proceedings of the National Academy of Sciences of the United States of America 104(9):3273–3277, doi:10.1073/pnas.0603798104

Legendre P, Legendre L (1998) Numerical Ecology, 2nd edn. Developments in environmental modelling ; 20., Elsevier, New York

Lindo Z, Gonzalez A (2010) The bryosphere: an integral and influential component of the Earth's biosphere. Ecosystems 13(4):612–627, doi:10.1007/s10021-010-9336-3

Lindo Z, Whiteley JA, Gonzalez A (2012) Traits explain community disassembly and trophic contraction following experimental environmental change. Global Change Biology 18(8):2448–2457, doi:10.1111/j.1365-2486.2012.02725.x

Loreau M (2000) Biodiversity and ecosystem functioning: recent theoretical advances. Oikos 91(1):3–17

Loreau M, Mouquet N, Gonzalez A (2003) Biodiversity as spatial insurance in heterogeneous landscapes. Proceedings of the National Academy of Sciences of the United States of America 100(22):12,765 – 12,770, doi:10.1073/pnas.2235465100

Mäkipää R, Karjalainen T, Pussinen A, Kellomäki S (1999) Effects of climate change and nitrogen deposition on the carbon sequestration of a forest ecosystem in the boreal zone. Canadian Journal of Forest Research 29(10):1490–1501, doi:10.1139/x99-123

Mantyka-Pringle CS, Martin TG, Rhodes JR (2012) Interactions between climate and habitat loss effects on biodiversity: a systematic review and meta-analysis. Global Change Biology 18(4):1239–1252, doi:10.1111/j.1365-2486.2011.02593.x

Ritchie ME (1998) Scale-dependent foraging and patch choice in fractal environments. Evolutionary Ecology 12(3):309–330

Sala OE, Chapin FS, Armesto JJ, Berlow E, Bloomfield J, Dirzo R, Huber-Sanwald E, Huenneke LF, Jackson RB, Kinzig A, Leemans R, Lodge DM, Mooney HA, Oesterheld M, Poff NL, Sykes MT, Walker BH, Walker M, Wall DH (2000) Global biodiversity scenarios for the year 2100. Science 287(5459):1770–1774, doi:10.1126/science.287.5459.1770

Soja AJ, Tchebakova NM, French NHF, Flannigan MD, Shugart HH, Stocks BJ, Sukhinin AI, Parfenova EI, Chapin FS, Stackhouse PW (2007) Climate-induced boreal forest change: Predictions versus current observations. Global

and Planetary Change 56(3-4):274–296, doi:10.1016/J.Glopacha.2006.07.028

Srivastava DS, Kolasa J, Bengtsson J, Gonzalez A, Lawler SP, Miller TE, Munguia P, Romanuk T, Schneider DC, Trzcinski MK (2004) Are natural microcosms useful model systems for ecology? Trends in Ecology & Evolution 19(7):379–384

Webb CT, Hoeting JA, Ames GM, Pyne MI, Poff NL (2010) A structured and dynamic framework to advance traits-based theory and prediction in ecology. Ecology Letters 13(3):267–283, doi:10.1111/J.1461-0248.2010.01444.X

Wiedermann MM, Gunnarsson U, Nilsson MB, Nordin A, Ericson L (2009) Can small-scale experiments predict ecosystem responses? An example from peatlands. Oikos 118(3):449–456, doi:10.1111/J.1600-0706.2008.17129.X