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Individual variation in plant traits drives species interactions, ecosystem functioning, and responses to global change

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To the Graduate Council:

I am submitting herewith a dissertation written by Quentin Daniel Read entitled "Individual variation in plant traits drives species interactions, ecosystem functioning, and responses to global change." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Ecology and Evolutionary Biology.

Nathan J. Sanders, Major Professor

We have read this dissertation and recommend its acceptance:

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Individual variation in plant traits drives species interactions, ecosystem functioning, and responses to global change

A Dissertation Presented for the
Doctor of Philosophy
Degree
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Quentin Daniel Read
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ABSTRACT

Ecologists have long sought to understand the processes that lead to the riotous diversity in communities of organisms that inhabit disparate climates and landscapes. Such a diversity of traits leads to a diversity of interactions among species in natural communities, which in turn generates a diversity of potential responses to ongoing global change. In this dissertation, I do three things: I explore the forces that structure plant communities and the ecosystem functions that they mediate, I describe patterns of variation among communities, species, and individual organisms across environmental contexts, and I disentangle the direct effects of global change from the indirect, cascading effects that result from disruptions of species interactions. I accomplish these goals through the synthesis of global data, the development of statistical and mathematical models, and the manipulation of global change drivers in field experiments. In the first chapter, I present a globe-spanning meta-analysis of plant functional trait patterns along elevational gradients. This meta-analysis shows that the plant traits that drive ecosystem function follow predictable trends with elevation due to climate filtering, and that much of this variation is at the level of the individual organism. In the second chapter, I present simulated data sets and illustrative experimental case studies that quantify how important individual-level variation is for explaining patterns in nature. In the third chapter, I present results from intensive plant sampling across a wide range of mountain environments; even in these harsh environments where only the hardiest species can survive, individual-level variation is so high that it makes predictions based on species identity nearly impossible. The fourth and fifth chapters consist of experimental evidence that ongoing human-caused global change is affecting montane plant communities, that species interactions mediate many of these effects, and that variation in the abiotic environment causes variation in both species interactions and in global change response. I demonstrate this through an experiment that combines nitrogen fertilization with removal of a dominant plant species in a montane meadow, and an experiment replicated at low and high elevations crossing dominant species removal with simulation of global warming.

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LIST OF ABBREVIATIONS

Abbreviation	***Term***
AIC	Akaike's information criterion
AICc	Akaike's corrected information criterion
aITV	Among-site intraspecific trait variability
ANOVA	Analysis of variance
BIC	Bayesian information criterion
CI	Confidence interval *or* Credible interval
CTI	Community thermal index
CWM	Community-weighted mean
CWV	Community-weighted variance
FD	Functional diversity
FDisp	Functional dispersion
GLM	General(ized) linear model
GPP	Gross primary productivity
LAI	Leaf area index
LDMC	Leaf dry matter content
LMA	Leaf mass:area ratio
MAP	Mean annual precipitation
MAT	Mean annual temperature
N_{area}	Nitrogen content per unit area
NEE	Net ecosystem carbon exchange
N_{mass}	Nitrogen content per unit mass
OTC	Open-top (warming) chamber
PERMANOVA	Permutational analysis of variance
R_e	Ecosystem respiration
RML	Root mass:length ratio
RMSE	Root mean squared error
SDM	Species distribution model
SEM	Structural equation model
SLA	Specific leaf area
SRL	Specific root length
SRTM	Shuttle radar topography mission
wITV	Within-site intraspecific trait variability

INTRODUCTION

Themes and key questions

Climate change and species losses threaten all ecosystems, and montane systems are especially at risk (Engler *et al.* 2011; Rangwala & Miller 2012; Pepin *et al.* 2015). Global change is affecting communities and ecosystems in mountains *directly* by changing the rates of physical processes, and *indirectly* by altering the functional composition of high-altitude plant communities. As the optimal temperature range for many plants moves to higher elevations with warming (Chen *et al.* 2011; Beckage *et al.* 2008), plant communities at different sites, and individuals with different traits within each plant community, are responding to warming at different rates (Saavedra *et al.* 2013; Alexander *et al.* 2015). Warming temperatures may increase environmental stress in some communities and decrease it in others—this shifting stress will likely lead to shifting dominance patterns (Gilman *et al.* 2010; Grassein *et al.* 2014; Michalet *et al.* 2014). Importantly, sensitivity to both warming and dominant species loss may be predictable from plant trait distributions (Suding *et al.* 2008). The leaf traits that drive carbon flux rates and predict responses to global change exhibit consistent patterns along climate gradients worldwide (Dubuis *et al.* 2013; Venn *et al.* 2011; Kunstler *et al.* 2016). Thus, plant functional traits link plant community ecology (Kraft *et al.* 2015, Ehrlén *et al.* 2015) and projections for future carbon sequestration potential (Sakschewski *et al.* 2015). The research presented here takes advantage of this trait-based link to explore the factors driving plant community composition and ecosystem functioning across biomes.

The research presented in this dissertation addresses the following key questions:

Are there general patterns of plant trait variation along elevational gradients across different biomes, and does this variation follow the patterns predicted by the leaf economics spectrum? What role does intraspecific variability play in the variation observed along elevational gradients? I present a meta-analysis in Chapter I that addresses these questions.

Under what conditions is it necessary to incorporate intraspecific variability into ecological studies? What are the consequences of ignoring intraspecific trait variation for ecological inference? In Chapter II, I present a simulation paired with several empirical case studies to illustrate how important intraspecific variation is for ecological inference.

How is functional trait variation partitioned within and among plant species along a climate gradient in a mountainous region? How well do functional traits alone predict species composition along this gradient? In Chapter III, I present observational data and a statistical model that attempts to predict species distributions from the relationship between plant traits and climate.

Are the effects of a dominant plant species on the plant community and on carbon cycling contingent on soil resource availability? Do soil resource availability and dominant plant species interact to determine the structure and function of the plant community? I conducted an experiment to explore interactions between species loss and nitrogen deposition, which I present in Chapter IV.

What effect does increased temperature have on the interactions between dominant plant species and other members of the community? How do these effects differ across the different climates associated with different elevations, and across multiple mountain ranges? In my fifth chapter, I present results from a multifactorial global change experiment replicated at low and high elevations.

My dissertation research focuses on a number of issues that both advance the field of community ecology and predict how global climate change will affect the structure and function of montane plant communities, both directly and through indirect trait-mediated effects. I present a meta-analysis, a simulation, a statistical model, and two field experiments, all of which inform one another. The research that is described here is grounded in a functional trait approach, extending that approach to incorporate individual-level variation. The theoretical, observational, and experimental results I present can be applied to inform models, to predict changes in mountain biodiversity and ecosystem functioning, and to test important theoretical questions dealing with sources of variation among organisms and how that variation affects ecosystems.

The functional trait approach. It is crucial to forecast biodiversity and ecosystem services under future environmental conditions. The fields of species distribution modeling (SDM; Araújo & Guisan 2006; Araújo & Peterson 2012) and ecosystem modeling have developed rapidly over the past few decades. However, a functional trait-based approach (Wright *et al.* 2004; Shipley *et al.* 2006a) has the potential to simultaneously refine ecosystem models and improve the utility and predictive power of SDMs (Guisan & Thuiller 2005; Stahl *et al.* 2014; Violle *et al.* 2014), uniting these two fields. If relationships are known between organismal traits, species identity and ecosystem function, we can use these forecasts to predict future ecosystem function.

Individual-level variation. In addition to the relationship between species identity and ecosystem properties, the functional trait approach allows individual-level variation to be incorporated into ecological models, improving our ability to determine the consequences of biotic and abiotic filters on community composition and ecosystem functioning. The observation that different species solve the problems presented by nature in different ways is what first spurred ecology to go beyond mere descriptive natural history (Warming 1909). Ecologists are increasingly recognizing what an important role variation among individuals within species plays—in some cases, the magnitude of intraspecific variation can dwarf variation among species. Incorporating functional traits, especially variation in traits among individuals, into the study of natural communities contributes to a synthesis of community ecology, biogeography, and ecosystem ecology (Weiher *et al.* 2011; Shipley *et al.* 2016).

Experiments that inform models. To date, few ecological studies have explicitly incorporated manipulative experiments into models (Diamond *et al.* 2012). Part of the reason for this is that manipulative experiments are necessarily focused on a relatively small spatial and temporal extent. Now with increased opportunities for international collaboration, it is possible to coordinate networks of manipulative ecological experiments that run simultaneously at macroecological scales (Nogués-Bravo & Rahbek 2011; Fraser *et al.* 2013; Borer *et al.* 2014). Data from such experiments are more suitable for larger-scale modeling approaches (Dukes *et al.* 2014), especially in community ecology. The work presented here incorporates plant community responses from a manipulative experiment and projections of species assemblages generated using functional traits into a unified research framework.

Predicting changes in mountain biodiversity. The research presented here consists of manipulative experiments conducted along elevational gradients in montane systems around the world. Mountains and alpine systems can be seen as a test bed for biodiversity research, and present unique challenges for ecological modeling (Carlson *et al.* 2013). Drivers of community assembly change with increasing elevation, caused both by decreasing temperature and changes in plant-plant interactions. While competitive interactions shape community structure at lower, warmer elevations, neutral or even positive interactions may dominate at higher elevations (Callaway *et al.* 2002). Different syndromes of plant functional traits prevail at low and high elevations, due to both the varying abiotic conditions and competitive environments. At a community level, these differences in average trait values lead to differences in ecosystem properties associated with the cycling of carbon and other nutrients (Suding *et al.* 2008).

In addition, montane systems are especially susceptible to global change (Engler *et al.* 2011). Rapidly shifting species ranges along elevational gradients due to climate change (Beckage *et al.* 2008) will cause unprecedented biological communities to assemble. It is critical to predict how these changing communities will drive changes in ecosystem properties. A functional trait-based approach, as is proposed here, will be instrumental in achieving this goal (Violle *et al.* 2014).

Testing community assembly theories. The research presented here tests ecological theory about the ways in which functional tradeoffs in plants structure communities along environmental gradients. The inclusion of root traits captures more variation in plant resource-use strategies and enables more accurate predictions of community composition and ecosystem properties relative to previous work (Freschet *et al.* 2010). The experimental response data I present allows more robust inferences about mechanisms, isolating biotic and abiotic factors that were previously lumped together in more correlative approaches. Finally, explicitly comparing intraspecific variation in traits along elevational gradients provides support for the stress-gradient hypothesis (Bertness & Callaway 1994; Maestre *et al.* 2009) and the importance of intraspecific variation for ecosystem functioning (Crutsinger *et al.* 2006; Siefert *et al.* 2015).

Chapter summaries

Chapter I of this dissertation is a meta-analysis that has been published in the journal *Functional Ecology*. For this meta-analysis, I drew upon existing work published in the literature to demonstrate that key functional traits show consistent patterns of variation with elevation. Along elevational gradients around the globe and across a stunning variety of plant taxa, leaf mass:area (LMA) tends to increase with elevation, as does leaf nitrogen content measured on a per-area basis. However, leaf nitrogen content measured per unit mass (N_{mass}) is as likely to increase with elevation as to decrease. These three traits are easily measured indices of whole-plant properties, including life history strategy, competitive ability, and per capita contribution to carbon cycling in ecosystems. This meta-analysis provides evidence that environmental filtering along elevational gradients determines the functional composition of plant communities: in particular, I show that variation within a single species along elevational gradients is equal in magnitude to variation measured at the community level across many species, spanning the same amount of difference in elevation. Changes in traits linked to life history have predictable consequences for the way plants interact with one another and the way that they contribute to the functioning of ecosystems. Because of these linkages, I hoped to be able to predict species distributions across the landscape based on traits (Chapter III) and to predict how species respond to increased temperature, dominant species loss, and changes in nitrogen availability across plant communities that vary greatly in their trait composition (Chapters IV & V).

The second chapter of this dissertation builds upon the meta-analysis that I present in Chapter I. Chapter II is a comparison of different ways of analyzing trait variation within and among species and populations; it has been published in the journal *Oikos*. While our current understanding of the way organisms interact with their environment is increasingly based on individual traits, many ecological studies still base their inference on species identity. To demonstrate that in many cases, this leads to incorrect inference or poor estimation of effect sizes, I simulated a large amount of trait data across many genotypes within different species. I fit two types of linear models to the simulated datasets: a non-nested linear model including only species as the fixed effect term, and a nested mixed model including a random effect term for genotype identity in addition to the species term. I varied the parameters of the simulation to encompass a wide range of heritability values, number of genotype markers, and absolute value of trait differences. The two types of statistical analysis differed in their ability to capture the true source of variation among individuals: the linear model tended to overestimate the proportion of variation attributed to species rather than genotype, as well as tending to lead to an incorrect inference, as I observed from the distribution of p-values. In addition to the simulated datasets, I present three case studies from *Eucalyptus*, *Populus*, and *Picea* common-garden experiments that further demonstrate my point. Fitting non-nested linear models to these datasets leads to a different inference about the source of trait variation among individuals across multiple populations. A key assumption of the functional trait-based approach has historically been that intraspecific variation in traits can be largely ignored (Shipley *et al.* 2016); my analysis of both simulated and experimental data highlights flaws in this assumption and informs the experimental and observational field methods and analytical techniques I use in Chapters III, IV, & V.

Chapter III, along with the following two chapters, presents results from studies conducted in the field. This work has not yet been published elsewhere. In the third chapter, I present a dataset consisting of plant community composition, plant functional trait composition, and environmental variables collected at 14 sites in Colorado, USA during the summer of 2015. Building upon the results of the preceding two chapters, I first demonstrate the large role played by intraspecific variation in shaping differences within and among plant communities in the region—in some cases greater than the role of species turnover across sites. Next, I set out to achieve an important goal of trait-based ecology: to use functional traits in a predictive framework. While much work exists on correlating functional traits to environmental conditions, few researchers have attempted to make predictions of species relative abundances based solely upon environmental conditions at a particular site. However, even if the precise mechanistic link between functional traits and environment is not known, it may be possible to parameterize a statistical model that can predict community structure from climate using functional trait data. I constructed just such a model, modified from an existing model known as Traitspace (Laughlin & Laughlin 2013). However, the statistical model did a relatively poor job of predicting species abundance based on functional traits. The most likely explanation for this disconnect is the large magnitude of intraspecific variation—ubiquitous in natural communities as my previous chapters show. However, changes in trait distributions in natural communities as a result of human-caused global change may still have important consequences for ecosystem functioning, as the following two chapters demonstrate.

In my fourth chapter, I present results from a manipulative experiment that crosses removal of a dominant grass species with addition of two different forms of nitrogen fertilizer to the soil. I show four years of community and ecosystem response to these global change treatments, which were established in 2012 in a montane meadow in Colorado, USA. This study addresses questions about the abiotic and biotic processes that structure plant communities: I ask whether environmental filtering drives communities toward an optimal distribution of functional traits across species, and whether this is dependent on the presence of a competitively dominant species, on the availability of a limiting resource, or both. In addition, the results from this study can be applied to make predictions about how plant communities and the ecosystem services they render will respond to drivers of global change, namely loss of foundation species and anthropogenic nitrogen deposition. After four years of species removal and nitrogen fertilization, I found that the functional trait makeup of the plant community was relatively resilient to the perturbation that occurred when the most abundant species, fescue, was lost. The leaf and root traits of fescue were at one extreme of the distribution of traits within the plant community, potentially indicating that competitive dominance results when a species possesses traits conferring higher relative fitness. Because of this, the remaining community cannot fully replace the functional role of the lost species. However, the remaining community showed resilience by convergence of its mean trait value on the value of the fescue-dominated community. This phenomenon was not dependent on whether or not additional nitrogen was added to the soil. In fact, the plant community was highly resistant to change under both organic and inorganic nitrogen addition. Nitrogen addition had impacts primarily on carbon cycling properties, causing both increased aboveground plant biomass and a temporary increase in soil carbon efflux.

While experiments that factorially manipulate multiple global change drivers in the field are rare enough, multifactorial experimental manipulations conducted in parallel at multiple field sites are rarer still. In my fifth chapter, I address this critical shortfall, presenting results from an experiment in which I simultaneously simulated both dominant species loss and anthropogenic warming at both a low-elevation and high-elevation site in Colorado, USA. I present results from the third field season of experimental manipulation. In 2013, I removed the dominant plant species from half the experimental plots at each elevation, then crossed the removal treatment with a warming treatment by placing hexagonal open-top warming chambers on half the plots. The warming chambers simulate approximately 2° C of growing-season warming. After the third year of manipulation, both warming and dominant species loss have affected the plant community and ecosystem-level carbon cycling both individually and in interaction with one another. Furthermore, these effects differ depending upon the elevation, and the effect of dominant species removal tends to be stronger than the effect of warming. Removing the dominant species causes the remaining community to become dominated by individuals that have faster-growing but less durable leaves and roots; this effect propagates through the plant community to have important consequences for carbon cycling. I present data collected at the peak growing season of 2015 showing that dominant species removal reduces the rate of carbon uptake by living biomass in plant communities across both sites; future work in this system will provide more insights into the long-term effects of warming and removal on carbon storage and turnover at low and high elevation.

This global change experiment conducted at two elevations provides important data to inform predictions and parameterize models that simulate community and ecosystem responses to ongoing global change. However, the results I present here in my fifth chapter, from a single site, are not adequate to allow broader inference about how the impacts of global change will differ by elevation in mountain ranges around the globe. The results from Chapter V represent preliminary data from a globally distributed network of experimental sites, at all of which an identical experiment has been established. I will contribute the results of the warming-by-removal experiment to a dataset being compiled by a global network of collaborators. In the future, my collaborators and I will be able to determine whether there are globally consistent elevation-dependent patterns in the response of communities and ecosystems to warming and to dominant species loss.

Study system

All of the fieldwork described here took place at and around the Rocky Mountain Biological Laboratory, located at the site of an abandoned mining settlement 10 km north of Crested Butte in western Colorado, USA (38° 57' N, 107° 0' W). The study sites are located in treeless areas along an elevational gradient spanning over 1000 m, including sagebrush grassland, montane meadow, and subalpine meadow habitats (Figure 1). Mean annual temperature decreases steeply with elevation, and precipitation increases with elevation. Warming experiments involving both active (Harte & Shaw 1995; Saleska *et al.* 1999) and passive designs (unpublished), as well as removals of plant species and functional groups (Cross & Harte 2007) are ongoing at nearby sites or have been conducted in the past. In addition, Bryant and others did an extensive survey of plant and microbial diversity (Bryant *et al.* 2008) along an elevational gradient including some of the sites for the work proposed here; plant functional traits and ecosystem-level properties have been measured along the same gradient (Brian Enquist, unpublished data).

Chapter III uses plant trait data collected from fourteen sites in the region to fit a statistical model to predict species relative abundances; these sites are located between 2480 and 3560 m above sea level. Chapter IV presents results from an experiment in which I removed a dominant plant species and added organic and inorganic N fertilizer to the soil; this site, Maxfield Meadow, is 2910 m above sea level. In Chapter V, I present results from an experimental manipulation crossing warming with species removal and conducted at two sites: Almont Triangle, 2740 m above sea level, and Cinnamon Mountain, 3460 m above sea level. The experiment I describe in Chapter V is embedded within a global network of experimental sites, all of which are paired and located at a high and low elevation. However, I am not presenting data from the other experimental sites in the network.

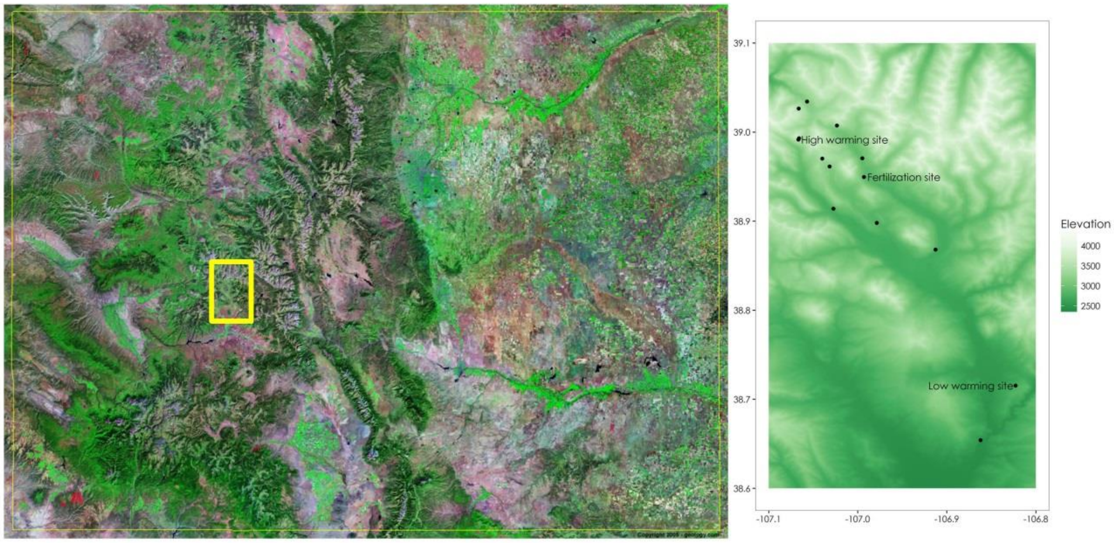

Figure 1. Satellite image of Colorado showing location of study region within yellow rectangle (left), and elevation-shaded map of study region with locations of study sites. Experimental sites are indicated with labels.

CHAPTER I

CONVERGENT EFFECTS OF ELEVATION ON FUNCTIONAL LEAF TRAITS WITHIN AND AMONG SPECIES

A version of this chapter was originally published by Quentin D. Read, Leigh C. Moorhead, Nathan G. Swenson, Joseph K. Bailey, and Nathan J. Sanders:

Read, Q. D., L. C. Moorhead, N. G. Swenson, J. K. Bailey, and N. J. Sanders. 2014. Convergent effects of elevation on functional leaf traits within and among species. *Functional Ecology* 28:37-45. DOI: 10.1111/1365-2435.12162

The research in this article was conducted by Q. Read, with help from L. Moorhead. Read wrote the manuscript, and all other co-authors assisted with writing and revision.

Abstract

1. Spatial variation in filters imposed by the abiotic environment causes variation in functional traits within and among plant species. This is abundantly clear for plant species along elevational gradients, where parallel abiotic selection pressures give rise to predictable variation in leaf phenotypes among ecosystems. Understanding the factors responsible for such patterns may provide insight into the current and future drivers of biodiversity, local community structure, and ecosystem function.

2. In order to explore patterns in trait variation along elevational gradients, we conducted a meta-analysis of published observational studies that measured three key leaf functional traits: leaf mass-area ratio (LMA), leaf nitrogen content per unit mass (N_{mass}), and N content per unit area (N_{area}). Importantly, these traits are associated with axes of variation in both competition for resources and stress tolerance. We used global climate data sets to model mean annual temperature (MAT) as a function of elevation for all study sites. To examine whether there may be evidence for a genetic basis underlying the trait variation, we conducted a review of published results from common garden experiments that measured the same leaf traits.

3. Within studies, LMA and N_{area} tended to decrease with MAT along elevation gradients, while N_{mass} did not vary systematically with MAT. Correlations among pairs of traits varied significantly with MAT: LMA was most strongly correlated with N_{mass} and N_{area} at sites at high elevation with relatively lower MAT. The strengths of the relationships were equal or greater within species relative to the relationships among species, suggesting parallel evolutionary dynamics along elevational gradients among disparate biomes. Evidence from common garden studies suggests that there is an underlying genetic basis to the functional trait variation that we documented along elevational gradients.

4. Taken together, these results indicate that environmental filtering both selects locally adapted genotypes within plant species and constrains species to elevational ranges based on the range of potential leaf trait values. If individual phenotypes are filtered from populations in the same way that species are filtered from regional species pools, changing climate may affect both the species and functional trait composition of plant communities.

Introduction

Understanding variation in functional traits among organisms enables ecologists to make predictions about community structure (McGill *et al.* 2006), to describe factors influencing the geographic ranges of species (Kelly 2003; Westoby & Wright 2006), and to infer why processes like nutrient cycling and plant productivity vary among systems (Díaz & Cabido 2001). In most ecosystems, the environment can act as a selective filter on plant species along abiotic gradients. This process, often referred to as abiotic filtering, mediates the assembly of plant communities so that species with similar functions tend to co-occur more often than would be expected by chance (Keddy 1992; Weiher, Clarke & Keddy 1998; Kraft, Valencia & Ackerly 2008; Swenson & Enquist 2009). For instance, to cope with abiotic gradients across elevations, plants in disparate taxa have evolved parallel morphological and physiological traits at high and low elevations (Clausen, Keck & Hiesey 1940; Ackerly & Reich 1999; Swenson & Enquist 2007). Despite the apparent ubiquity of variation in plant functional traits with elevation, and the growing literature documenting patterns along single elevational gradients, global-scale analyses are needed to show repeated elevational gradients in form and function within and among species across systems that may point to universal underlying mechanisms (Poorter *et al.* 2009; Körner 2012). To assess whether such patterns in form and function are similar, both within and among species and across disparate systems, we conducted a meta-analysis that focused on a few key leaf functional traits from the leaf economics spectrum (Reich, Walters & Ellsworth 1997; Wright *et al.* 2004, 2005).

Key functional traits along elevational gradients

A global spectrum of morphological and chemical leaf traits, often referred to as the leaf economics spectrum, spans a continuum of plant life forms and life histories (Reich, Walters & Ellsworth 1997; Wright *et al.* 2004, 2005). Generally speaking, fast-growing species with a resource-acquisitive life strategy tend to have short-lived leaves, while slow-growing, conservative species invest more resources into thick, durable leaves. Leaf mass:area ratio (LMA) and leaf nitrogen (N) content per mass (N_{mass}) and per area (N_{area}) are correlated with relative growth rate and serve as cornerstones of this trait spectrum. Species exhibiting the acquisitive syndrome tend to have lower LMA, higher N_{mass}, and lower N_{area} than conservative species (Shipley *et al.* 2006a). A principal component analysis of the GLOPNET database, including plant trait values from a variety of ecoregions and growth forms, showed that roughly three-quarters of trait variation was loaded onto a single axis which differentiated acquisitive species from conservative species, including significant loadings on LMA and N_{mass} (Wright *et al.* 2004). The GLOPNET analysis suggests that easily measured traits on which a number of researchers have collected data can provide ecologically relevant information on plants' life strategies and their contribution to ecosystem functioning.

The trade-off between competitiveness for limiting resources and stress tolerance mediates the assembly of at least some plant communities and may be responsible for functional trait gradients observed along latitudinal (Stott & Loehle 1998) and elevational gradients around the globe (Cornwell & Ackerly 2009; Körner 2012). Of course, the plants are not responding to latitude or elevation directly, but are instead responding to a suite of factors that covary with latitude and elevation. For instance, as elevation increases, temperature and atmospheric pressure decrease, and clear-sky solar radiation increases, although mountain ranges around the world exhibit different trends of moisture, growing season length, and cloudiness (Körner 2007). Despite the variability in elevation-climate relationships, it may be that at lower elevations, conditions tend to favour acquisitive species that can take advantage of high resource levels, since higher temperature stimulates microbial activity and increases resource availability (Raich & Schlesinger 1992). Conversely, at higher elevations, harsh environmental conditions and lower resource availability promote stress-tolerant species that invest more carbon on a per-leaf basis (Körner *et al.* 1989, Körner 2012). Therefore, we expect individual plants at higher elevations to have increased LMA, increased N_{area}, and decreased N_{mass} relative to low-elevation individuals.

Variation in plant functional traits along elevational gradients, whether caused by phenotypic plasticity or genetic divergence, may influence how communities and ecosystems respond to global change. The resource conservation-acquisition trade-off, for which LMA and leaf N may represent useful proxies, is critical in predicting the responses of plant species to changing environmental conditions (Suding *et al.* 2008; Bardgett & Wardle 2010; Reu *et al.* 2011). Spatial variation in plant traits along elevational gradients may parallel trends associated with on-going and projected anthropogenic climate warming (Dunne *et al.* 2004; Fukami & Wardle 2005), which is predicted to affect the functional trait composition of plant communities (Suding *et al.* 2008). The degree to which genetic variation and phenotypic plasticity are responsible for variation in plant traits will influence the responses of plants to climate change: phenotypic plasticity will allow short-term responses to abiotic changes, while genetic variation may permit evolutionary responses to abiotic changes.

Based on predictions made by leaf economics spectrum theory, LMA and N_{area} should increase with elevation and that N_{mass} should decrease, and that the mechanisms contributing to these patterns would include phenotypic plasticity, genetic divergence within a species, and changing species composition along gradients of elevation. We hypothesized that intraspecific and interspecific variation would contribute roughly equally to elevational patterns in functional traits, because particular species and functional trait compositions are ultimately the result of filtering processes acting on individuals (Lavorel & Garnier 2002). We further hypothesized that parallel evolution, the independent evolution of similar phenotypes in response to similar selective pressures, is largely responsible for parallel trait patterns along elevational gradients. Previous studies have documented parallel evolution in a variety of organisms both within and across species (Schluter & McPhail 1992); parallel genetic changes in different populations often gives rise to parallel changes in interspecific interactions and ecosystem properties (Fussmann, Loreau & Abrams 2007; Harmon *et al.* 2009; Agrawal *et al.* 2013). The role of parallel genetic divergence relative to phenotypic plasticity in causing patterns of trait variation that are similar across plant functional types and biomes can be determined using common garden experiments, reciprocal transplants, or hybridization studies (Clausen, Keck & Hiesey 1940; Whitham *et al.* 2006).

Using a meta-analysis approach (Borenstein *et al.* 2009) we assessed (a) whether general patterns of variation exist in key plant functional traits along elevational gradients. Trait values that vary consistently with elevation would suggest that abiotic factors associated with elevation represent a selective gradient to which plants respond consistently. In addition, we asked (b) whether variation within species was greater than variation across species assemblages. Finally, we conducted a qualitative review of experimental common garden studies to test the hypothesis

(c) that genetic divergence explains a significant proportion of intraspecific variation in important plant functional traits.

Materials and methods

Trait selection

We selected three leaf functional traits for analysis based on the availability of data and their significant association with the resource conservation-acquisition tradeoff axis. LMA, N_{mass}, and N_{area} are relatively easy to measure (Pérez-Harguindeguy *et al.* 2013) and are associated with plant resource acquisitiveness and stress-tolerance (Grime 1977; Shipley *et al.* 2006a; Poorter *et al.* 2008, 2009), composite traits that are difficult or impossible to measure (Wright *et al.* 2004, 2005). While natural selection does not operate at the level of traits or even trait syndromes, functional trait approaches provide insight into selective processes occurring at the individual-fitness level that scale up to higher levels of organisation (Violle *et al.* 2007). LMA is the product of leaf thickness and density, increasing with the proportion of leaf biomass that consists of cell wall (Niinemets 2001; Poorter *et al.* 2009). Leaf N content corresponds to the amount of protein and other secondary compounds present within the leaf, which are necessary for photosynthesis and growth (Wright *et al.* 2004). Leaves with high LMA are more tolerant of abiotic stress including cold temperatures (Poorter *et al.* 2009). However, high-elevation plants that have high LMA due to increased leaf tissue thickness experience constraints on their maximum photosynthetic rate per unit area due to diffusion and shading constraints in the interior of the leaf (Körner & Diemer 1987; Poorter *et al.* 2009). Therefore, high-LMA leaves are constrained to have low N content per unit mass and long lifespans to repay leaf construction costs (Reich, Walters & Ellsworth 1997). For these reasons, we selected studies where LMA, N_{mass}, or N_{area} were sampled along elevational gradients for the meta-analysis.

Literature search

In March 2012, we conducted a literature search on Web of Knowledge and Google Scholar, using combinations of the search terms plant, trait, altitude, and elevation. After screening the initial list of over 10,000 articles generated by our database searches and retaining only studies that measured plant functional traits at multiple elevations, we selected additional literature from parent and child citations, i.e., articles that cited or were cited by articles on the reduced list. For each published study, we recorded the identities of the focal species, the traits measured, whether variation was examined within or among species, the number and elevations of sites sampled, the mean latitude of the gradient, the number of individuals sampled at each site, and the plant functional types represented in the study: angiosperm tree, conifer, fern, forb, graminoid, legume, or shrub. In addition, we extracted the raw trait data where possible.

We modelled mean annual temperature (MAT) at all the study sites by georeferencing all site coordinates, extracting elevation and temperature data from the SRTM (Jarvis *et al.* 2008) and Bioclim (Hijmans *et al.* 2005) datasets over a rectangular area spanning 1° × 1° at 0.5' resolution, then generating functions relating elevation and MAT (McCain & Colwell 2011). We analysed 46 independent regressions of LMA on MAT modelled as a function of elevation using data extracted from 29 papers, 39 regressions of N_{mass} (23 papers), and 29 regressions of N_{area} (16 papers; see Appendix 1). Our analysis was global in scope, including study sites distributed across a wide range of latitudes and both hemispheres (see Figure 5, given as an appendix to this chapter).

We conducted an additional literature search using the terms common garden, genetic, plant, trait, leaf nitrogen, and leaf area. We compiled a second database of studies in which plants from a single species collected at multiple sites along an elevational or latitudinal gradient were grown under controlled environmental conditions in a common garden, and where the investigators measured LMA, N_{mass}, and N_{area}. This database included the focal species, the traits measured, the number of sites sampled, and the statistical technique used to test the hypothesis that populations at different elevations vary genetically (see Appendix 2).

Meta-analysis of trait-elevation relationships

For each study, we obtained the correlation coefficient, Pearson's r, of the trait regressed on MAT along the elevational gradient and used it to calculate the effect size for that study. The effect that each study estimated was a correlation or relationship between MAT and a leaf trait. The Pearson's r is a standardized estimate of the strength of that relationship. When transformed to an effect size z, it can be compared among studies, and the mean effect size among many studies can be estimated.If necessary, we extracted raw elevation and mean trait values from tables or scatter plots using GetData Graph Digitizer 2.24 software (Fedorov 2008) and calculated r from the raw data. We calculated degrees of freedom from the number of sampling sites along the elevational gradient, instead of treating each sampled individual as independent. Averaging trait values at each site in this way ignores variation within a site, avoiding inflation of degrees of freedom at the expense of increasing the absolute value of the effect size estimate. We calculated the variance around each effect size estimate using the formula $v(z_i) = \frac{1}{n_i - 3}$, where n_i is the number of sites sampled in study i (Zar 1999).

We transformed effect sizes using Fisher's r-to-z transformation, $z_i = tanh^{-1} r_i$, to ensure that the distribution of effect sizes approximated a normal (DeCoster 2004), and conducted a random effects meta-analysis on the transformed correlation coefficients (Field 2001). A random effects meta-analysis assumes that the true effect size differs among studies and weights each effect size with a parameter accounting for variance across effect sizes (see Appendix 3). We back-transformed all mean effect sizes to r values for ease of interpretation.

We also investigated trends in the pairwise relationships among all three trait pairs using a moving-window regression analysis (Loader 1999). The pairwise data included all studies within the meta-analysis that measured two or more traits at each site (16 studies for LMA:N_{area}, 19 for LMA:N_{mass}, and 15 for N_{mass}:N_{area}). We sorted data points by modelled MAT and we calculated the correlation coefficient r between the two traits at each point within a surrounding bandwidth of 10 data points. The moving-window regressions used estimated mean annual temperature values as a predictor instead of elevation so that we could compare elevational gradients across all latitudes. We fit linear and quadratic regression models to the moving-window coefficients for each trait pair and selected the best model using Akaike's Information Criterion (AIC).

Sources of variation in effect size

We constructed generalized linear models with plant functional type, latitude, elevational range, minimum elevation, and type of variation (within or among species) as predictors, then used a stepwise model selection procedure based on AIC to find the best reduced models. We conducted Z-tests for effect size heterogeneity (Borenstein *et al.* 2009) to compare the weighted mean effect sizes among groups of studies. Finally, we assessed publication bias using a number of tests. We found only limited evidence for publication bias in favour of positive results in LMA studies, and no evidence for bias in N_{mass} or N_{area} studies (See Appendix 4). All analyses were done using R 2.14.1 (R Development Core Team 2011), including the packages *meta* (Schwarzer 2012) and *raster* (Hijmans & van Etten 2013).

Review of experimental studies

We did not conduct a quantitative meta-analysis of the common garden and reciprocal transplant studies due to low availability of published data. Instead, we determined whether each study reported significant genetic effects across elevations, using mean square values from analyses of variance or correlation coefficients from linear regressions. We used a vote-counting approach (DeCoster 2004) to qualitatively assess the genetic basis of variation in LMA, N_{mass}, and N_{area} across elevations.

Results

Overall, we found that (a) LMA and leaf N content varied with mean annual temperature along elevational gradients in similar fashion among plant species, (b) both intraspecific and interspecific variation in these traits are of similar magnitude across disparate and extensive elevational gradients and (c) much intraspecific variation in leaf traits along elevational gradients may be explained by convergent evolution.

Meta-analysis of trait-temperature relationships

Across 46 elevational gradients spanning a total of over 4800 meters, the mean effect of modelled MAT on LMA was negative (mean r = -0.51, 95% CI = [-0.30, -0.68], $P = 1\times10^{-6}$, Figure 2a). For N_{mass}, the mean effect size did not differ significantly from zero (P = 0.84, Figure 2b). On average for each gradient, there was a significantly negative relationship between N_{area} and MAT (mean r = -0.55, 95% CI = [-0.40, -0.67], $P < 1\times10^{-6}$, Figure 2c). The absolute trait values showed only weak trends with MAT when compared among all gradients (Figure 6, given as an appendix to this chapter).

The strengths of the correlations among each of the three trait pairs changed significantly with increasing mean annual temperature, as revealed by moving-window regression analyses (Figure 3). A change in the magnitude or direction of pairwise trait relationships across different environments represents strong evidence for environmental filtering across elevations. A quadratic least-squares regression model fit the pairwise trait correlation data best for all three pairs. At sites with lower mean annual temperature, generally corresponding to high elevations, LMA and N_{area} tended to be positively correlated, but the positive relationship decreased with increasing temperature ($R^2 = 0.77$, $P < 1\times10^{-6}$, Figure 3a). Conversely, at sites with relatively lower temperatures, LMA and N_{mass} tended to be negatively correlated, but the correlation tended to approach zero as temperature increased ($R^2 = 0.24$, $P < 1\times10^{-6}$, Figure 3b). The relationship between N_{mass}:N_{area} correlation and mean annual temperature tended to be most positive at intermediate temperature ($R^2 = 0.39$, $P < 1x10^{-6}$, Figure 3c).

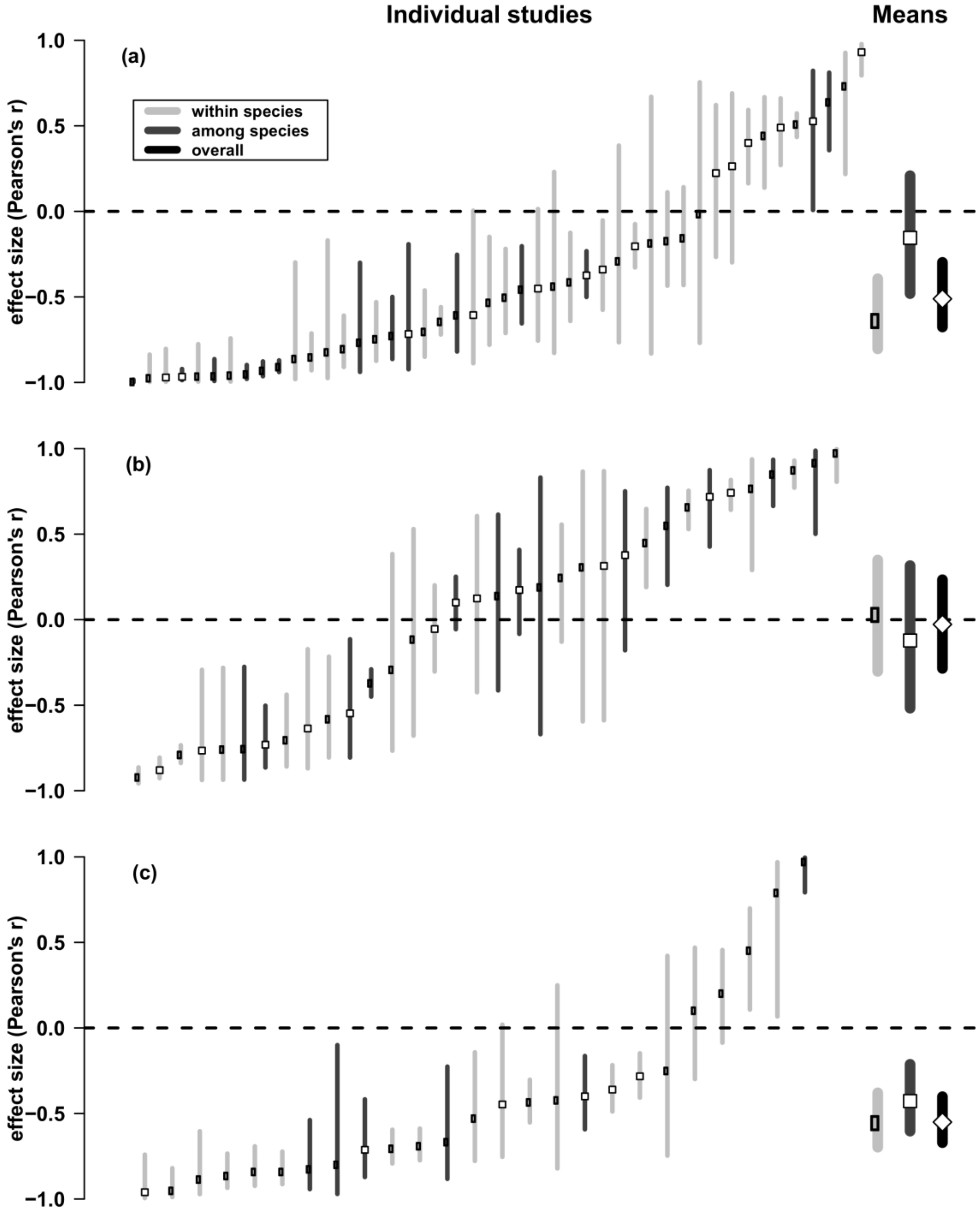

Figure 2. Effect-size plots for (a) LMA, (b) N_{mass}, and (c) N_{area}. Points represent Pearson's r values from each independent regression of trait on modelled mean annual temperature with asymmetrical 95% confidence intervals. Within-species studies are circular points with light-shaded confidence bars, and among-species studies are square points with dark-shaded confidence intervals. The large points at left shows the weighted mean effect size with 95% confidence bar from a random-effects meta-analysis of within-species studies, among-species studies, and overall (diamond point with black-shaded confidence bar; LMA, n =46, r = 0.51; N_{mass}, n = 39, r = 0.03, N_{area}, n = 29, r = 0.55).

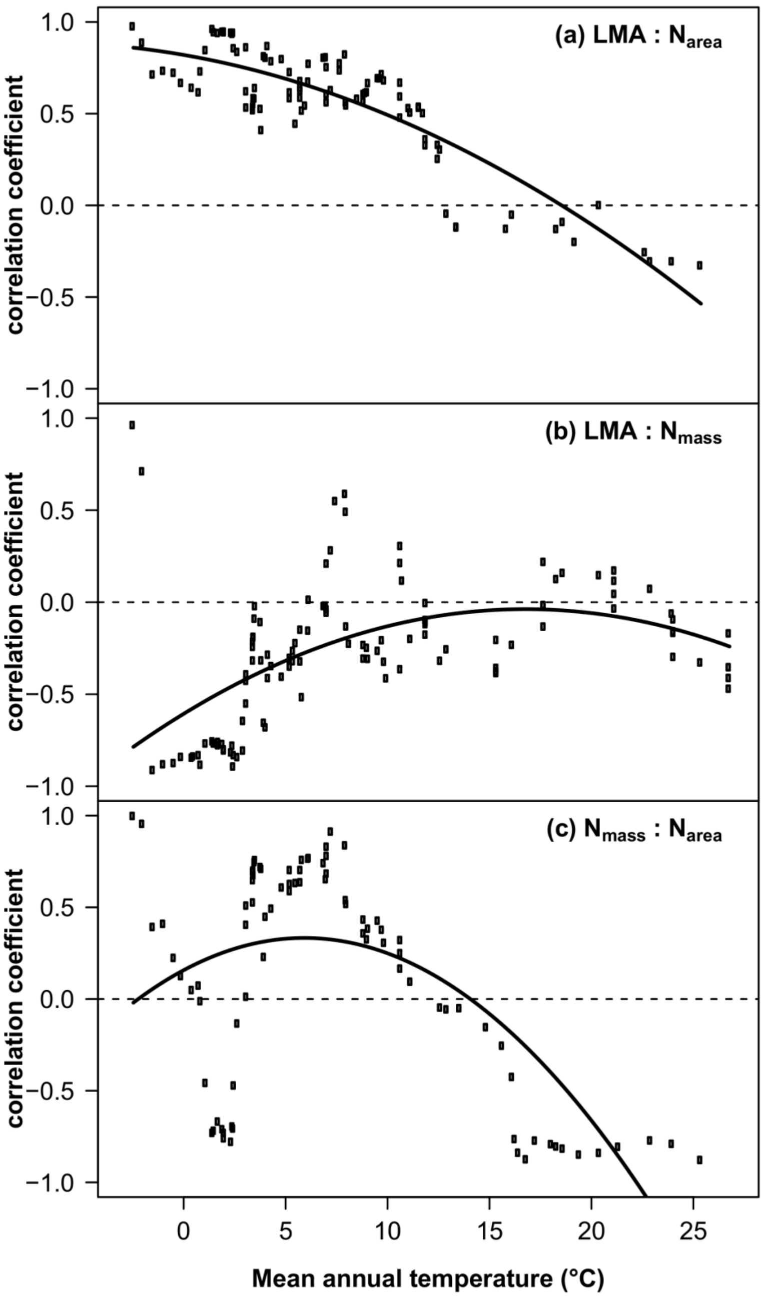

Figure 3. Moving-window regression plots for (a) LMA:N_{area} (R^2 = 0.77), (b) LMA:N_{mass} (R^2 = 0.24), and (c) N_{mass}:N_{area} (R^2 = 0.39). Points represent the correlation coefficient between the two traits at a particular site and the ten surrounding data points, sorted by estimated mean annual temperature. A quadratic trendline was fit to each pairwise comparison.

Sources of variation in effect size

Studies that measured variation in LMA within species had a significantly more negative mean effect size than did studies that measured community-level variation ($Z = 2.35$, $P = 0.02$). For N_{mass}, there was no significant difference between intraspecific and interspecific studies, neither of which had an overall mean effect size different from zero ($Z = 0.52$, $P = 0.60$). Mean effect sizes for N_{area} within species and among species were both significantly negative ($P < 1x10^{-6}$, $P = 1x10^{-6}$) and did not differ from one another ($Z = 0.31$, $P = 0.95$).

Plant functional types varied in their average trait response to decreasing MAT with increasing elevation. LMA decreased with increasing MAT in forbs (mean r = -0.47, 95% CI = [-0.24, -0.65], $P = 0.002$) and angiosperm trees (mean r = -0.74, 95% CI = [-0.44, -0.90], $P = 0.0001$), with no significant trend in conifers ($P = 0.81$). N_{area} was negatively correlated with MAT in forbs (mean r = -0.46, 95% CI = [-0.27, -0.62], $P = 2\times10^{-5}$) and angiosperm trees (mean r = -0.65, 95% CI = [-0.46, -0.79], $P < 1\times10^{-6}$); as before, conifers showed no trend. No individual functional type showed a significant relationship between MAT and N_{mass}.

Model selection did not show a consistent effect of any one factor in determining variation in effect size across the three traits (see Table 1). For LMA, type of variation (within-species versus among-species) explained the most variation in effect size in the best models, with within-species studies having a more negative mean effect size. For N_{mass}, plant functional type was retained as a significant predictor in the best models because conifers tended to have a weaker relationship between N_{mass} and MAT, while other functional groups tended to have greater N_{mass} at sites with lower MAT at higher elevations. Finally, for N_{area}, only gradient length was retained as a significant predictor in the best models, indicating that studies conducted over a wider range of elevations tended to have larger absolute effect sizes, as expected.

Review of experimental studies

In a majority of the common garden studies we reviewed, genetic divergence among populations from different elevations or latitudes was a significant driver of variation (Figure 4). Of 17 studies measuring LMA, 13 (76%) showed significant genetic effects. Of 12 studies measuring N_{mass}, 9 (75%) showed genetic effects, and 4 of 5 (80%) studies measuring N_{area} showed these effects.

The most salient results of our meta-analysis are (a) general patterns emerge with elevation in leaf economic traits due to consistent abiotic gradients associated with elevation; (b) trait variation within plant species is equal to or greater than community-level variation, supporting the view that plant community composition is the result of a hierarchy of processes acting on individuals (Sundqvist, Sanders & Wardle 2013); (c) a significant proportion of the variation observed in the field is driven by genetic effects, consistent with our expectation that parallel evolutionary processes and phenotypic plasticity act in concert to produce functionally meaningful patterns in plant traits.

Across many taxa and systems, LMA was positively associated with elevation; previous studies have found that LMA increases with elevation in alpine plants (Körner *et al.* 1989) and trees (Körner 2012). Furthermore, LMA varies significantly with light, temperature, CO_2 concentration, and nutrient stress across plant taxa (Poorter *et al.* 2009), all variables that change with elevation above sea level. Although some functional groups responded positively to elevation (forbs, angiosperm trees) and others did not respond (conifers), no functional group

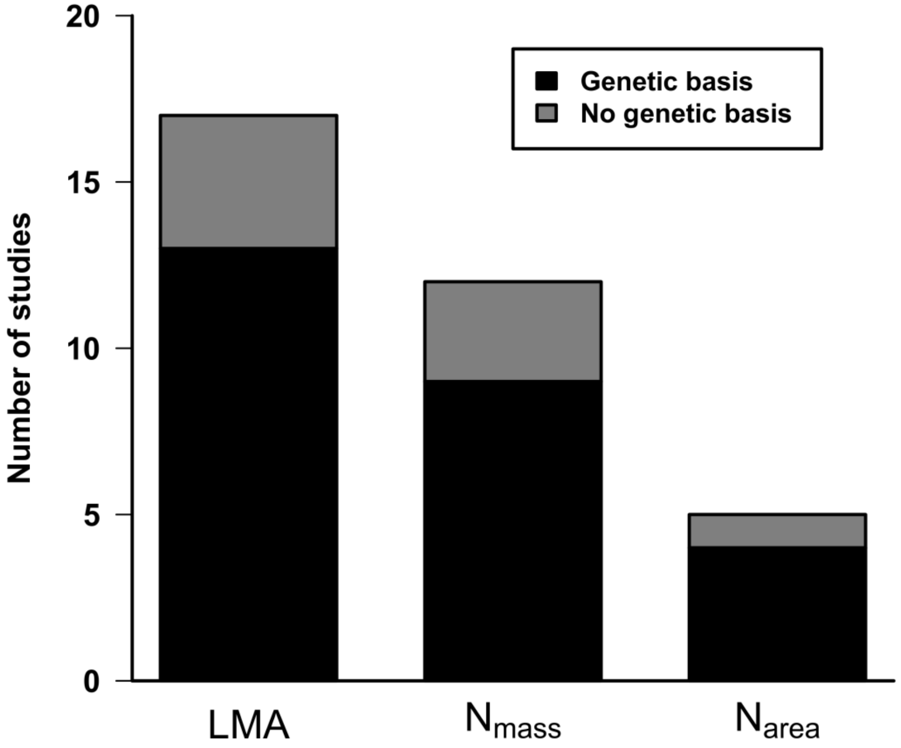

Figure 4. Bar plot showing the proportion of studies that found a significant genetic basis to variation along an elevational or latitudinal gradient from the quantitative reviews of common garden experiments measuring each of the three traits (LMA, 13/17 studies; N_{mass}, 9/12 studies; N_{area}, 4/5 studies).

declined significantly in LMA as elevation increased. Similarly, the meta-analysis of Poorter *et al.* (2009) found that plant functional groups differed in their plasticity with respect to environmental gradients. We found significantly positive trends in LMA not only among populations of the same species, but among species assemblages at different elevations along a gradient. Increased leaf density that often accompanies increased LMA is associated with a higher percentage of biomass in N-poor cell walls (Craine 2009; Poorter *et al.* 2009). Nonetheless, N_{area} increased with LMA as expected. The lack of response in N_{mass}, contrary to our hypothesis, suggests that variation in N_{mass} is limited by physiological constraints that vary independently of elevation. Coupled with an increase in LMA and N_{area} with elevation, constant N_{mass} leads to higher C:N ratios in leaf tissue. Elevated C:N ratios in leaves should, in turn, influence foliar herbivory, decomposition, nutrient cycling, and transpiration, ultimately feeding back into plant community structure (Bardgett & Wardle 2010). Our pairwise correlation analysis showed that at sites with relatively lower mean annual temperature (at high elevations), LMA and N_{area} were more positively correlated and LMA and N_{mass} were more negatively correlated, although the trend was relatively weaker for LMA:N_{mass}. The tightness of the relationship among traits tends to change along with their absolute values, providing additional support for the hypothesis that the strength of environmental filters changes along environmental gradients.

Discussion

We found that N_{mass} was as likely to decrease with elevation as increase, which may be explained by biological constraints on the range of leaf N content within a species or local community coupled with different optimum N concentrations in different environmental contexts. Although N content in plant tissue is often closely linked with soil N availability, which shows no global trend with elevation (Körner 2007), developmental constraints on high-elevation plants may decouple N_{mass} and N_{area} from soil N content (Körner 1989), contributing to the trends observed here. In particular, constraints on tissue formation in high-elevation plants may inhibit the dilution of N and other nutrients in leaf tissue (Körner 1989), leading to higher observed Narea values and higher metabolic activity per leaf area at high elevations, regardless of the degree of soil nutrient limitation. Belowground functional traits, such as specific root length or root nutrient content, show similar patterns due to similar constraints on tissue formation and growth resulting from stressful environments at high elevations (Körner & Renhardt 1987, Álvarez-Uria & Körner 2011). Unfortunately, sufficient data do not yet exist to conduct a meta-analysis for belowground traits.

While the overall relationship between elevation and N_{mass} was not negative as we predicted, we found that at least two traits, LMA and N_{area}, that are associated with plant life-history strategies (Wright *et al.* 2004) varied predictably with elevation. This finding lends support to the hypothesis that selection imposed by the environment on linked traits leads to trait convergence along similar environmental gradients. Specifically, selective pressures associated with harsher environmental conditions at higher elevations promote leaf trait syndromes associated with superior stress tolerance but inferior competitiveness; this supports the hypothesis that the role of environmental filtering in community assembly increases with elevation (Callaway *et al.* 2002). However, in many cases, LMA and leaf N content do not fully capture the syndrome of responses exhibited by high-elevation plants; for example, due to colder temperatures and shorter growing seasons at higher elevations, tissue formation is highly constrained (Körner *et al.* 1989). As a result, leaf size tends to decrease with elevation (Körner, Bannister & Mark 1986; Kouwenberg *et al.* 2007; Bresson *et al.* 2011), causing allometric responses in leaf morphological traits including LMA. Although abiotic constraints at high elevations may explain some of the variation we observed without invoking adaptation, our review of common-garden studies provides additional support for the existence of adaptive trade-offs along elevational gradients.

In our meta-analysis, population-level variation equalled or exceeded community-level variation along elevational gradients, suggesting a consistent selective effect of environmental factors associated with elevation across multiple levels of organisation. The composition of a plant assemblage is the result of a hierarchy of filters that select species and traits from a regional pool over both ecological and evolutionary time, comprising both abiotic filters and biotic interactions (Lavorel & Garnier 2002). Intraspecific variability enables plants to pass through abiotic filters across a wider range of elevations (Jung *et al.* 2010). Intraspecific variation was equal to or greater than interspecific variation, suggesting that the filter imposed by elevation on individual plants may dictate the composition of local communities found along elevational gradients, and that intraspecific variation is an important driver of community structure and ecosystem function.

In general, the consistent responses we observed can be explained by a combination of phenotypic plasticity and genetic variation. Our qualitative review of common garden studies using plants from multiple sites along elevational and latitudinal gradients showed that genetic divergence often explains a significant amount of variation in our three functionally important leaf traits, LMA, N_{mass}, and N_{area}. Genetic variation is essential for plants to adapt to long-term climate change. Phenotypic plasticity is the most important mechanism by which plants can react to short-term environmental changes (Agrawal 2001; Matesanz, Gianoli & Valladares 2010), but if the magnitude of change is severe enough, plastic responses will be insufficient to cope with change (Valladares, Gianoli & Gómez 2007). Unless the reaction norm evolves to fit the new environmental conditions, the species will suffer long-term fitness consequences or become locally extinct (Sultan 2000). It is important to note that plasticity is itself a trait under genetic control (Schlichting & Pigliucci 1993) and that evolution by natural selection may lead to increased plasticity for important plant functional traits in variable environments and in a changing climate (Agrawal 2001; Matesanz *et al.* 2010).

With climate change, plants are being forced to evolve, move, be plastic, or go locally extinct (Bellard *et al.* 2012). For example, movement toward mountaintops and toward the poles is taking place, resulting in the reshuffling of plant genotypes, species, and communities on the landscape (Parmesan & Yohe 2003; Beckage *et al.* 2008; Lenoir *et al.* 2008; but see Crimmins *et al.* 2011). Global patterns of plant functional traits with elevation may be useful as a space-for-time substitution to provide insights into the responses of plant species and communities to temporal change caused by humans (Dunne *et al.* 2004; Fukami & Wardle 2005). Plant taxa that show relatively higher levels of genetic and phenotypic variation along elevational gradients may have a higher capacity to respond to global change, in addition to expected uphill or poleward dispersal (Beckage *et al.* 2008; Rapp *et al.* 2012). Physiological changes driven by both genetic divergence and phenotypic plasticity may contribute to the overall response of plant communities to the selective agent of climate change (Bellard *et al.* 2012), just as they determine the ability of plants to pass through existing environmental filters. Regardless of the mechanism, our results indicate that different locally adapted ecotypes, and different species within assemblages, are associated with changes in the abiotic environment along elevational gradients. Our results speak to the paramount role of abiotic filtering in community assembly, with potential implications for changing community structure and ecosystem function on a warming planet.

Acknowledgments

The authors would like to thank J. Schweitzer and A. Classen for insightful comments during the writing process.

Appendices

Appendix 1: References of studies included in meta-analysis

We extracted data from these studies for the meta-analysis. However, they are not cited in the text of the chapter.

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Appendix 2: References for studies used in review of experimental studies

We extracted data from these studies for a review of common-garden experiments. However, they are not cited in the text of the chapter.

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Appendix 3: Equations

The following equations were used to calculate the mean and standard error of the effect size for each trait. Here, k = the number of studies in the meta-analysis for the trait, n_i = the sample size of study i, and r_i = the correlation between elevation and the trait estimated in study i. We used Fisher's transformation (Eq. 1) to estimate the effect size z_i from each study. The variance v_i of each effect size is inversely proportional to sample size (Eq. 2). $\overline{z}_{fixed}$ is the mean effect size from the fixed effect model (Eq. 3), estimated using the inverse-variance weights, $(n_i - 3)$. $\overline{z}_{fixed}$ is used to estimate the heterogeneity parameter Q (Eq. 4). The effect size weights from the fixed-effect model are also used to calculate the constant c (Eq. 5). Q, k, and c are used to estimate τ^2, the parameter that accounts for variance in effect size across studies (Eq. 6). Random-effect weights w_i^* are proportional to sample size and are additionally weighted with the τ^2 parameter (Eq. 7). Finally, $\overline{z}$, the mean effect size from the random-effects model, is estimated using the random-effects weights (Eq. 8), as well as its standard error $SE(\overline{z})$ (Eq. 9).

Equation 1. $z_i = tanh^{-1} r_i$

Equation 2. $$v(z_i) = \frac{1}{n_i - 3}$$

Equation 3. $$\bar{z}_{fixed} = \frac{\sum_{i=1}^{k}(n_i - 3)z_i}{\sum_{i=1}^{k}(n_i - 3)}$$

Equation 4. $$Q = \sum_{i=1}^{k}(n_i - 3)(z_i - \bar{z}_{fixed})^2$$

Equation 5. $$c = \sum_{i=1}^{k}(n_i - 3) - \frac{\sum_{i=1}^{k}(n_i - 3)^2}{\sum_{i=1}^{k}(n_i - 3)}$$

Equation 6. $$\tau^2 = \frac{Q - (k - 1)}{c}$$

Equation 7. $$w_i^* = (\frac{1}{n_i - 3} + \tau^2)^{-1}$$

Equation 8. $$\bar{z} = \frac{\sum_{i=1}^{k} w_i^* z_i}{\sum_{i=1}^{k} w_i^*}$$

$$SE(\bar{z}) = \sqrt{\frac{1}{\sum_{i=1}^{k} w_i^*}}$$

Equation 9.

Appendix 4: Assessment of publication bias

We used the rank-correlation method (Begg & Mazumdar 1994) and Egger's linear regression test (Egger 1997) to test for publication bias; a disproportionate number of small studies with large positive effect would indicate that some non-significant results were withheld from publication. Rank correlation tests detected no significant asymmetry in funnel plots of study size versus effect size, indicating no significant publication bias for any trait ($P > 0.05$ in all cases). However, Egger's tests indicated a significant increase in variance of effect sizes as the standardized effect size increased for LMA ($P = 0.01$), but not for N_{mass} or N_{area} ($P > 0.05$). Therefore, one of two commonly used tests for publication bias in meta-analyses provided evidence that some investigators measuring LMA along an elevational gradient may have found no relationship with elevation and not published their results; the findings here should be considered in this light.

Table 1. Model selection criteria for models exploring sources of variation in effect size for three functional traits.

LMA	
predictors	ΔAIC
variation type, minimum elevation, latitude	0
variation type, minimum elevation, elevation range	0.39
variation type, minimum elevation	0.44
leaf N_{mass}	
predictors	ΔAIC
latitude, minimum elevation, PFT, variation type	0
latitude, minimum elevation, PFT	0.03
latitude, minimum elevation, variation type	1.11
leaf N_{area}	
predictors	ΔAIC
elevation range	0
elevation range, latitude	0.96
elevation range, variation type	1.01

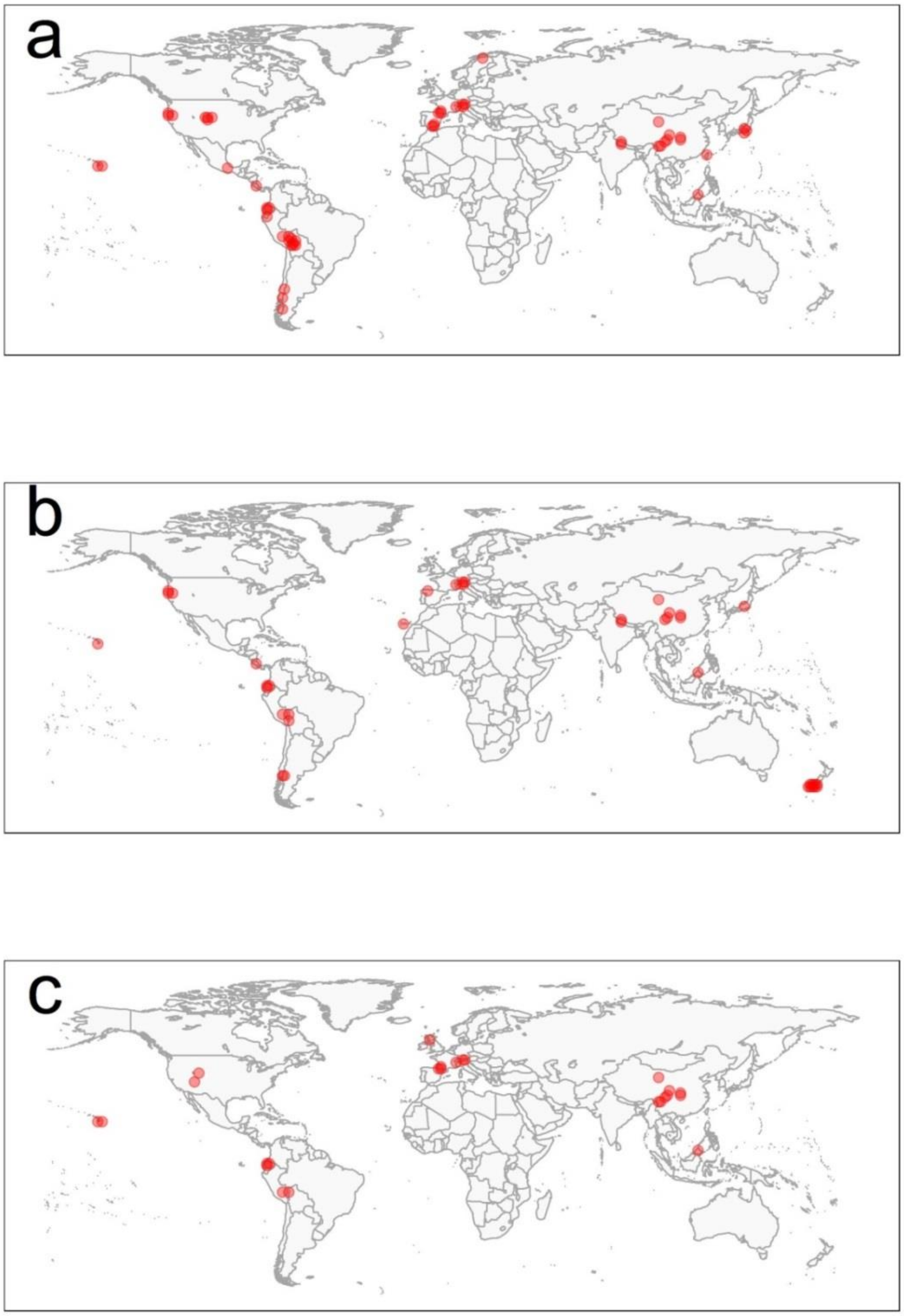


Figure 5. Study sites where one or more elevational gradients included in the meta-analysis were sampled for (a) LMA, (b) N_{mass}, and (c) N_{area}.

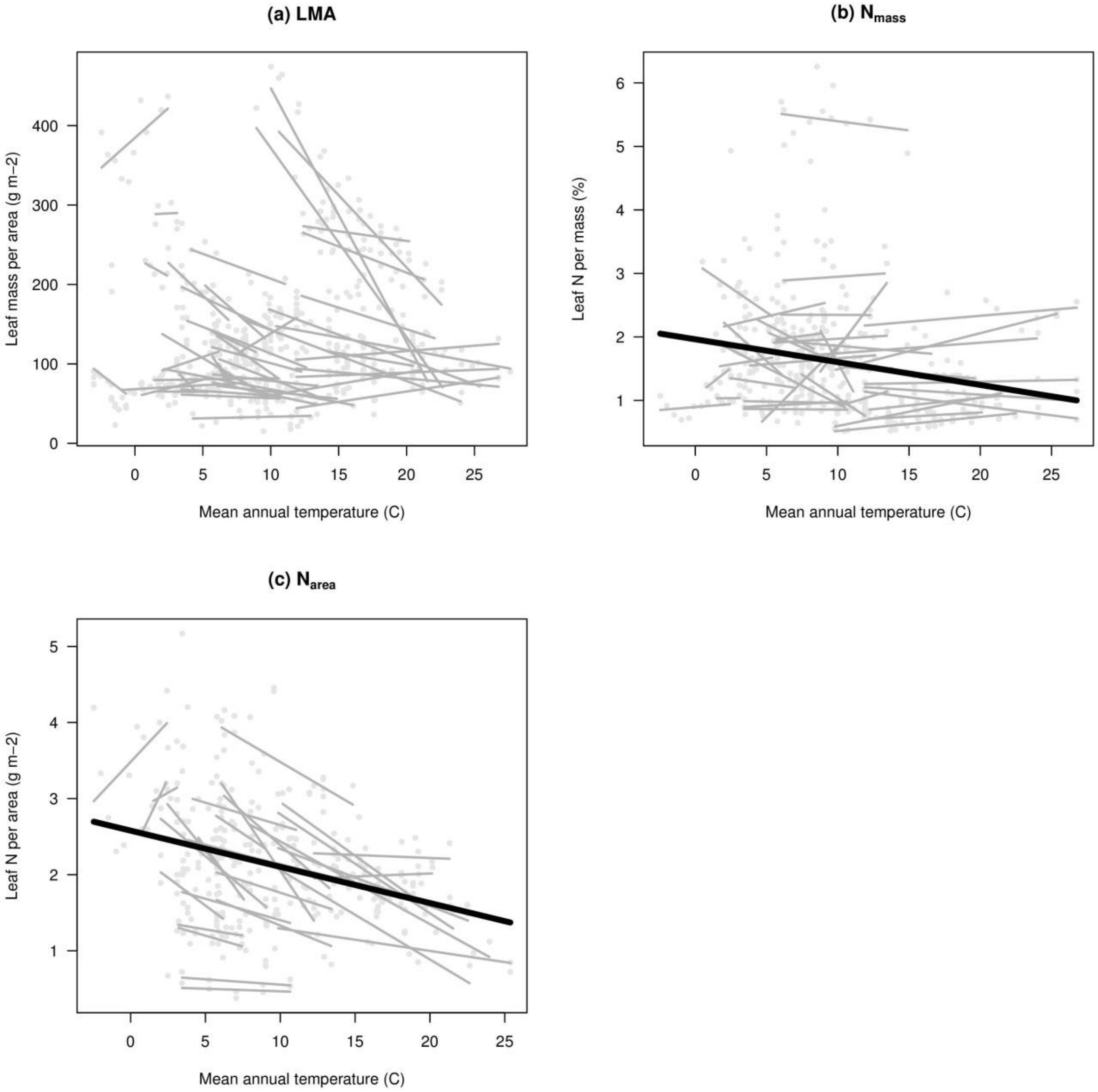


Figure 6. Meta-regression plots for (a) LMA, (b) N_{mass}, and (c) N_{area}. Grey lines represent least-squares regression fits for each study, grey points represent raw site mean data from the individual studies, and bold black lines represent a least-squares meta-regression fit for all studies combined. A small but significant amount of variation was explained by modeled mean annual temperature in N_{mass} ($R^2 = 0.04$) and N_{area} ($R^2 = 0.10$).

CHAPTER II

ACCOUNTING FOR THE NESTED NATURE OF GENETIC VARIATION ACROSS LEVELS OF ORGANIZATION IMPROVES OUR UNDERSTANDING OF BIODIVERSITY AND COMMUNITY ECOLOGY

A version of this chapter has already been published by Quentin D. Read, Sean M. Hoban, Maarten B. Eppinga, Jennifer A. Schweitzer, and Joseph K. Bailey:

Read, Q. D., S. M. Hoban, M. B. Eppinga, J. A. Schweitzer, and J. K. Bailey. Accounting for the nested nature of genetic variation across levels of organization improves our understanding of biodiversity and community ecology. *Oikos*: accepted. DOI: 10.1111/oik.02760

Quentin Read both conducted the study and wrote and revised the manuscript; other co-authors assisted with developing the idea, helping with some statistical analysis, and aiding in the writing and revision process.

Abstract

Recent work has demonstrated that the presence or abundance of specific genotypes, populations, species and phylogenetic clades may influence community and ecosystem properties such as resilience or productivity. Many ecological studies, however, use simple linear models to test for such relationships, including species identity as the predictor variable and some measured trait or function as the response variable without accounting for the nestedness of genetic variation across levels of organization. This omission may lead to incorrect inference about which source of variation influences community and ecosystem properties. Here, we explicitly compare this common approach to alternative ways of modeling variation in trait data, using simulated trait data and empirical results of common-garden trials using multiple levels of genetic variation within *Eucalyptus, Populus,* and *Picea*. We show that: (1) when nested variation is ignored, an incorrect conclusion of species effect is drawn in up to 20% of cases; (2) overestimation of the species effect increases—up to 60% in some scenarios—as the nested term explains more of the variation; and (3) the sample sizes needed to overcome these potential problems associated with aggregating nested hierarchical variation may be impractically large. In common-garden trials, incorporating nested models increased explanatory power twofold for mammal browsing rate in *Eucalyptus*, threefold for leaf area in *Populus*, and tenfold for branch number in *Picea*. Thoroughly measuring intraspecific variation and characterizing hierarchical genetic variation beyond the species level has implications for developing more robust theory in community ecology, managing invaded natural systems, and improving inference in biodiversity-ecosystem functioning research.

Introduction

Biodiversity is inherently nested. Genetic variation is differentially expressed within sub-populations and populations, within and among species, and, most broadly, across phylogenetic clades. At any level of biological organization, this variation may be expressed in traits that may in turn influence community structure or ecosystem properties. For example, two recent experimental studies showed that population-level genetic differentiation drives community structure in natural arthropod communities (Barbour *et al.* 2009), and that trait variation among species and phylogenetic clades influence a diversity-stability relationship in the overall accumulation of plant biomass in artificial communities (Cadotte *et al.* 2012). Understanding the relationship between genetic diversity, species diversity, and ecosystem properties has been fundamental to basic research linking ecological and evolutionary disciplines for decades (Tilman *et al.* 1997; Hooper *et al.* 2005; Fargione *et al.* 2007), including the growing field of community and ecosystem genetics (Whitham *et al.* 2006; Hughes *et al.* 2008; Genung *et al.* 2012; Bailey *et al.* 2014). Recently, studies in eco-evolutionary dynamics have shown experimentally that genotype changes in response to selective environmental pressures often have ecosystem consequences (Turcotte *et al.* 2013; Hiltunen & Becks 2014). This knowledge is critical for predicting the consequences of climate change (Singer & Thomas 1996; Savolainen *et al.* 2007; Visser 2008), assessing the effects of exotic species (Hobbs & Huenneke 1992; Levine & D'Antonio 1999; Levine *et al.* 2003; Fargione & Tilman 2005), and boosting food production, among other ecosystem services (Hoehn *et al.* 2008; Feld *et al.* 2009; de Bello *et al.* 2010; Cardinale *et al.* 2012). Correct inference regarding the source of genetic and trait variation, and its consequences, is critical for making predictions grounded in theory about how biodiversity changes will affect ecosystem functioning.

Accounting for the nested nature of biodiversity (e.g., that genotypes are nested within populations, populations exist within species, and species are nested within phylogenies) is rarely undertaken in studies measuring the functional consequences of biodiversity (but see Cook-Patton *et al.* 2011; Schöb *et al.* 2015). Without considering variation in a trait at a lower level (e.g., genotype), estimates of differences at a higher level (e.g., species) may potentially be inflated. Similarly, examining trait differences among phylogenetic clades without accounting for variation among species would yield similarly increased estimates of effect size. In both cases, this is simply because only considering higher-order variation (i.e., species or phylogenetic clade, respectively) means that much of the variation among individuals is assigned incorrectly, either to higher-level differences or to residual variation (i.e., variation among species may simply be due to variation among genotypes or populations and not reflect true differences among species). Despite the potential for inflated effect sizes and incorrect inference, estimates of differences in traits or functions among populations, or species, in a range of contexts routinely do not account for the nested nature of biodiversity. For example, many landmark studies in biodiversity-ecosystem functioning relationships have treated species richness as synonymous with diversity, neglecting any genotypically driven or within-species variation (e.g., Tilman *et al.* 1997; Caliman *et al.* 2013; Isbell *et al.* 2013). One alternative in biodiversity-ecosystem functioning experiments is to manipulate genotype identity or diversity within species, as several recent experimental studies have done (Hiltunen & Becks 2014; Rudman *et al.* 2015). However, this labor-intensive approach is admittedly prohibitive in some systems. In such cases, we recommend either indirectly addressing intraspecific variation with a functional trait approach (Díaz *et al.* 2007) or, at the minimum, explicitly stating that taking into account variation at lower levels could change the magnitude or significance of the findings.

Trait distributions of different species within an assemblage can overlap greatly; therefore, genotypic variation within species may represent the bulk of variation in traits that drives variation in ecosystem function (Crutsinger *et al.* 2006, 2014). Unfortunately, variation between genotypes is not captured in an aggregated estimate of species-level variation. Appropriate aggregation requires much larger sample sizes than would be expected from sampling a single distribution. Hence, stratifying trait samples only at the species level may lead to an increased chance of the investigator concluding that there is an effect of species identity on a response trait or ecosystem process. In many cases, the result is more properly attributed to variation at the within-species genetic level. If sampled individuals are only binned by species, the uneven sampling of variation at levels lower than species can lead to Type I error where trait variation is attributed solely to species identity. This phenomenon is not merely statistical nuance – this misleading and incorrect inference may have profound effects on how we interpret ecological patterns and how theory is developed. For example, Schöb *et al.* (2015) found that for barley plants and associated weed species that were planted in monoculture and mixture, the effect on overall productivity of increasing genotypic variation in barley was qualitatively different than the effect of increasing species richness of associated weeds. This result is different from what one would predict from a non-nested model. It suggests, as do other similar studies (e.g., Pante *et al.* 2015), that aggregating or averaging out intraspecific variation can lead investigators to overlook critical processes that structure natural systems.

An approach that goes beyond a narrow focus on species identity has important implications for extending the scope and power of numerous fields of ecological research. For example, in invasion biology, genetically based trait variation can explain variation in the success of invasive species (Lindholm *et al.* 2005; Roman & Darling 2007), as well as variation in the ability of resident communities to resist invaders (Crutsinger *et al.* 2008). More generally for community ecology, the inclusion of genetically based intraspecific variation is vital for increasing our understanding of the forces driving species coexistence. For example, individual-level trait diversity is necessary for species coexistence in temperate forests (Clark 2010), and underlies the relationship between environmental stress and the net balance between competition and facilitation among tree species (Coyle *et al.* 2014). Predictions of species responses to ongoing global change may also be made more accurate by incorporating genotypic and phenotypic trait variation within species, which has been shown in the context of trait variation within a single species (Anderson & Gezon 2015) as well as across species at the landscape level (Fitzpatrick & Keller 2015). Finally, meta-analyses (Cardinale *et al.* 2012) and reviews (Hooper *et al.* 2005) in the biodiversity-ecosystem functioning field have synthesized studies assessing the consequences of changes to biodiversity. However, diversity metrics that are partitioned more finely than at the species level have been largely neglected in the studies that are synthesized in these meta-analyses. If the published studies from which estimates of effect size in these meta-analyses are derived fail to account for the nested nature of biodiversity, the grand-mean effect size estimates may be inflated or incorrect (Borenstein *et al.* 2009).

Using simulated trait data and three empirical examples as case studies, we examine how inferences drawn from models including nested hierarchical variation differ from those drawn using models that do not incorporate this variation. We show that: (1) when hierarchically nested variation is ignored and only one level of variation, such as the species level, is considered, an incorrect conclusion of a species identity effect where one does not exist is drawn in up to 20% of cases (i.e., Type I error); (2) overestimation of the proportion of variation explained by species identity increases up to 60% under some scenarios; and (3) the sample sizes needed to overcome these potential problems associated with aggregating nested hierarchical variation may be too large to feasibly achieve, demonstrating the importance of partitioning variation among individuals at multiple levels of diversity. Such potentially high rates of type I error and inflated effect size suggest that taking the hierarchically nested nature of biodiversity into account is an important step in biodiversity research and should be considered in future theoretical and empirical studies.

Simulated data sets

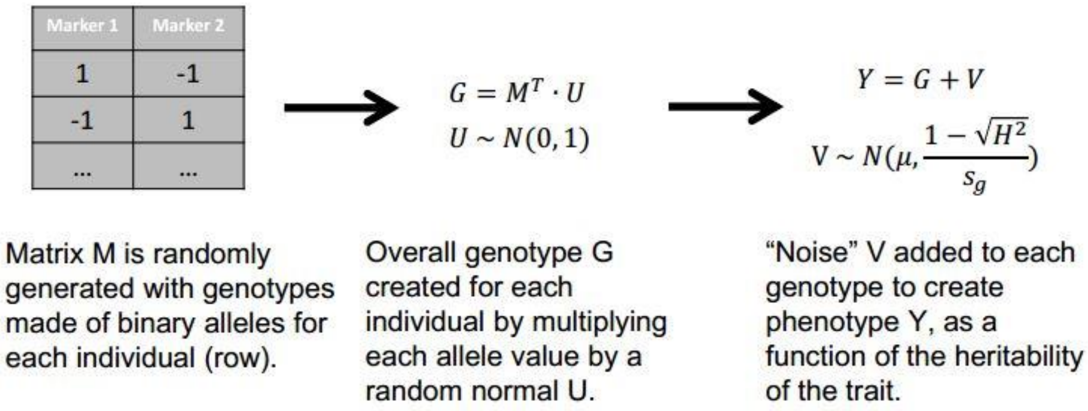
Generation of simulated data

Our goal was to determine the statistical effect of nesting compared to an approach without nesting when a genetic hierarchy (i.e., genotypes nested within species) exists and is available to incorporate into ecological field or laboratory studies. Specifically, we examined variation in genotypes when comparing trait means in a system in which some variation in a continuous quantitative trait is due to among-genotype differences within a species and some is due to among-species differences. The hierarchical levels simulated need not necessarily be limited to genotype nested within species; our results could equally apply to any other level of genetic hierarchy, such as species nested within phylogenetic clades.

We simulated trait data as follows. First, we generated an allele matrix *M* with variable numbers of binary loci with a value of either 1 or -1 that together represent a simulated genotype for each individual within a species. In each replicate simulation, *M* is an $m \times n$ matrix, where *m* is the number of individuals in the simulated population and *n* is the number of genetic loci. The total additive genetic variation *G* for each individual was calculated by taking the cross product of the transposed matrix containing allele values and a vector with length *m* of normally distributed random variables *U* representing variation across genotypes: $G = M^T \cdot U; U \sim N(0, \sigma_g)$. This results in a vector of length *m*. Each individual was assigned a phenotype or trait value *Y* by adding a normally distributed environmental noise term *V* (also a vector of length *m*) to its genotype value: $Y = G + V; V \sim N(\mu_s, \frac{1-\sqrt{H^2}}{\sigma_g})$. In this way, individuals of the same genotype may have differing phenotypes. For each replicate simulation run, we generated a new allele matrix *M* and vectors *U* and *V*. The heritability value H^2 represents the proportion of variation in the trait value due to variation in the genotype (i.e., broad-sense heritability). The simulation procedure is outlined in Figure 7.

All simulations consisted of two species. The parameters that were varied across simulations included the standard deviation σ_g of the genotype value, the mean trait value μ_s for each species, the heritability H^2, the number of individuals, and the number of binary loci. We simulated 100 datasets for each combination of parameters, including 10, 50, and 100 individuals per species; 1, 2, and 3 loci (yielding 2, 4, and 8 possible genotypes per species); heritability values $H^2 = \{0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9\}$; species means $\mu_1 = 0$ and $\mu_2 = \{0, 0.1, 0.25, 0.5, 1, 2, 5, 10\}$; and genotype standard deviation $\sigma_g = \{0.1, 0.25, 0.5, 1, 2, 5, 10\}$. In addition, to assess the influence of increasing the number of loci per individual while heritability is held constant, we conducted an additional simulation with 10 datasets for each of the following combinations of parameters: 100 individuals per species; each possible number of loci from 1 to 10; $H^2 = 0.5$; species means $\mu_1 = 0$ and $\mu_2 = \{0, 1, 5, 10\}$; and genotype standard deviation $\sigma_g = \{0.1, 1, 5, 10\}$. Data were simulated in the R 3.0.1 statistical programming language (R Core Team 2013).

We were additionally interested in assessing the sample size needed to appropriately aggregate trait differences at the species level while assuming different magnitudes of genotype-level variation. We considered the specific case in which two species had identical mean trait



Marker 1	Marker 2
1	-1
-1	1
...	...

$$G = M^T \cdot U$$
$$U \sim N(0,1)$$

$$Y = G + V$$
$$V \sim N(\mu, \frac{1-\sqrt{H^2}}{s_g})$$

Matrix M is randomly generated with genotypes made of binary alleles for each individual (row).

Overall genotype G created for each individual by multiplying each allele value by a random normal U.

“Noise” V added to each genotype to create phenotype Y, as a function of the heritability of the trait.

Figure 7. Diagram outlining the procedure we used to generate simulated trait data, including generating sets of alleles for each individual and drawing trait values from hierarchically nested normal distributions.

values, but each species consisted of eight genotypes that differed in trait means, with heritability $H^2 = 0.5$, and genotype standard deviation $\sigma_g = \{0.1, 0.25, 0.5, 1, 2, 5, 10\}$. The more appropriate (i.e., larger) the sample size, the closer to zero the difference in estimated species means, $|\mu_1 - \mu_2|$, should be. We conducted this separate simulation with larger variation of sample sizes (10^1, 10^2, 10^3, 10^4, and 10^5 individuals per species). We generated 100 replicate datasets for each of the above combinations of sample size and genotype standard deviation. For each replicated dataset, we calculated the observed difference between the species-level sample means and compared them to the expected difference of zero, assuming a normal distribution, $N(0, \sigma_g)$, to estimate the standard error of the difference between means across datasets. The sample size simulation was carried out in the MATLAB v8.4 programming language (MathWorks, Natick, MA, USA, 2014).

Statistical analysis of simulated data

We used two different approaches to statistically model the variation in trait means within each data set: (1) non-nested linear models with the trait as response variable and species as a categorical fixed effect, a common method of analyzing trait data across a broad range of studies and sub-disciplines, and (2) nested mixed models with the trait as response variable, species as a categorical fixed effect, and genotype as a categorical random effect nested within species. It is important to note that we did not explicitly use species or genotype diversity as an effect in either model; we concerned ourselves only with the way individuals were binned when statistically modelling variation in trait values. The models were fit using the R language (R Core Team 2013); in particular, mixed models were fit with the *lmer* function in the *lme4* package (Bates *et al.* 2015) using the restricted maximum likelihood method.

The coefficient of determination (R^2) and Akaike's Information Criterion (AIC) were calculated for both types of model. We calculated the R^2 for nested mixed models using a variance decomposition technique (Nakagawa & Schielzeth 2013); we partitioned the total variance estimated by the model into the variance of the random effects and of the fixed effects, and the residual variance. The R^2 value is decomposed into two components: the marginal R^2, or the percent variation explained by fixed effects, is the variance of the fixed effects divided by the total variance (fixed + random + residual variance). The conditional R^2, accounting for both fixed and random effects, is the sum of the fixed and random effect variance divided by the total variance term. We compared the estimates of the proportion of variation explained by species in non-nested models and nested mixed models by subtracting the marginal R^2 of the mixed model from the R^2 value of the non-nested model. We calculated the variance components with the *rsquared.GLMM* function in the *MuMIn* package (Kamil Bartoń 2015). For both the non-nested and the mixed nested models, we calculated the *p*-value using a likelihood ratio test, comparing the full fitted model with a model only fitting an intercept for the non-nested models, and a model fitting the intercept and the random effect for the nested models (*lmtest* package; Achim Zeileis & Torsten Hothorn 2002).

We quantified how each parameter of the simulation influenced (1) the R^2 value associated with the species term in each model type, (2) the difference in AIC scores (hereafter ΔAIC) between the nested and non-nested models, and (3) the proportion of times that each model included a species coefficient significantly different from zero at $\alpha = 0.05$. Scripts from all of our analyses are included as supplementary information.

Influence of variance in parameters on inference drawn from simulated datasets

Fitting both non-nested and nested mixed models to our simulated datasets revealed that non-nested models uniformly overestimate the proportion of variation explained by species (ΔR^2, the R^2 of the non-nested model subtracted from the marginal R^2 of the nested model) if genotype-level variation is ignored, with overestimates of marginal R^2 ranging from 0 to 0.62. Overestimates of species-level variation decreased with increasing sample size and increased with heritability (Figure 8a), although ΔR^2 values varied most at the lowest sample size of 10 individuals, occasionally exceeding 0.6 in some simulation runs (Figure 14, see chapter appendix). In addition, overestimates were highest when the variance in genotype means was similar to the difference between species means (i.e., both very small or both very large; Figure 8b). When either variance parameter was much larger than the other, the non-nested model estimated the proportion of variation due to species accurately, even at high heritability values (Table 2; Figure 14, see chapter appendix).

In addition, despite the increased number of parameters required to model variation by genotype and the penalty assessed for model complexity by Akaike's Information Criterion, model selection most often chose the more complex model incorporating genotype; ΔAIC between non-nested and nested models was positive 83% of the time and increased with heritability (Figure 9). The ΔAIC increased with increasing sample sizes from 10 to 100 individuals, but did not systematically change with the absolute difference in species means or the among-genotype standard deviation (Table 2; Figure 15 in chapter appendix). This indicates that a nested model is a more parsimonious fit to the data regardless of the differences in trait values.

Regardless of the actual proportion of variation explained, many studies draw biological inferences solely from the p-value of a null-hypothesis significance test. When the trait variation is modeled using only the fixed factor of species, an investigator would draw a different inference compared to the nested mixed model. When we evaluated the statistical significance of the likelihood ratio of each model fit at $\alpha = 0.05$, a significant effect of species on the trait mean was found much more often in the non-nested model than the nested model (44% of cases versus 27%, respectively, for all parameter combinations). As heritability increased, the proportion of discrepancies in null hypothesis rejection between the non-nested and nested mixed model increased, ranging from 14% at $H^2 = 0$ to 20% at $H^2 = 0.9$ (Figure 10a). As before, the discrepancies among models were most frequent when the species and genotype-level variance terms were either both small or both large; both statistical techniques generally agreed when one variance term was much larger than the other (Figure 10b and c). Finally, increasing sample size tended to cause models to support opposing inference more often, as increased statistical power meant that p-values for the non-nested model were more likely to drop below the significance threshold, all else being held constant (Table 2; Figure 16 in chapter appendix).

Table 2. Effect of variation in simulation parameters on explanatory power and parsimony of nested mixed versus non-nested models.

Parameter	**Effect on ΔR^2**	**Effect on ΔAIC**
Population sample size *n*	negative	positive
Heritability H^2	positive	positive
Genotype variance σ_g	variable	none
Species mean difference μ_1 - μ_2	unimodal	none
Number of loci	negative	unimodal

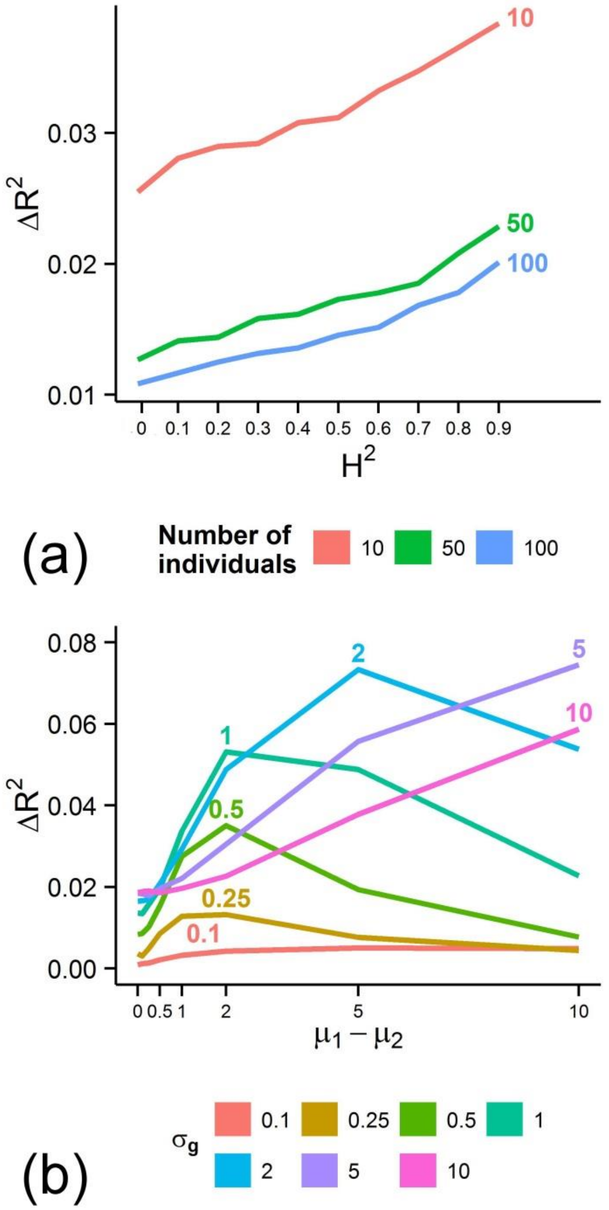


Figure 8. Mean differences between the R^2 value of the non-nested model and the marginal R^2 value of the species effect in the nested mixed model (y-axis) for different combinations of parameters in the simulated data. In panel (a) at top, the heritability parameter is on the x-axis, and each line shows the mean ΔR^2 value at each combination of heritability and sample size (colored labels associated with each line). In panel (b) at bottom, the preset difference between species means is on the x-axis, and each line shows the mean ΔR^2 value at each combination of species mean difference and among-genotype variance (colored labels associated with each line).

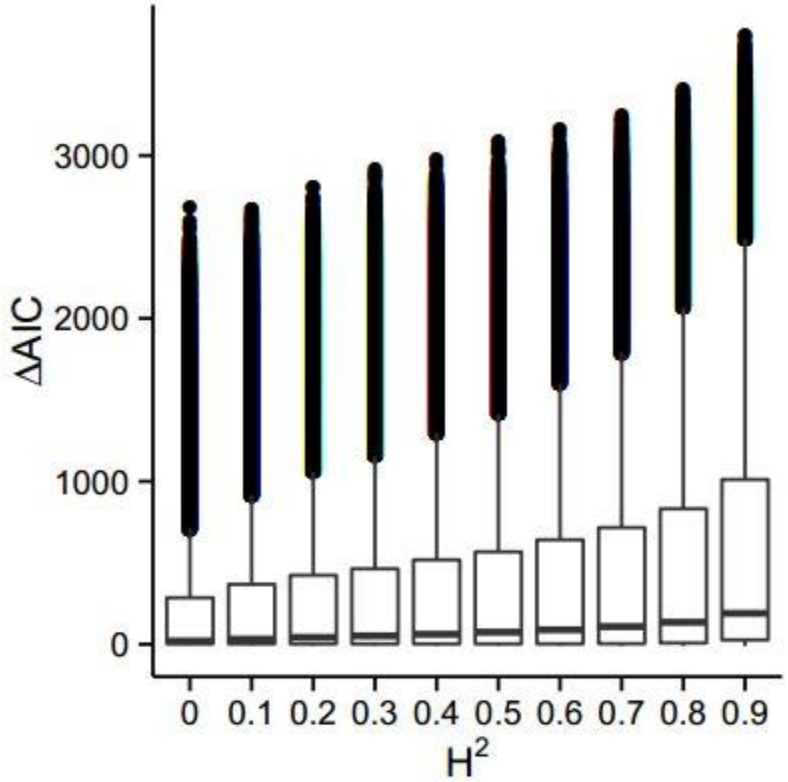


Figure 9. Boxplots showing information criteria versus heritability. As the heritability parameter (x-axis) of simulated trait data sets increased, there was a corresponding increase in the ΔAIC value (y-axis) comparing the nested mixed model to the non-nested linear model, indicating that the nested mixed model is more superior as heritability increases.

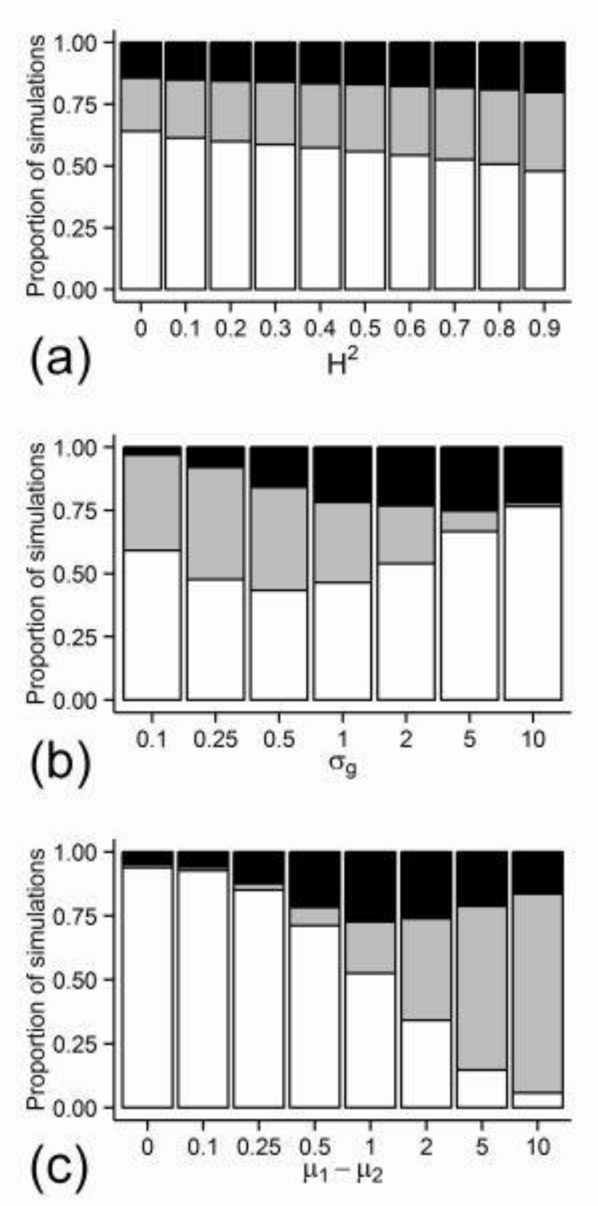


Figure 10. Discrepancy among models considering α = 0.05 significance threshold from simulation results. Bars are shaded based on the proportion of simulation outcomes. White-shaded bars indicate that neither model rejected the null hypothesis that species means are equivalent, grey shading indicates that both rejected this hypothesis, and black indicates that only the non-nested model rejected this hypothesis. The proportion of simulations in which both models reached the same conclusion decreased as the heritability parameter increased (a), was lowest at intermediate among-genotype variance (b), and was lowest at intermediate among-species trait mean differences (c).

The previous datasets were generated from simulated genotypes with few loci (1, 2, or 3); the additional simulations we ran with number of loci ranging from 1 to 10 showed that increasing the number of loci decreased the effect of accounting for nested variation on ecological inference (Table 2; Figure 11). Increasing the number of loci had little effect on the model selection criteria at low numbers of loci: across all combinations of parameter values, median ΔAIC was highest (1153.9) with 3 loci. At higher numbers of loci, median ΔAIC steadily decreased: the median value with 10 loci was 75.1 (Figure 11a). While this difference is still large enough to signify that a nested mixed model is more parsimonious than a non-nested linear model, the increase in explanatory power may not be great enough to justify the additional effort in some cases, as both the ΔR^2 value and proportion of false positive results both approached zero as number of loci increased (Figure 11b and c). Finally, our simulation of observed difference in sample means with increasing sample size showed the pattern that as sample size increased, the effect size associated with species decreased (Figure 12). The absolute value of the mean effect size decreased despite no change in true difference in sample means as sample size increased. In our particular example, the spuriously high observed difference in species-level means was present even at relatively high levels of within-genotype variance, and did not disappear until impractically large sample sizes were assumed (Figure 12).

Empirical data sets

Common-garden studies

We used data from three previously conducted common-garden studies to illustrate similar patterns to the ones we observed in simulated datasets. Each data set represents different levels of hierarchically nested biological variation (for the first, family nested within species, and for the second two, genotype nested within locality), illustrating that the nestedness of variation—and potential pitfalls for inference—are independent of the particular level examined. The first, established in Tasmania, was a controlled cross trial of multiple *Eucalyptus* species in three clades within the subgenus *Symphyomyrtus*, with a total of 332 individuals measured across 85 families nested within 16 cross types (Gorman *et al.* 2014). The second study consisted of genotypes of cottonwood (*Populus angustifolia*) distributed in the arid Southwestern United States, with a total of 74 individuals measured across 37 genotypes nested within six localities along an elevation gradient (Whitham *et al.* 2006 and references therein). See the above two references for specific methods. Finally, in the third study, which consists of previously unpublished data, seeds from 10 red spruce (*Picea rubens*) genotypes from 6 different mountaintop populations were collected in Great Smoky Mountains National Park (TN/NC, USA). The seeds were cold-stratified, germinated, and potted in a randomized single-block design in a greenhouse. After one year, the seedlings were measured for a number of properties relating to plant architecture. Here, we present one response variable from each set of trials: level of mammal browsing from the *Eucalyptus* trials, leaf area measurements from the *Populus* trials, and number of branches from the *Picea* trials. Each of the traits we selected represents an easily measured property of a plant; the traits were chosen to demonstrate patterns of variation similar to those observed in the simulated data.

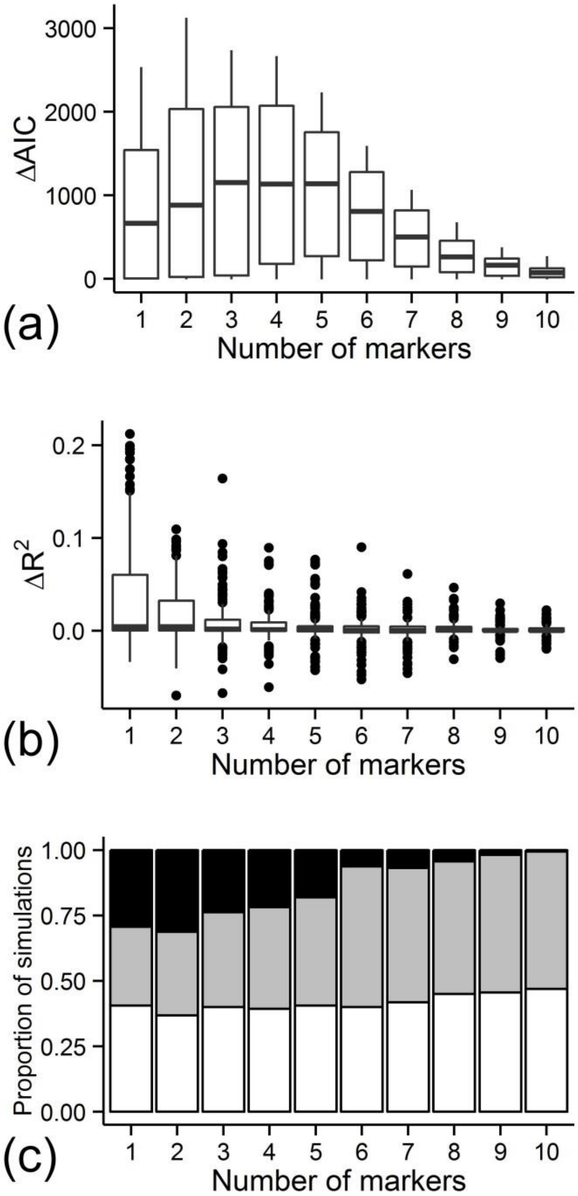


Figure 11. Effect of increasing the number of loci, displayed on the x-axis in all three panels, on (a) ΔAIC comparing the non-nested linear model and nested mixed model; (b) ΔR^2 comparing the two models; and (c) discrepancy between the two models considering the $\alpha = 0.05$ significance threshold. As in Figure 10, white-shaded bars indicate that neither model rejected the null hypothesis that species means are equivalent, grey shading indicates that both rejected this hypothesis, and black indicates that only the non-nested model rejected this hypothesis. The ΔAIC value was highest at 3 loci, but the ΔR^2 and the proportion of discrepancies both decreased with increasing numbers of loci.

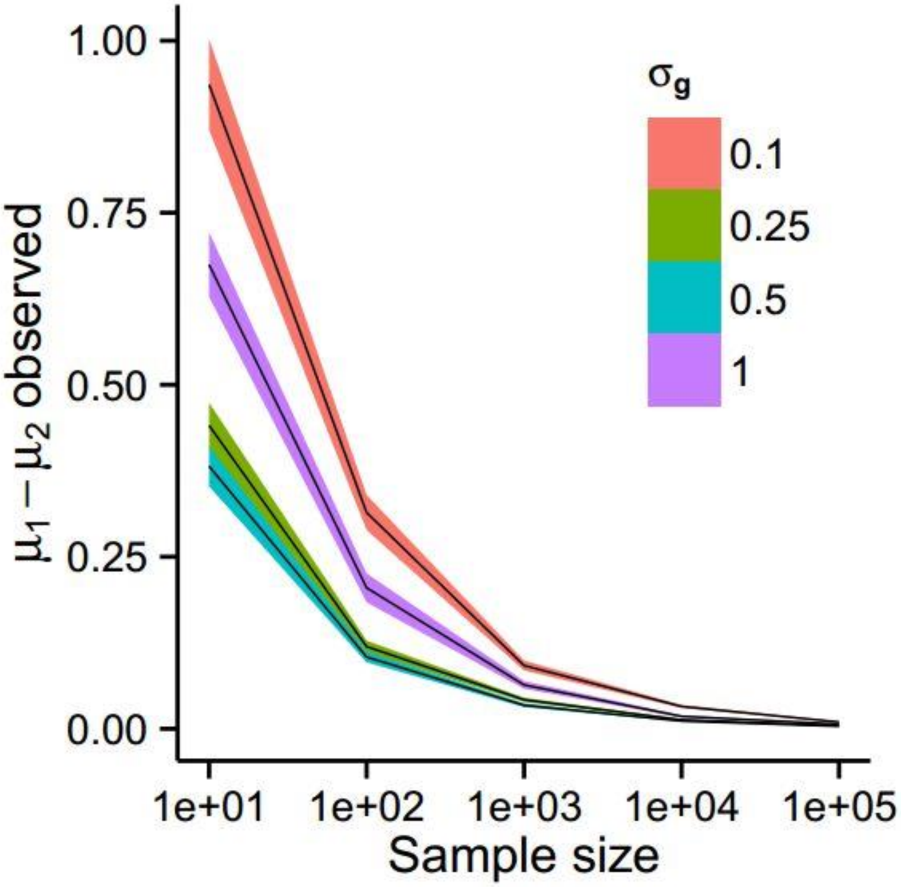


Figure 12. Absolute value of observed difference in species means plotted on the y-axis against the logarithm of sample size on the x-axis. Each of the simulated datasets was drawn from a distribution with a mean of zero, so the expected $\mu_1 - \mu_2 = 0$. The width of the shaded region around each line is the standard error of the difference between means, and the color of each shaded region represents the value of σ_g, genotype-level standard deviation.

We analyzed each of the empirical data sets in the same way as the simulated data sets, modeling each one using a non-nested model with species or locality as a categorical fixed effect and a nested mixed model with family or genotype nested within species or locality (see above). We generated bootstrapped standard errors for each estimate of R^2 by drawing with replacement from the trait values until reaching the original sample size, calculating the marginal and conditional R^2 values, and repeating the procedure 9999 times (*boot* package; Angelo Canty & Brian Ripley 2015).

Application of different statistical models: **Eucalyptus**

A significant amount of variation in mammal browsing percentage in *Eucalyptus spp.* was explained by species in the non-nested ANOVA model. However, accounting for family in a mixed model reveals that the species term accounts for a relatively small proportion of the total variation explained by the nested mixed model (non-nested model $R^2 = 0.13$, mixed-model conditional $R^2 = 0.28$; Figure 13). This indicates an approximate twofold increase in explanatory power for the mammal browsing trait in *Eucalyptus*. Additionally, both the non-nested and the nested mixed model indicate that roughly the same proportion of variation is explained by the non-nested factor, species, across both models. Despite this, each model would lead to a different inference from a frequentist standpoint because the species effect was significant at $\alpha = 0.05$ in the non-nested model but not in the nested mixed model (non-nested model, $p = 0.00010$; nested mixed model, $p = 0.06$).

Application of different statistical models: **Populus**

A significant proportion of variation in leaf area was explained by locality of origin in the *Populus* common-garden trial. When genotype was accounted for in a nested mixed model, the explanatory power increased roughly threefold relative to the non-nested model (non-nested model, $R^2 = 0.25$; nested mixed model, conditional $R^2 = 0.70$; Figure 13). As in the previous case, although the non-nested and nested mixed models both apportion a similar amount of variation to the outermost level – in this case locality – the locality effect was not significant at $\alpha = 0.05$ in the nested mixed model (non-nested model, $p = 0.0086$; nested mixed model, $p = 0.22$).

Application of different statistical models: **Picea**

As in the previous examples, a significant proportion of variation was explained by locality of origin, but when genotype was also accounted for in a nested mixed model, explanatory power increased by an order of magnitude (non-nested model, $R^2 = 0.03$; nested mixed model, $R^2 = 0.24$; Figure 13). However, both would lead to a similar qualitative inference, albeit stronger for the non-nested model (non-nested model, $p = 2 \times 10^{-8}$; nested mixed model, $p = 0.02$). These three empirical results collectively suggest that the non-nested model leads to an incorrect inference in a traditional null hypothesis significance testing framework, as is commonly employed.

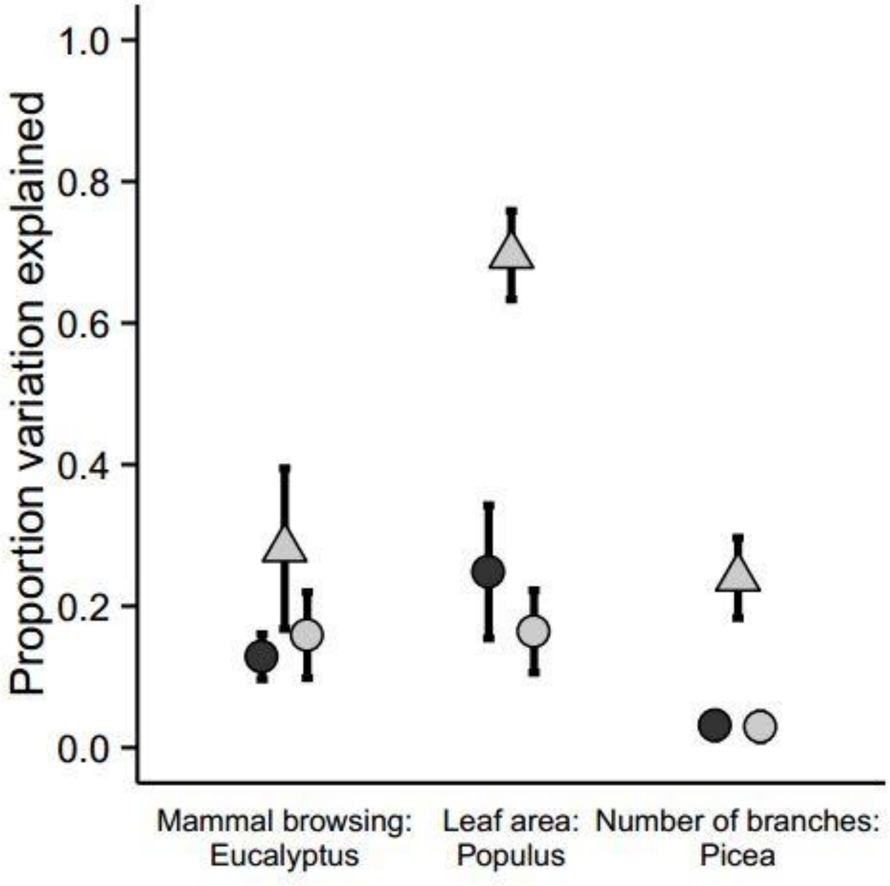


Figure 13. Coefficient of determination (R^2) values from case studies. Values are calculated from *Eucalyptus* mammal browsing, *Populus* leaf area, and *Picea* branch number data sets, respectively. Black circles represent the non-nested model R^2 values, grey circles represent the marginal R^2 value of the nested mixed models, and grey triangles represent the conditional R^2 values of the nested mixed models. Error bars represent bootstrapped standard errors of the R^2 values.

Discussion and Implications

Here we show that in a simulated system, a nested mixed model incorporating genotypic variation to partition variation in trait means is more appropriate than a non-nested model that does not nest other levels of genetic variation. The simulated system was constructed with no interactions among species or genotype and no environmental effects, which could either mask or amplify the biodiversity signal in natural systems. Specifically, the proportion of detected significant species effects was up to 20% greater in non-nested versus nested models. In other words, one would conclude that there are species effects when in fact those effects were related to lower-order levels of variation. Non-nested linear models resulted in effect sizes associated with the species term from 20% to 50% greater than the nested models; additionally, the model selection criterion showed that nested models were superior regardless of parameter values.

The results of our simulations demonstrate the need to employ a nested approach. Even if data are not available to do so explicitly, the binning of individuals into species should be acknowledged as one choice among multiple ways of partitioning variation among individuals, instead of being regarded as synonymous with diversity itself. By the same reasoning, genotype identity should be acknowledged as an important basis for inference in its own right. We also show the utility of the nested approach for analyzing data in field experiments. When fitting nested and non-nested models to empirical data taken from *Eucalyptus*, *Populus*, and *Picea* common-garden trials, the nested and non-nested models performed similarly in terms of estimating the proportion of total variation due to species, but the likelihood ratio test showed that the non-nested models would erroneously conclude the species effect to be significant in two of the three cases. Whether the system is experimental, natural, or simulated, a hierarchical nesting approach should help reduce the false attribution of variation and improve our understanding of the sources of variation among species or among genotypes. One caveat to our findings is that adding additional parameters to a model – in this case by including an additional nested level of genetic variation – will raise the proportion of variation explained, potentially leading to overfitting (Ginzburg & Jensen 2004). However, in our study, analysis of AIC values showed that nested models are much more parsimonious despite the additional parameters, relaxing the above caveat. The conclusions we draw here on the importance of nesting apply not only to comparisons of intraspecific and interspecific trait variation, but to any system with hierarchically nested variation, including at the genotype, population, species, or phylogenetic level.

Importantly, our conclusions on the importance of placing individuals in a properly nested hierarchy of bins are independent of the statistical approach chosen by the investigator (such as frequentist, Bayesian, or maximum-likelihood methods). Although we employ frequentist methods to support our point, neglecting to incorporate a particular level of variation into any type of statistical model will result in impoverished inference, regardless of the method. Along these lines, we found that a species effect may be incorrectly detected at relatively large sample sizes (Figure 12). As increasing sample size does not tend to decrease the incidence of Type I errors, this indicates that the most effective way to avoid such errors is to distinguish species-level from genotype-level variation. The sample sizes required to alleviate the problem of falsely attributing a pattern to the higher nested level may be several orders of magnitude higher than what is feasible to collect in natural systems. Explicitly accounting for variation at different nested levels of genetic variation would help solve this problem even at the relatively low sample sizes characteristic of most ecological studies.

Our simulated datasets also showed cases in which incorporating nested genetic variation may not be as important to consider from a functional perspective. In the algorithm we used to generate trait values from genetic loci, increasing the number of loci means that more extreme trait values are possible within a species. This effectively yields a wider distribution of trait values around the mean, with a larger proportion of values concentrated in the tails of the distribution. However, if more extreme trait values, both positive and negative, are possible in both species, both the nested and non-nested models are less likely to detect a significant effect of intraspecific variation. As the effect of intraspecific genetic variation on phenotype decreases, accounting for nested genetic variation at levels below species may be less useful for characterizing the ecological function of a population as measured by its mean trait value (Figure 11). However, in many cases the mean trait value may not be what drives community assembly or ecosystem function; instead, the variance in trait values or the extremes of the trait distribution may be more important (Violle *et al.* 2012). In those cases, other statistical methods than the ones we consider here may be more appropriate (e.g. Violle *et al.* 2012; Siefert *et al.* 2015).

It is becoming increasingly clear that genotype identity can be as important as species identity for determining the outcome of ecological processes. In the field of biodiversity-ecosystem function research, experimental and observational tests (Bailey *et al.* 2009; Latta *et al.* 2011; Avolio & Smith 2013; Crawford & Rudgers 2013; Genung *et al.* 2013) have shown that genotypic diversity within foundational species may explain as much variation in important ecosystem functions as variation in species diversity, further emphasizing the importance of taking a nested approach. Similar techniques that account for identity and diversity of both species and genotypes have been recently applied in fields including invasion biology (Bossdorf *et al.* 2005; Dlugosch & Parker 2008; Kimbro *et al.* 2013) and sustainable agriculture (Newton *et al.* 2010; Tester & Langridge 2010). Analogous to the acknowledgement that sampling effects and genotypic effects can mechanistically explain many ecological patterns and therefore should be accounted for, hierarchically partitioning variation also significantly changes how we interpret data when nested genetic variation is taken into account.

Accounting for nested levels of variation in functional traits and for the effects of different levels of diversity is important for theoretical and applied work. Historically, the approach has been more common in applied contexts such as production forestry (Jordan *et al.* 1993; Hartley 2002). However, because nested genetic effects have often been neglected in basic biodiversity research, we should acknowledge that existing syntheses and estimates of biodiversity effect sizes in meta-analyses may not tell the whole story. If the full range of variation at all levels were incorporated and nested properly, we might reach different conclusions about the mechanisms by which biodiversity influences ecosystem functioning and the strength of those effects, as our simple model suggests that effect sizes associated with non-nested models may be inflated by 20% or more.

Our results clearly demonstrate the changes in interpretation of biological relationships that can result from incorporating nested, genetically based variation into studies of natural systems. However, we admit that some biological systems are more amenable to incorporating genetic variation into experimental or observational studies than others. We therefore strongly recommend that researchers take one of the following three approaches. First, if possible, genetic variation should be quantified at the within- and among-population level and either experimentally manipulated or accounted for as a random effect in observational studies. If this is not possible, however, a promising alternative is to address intraspecific variation with a functional trait-based approach -- recent work has shown that intraspecific trait variability accounts for a large proportion of the variation in functional traits both within and among communities (Siefert *et al.* 2015), and that this variability has important consequences for both species abundance and species performance (Umaña *et al.* 2015). Finally, if neither of these approaches is tractable and intraspecific variability is neither quantified directly at the genetic level nor indirectly via functional traits, we feel that researchers should qualify their results with the explicit statement that variation at levels below species was assumed *a priori* not to affect the outcome of their study.

As a discipline, it is important to recognize the importance of hierarchically nested genetic variation and incorporate it into analyses to advance ecological research, if we are to fully understand which of the many facets of diversity have important consequences for ecosystem function. Given the growing research emphasis linking ecology and evolutionary biology (Schoener 2011), it is crucial to ensure that quantitative or molecular genetic variation is accounted for. The approach demonstrated here is particularly important for studies that examine quantitative or molecular genetic divergence, as well as species mixture effects, as we demonstrate that incorporating a properly nested variance structure may dramatically affect the way the results are interpreted. From an applied perspective, it is possible that if managers take local adaptation and intraspecific trait variation into account when managing or restoring communities affected by anthropogenically driven species loss or gain (Zenni *et al.* 2014), the functional importance of variation within species may be as important as the presence or absence of particular species for the success of management and restoration efforts. Finally, our results suggest that the response of ecological communities and the ecosystem services they provide to species gains and losses are likely more subtle than we would expect by simply observing which species invade or are extirpated. Correctly determining the nature of the relationship between biodiversity and ecosystem functioning is critical for understanding the direct and indirect effects of losses in species diversity and genetic diversity in natural systems.

Acknowledgments

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Appendices

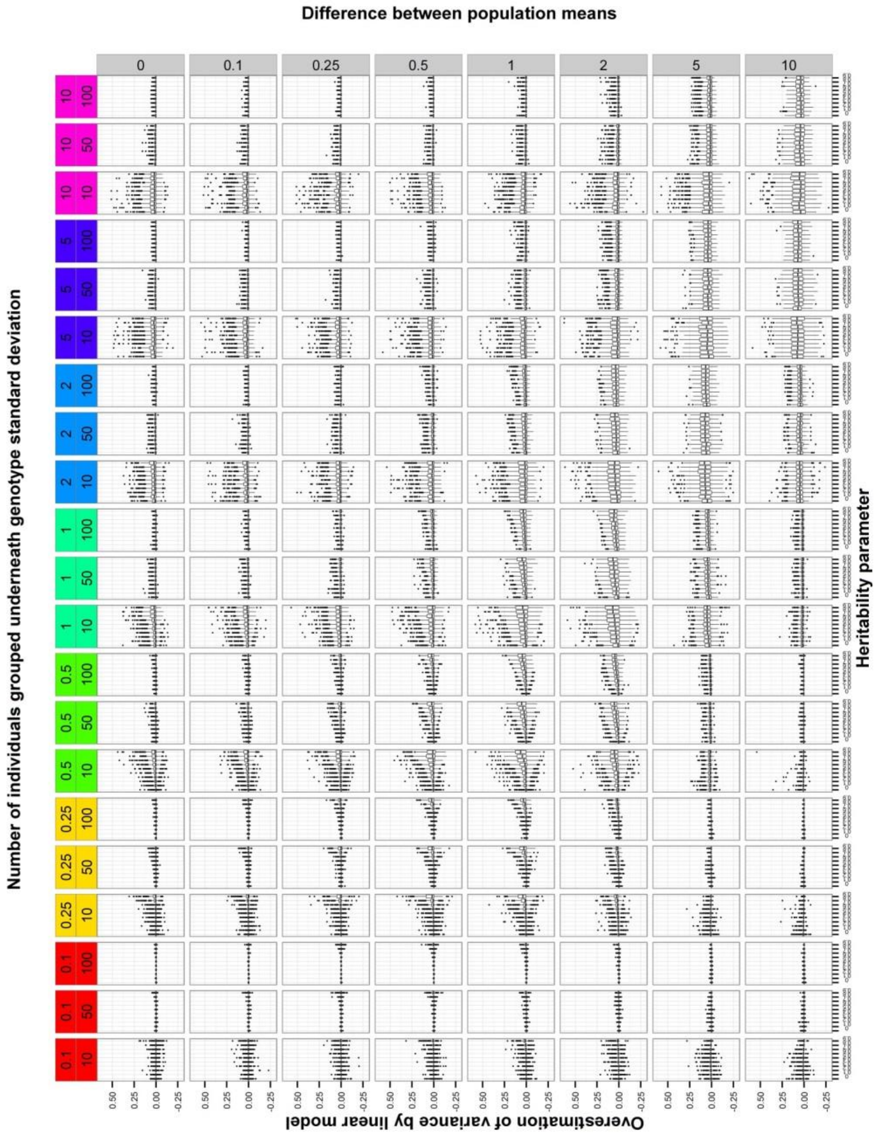

Figure 14. Overestimation of partial R^2 associated with the species-level term when comparing linear models to nested models. A boxplot depicts the variation in ΔR^2 for many combinations of parameter values. Within each panel, the heritability parameter is on the x-axis, and the overestimation value is on the y-axis. Proceeding from top to bottom on the page, the difference between species means increases. Proceeding from left to right, the among-genotype variance increases. Within each variance level, three sample size levels are shown, increasing from left to right.

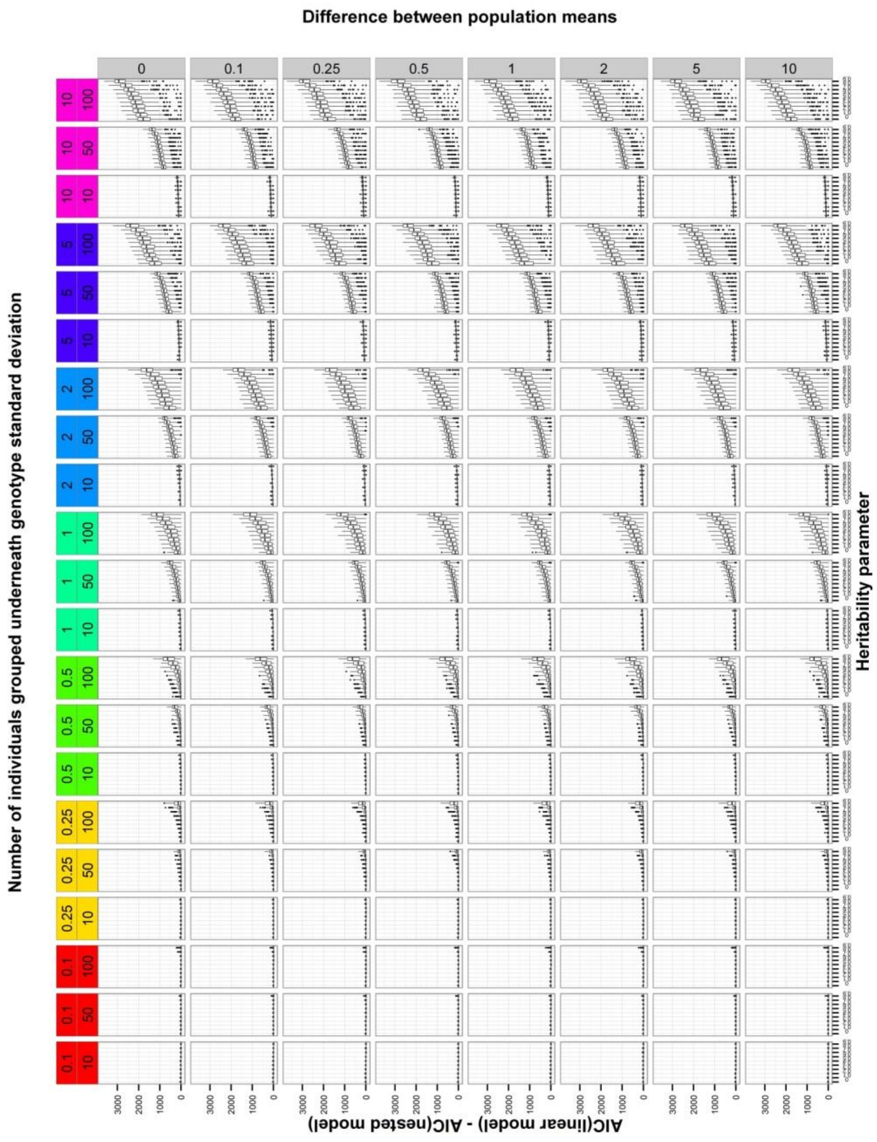

Figure 15. ∆AIC values comparing linear models to nested models for many combinations of parameter values, arranged as in Figure 14.

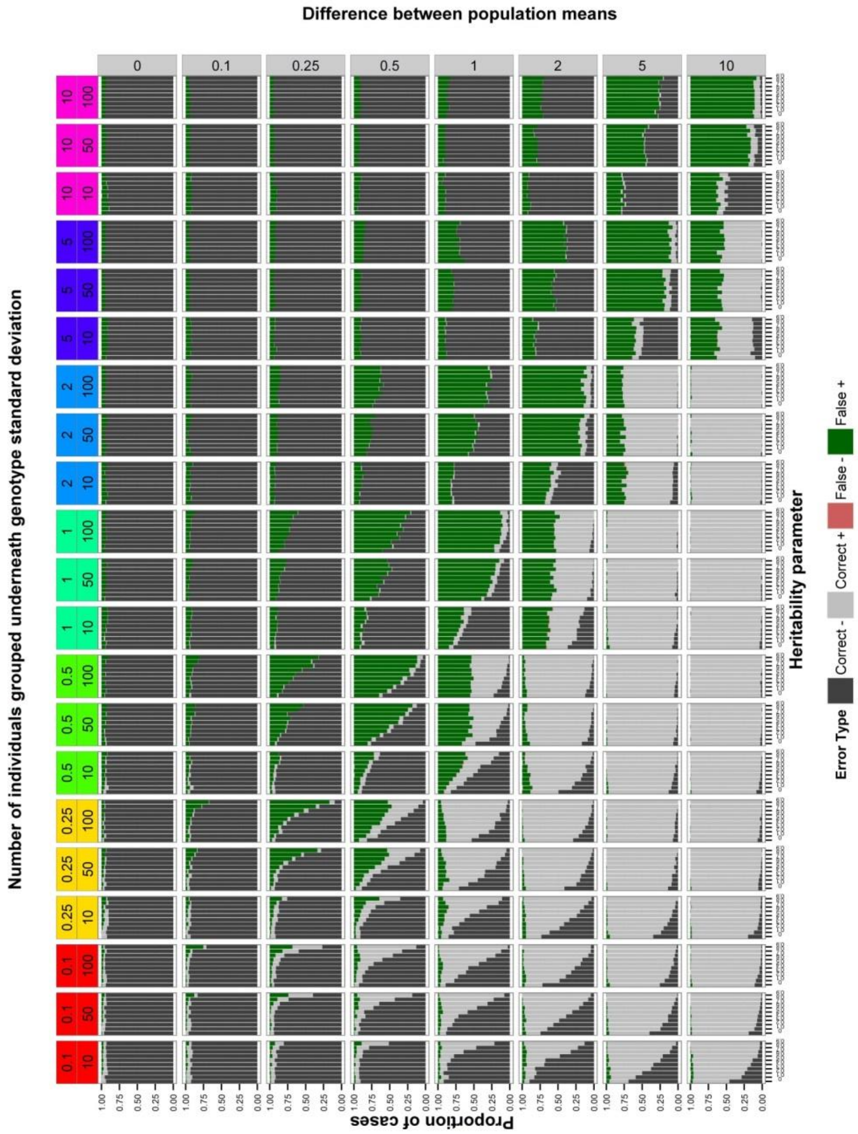

Figure 16. Proportions of simulated cases in which the linear models and nested models agreed in detecting a significant effect of species-level variation; stacked-bar plots are arranged as in Figure 14. Here, light gray shading indicates that neither model detected a significant effect, dark gray shading indicates that both models detected a significant effect, green shading indicates that only the linear model detected a significant effect, and red shading indicates that only the nested model detected a significant effect (found in virtually no cases).

CHAPTER III

TRAIT-BASED MODELS OF COMMUNITY ASSEMBLY FAIL IN THE FACE OF HIGH INTRASPECIFIC VARIABILITY

Abstract

Questions: The holy grail of functional trait ecology is to predict community assembly at the local scale directly from functional traits. Predicting species abundances from functional traits given known environmental conditions should be possible if natural communities are structured by deterministic processes including environmental filtering from the regional species pool and niche differentiation. In this study, I asked how much intraspecific variation contributes to trait distributions observed within and among plant communities, and how well trait distributions resulting from environmental filtering and niche differentiation predict relative species abundances given climate.

Location: West Elk Mountains, Colorado, USA

Methods: I collected functional trait, species composition, and environmental data from 14 observational sites along a broad climate gradient in Colorado, USA. I partitioned the variation within and among sites into intraspecific and interspecific components, and compared the results to values from a recent global meta-analysis. I also used these data to parameterize statistical models that can reproduce patterns associated with the processes of environmental filtering and niche differentiation. I fit two models to the data, one assuming that niche differentiation is equal across sites, and another assuming that it differs across sites.

Results and Conclusion: I found that the models performed worse at predicting species relative abundance than a null model assuming equal abundance of all species. This may be due to intraspecific variation in functional traits, which in this study system was higher than the global averages documented in the meta-analysis. In particular, almost all root trait variation was within species, even across sites, indicating that belowground traits may not be useful for predicting species abundance. These results suggest that a greater focus be placed on measuring intraspecific trait variability and determining its consequences for community assembly and ecosystem properties.

Introduction

Community ecologists seek to describe the ways in which environmental conditions and interactions among organisms influence patterns of species distribution across space and time. Both the abiotic and biotic processes that structure communities are correlated with, and also possibly driven by, easily measured properties of organisms (Lavorel & Garnier 2002; Lavorel 2013; Díaz *et al.* 2015). This key insight has led to a massive research effort aimed at linking functional traits to ecosystem function and community assembly. Here, functional traits are defined as any property of an organism that is correlated either with its niche or its fitness. The functional trait approach offers a way to deal with a bugbear of community ecologists: the fact that results of community ecology studies are often difficult to interpret or generalize. Because community assembly processes are so contingent on the specific local composition of the species pool and on small-scale environmental variation, hardly any two communities are the same (Lawton 1999; Ricklefs 2008). By measuring functional traits instead of focusing on species identity, ecologists can make quantitative comparisons among species assemblages (McGill *et al.*

2006; Webb *et al.* 2010). Furthermore, functional traits are not only correlated with climate but also with emergent ecosystem-level properties such as carbon turnover (de Deyn *et al.* 2008; Reichstein 2014). The ultimate goal of functional trait ecology, which has been described as the holy grail of the field (Lavorel & Garnier 2002), should be to predict variation and change in community composition and ecosystem function from functional traits.

Despite its promise, the functional trait approach has drawn several key critiques (Violle *et al.* 2007). In particular, the correlational nature of many trait studies is problematic because the correlations between environment and traits are often examined in isolation, ignoring the processes that have generated the observed trait and species distributions. Too much effort is being focused on traits that are correlated with environment but do not matter for how species actually respond to environmental change or how they interact with one another (Messier *et al.* 2010). However, some recent work has explicitly linked functional traits to unambiguous fitness metrics that drive community assembly, including growth and mortality of forest trees (Wright *et al.* 2010) and demographic parameters from a global dataset of plant species (Adler *et al.* 2014).

In previous trait-based ecological studies, functional traits have often been used as proxies for species, but they should instead be thought of as ways to represent processes occurring at the organism level. Traits are poor proxies for species identity for three reasons: the multidimensional nature of traits (Albert *et al.* 2010), the strong relationship between traits and environmental conditions (Laughlin & Messier 2015), and high levels of intraspecific variability even in similar environments. Ecological outcomes such as herbivore damage can be predicted only when accounting for complex interactions of traits along multidimensional axes (Loranger *et al.* 2013). In addition, individual responses to environmental gradients are necessary to explain species distributions and diversity patterns, as has been shown for forest trees (Clark *et al.* 2011). The relationship between traits and fitness should be examined accounting for the trait-environment interaction (McGill *et al.* 2006; Shipley *et al.* 2016). In addition, there has been too little attention paid to intraspecific variation in traits. For example, earlier trait-based assembly models such as MaxEnt (Shipley *et al.* 2006, 2011) are based solely on species means.

In this study, I suggest new approaches to move the field of functional trait ecology forward in three key ways. First, I explicitly incorporate intraspecific variation into our statistical models, building on previous work (Laughlin & Laughlin 2013; D'Amen *et al.* 2015). Second, I quantified how much the variation in trait values within and among species contributes to observed species distributions. I accomplished this by putting traits into a predictive framework that incorporates both filtering (i.e., relative fitness differences) and niche-differentiation processes (HilleRisLambers *et al.* 2012) and includes variation in these processes along environmental gradients. Both relative fitness differences and stabilizing niche differences contribute to the realized abundance distribution at a given site. Finally, I used root morphology as a predictor, which has rarely been included in previous studies. Most of the traits considered for plants have been aboveground leaf and stem traits although it is increasingly recognized that root traits may be more important (Freschet *et al.* 2010, 2015; Bardgett *et al.* 2014). Addressing these issues is crucial to enable the functional trait-based approach to community ecology to fulfill its promise as a way to describe the linkages between environment and species and to predict future change in those linkages.

I established plots at fourteen observational sites along a transect, where I collected data on relative species abundance and functional traits of the most abundant species. I used functional trait, species abundance, and environmental data from these observational plots to ask a series of related questions:

- How much does intraspecific variation contribute to trait distributions observed within and among plant communities? How does this differ aboveground and belowground, and what does this mean for the processes that drive community assembly?
- How do environmental filtering and niche differentiation interact to structure plant communities across a landscape, and can we use the trait distributions resulting from these processes to predict relative species abundances given climate?

Methods

Site description

The study region is in the West Elk range of the southern Rocky Mountains in Colorado, USA, in the vicinity of the Rocky Mountain Biological Laboratory. Elevations of the study sites range from 2450 to 3500 m above sea level. Mean annual temperatures, derived from the Worldclim dataset (Hijmans *et al.* 2005) range from -1.8 to 2.7 °C, and mean annual precipitation from 355 to 679 mm y^{-1}. The study sites are located on an elevational gradient along which temperature decreases (Figure 17) and precipitation increases (Figure 18) with increasing elevation. In addition, due to more recent glaciation, soils are younger and have increased available phosphorus and decreased available nitrogen at higher elevation (Joshua Lynn, unpublished data); there tends to be decreased cattle grazing pressure at higher elevations as well. I selected open meadow sites without tree cover. At each of 14 study sites, I established a permanently marked 10 × 10 m plot.

Data collection

Plant community: In the summer of 2015, I measured the relative abundance of plant species at each site. I placed a 0.25 m^2 quadrat at ten random locations in the 10 × 10 m plot, and visually estimated the aboveground cover of all vascular plant species within each quadrat, identifying them to species. Species with less than 10% relative cover were estimated to the nearest 1%, while species with over 10% were estimated to the nearest 5%. At each plot, both an early-season and peak-season relative abundance survey was done. I used the relative cover of the plant species to determine which plant species to collect for plant traits, as well as to validate model predictions of relative species abundances.

Plant traits: In 2014 and 2015, I collected leaves and roots from at least five individuals of at least five of the most abundant species at each site within the marked plot. At a subset of sites, I collected leaves and roots from 10-20 individuals of 5-15 species. For most sites, there is plant trait information for the species that make up at least 80% of the aboveground plant cover within the sites. I measured plant height on the collected individuals, from the base to the tip of the topmost leaf blade.

I collected at least three mature and fully expanded leaves from each individual that was sampled for leaf traits. I transported the leaves on moist paper towels and scanned them on an Epson flatbed scanner. I weighed the scanned leaf material, dried it for 48 hours at 60° C, then weighed it again. I pooled the dried leaf material with additional leaves that were collected for chemical analysis.

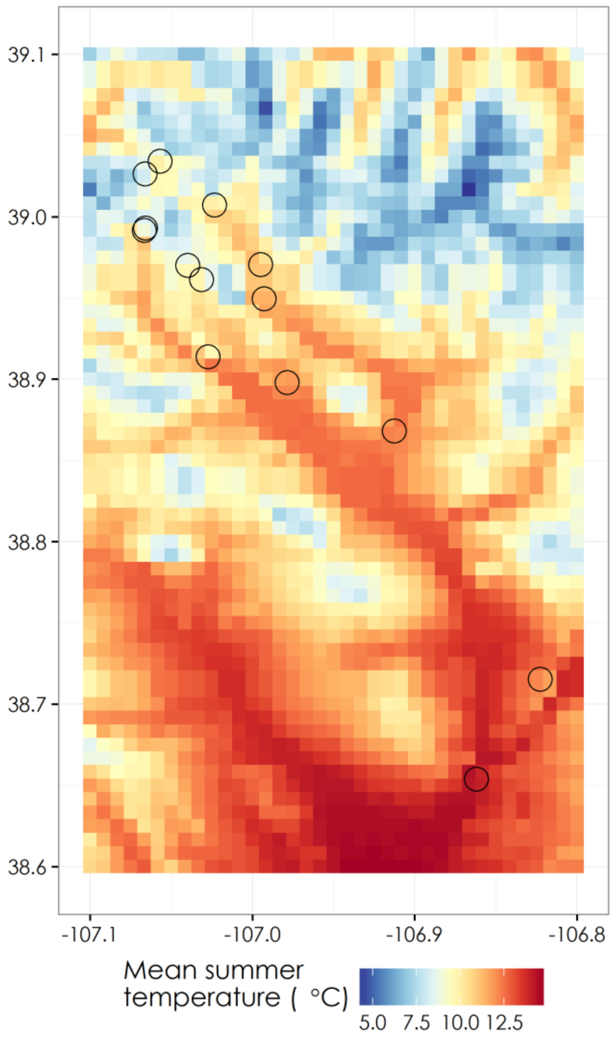

Figure 17. Gridded (0.5') mean temperature in the warmest three months in the study area. The black circles are the locations of the study sites.

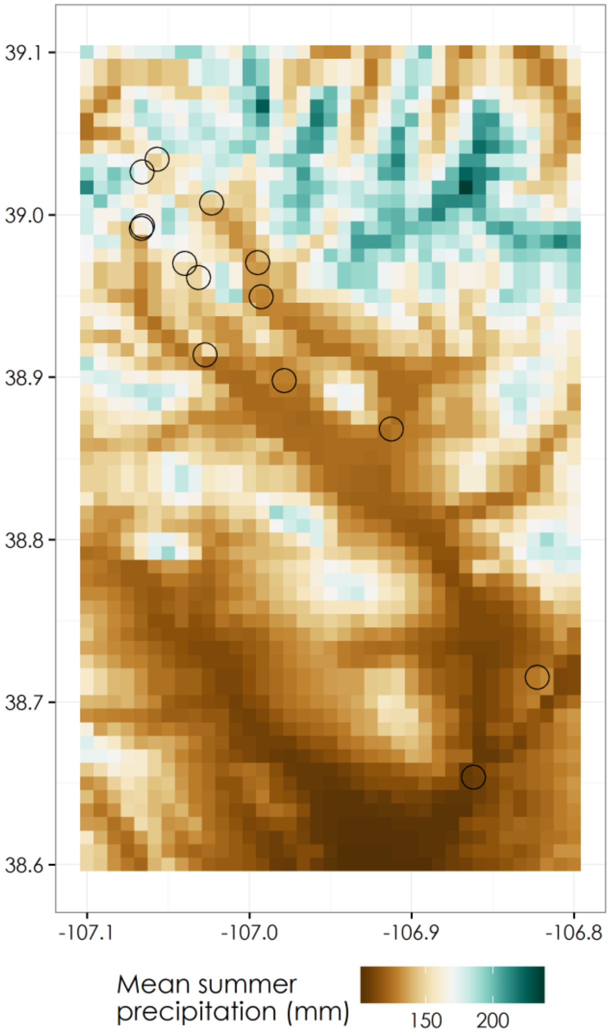

Figure 18. Gridded (0.5') total precipitation of the warmest three months in the study area. The black circles are the locations of the study sites.

I collected two to four 3-cm diameter, 15-cm long soil cores from directly below the base of each plant that was sampled for root traits. I extracted as much fine root material as possible from the soil cores, washing the soil over a 2-mm sieve if necessary. I scanned 10 to 20 intact pieces of fine root from each individual on an Epson flatbed scanner after floating the root pieces in a transparent tray of water. I dried the root material for 48 hours at 60° C and weighed it. I pooled the dried root material with additional collected roots for chemical analysis.

To measure nitrogen and phosphorus content by mass (total Kjeldahl N and P) of the dried tissue samples (both leaf and root), I first ground the tissue samples with a mortar and pestle. I weighed 75 mg, or as much as was available, of the ground sample and folded it into a piece of adhesive-free cigarette paper. I digested the sample for 5 h at 350° C in 5 mL H_2SO_4 in a Kjeldatherm digestion block (Gerhardt, Königswinter, Germany); in each digestion run I included a blank with no sample, and one sample of apple leaf standard with known N and P content. After the digests cooled, I added 45 mL deionized water to each one. I used a SmartChem 200 discrete analyser (Unity Scientific, Brookfield, CT, USA) to measure total Kjeldahl N and P, expressed as mg/g tissue.

I measured the area of the scanned leaf images, and the total length of the scanned root images, with ImageJ software (version 1.45s; Schneider *et al.* 2012), using the IJRhizo macro (Pierret *et al.* 2013) to automatically trace all the roots in each image. I calculated LMA for each individual plant by summing the mass and area of each individual and dividing the dry mass by the scanned area. LDMC was calculated as the dry leaf mass divided by the leaf mass before drying. I calculated RML for each individual plant by dividing dry root mass by the total root length.

Partitioning of intraspecific variability

All analyses were conducted in R (version 3.2.3, R Core Team, https://www.r-project.org/), with packages listed where appropriate. To determine the contribution of intraspecific trait variation to total trait variation both within our study communities and among communities, I used variance partitioning equations (de Bello *et al.* 2011) identical to those used by Siefert *et al.* (2015). For each trait at each site, I calculated within-community intraspecific trait variability (wITV), the relative contribution of intraspecific trait variability to total within-site trait variability. Also, for each trait across all sites, I calculated among-community intraspecific trait variability (aITV), the log ratio of variance due to intraspecific trait variability to variance due to species turnover across sites. I report wITV as a proportion between 0 and 1, where higher values indicate greater contribution of intraspecific variability to the total, and I report aITV as the natural logarithm of a ratio, where a positive number indicates that intraspecific variability is relatively more important than species turnover across sites. I used ANOVA to compare wITV values among traits and sites, and I also compared the wITV and aITV values to the global distributions of values reported by Siefert *et al.* (2015). Unfortunately, no root traits were reported in the meta-analysis.

Predictions of species abundances

I used a model that mathematically represents assembly of local communities from a regional species pool through the selective processes of environmental filtering and niche differentiation. The model is modified from the Traitspace model (Laughlin *et al.* 2012). The model predicts species abundances at locations with differing environmental conditions, using species traits to connect species identities with environmental conditions. The Traitspace model captures two key processes that contribute to patterns of species abundance at the landscape scale. First, in order for a species from the regional species pool to be able to establish at a particular site, it must possess traits that enable it to tolerate the environmental conditions at that site. Species with traits conferring higher fitness given a particular set of environmental conditions should have higher abundance. Second, in addition to these relative fitness differences, niche differentiation resulting from interactions among individuals is an additional filter that determines the species composition and abundance distribution at a given site. Biotic processes might contribute to increased trait dispersion both within and among species.

I fit Traitspace models with two types of priors: uninformative priors and priors that used each species' global range size as a prior estimate of its local abundance. Across both types of prior, we fit models with two structures of trait variance: one in which trait variance, and thus the strength of niche differentiation was assumed constant across sites, and one in which it was assumed to vary across sites. I fit these four variants of the Traitspace model to all 31 possible subsets of the five plant traits I measured, for a total of 124 model fits (Table 3 summarizes the different models that were fit.) For each of these model fits, we output predictions of relative species abundances at each of the study sites and compared them to the observed abundances.

The model fitting process is described in detail below:

First, we fit trait-by-environment regressions using trait and environmental data from each of the fourteen observational sites. For each trait, we combined all the individual measurements, without information on species, and fit a multiple quadratic regression model with environmental variables as predictors. Next, we calculated the trait distribution for each species across all sites where it occurred. We parameterized a set of multivariate normal distributions for each species. The distributions were fit with the *mvnXII* function in the *mclust* package in R (version 5.1; Fraley & Raftery 2002).

For each of the study sites, we took the mean predicted value of each trait at that grid cell given the local environment and the parameters of our trait-by-environment regressions. In a subset of the model fits, we used the standard error of the residuals of the regression to estimate the baseline variation in each trait at each site, which assumes that the effect of processes that would cause spread in trait distributions is constant across sites. In another subset of model fits, to model variation in the strength of niche-differentiation processes across the landscape, we scaled the standard deviation of the residuals across sites by the standard deviation at each site. We took 9999 samples (which we found to be more than adequate to reach convergence) of each trait at each site, sampling from a normal distribution around the mean predicted value at each site, and with the scaled standard deviation described above. This allowed us to simultaneously account for environmental filtering and niche differentiation. The sampling distribution of traits at each site, $P(T|E)$, is independent of species.

Next, we used the sampled trait data and the previously fit trait distributions for each species to calculate the likelihood, or the probability of each species being present given each trait sample. We applied Bayes' theorem to estimate the probability of each species given traits and

Table 3. Parameters varied across different Traitspace model fits.

Factor	*Levels used in different model fits*	
Prior information	Uninformative prior	Prior for each species proportional to its global range size
Trait variance	Variance of each trait assumed constant across sites	Variance of each trait assumed to vary across site
Traits used	All 31 possible subsets of the following traits: leaf mass:area ratio, root mass:length ratio, height, leaf dry matter content, leaf N:P ratio	

environment, or $P(S|T,E)$. In a subset of the models, we used a flat discrete prior, and in another subset of the models, I scaled the prior for each species by the global range size of that species, estimated from publicly available global occurrence data (see Appendix 1 for how these range sizes were estimated). Finally, I integrated out the traits using Monte Carlo integration to get the posterior predictions of relative species abundances, $P(S|E)$. The predictions were normalized to sum to 1, corresponding to the predicted proportion of aboveground cover of each species at each study site.

I fit the models using all possible subsets of these five traits: plant height, leaf mass:area ratio (LMA; the ratio of dry mass to scanned leaf area), root mass:length ratio (RML; the ratio of root mass to root length), leaf dry matter content (LDMC; the ratio of dry leaf mass to wet leaf mass), and leaf tissue N:P ratio. The environmental variables included are mean temperature of the warmest quarter of the year, and total precipitation during the warmest quarter of the year; these variables are derived from the Bioclim dataset, a set of transformed, biologically relevant variables generated from the Worldclim dataset (Hijmans *et al.* 2005).

I assessed model predictive accuracy using root mean squared error (RMSE). For each site, the root mean squared error is defined as $RMSE = \sqrt{\frac{\sum_{i=1}^{n}(\hat{p}_i - p_i)^2}{n}}$, where $\hat{p}_i$ is the predicted relative abundance of each species i, and p_i is the observed relative abundance. I compared the accuracy of model predictions with all the possible subsets of traits, with and without variation in niche-differentiation processes across the landscape, and with and without the incorporation of prior information on species range sizes (Table 1). In addition, I compared all these model predictions to a null model for which all 46 species in the dataset were assumed to have equal relative abundances at each site.

Results

Partitioning of intraspecific variability

Within sites, intraspecific trait variation (wITV) varied widely but unpredictably for aboveground traits, with proportions of variation explained by intraspecific variation at a particular site ranging from under 0.2 to over 0.95. The magnitude of intraspecific variation across sites was unrelated to any climatic variable. Variation in root mass:length ratio within sites was uniformly dominated by within-species variation, with proportions ranging from 0.66 to 0.96 (Figure 19). Among sites, root mass:length ratio, leaf N:P ratio, and leaf dry matter content had positive aITV values, indicating that intraspecific variability caused more variation in those traits among sites than did species turnover. LMA, root N:P ratio, and plant height had negative aITV values (Figure 19), demonstrating that species turnover among sites made up the majority of variation in LMA and plant height among sites, with intraspecific variation making up a smaller proportion. Taken together, these results indicate that LMA, root N:P ratio and plant height determine which species from the regional species pool pass through environmental filters and become established at each site, but that RML may be involved with niche differentiation among individuals.

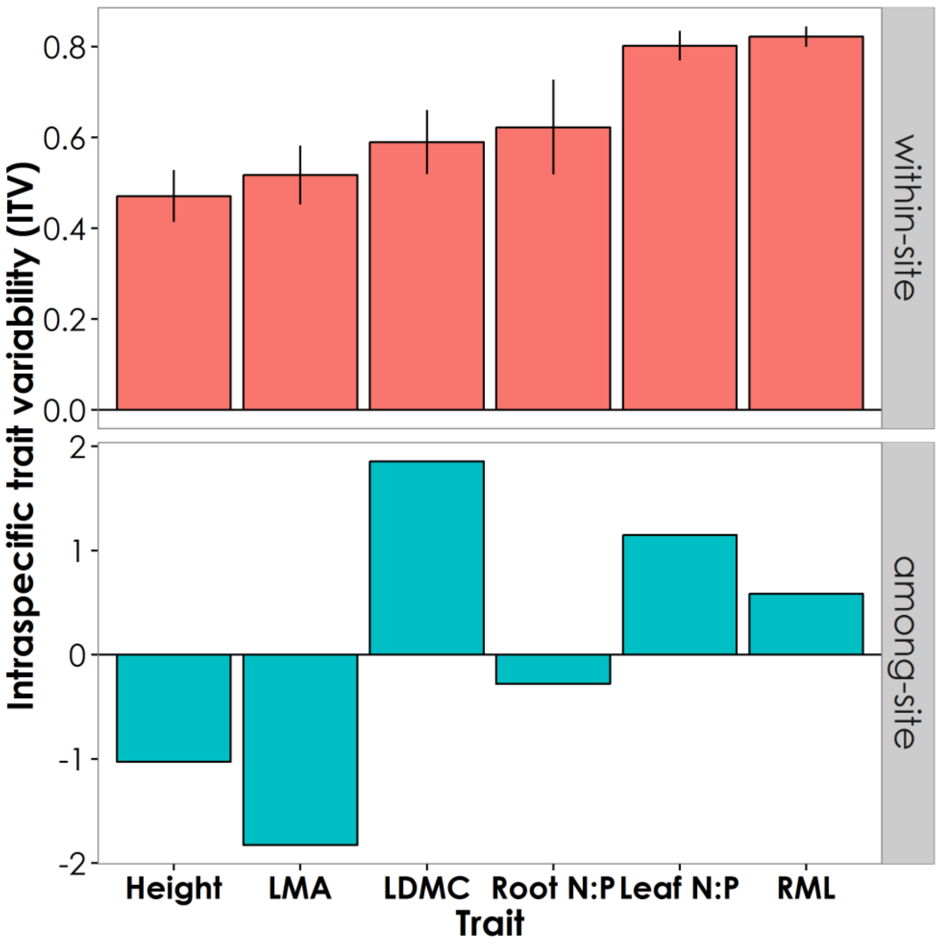

Figure 19. Bar plots of intraspecific trait variability. The top panel shows a bar plot of the proportion of intraspecific variability (wITV) at each site for each of the four traits; the error bars represent standard error of the mean. A value of 0 would indicate no intraspecific variability. The bottom panel shows, for each trait, the log ratio of variation among sites due to intraspecific variability to variation among sites due to species turnover (aITV). A positive number means that intraspecific variability contributes more than species turnover, and 0 would mean the effects are of the same magnitude.

I also compared our wITV and aITV values to those recorded in a global meta-analysis and found that the values for intraspecific trait variability in this study were generally higher than the global medians in the meta-analysis (Siefert *et al.* 2015). The global median wITV values for LMA, plant height, and LDMC were all between 0.25 and 0.3, albeit with high variability among studies. In contrast, the mean wITV values were greater than 0.4 for all traits, indicating that the role of within-site intraspecific variability was greater than the global median in the study region. The three median aITV values were all negative in the global meta-analysis and ranged between -0.3 and -0.7, although none significantly differed from zero. In this study, the aITV values for LMA and plant height were more negative than the global median, but the aITV value for LDMC was positive. This result indicates that species vary more in their LMA and height among sites than the global median, but that differences in LDMC across sites are driven by individual-level variation to a greater degree than is typical globally.

Trait-by-environment regressions

Across all sites, plant height had a unimodal relationship with both summer precipitation and summer temperature, while leaf mass:area ratio was highest at sites with high temperature and low precipitation. Leaf dry matter content was lowest at sites with intermediate temperature and precipitation, and leaf N:P ratio had a moderate peak at intermediate sites. As root mass:length ratio varied so widely within sites, there were no significant across-site relationships with climatic variables. Figure 20 shows regression plots with the trait value of each sampled individual plotted, each with a confidence band representing the standard error of the regression fits.

Predictions of species abundances

The performance of all models, whether incorporating variation in functional diversity among sites or not, whether incorporating information about global range sizes, and regardless of which traits were included, was remarkably poor (Figure 21). Model performance was not affected by the assumption of variable trait variances across sites, nor by the incorporation of prior information (Figure 21). In fact, essentially all models performed worse at predicting relative species abundances than a null model assuming equal species abundances across all sites: The RMSE of the null model was 0.056, and the lowest RMSE across all 124 model fits was also 0.056. The poor performance of trait-based models indicates that relative abundance distributions of species in the study region are not driven by the relationship between environment and species traits for the traits and environmental conditions that were considered. Figure 22 shows predicted versus observed abundance for each species at each of the fourteen study sites for one of the model runs; this individual fit, which assumed that trait variances are different across sites, incorporated prior information, and was fit with LMA, RML, and height data, is characteristic of most of the runs.

In general, the models underpredicted the abundance of most of the common species and predicted that some species that were present but rare would be absent. In addition, the predicted species abundance distributions were much more even than the observed distributions: median Simpson’s evenness across sites was 0.14, but across all model runs median evenness across sites was 0.61 (between 0.46 and 0.92 in 95% of runs). Globally, the models tended to underpredict the abundances of exotic species, including *Bromopsis inermis* (Leyss.) Holub and *Achillea*

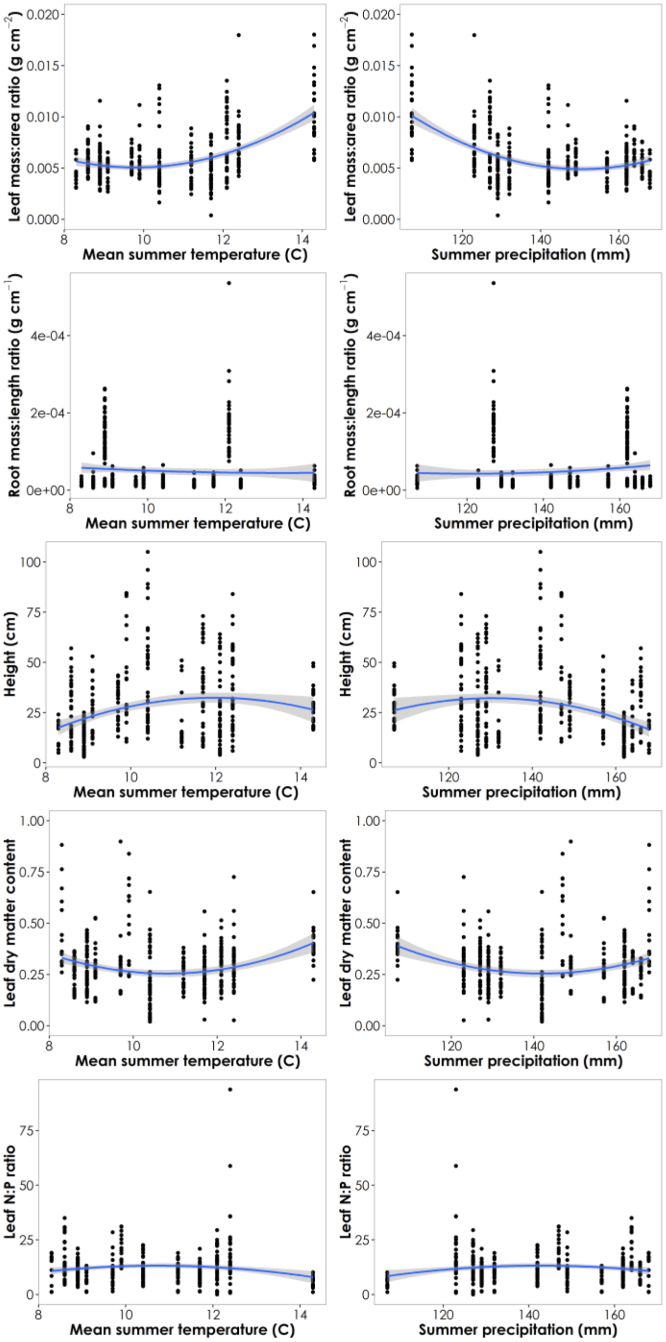

Figure 20. Trait-by-environment scatterplots showing, from top to bottom, leaf mass:area ratio, root mass:length ratio, plant height, leaf dry matter content, and leaf N:P ratio plotted against mean summer temperature (left panels) and total summer precipitation (right panels). Each point represents a trait value for an individual plant at a particular site, ignoring species identity. Quadratic regression fits are plotted, along with a band representing the standard error of the fits.

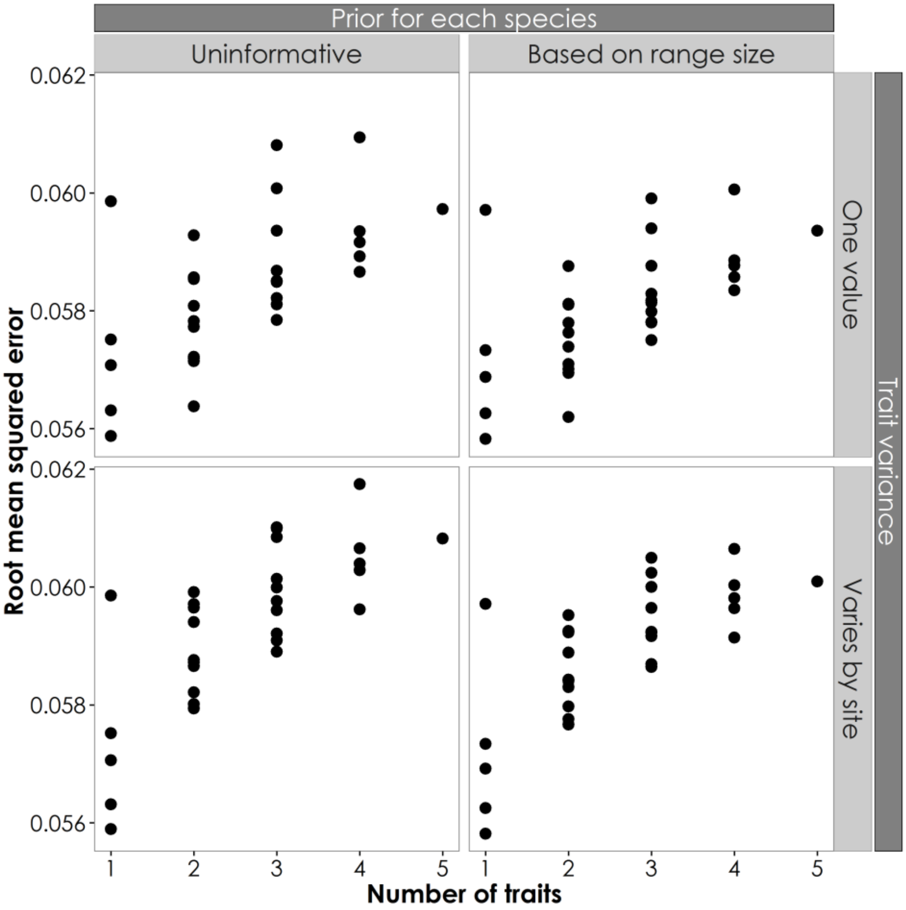

Figure 21. Root mean squared errors (RMSE) of all model fits. Within each panel, RMSE is plotted against the number of traits used to fit the model. The top two panels show RMSE of models with a single trait variance value used across sites, and the bottom two panels show RMSE of models in which trait variance was allowed to vary across sites. The left two panels show RMSE of models with uninformative priors, and the right two panels show RMSE of models with informative priors based on species global range sizes.

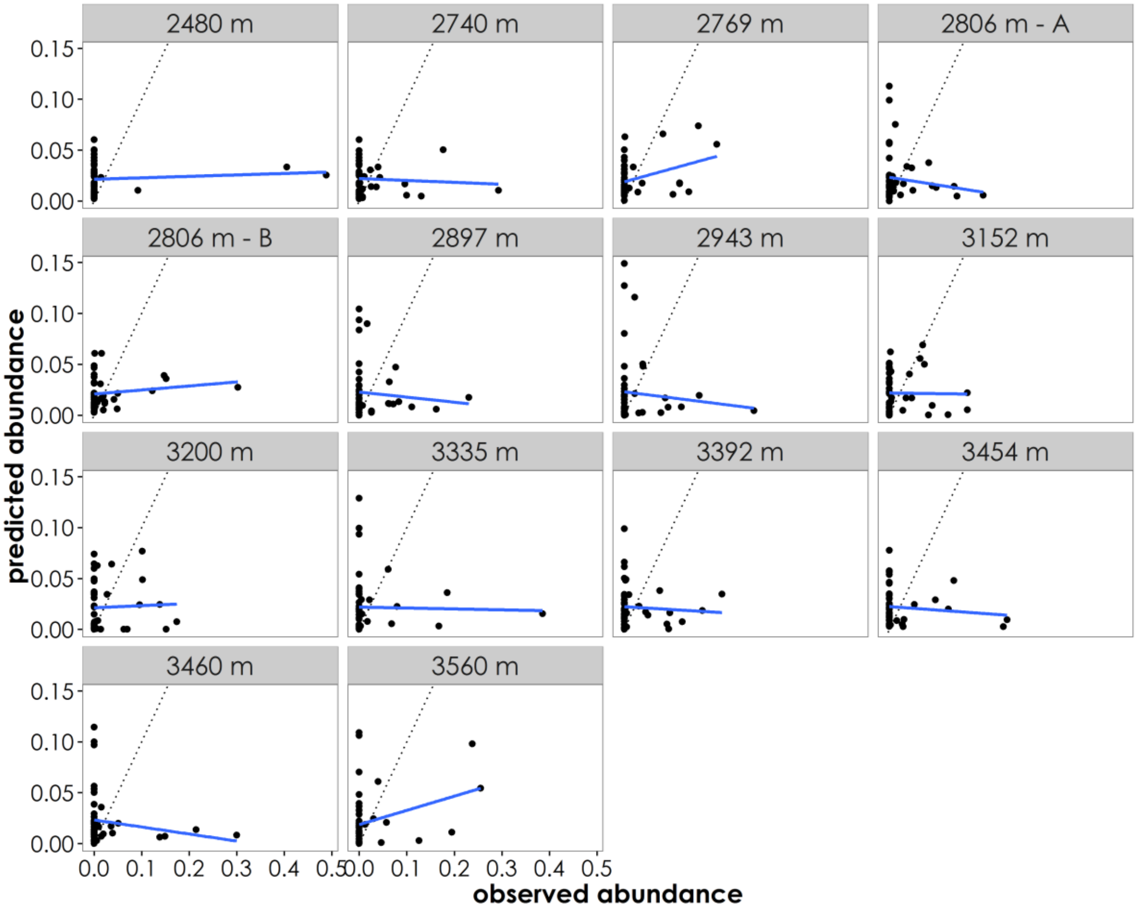

Figure 22. Predicted versus observed relative abundance for all 14 study sites for one model fit. The model fit shown here assumes variation in trait spread across sites, incorporates prior information on species range sizes, and is fit with LMA, RML, and height data. Each point represents a species at a site. Elevations of each study site are labeled. The dotted line represents perfect correspondence between observed and predicted values, and the solid blue line represents the trend in deviation of the observed relative abundance values from the predicted values.

millefolium L. The models overpredicted the abundances of some species that were locally common at one or two sites, but not found elsewhere, including *Poa* spp. L., *Valeriana occidentalis* A. Heller, *Alopecurus pratensis* L. and *Wyethia amplexicaulis* (Nutt.) Nutt. When considering sites where species were present, the local abundance of grass species, including *Poa, Elymus,* and *Festuca* spp., tended to be underestimated.

Discussion

My results show that different traits are involved with different processes of community assembly, suggesting that root traits vary more among individuals within sites and might be involved with niche differentiation, while leaf traits are less variable within and among species and might be involved with filtering. The predictive model shows that the functional traits I measured are not correlated with species relative abundance across sites. Incorporating functional diversity to account for niche spread does not improve predictive accuracy at all. Overall, the poor performance of traits in predicting species distributions indicates that more than just trait-based filtering from the regional species pool determines which species establish at a site and what relative abundance they attain. Observed species distributions were much less even than modeled distributions, indicating that the model does not adequately account for the effects of competition and niche differentiation processes in local community assembly. The high level of intraspecific trait variability in our study system is a plausible explanation for the lack of predictive power of my models. In addition, a variety of other processes may be more important than trait-based filtering. Among these processes may be historical and biogeographic effects, effects of spatial scale, or the fact that functional traits may not capture the processes leading to community assembly patterns in the study region.

Intraspecific variability

It is notable that the intraspecific variation in root traits within sites was higher than for the leaf and height traits. This may indicate that there is greater competition among individuals, regardless of species, belowground that is driving niche differentiation (Westoby & Wright 2006). Further, the high variability in root traits may mean that root traits are not very useful for predicting species abundances at a given site, because most of the variation is within species. Individual plants exhibit morphological plasticity in roots in response to environmental change, presumably to achieve optimal resource colimitation (Freschet *et al.* 2015). Adjustments in root mass:length ratio and root biomass allocation may explain most of the community-level variation in root morphology that I recorded.

In comparison to the values from the global meta-analysis of Siefert *et al.* (2015), the wITV values from the study region were higher, while the aITV values for LMA and height were comparable. This shows that leaf dry matter content and root mass:length ratio are explained more by the individual variability than by species turnover across sites, but LMA and height are explained more by species turnover. The correlation of LMA and height with species turnover across sites appears to suggest that those traits would be the most useful for predicting species presence across the study sites. However, LMA and height are not useful for predicting relative abundance due to high intraspecific variability, which helps explain the poor model performance we observed. My results lend support to the calls for an increased appreciation of individual trait variability in ecology (Bolnick *et al.* 2011; Violle *et al.* 2012; Rosindell *et al.* 2015). They suggest, confirming previous work, that intraspecific variation is a crucial driver of global change response (Siefert *et al.* 2014; Moran *et al.* 2016). The high intraspecific trait variability in this study system would suggest that species at a given site could approach functional equivalence (Hubbell 2005), making it difficult to predict which species should achieve the highest abundance at a given site from their traits alone. As my models failed to predict species dominance patterns, I observed a mismatch between predicted even distributions of species abundance and the observed distributions dominated by one or a few species at each site. I discuss potential reasons for this mismatch below.

Biogeographic and scale effects

One potential reason that my models failed to predict species relative abundance is that differences in relative abundance of species among sites separated by hundreds of meters of elevation is controlled by historical and biogeographic factors. These contingent historical events have interfered with the deterministic outcomes of filtering and niche differentiation. There may be dispersal barriers that prevent plants with appropriate traits from reaching optimal sites (Clark *et al.* 2002), especially due to topographic variation in the mountainous study region (Engler *et al.* 2009). In addition, species relative abundance may shift from year to year due to temporal stochasticity (Alonso *et al.* 2006). Another potential explanation for the mismatch between traits and environment is that I measured species composition at a single time point and attempted to correlate it with static measures of climate (mean annual temperature and precipitation). However, plant species relative abundance may respond more sensitively to climatic fluctuations at a rapid time scale (Fukami & Nakajima 2011), even leading to annual turnover in dominant species identity (Allan *et al.* 2011). Furthermore, coarse-scale measurements of climatic factors may not adequately account for microclimate heterogeneity, which may act as a strong determinant of relative abundance at the plot scale (Levin 1992).

Mismatch between traits and community assembly processes

A further reason for the poor performance of the trait-based models may be that while community assembly is driven by processes that are measurable by plant traits, I failed to measure the plant properties or environmental factors that are driving community assembly. Interestingly, the models tended to predict species distributions that were much more even than those I observed, predicting the presence of many species not found at particular study sites, even when both filtering and niche differentiation were accounted for (Figure 22). The underprediction of abundance of common species may indicate that the models do not capture processes that generate uneven abundance distributions. Instead, they inflate predicted abundance for species with trait values close to the site optimum but that are excluded for other unknown reasons. Ecologists that have modeled species abundance distributions both statistically (Magurran & Henderson 2003) and mechanistically (McGill *et al.* 2007) have disagreed on the appropriate way to model the process that generates diverse communities with many rare species. In addition, empirical studies have often failed to find significant functional differences among dominant and minor species (Walker *et al.* 1999), indicating that neutral processes may be more important for generating relative abundance distributions (Hubbell 2005) and that some of the observed correlation between species abundances and environmental conditions is a result of temporal fluctuation in abundance (Fukami & Nakajima 2011).

The species whose abundances were most underpredicted by the models included both of the most common non-native species in the study region. Exotic species tend to be able to colonize relatively extreme environments despite apparently lacking specialized adaptations for stress tolerance (Alexander *et al.* 2011). If the traits that confer abundance in exotic species are not related to climatic filtering (Levine *et al.* 2003), these community-level models will fail to predict their abundance accurately.

In addition, although I included belowground traits in our model, which previous studies have not done, I did not include soil nutrient availability as a site-level predictor. Previous work has shown that filtering along steep gradients of nutrient availability can lead to species abundance distributions that correlate well with functional traits (Laughlin & Laughlin 2013). The elevational gradient is also characterized by a gradient in nutrient availability: low-elevation sites with older soils have a higher N:P ratio than high-elevation sites with younger soils (Joshua Lynn, unpublished data). It may be that this environmental filter is more important than climate variation in this system, and that leaf and root nutrient concentrations would do better at predicting relative abundances. Incorporating soil N availability might also improve the models' ability to accurately predict legume abundance, which was significantly underpredicted by both models.

Conclusion

In order for functional trait ecology to fulfill its promise, traits must be used to predict, rather than solely describe (Laughlin *et al.* 2012; Violle *et al.* 2014). However, because trait distributions reflect the outcome of selective processes acting at the level of the individual organism, they may not be good predictors of species abundances within communities, especially when trait plasticity is high (Messier *et al.* 2010; Siefert *et al.* 2015). I recommend that future model development focus on accounting for individual variation and plasticity and its consequences for community assembly. In addition, I suggest that researchers measure root traits that capture tradeoffs in resource acquisition belowground, as this may be the hidden driver of species abundance in plant communities.

Appendices

Appendix 1: Estimation of global range sizes

Global range sizes were estimated using data from the GBIF database (http://www.gbif.org). I downloaded all georeferenced occurrence data for each of the species in the dataset on 19 November 2015, and extracted species name, latitude, and longitude from the downloaded .txt files. I binned all the occurrence data into 1° longitude × 1° latitude bins. I counted the number of bins in which each species occurred, resulting in a relative estimate of range size for each single species in the dataset. This method is relatively robust to sampling effort within a species' range, as it ignores the density of observations within a bin. However, it assumes that there are at least some records distributed evenly across each species' range. For plants that were only identified to the genus level in the field, I used the mean range size for the other species in that genus. R code to process the .txt files that are publicly available on the GBIF website is available from the author upon request.

CHAPTER IV

ABOVEGROUND RESILIENCE AND BELOWGROUND RESISTANCE: LOSS OF A DOMINANT GRASS SPECIES CAUSES NICHE FILLING BY MONTANE MEADOW PLANT SPECIES, WHILE NITROGEN FERTILIZATION AFFECTS CARBON CYCLING

Abstract

1. Species differ in their traits and thus in their ability to tolerate environmental conditions and take up resources from the environment. In addition, theoretical and empirical work has repeatedly demonstrated that biotic interactions play a large role in structuring plant communities. Taking a functional trait approach could help us predict how interactions among species shape species abundance distributions and determine ecosystem processes. However, these trait-based predictions have not often been tested in multifactorial field experiments.

2. Here, I aimed to test how resource availability and species dominance influence—separately or together—the structure and function of a montane meadow plant community. Specifically, I added nitrogen to the soil (control, + organic N, + inorganic N) and removed the dominant species (control, - dominant species, - random biomass) in a fully factorial design. I hypothesized that traits related to plants' ability to take up different forms of soil N would be related to species abundance distributions, resulting in interactive effects of dominant species loss and N fertilization on community structure and function.

3. After four years of manipulation, the plant community in removal plots converged toward species whose leaf and root functional traits resembled those of the dominant species. Soil carbon efflux was at least 57% greater under both organic and inorganic nitrogen addition, and inorganic N addition increased aboveground biomass production by 58% relative to controls. Surprisingly, there were no interactive effects of N fertilization and dominant species loss on structure or function.

4. The montane meadow community in this study was generally resistant to change. It responded little to soil N addition and, given the functional convergence I observed, the community seemed resilient to species loss. The magnitude of change may have been dampened by shifts in relative species abundance related to yearly fluctuations in climate.

5. I experimentally manipulated multiple global change drivers, tested ecological niche theory, and united global-change ecology with a trait-based approach. My results indicate that the ability of species to compensate functionally for species loss confers resilience and maintains diversity in montane meadow communities.

Introduction

The structure of plant communities and the traits of the individuals that make them up are important predictors of ecosystem function (Lavorel 2013). For example, plants may differ in their ability to access the resource that most limits growth, which may lead to partitioning of the limiting resource into pools accessible by different mechanisms or at different times (Suding *et al.* 2004; Farrior *et al.* 2013). The species that is the best competitor for the most limiting resource has the potential to achieve the highest abundance within the community (Tilman 1977; Gilman *et al.* 2010). Unfortunately, it is not generally possible to directly measure the strength of interactions among individual plants in the field to determine how competitive dominance for a particular resource arises (Levine & HilleRisLambers 2009). However, measuring plant functional traits such as leaf and root morphology, plant size, and nutrient content captures variation among plants in life history, tolerance of varying environmental conditions, and resource uptake strategy (Violle *et al.* 2007; Bardgett *et al.* 2014; Reich 2014). Life history strategies, tolerance of variation in the environment, and rates of resource uptake determine the outcome of processes such as environmental filtering (Pavoine *et al.* 2011; Stahl, Reu & Wirth 2014) and interactions including competition for limiting resources (Freschet, Swart & Cornelissen 2015). Ultimately, the outcomes of such interactions contribute to shape abundance distributions of plant communities (Adler *et al.* 2014), with predictable effects on ecosystem functions such as productivity and carbon storage (Cornelissen *et al.* 2007; Reichstein 2014). In this study, I aim to test how resource availability and species dominance influence—separately or together—the structure and function of a montane meadow plant community.

Theoretical and empirical work has repeatedly demonstrated that biotic interactions play a large role in structuring plant communities (Pigot & Tobias 2013; Wisz *et al.* 2013), whether they reinforce or undermine the role of environmental filtering. Differences in relative abundance within communities are often attributed to relative fitness differences, or a fitness hierarchy among species (HilleRisLambers *et al.* 2012). However, maintenance of diverse plant communities may not be possible without sufficient stabilizing niche differences among species. The net outcome of biotic interactions in communities often results in abundance distributions dominated by a few species. Small changes in the relative biomass of these abundant species tend to have large effects on ecosystem functioning (Grime 1998; Gaston & Fuller 2008; Umaña *et al.* 2015). However, the response of subordinate plant species to removal of competitively dominant plant species has rarely been assessed (Wardle *et al.* 1999; Díaz 2003; Wardle & Zackrisson 2005). To address this, I removed the most abundant plant species, *Festuca thurberi* Vasey (Thurber's fescue), from a subset of experimental plots to test whether suppression by this species limits diversity and mediates ecosystem function in this system. *Festuca thurberi* is functionally distinct from all the other common species in the montane meadow community where I conducted this experiment, having much higher leaf mass:area ratio (LMA) and higher root mass:length ratio (RML) than any of the other common species (Figure 23).

Recent work has also shown that the availability of multiple soil resources can promote coexistence in and determine the structure of plant communities (Harpole & Tilman 2007, Harpole & Suding 2011; Eskelinen & Harrison 2015). In particular, coexistence within diverse plant communities such as montane meadows may be possible because different species may be capable of exploiting different forms of soil nitrogen (N), which likely limits plant growth in the study region (Suding *et al.* 2005). Depending on root physiology and root microbiome, a given species might be better than others at accessing inorganic nitrogen or at cultivating symbiotic microbes that can break down organic N molecules in the soil (Cantarel *et al.* 2015). To test whether plant community structure and ecosystem function in montane meadows might be at least partly driven by partitioning among different forms of soil N, I added both organic and inorganic N fertilizer to a subset of plots annually.

While many experimental and observational studies have focused either on the role of competitive interactions in structuring plant communities (Choler, Michalet & Callaway 2001; Callaway *et al.* 2002; Levine & HilleRisLambers 2009) or on the effects of varying soil resource availability on community and ecosystem processes (Suding *et al.* 2005, 2006; Baribault & Kobe 2011), few studies have experimentally tested whether these two processes interact (Gundale *et al.* 2012; Wardle *et al.* 2013). To address this, the experimental setup consisted of dominant species removal (3 levels) crossed with N addition (3 levels). Differences in plant community response to the presence of a dominant species across levels of nitrogen fertilization would indicate that species dominance is related to the ability to most efficiently take up limiting soil

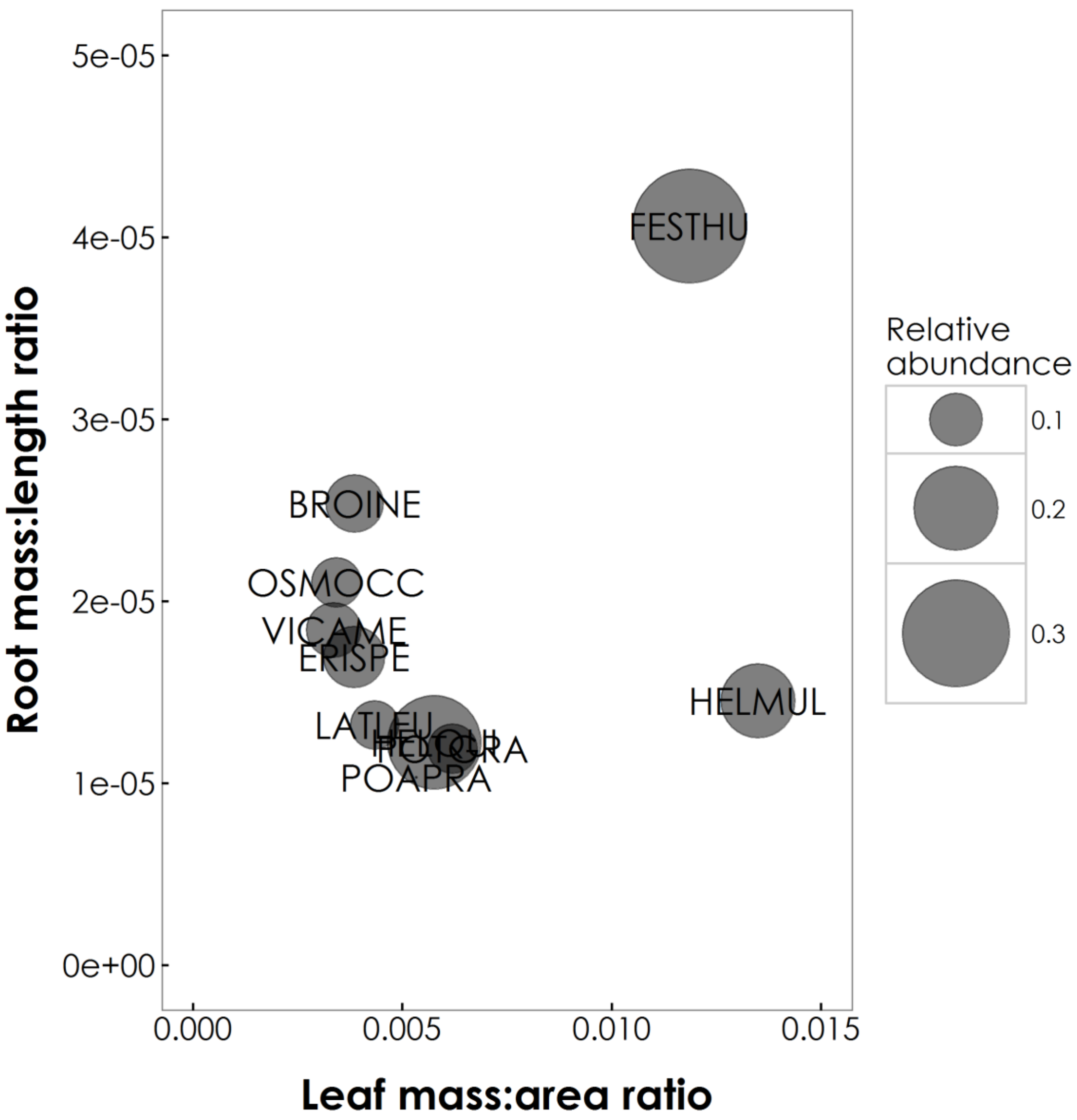

Figure 23. The ten most abundant plant species in the Maxfield Meadow plant community. Species are plotted with their leaf mass:area ratio on the x-axis and root mass:length ratio on the y-axis. The size of each point corresponds to the relative abundance of the plant species within the community in untreated plots in July 2015. Species codes are as follows: BROINE, Bromopsis inermis; ERISPE, Erigeron speciosus, FESTHU, Festuca thurberi; HELMUL, Heliomeris multiflora; HELQUI, Helianthella quinquenervis; LATLEU, Lathyrus leucanthus; OSMOCC, Osmorhiza occidentalis; POAPRA, Poa pratensis, POTGRA, Potentilla gracilis; VICAME, Vicia americana.

resources. However, if the community response to the dominant species does not depend on soil N addition, it would indicate that the processes that generate abundance distributions in the community are not dependent on traits that determine plants' ability to take up soil N. In addition to the ability to test predictions made by theory, experiments such as this that last multiple years and simulate multiple drivers of anthropogenic change are important to help generate predictions about how natural communities and the services they render are responding to ongoing global change (Mikkelsen *et al.* 2008; Rustad *et al.* 2008; Dukes *et al.* 2014). Specifically, in this study, I asked the following questions:

- Do organic and inorganic N fertilization differ in their effects on plant species diversity, community-level functional trait composition, and C storage and turnover in a montane meadow?
- Does the presence of a dominant tussock grass species, *Festuca thurberi*, affect the species diversity and functional trait composition of the remaining plant species in the community, and does this species drive C dynamics in the community?
- Are the effects of the dominant grass species on the plant community and on C cycling contingent on soil N addition? Do soil N addition and dominant plant species interact to determine the structure and function of the plant community?

Methods

Site description

In the summer of 2012, I established 36 permanent 1.5 × 1.5 m plots in Maxfield Meadow, a montane meadow in the West Elk Mountains near the Rocky Mountain Biological Laboratory in Gothic, Colorado, USA, at 2910 m above sea level. The site is subject to mild grazing by cattle during late summer and avalanche activity during the winter. The most abundant plant species found in the meadow is *Festuca thurberi*, a large, sturdy perennial tussock grass (Poaceae). Other abundant species include *Helianthella quinquenervis* (Hook.) A. Gray, *Erigeron speciosus* (Lindl.) DC., *Heliomeris multiflora* Nutt. (Asteraceae), and *Bromopsis inermis* (Leyss.) Holub (Poaceae).

Study design and treatments

I did a full factorial cross (n = 4) of N addition and dominant species removal over a three-year period. The three levels of the N treatment were the addition of 10 g organic N m^{-2} y^{-1} (as 21.7 g urea, $CO(NH_2)_2$), addition of 10 g inorganic N m^{-2} y^{-1} (as 29.4 g ammonium nitrate, NH_4NO_3), and no N addition. In 2013 and 2014, I watered all plots, including controls, as I fertilized them, but in 2015 I synchronized fertilization with a rain event. There were three levels of the species removal treatment: fescue removal, random biomass removal, and intact control plots. In the fescue removal treatment, I removed all aboveground biomass of the most abundant species (*F. thurberi*) within the 1.5 × 1.5 m plot. The random biomass removal plots control for any potential effects of removing aboveground plant biomass that are not specific to fescue. In the random biomass control plots, percentage fescue cover was recorded before removal, and an equivalent amount of vegetative cover was randomly selected from among all species in the plot, including fescue, and removed. In plots assigned to the intact control treatment, I removed no biomass. After removing and bagging aboveground fescue biomass, I applied a dilute mixture of glyphosate herbicide to the clipped *Festuca* stem bases with a paintbrush to kill the belowground portion of the plant.

Confirmation of the effectiveness of removal. Dry mass of removed vegetation did not differ between fescue removal and random removal plots (unpaired t-test: $t_{17.6} = 1.235$, $p = 0.233$). In addition, while removal treatments initially decreased vegetative cover, other species replaced *Festuca*. No *Festuca* was observed to have grown back in fescue removal plots. In fact, percent plant cover was significantly greater in *Festuca* removal plots by 2015, three years after the initiation of the experiment (147% versus 129% in peak season; $p = 0.03$; ΔAICc of best model = 4.22 less than null model).

Plant community structure and function

Community composition and diversity. I measured all response variables within the central 1 × 1 m area of each plot, including plant community composition, soil moisture, soil respiration, and leaf area index (LAI). I used percentage aboveground cover of each plant species, estimated visually (to within 1% if ≤10%, and to within 5% if greater), as a proxy for abundance in the plant community composition measurements. Composition was recorded once in June 2012 prior to fescue removal, twice after removal during the growing season of 2012, and twice during the growing seasons of 2013, 2014, and 2015.

Plant functional trait composition. In 2015, I collected leaf samples from 10 individuals of each of the ten most common species at the site, from individuals not in the experimental plots but within 20 m of the plots. I also measured the heights of all individuals perpendicular to the ground from the base of the stem to the tip of the topmost leaf. From five of these individuals in each species, I also collected root samples. The plant tissue samples were used to measure LMA, RML, and leaf dry matter content (LDMC).

I collected at least three mature and fully expanded leaves from each of the individuals that were sampled for leaf traits. I transported the leaves on moist paper towels and scanned them on an Epson flatbed scanner. I weighed the scanned leaf material, dried it for 48 hours at 60° C, and weighed it again. I saved the dried leaf material and pooled it with additional leaves that were collected for chemical analysis.

I took two to four 3-cm diameter, 15-cm long soil cores from directly below the base of each individual plant that was sampled for root traits. I extracted as much fine root material as possible from the soil cores, washing the soil over a 2-mm sieve if necessary. I scanned 10 to 20 intact pieces of fine root from each individual on an Epson flatbed scanner after floating the root pieces in a transparent tray of water. I dried the root material for 48 hours at 60° C and weighed it. I saved the dried root material and pooled it with additional collected roots for chemical analysis.

To measure nitrogen and phosphorus content by mass (total Kjeldahl N and P) of the dried tissue samples (both leaf and root), I first ground the tissue samples with a mortar and pestle. I weighed 75 mg, or as much as was available, of the ground sample and folded it into a piece of adhesive-free cigarette paper. I digested the sample for 5 h at 350° C in 5 mL H_2SO_4 in a Kjeldatherm digestion block (Gerhardt, Königswinter, Germany); in each digestion run I included a blank with no sample, and one sample of apple leaf standard with known N and P content. After the digests cooled, I added 45 mL deionized water to each one. I used a

SmartChem 200 discrete analyser (Unity Scientific, Brookfield, CT, USA) to measure total Kjeldahl N and P, expressed as mg/g tissue.

I measured the area of the scanned leaf images, and the total length of the scanned root images, with ImageJ software (Schneider, Rasband & Eliceiri 2012), using the IJRhizo macro (Pierret *et al.* 2013) to automatically trace all the roots in each image. I calculated LMA for each individual plant by summing the mass and area of each individual and dividing the dry mass by the scanned area. LDMC was calculated as the dry leaf mass divided by the leaf mass before drying. I calculated RML for each individual plant by dividing dry root mass by the total root length.

Carbon storage and turnover

I used a LI-6400 gas analyser (Li-Cor Corporation, Lincoln, NE, USA) to measure soil respiration three times during the growing season of 2012 and twice during 2013, 2014, and 2015. Permanent PVC soil collars for respiration measurement were placed inside each plot near the edge of the central area. I measured LAI twice during 2013, 2014, and 2015. The LAI results presented here from 2015 were taken with an Apogee MQ-200 light sensor (Apogee Instruments, Logan, UT, USA). To confirm that LAI is a good proxy for standing aboveground plant biomass, I collected standing aboveground biomass from 6 sites in the region, including our study site. I selected these sites to capture most of the regional variation in LAI. At each site, I measured the leaf area index in 5 randomly selected plots 0.25 m^2 in area, removed all aboveground plant biomass from the plots, dried the biomass for 48 h at 60° C, and weighed it. The biomass across sites varied approximately sevenfold; the mean biomass at a site was very closely correlated to the mean LAI at that site ($R^2 = 0.92$). For all environmental response variables, I took measurements at multiple times during the growing season; however, I primarily present measurements from the peak of the growing season (mid-July) here, following standard practice.

Data processing and analysis

All analyses were conducted in R 3.2.3 (R Core Team 2015), with packages listed where appropriate. I excluded *Festuca* from the diversity metric calculation because it was physically removed from some of the plots, and I was interested in assessing the diversity of the remaining community. However, for the functional diversity, community-weighted trait mean, and community-weighted trait variance calculations, *Festuca* was included to investigate how the entire community's function changed with the removal of *Festuca* and whether communities where *Festuca* was removed would converge functionally on intact communities. Although there is a relatively large amount of turnover in plant species between the early growing season (early June) and the peak growing season (mid-July), I based all the following analyses on peak-season measurements (July); this follows standard practice. Finally, all analyses are based on data collected in 2015 except for the analysis of trait convergence through time.

Calculation of diversity metrics. I calculated species diversity metrics for each treatment combination (Jost 2007). I converted Shannon's alpha diversity (H') to first-order effective species number (d_1) using the formula $d_1 = e^{H'}$. For a particular community, the effective species number is the number of species in a community of maximum evenness that would be required to attain an equivalent value of Shannon's diversity. I excluded *Festuca* from the calculations of effective species number.

I also calculated functional diversity by taking the mean trait value for all species in each plot for which trait data exist (10 species, comprising a median of 88% of the plant cover in each plot, and for over 50% in all plots). For each of these species, I used the trait values of LMA, RML, LDMC, leaf N:P ratio, root N:P ratio, and plant height. I calculated the pairwise Gower distance between each pair of species and used these distances to estimate an index of functional dispersion (FDisp) for each plot. For this calculation, I used the *gowdis* and *fdisp* functions in the *FD* package in R (Laliberté *et al.* 2014).

Calculation of community-weighted trait means. I calculated community-weighted means (CWM) and community-weighted variances (CWV) for LMA, RML, LDMC, leaf N:P ratio, root N:P ratio, and plant height for each of the 36 experimental plots. In addition, I ran a principal components analysis on the six traits using the *prcomp* function in R, and calculated CWM and CWV for the first principal component axis. Community-weighted means are calculated by taking the mean trait value for the 10 species for which trait data exist and calculating a mean trait value for each of the 36 plots weighted by the relative cover of each plant species; variance is calculated in a similar way.

Statistical analysis. I assessed the effect of the fertilization treatments, the removal treatments, and their interaction with a linear model (ANOVA). I visually examined all residual plots for normality to ensure that parametric analysis of untransformed data was appropriate. In each case, I fit the full model with both main effects and their interactions, then fit all possible subsets of that model and compared their AICc scores using the *dredge* function from the *MuMIn* package (Bartoń 2016). Models with an AICc value within 2 of the lowest value were considered to be the best models. As a post hoc test for differences among treatment means, I calculated the least-square means for each treatment combination and marginal least-square means for each single treatment, along with their standard errors and 95% confidence intervals using the *lsmeans* function from the *lsmeans* package (Lenth 2015).

To determine whether variation in community structure was explained by the treatments, I ran 9999 iterations of a permutational analysis of variance (PERMANOVA; Anderson 2001) for both the early-season and peak-season time points. This test, implemented in the *adonis* function in the *vegan* R package (Oksanen *et al.* 2016), partitions variance in ecological community datasets similarly to a multivariate analysis of variance.

In addition to comparing means across treatment groups for the 2015 growing season, I also investigated whether the plant community in plots where *Festuca* was removed converged functionally over time toward intact control plots where no species was removed. I took the median plot-level values of the six trait CWMs of each removal treatment group (intact control, *Festuca* removal, and random biomass removal), pooled across all fertilization treatments, for the peak growing seasons in each of the four years since treatments were established (2012-2015), as well as the first principal components axis. I subtracted the median CWM value of the intact control group from the *Festuca* removal group and from the random removal group. For each of the two removal groups, I regressed the difference relative to the control against the number of years since treatments were established. A slope trending toward zero over time would indicate that the mean trait value of the community without *Festuca* is converging on the mean trait value of the community with *Festuca*, presumably because other species are increasing in relative abundance to fill the niche space left by the removal of *Festuca*.

Results

Plant community structure and function

Community composition and diversity. There was no significant difference in species diversity among any of the treatments in July 2015 (null model was selected by AICc). In addition, there was no significant response of community structure, as measured by turnover in species identity, to fertilization or removal (PERMANOVA; $p > 0.1$ for both fertilization and removal treatments). In contrast to the lack of response in community structure, functional diversity as measured by FDisp was 21% lower in *Festuca* removal plots relative to intact control plots ($p = 0.0001$, $R^2 = 0.367$, ΔAICc of best model = -11.42 less than null model; Figure 24). However, functional diversity was not significantly lower in random biomass removal plots compared to intact control plots. There was no interactive effect of the treatments on species diversity or functional diversity.

Plant functional trait composition. The functional composition of plant communities in July 2015 differed across removal treatments, but not fertilization treatments. Dominant species removal did not cause a change in the CWM of leaf mass:area ratio (Figure 25). Dominant species removal was retained as a predictor in all the best models for the CWM of root mass:length ratio (ΔAICc = -15.34; Figure 26), height (ΔAICc = -2.54; Figure 27), and leaf dry matter content (ΔAICc = -9.03; Figure 28). Removing *Festuca* caused the plant community to have a 23% lower mean root mass:length ratio ($p = 2 \times 10^{-5}$; $R^2 = 0.431$), a 15% shorter mean height $p = 0.02$; $R^2 = 0.187$), and a 20% lower leaf dry matter content ($p = 0.001$; $R^2 = 0.321$). In all three cases, the coefficient associated with *Festuca* removal was of greater magnitude than the coefficient associated with random biomass removal, although the plant community also had lower mean root mass:length ratio, shorter mean height, and lower leaf dry matter content in random removal plots relative to intact controls. Removal and fertilization had no interactive effect on any trait mean.

Paralleling the change in functional diversity, the variance in both root mass:length ratio and leaf dry matter content decreased in the absence of *Festuca*. CWV of root mass:length ratio decreased by 70% ($p = 9 \times 10^{-6}$; Figure 26), and variance in leaf dry matter content decreased by 57% ($p = 0.0002$; Figure 28). However, as with the community-weighted means, the variances in root mass:length ratio and leaf dry matter content were also decreased in random removal plots but to a lesser degree (by 32% and 19%, respectively).

The means and variances of root and leaf N:P ratios were also affected by removal of *Festuca*, but not by random biomass removal. Leaf N:P ratio was 4% lower when *Festuca* was removed, although this was a marginally significant difference ($p = 0.7$; $R^2 = 0.225$; ΔAICc = 4.23; Figure 29). The CWV of leaf N:P ratio was the same across all treatments. In contrast, the CWM of root N:P ratio was unchanged across treatments, but the CWV was 43% higher when *Festuca* was removed ($p = 0.0007$; $R^2 = 0.304$; ΔAICc = 8.13; Figure 30). There was no interactive effect of treatments on any of the functional trait means.

Despite these differences among removal treatments, it is important to note here that the differences in trait mean and variance were caused primarily by the removal of *Festuca*, not by differences in the trait composition of the subordinate community across treatments. When I compared CWM and CWV across treatments excluding *Festuca* from the calculations, there was no significant difference between the CWM or CWV of any trait between *Festuca* removal

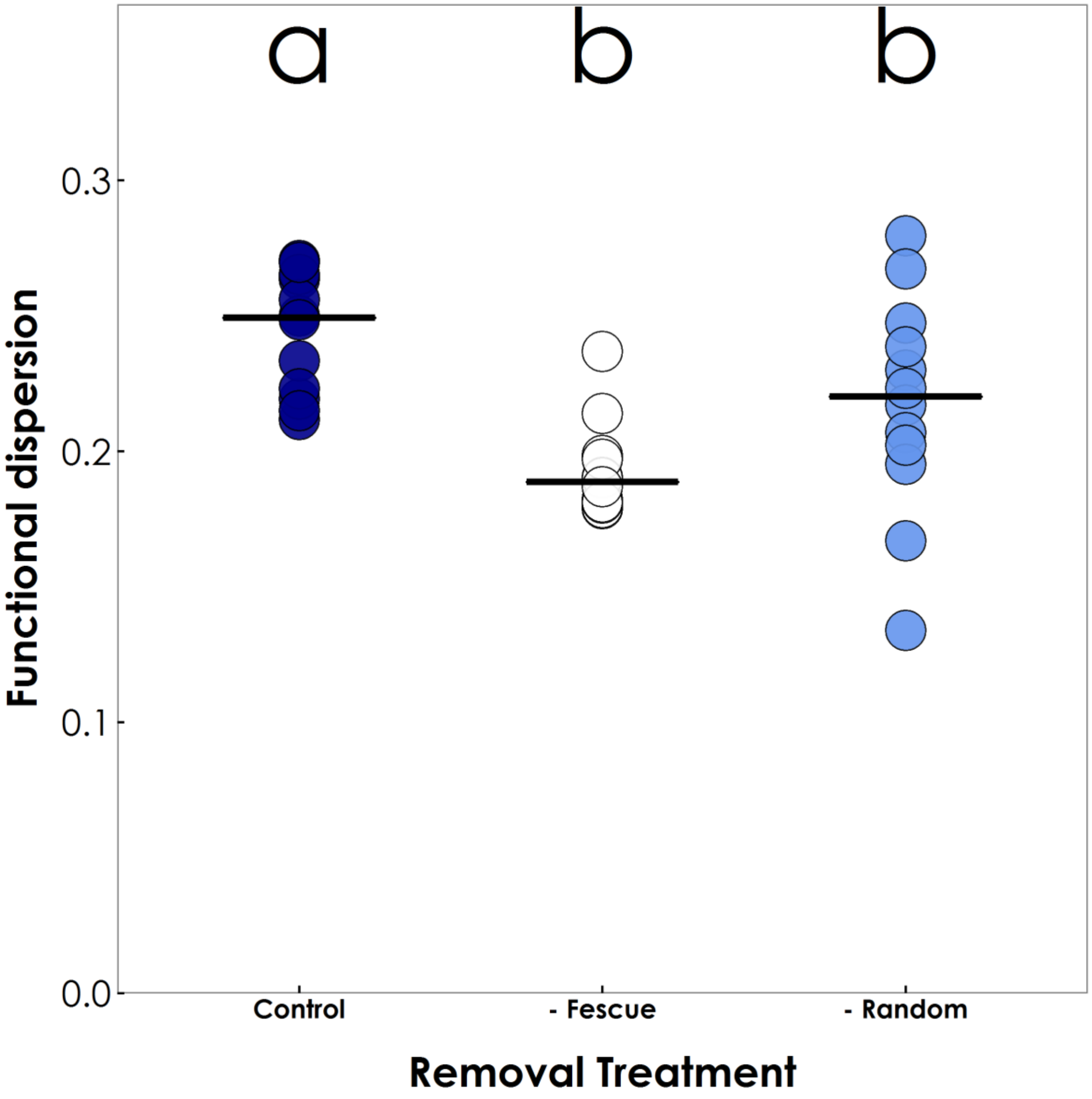

Figure 24. Functional dispersion (on y-axis) by removal treatment (x-axis) in July 2015. The intact-community control is shown in dark blue, fescue removal treatment in white, and random removal treatment in light blue. Each point represents the value from a single experimental plot, and the horizontal bars are the median value from each removal treatment group, across all three fertilization treatment groups. Different letters indicate removal treatments that significantly differ from one another (*post hoc* comparison of least-square means across all levels of fertilization treatment).

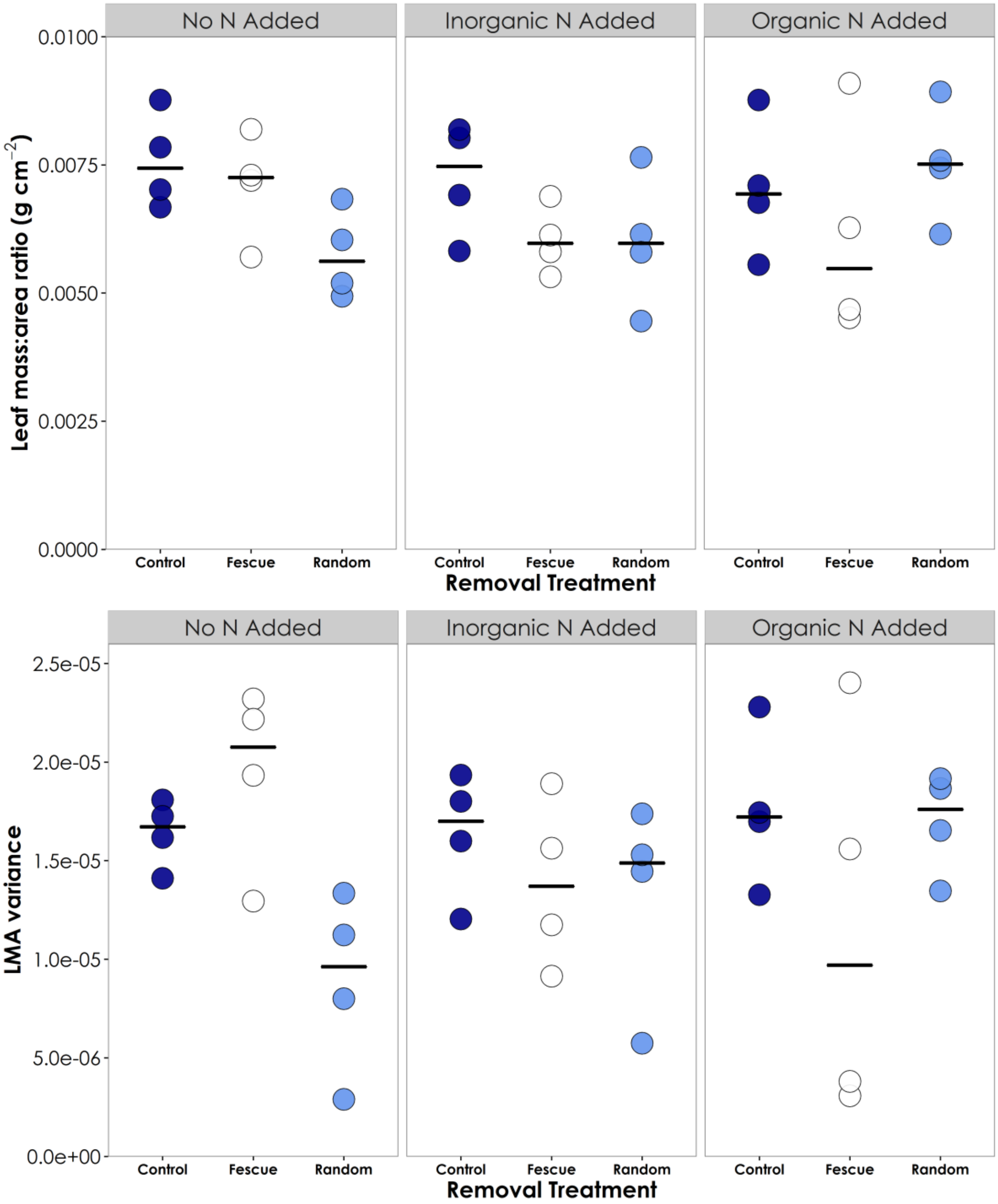

Figure 25. Leaf mass:area ratio community-weighted means (top panel) and community-weighted variances (bottom panel) across all 9 treatment combinations in July 2015.

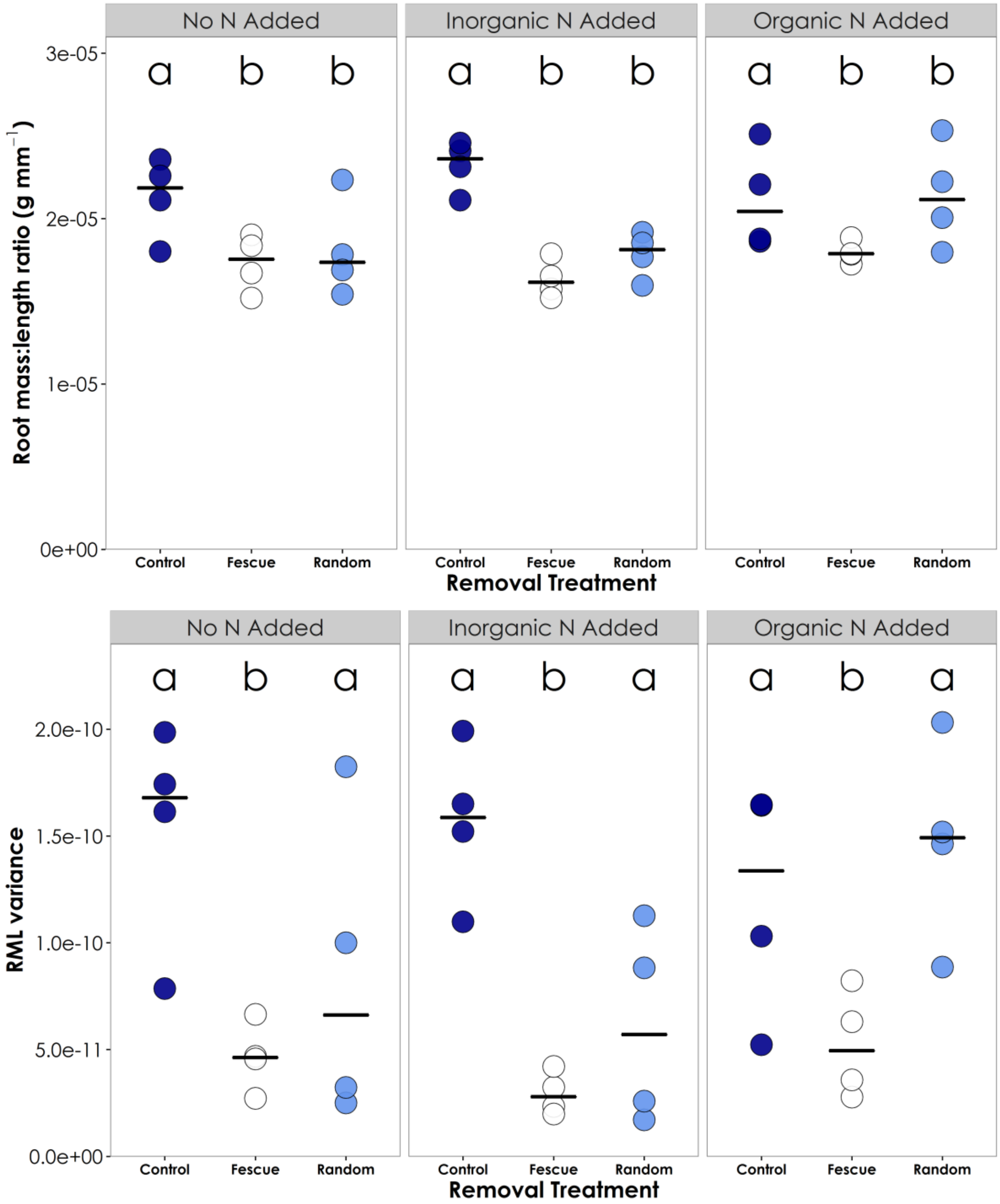

Figure 26. Root mass:length ratio community-weighted means (top panel) and community-weighted variances (bottom panel) across all 9 treatment combinations in July 2015.

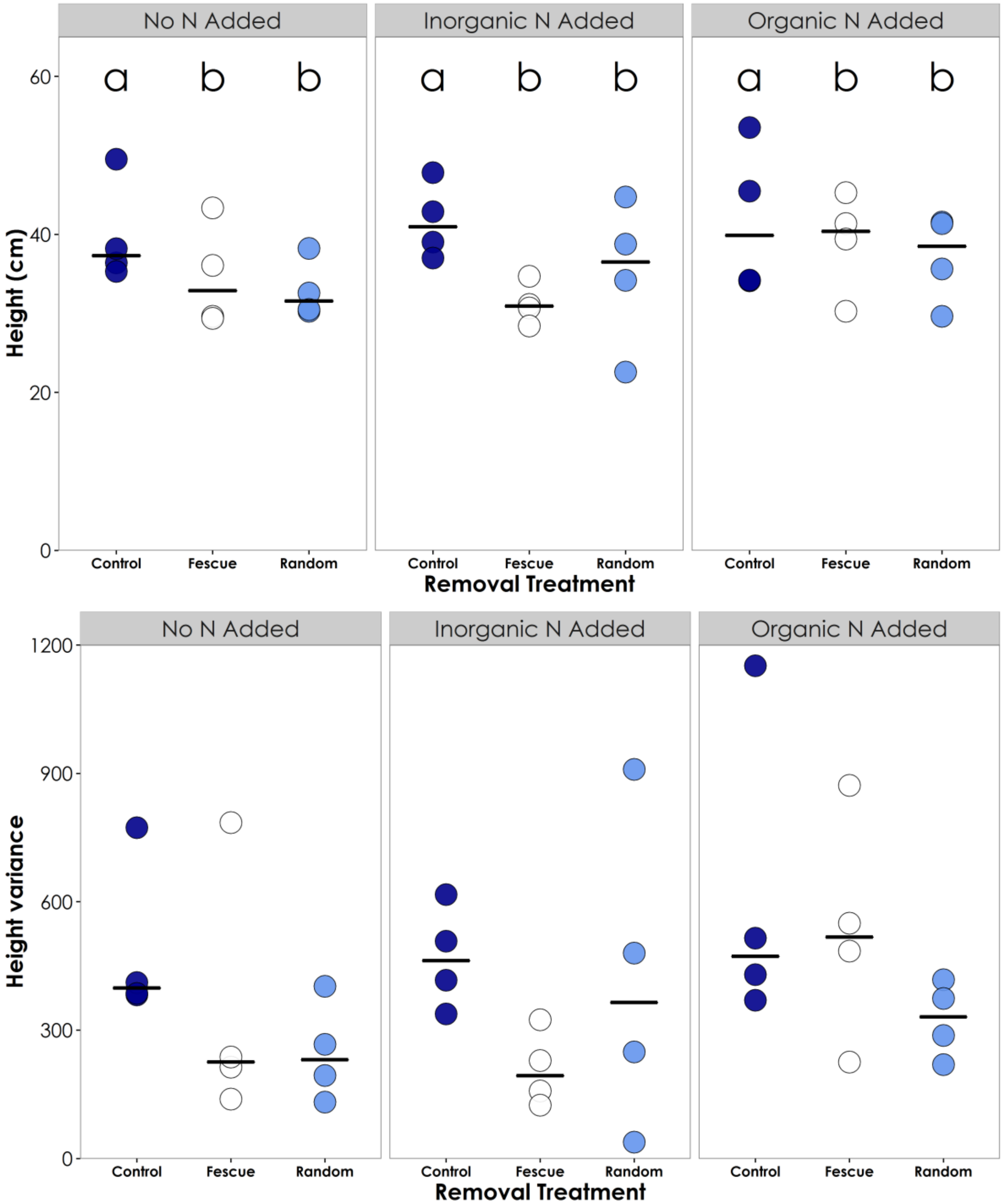

Figure 27. Plant height community-weighted means (top panel) and community-weighted variances (bottom panel) across all 9 treatment combinations in July 2015.

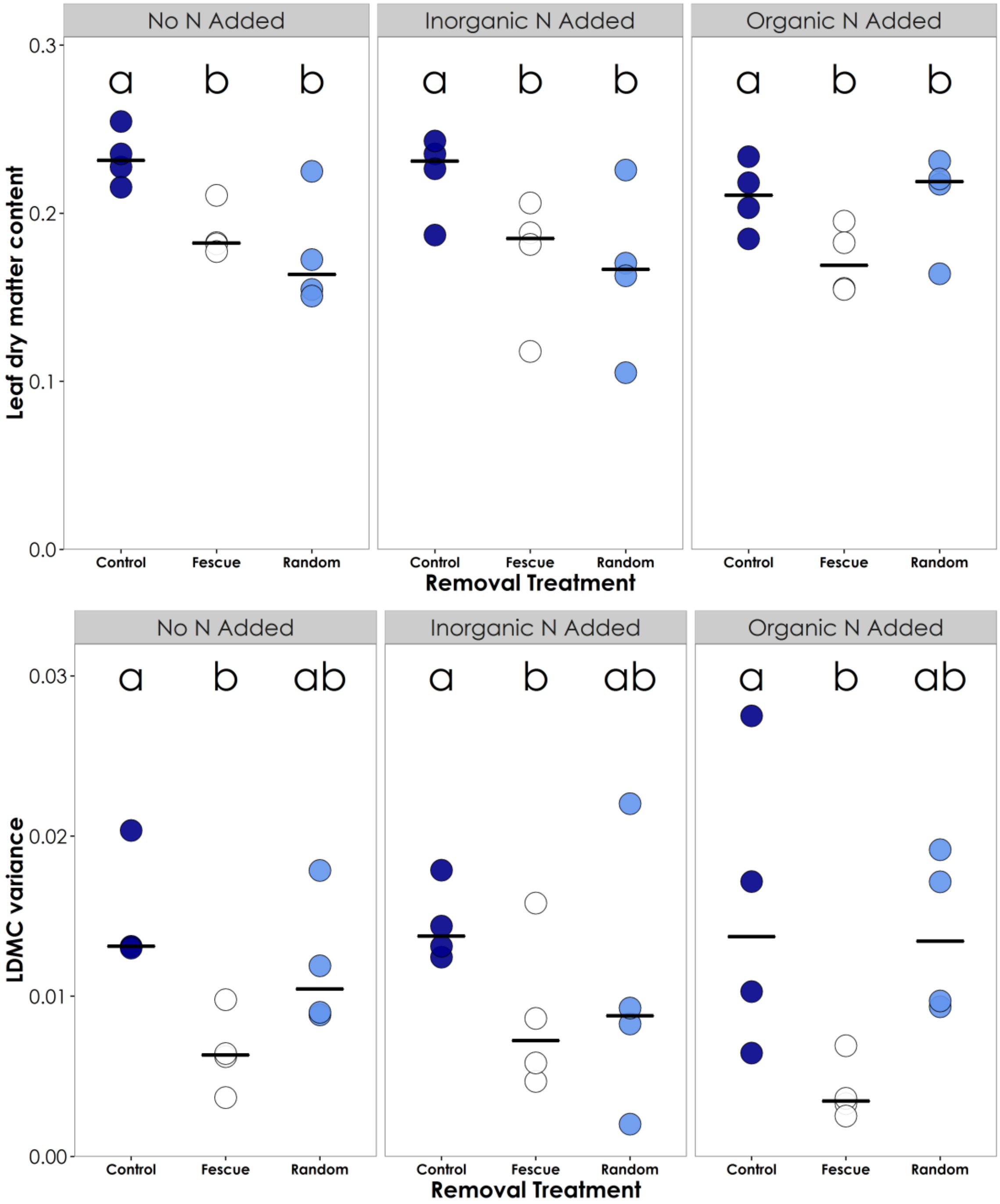

Figure 28. Leaf dry matter content community-weighted means (top panel) and community-weighted variances (bottom panel) across all 9 treatment combinations in July 2015.

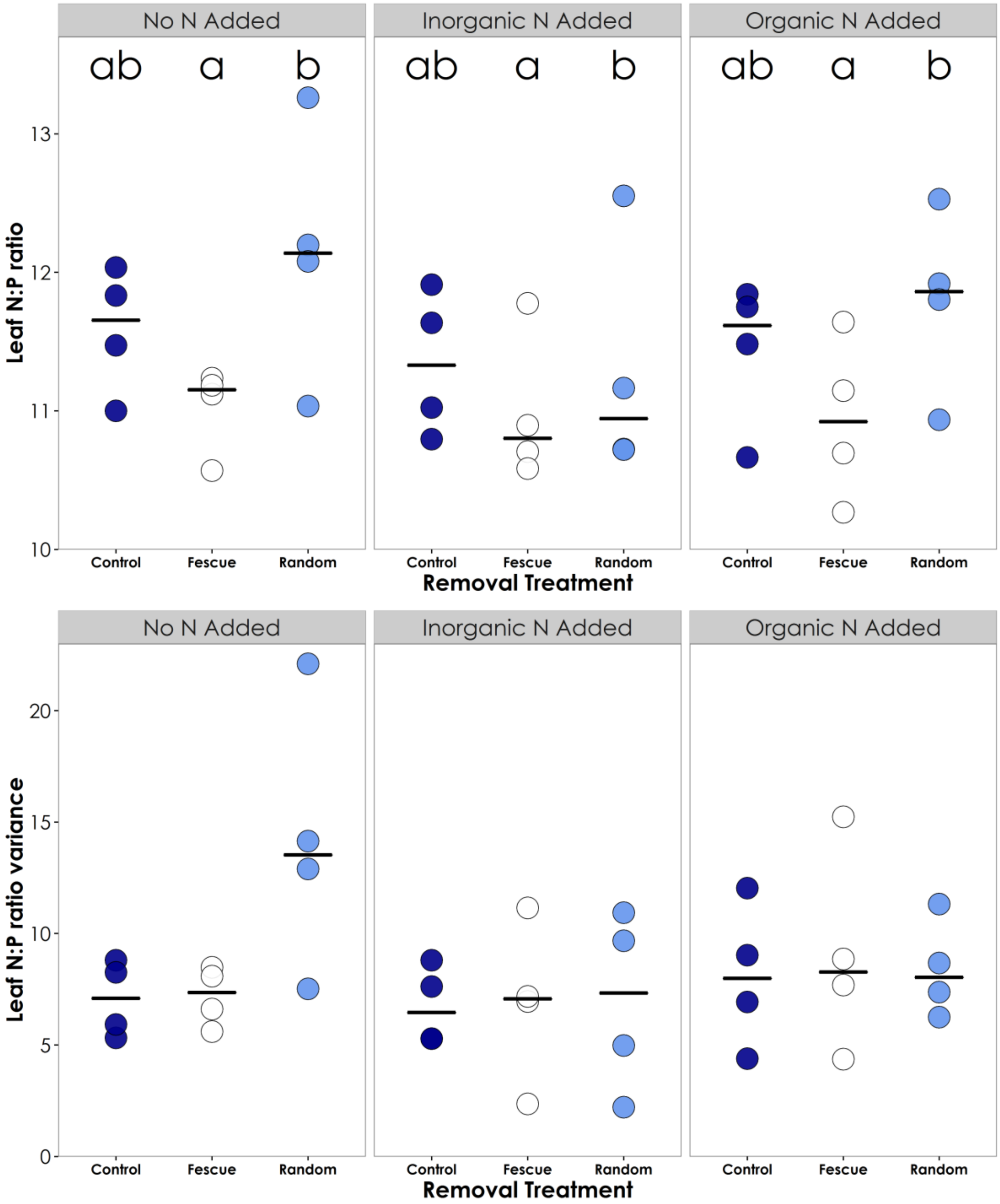

Figure 29. Leaf nitrogen:phosphorus ratio community-weighted means (top panel) and community-weighted variances (bottom panel) across all 9 treatment combinations in July 2015.

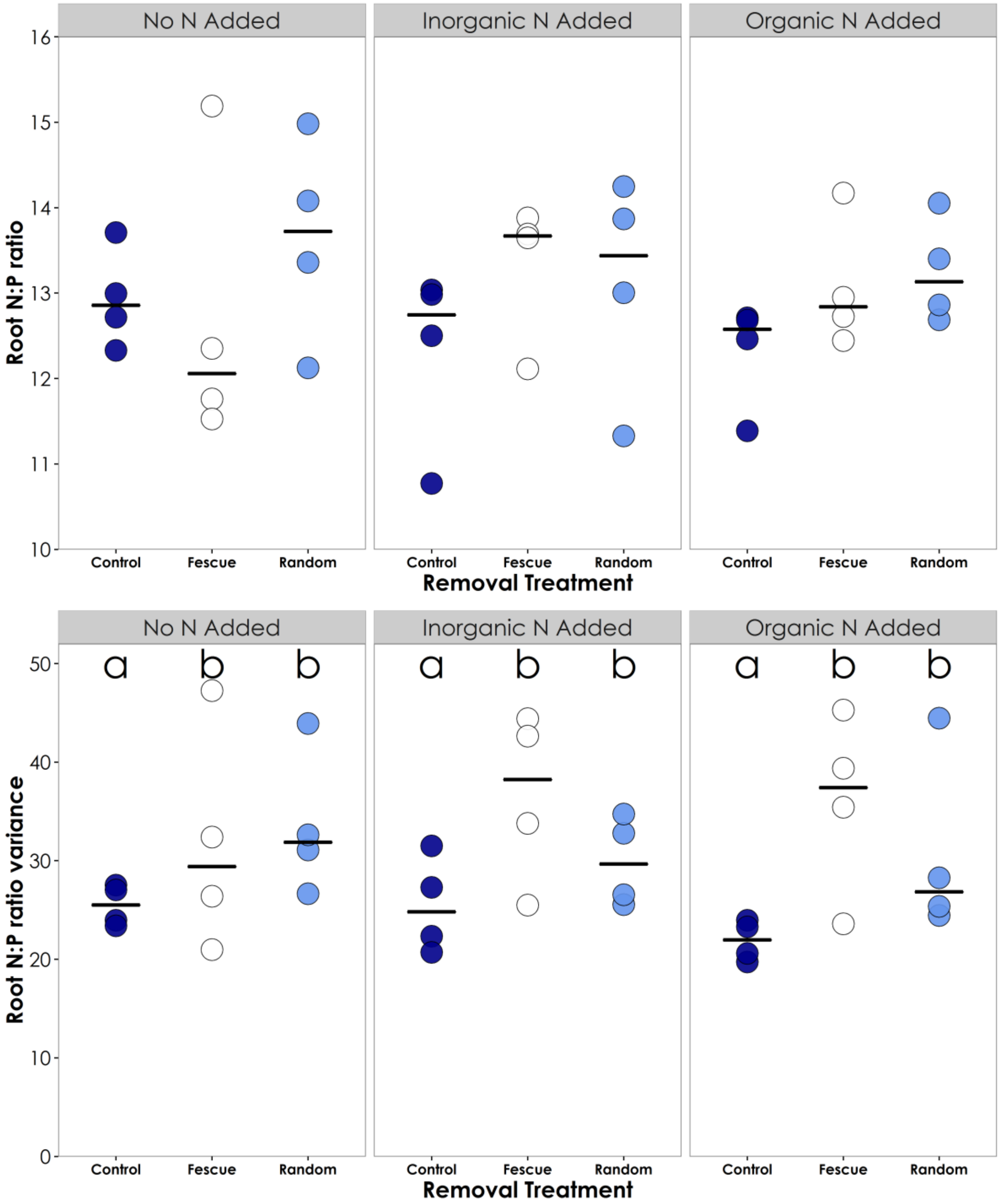

Figure 30. Root nitrogen:phosphorus ratio community-weighted means (top panel) and community-weighted variances (bottom panel) across all 9 treatment combinations in July 2015.

plots and intact control plots, with the single exception of a marginally higher variance in root N:P ratio in the absence of *Festuca*. N addition had no effect on CWM or CWV in either case. I hypothesized that some of this lack of response was due to the fact that the remaining plant community had begun to converge toward the trait value of *Festuca* in the years since removal.

Trait convergence: The community-weighted mean of the principal trait axis in *Festuca* removal plots increased relative to control plots from 2012, just after *Festuca* removal, to 2015, three years after removal (Figure 31; $R^2 = 0.96$; $p = 0.02$). No significant trend in the community-weighted mean of random biomass removal plots was observed (Figure 31). The trait means for the control group were higher than the *Festuca* removal group in all cases, indicating that the community trait value in *Festuca* removal plots was converging on the pre-removal value. Five of the six individual traits also showed this pattern (Figure 32). The R^2 value was greater than 0.87 for these five traits; the convergent trend was significant at $\alpha = 0.05$ for LMA, RML, and LDMC, but only at $\alpha = 0.1$ for height. In contrast, leaf N:P ratio showed no convergent trend over time. The three species that increased most over time in absolute cover with *Festuca* removal were *Bromopsis inermis,* which had the closest root mass:length ratio to *Festuca*, *Heliomeris multiflora*, which had the closest leaf mass:area ratio to *Festuca,* and *Elymus glaucus* Buckley (traits not measured), similar in growth habit to *Bromopsis*. I did not expect any significant trends over time in random biomass removal plots; however, the CWM of leaf dry matter content in random biomass removal plots increased over time relative to the control group ($p = 0.04$).

Carbon storage and turnover

Leaf area index. In July 2015, leaf area index, a reliable proxy for standing aboveground biomass in this study system, did not differ among species removal treatments, nor did species removal treatments interact with fertilization treatments to affect LAI (Figure 33). However, ammonium nitrate fertilization, but not urea fertilization, caused a significant increase in LAI (ΔAICc of fertilization-only model = -5.3; $p = 0.0076$, $R^2 = 0.247$; Figure 33). Averaged across all levels of removal treatment, the least-square mean of leaf area index in unfertilized control plots was 2.38, with 95% CI [1.69, 3.07], compared to 3.75 [3.05, 4.44] with ammonium nitrate fertilization and 2.38 [1.68, 3.07] with urea fertilization.

Soil respiration. Soil carbon efflux increased with the addition of both ammonium nitrate and urea fertilizer, but only in June 2015 shortly after fertilizer was added (Figure 34a); there were no significant differences among treatments in July 2015 (Figure 34b). In June, Soil respiration increased by 68% ($p = 0.005$) with ammonium nitrate addition relative to the control, and by 57% with urea addition relative to the control ($p = 0.02$; $R^2 = 0.369$; Figure 34a). However, soil respiration did not vary with the presence or absence of *Festuca* after four growing seasons of removal, despite a moderate but nonsignificant pulse in the respiration rate shortly after removal in the *Festuca* removal plots but not the random biomass removal plots.

Discussion

After the fourth growing season since treatments were established, the effects of yearly organic and inorganic N addition and loss of the dominant grass species differed dramatically. Both species and functional diversity of the plant community were resistant to change in response to species loss and N addition. The remaining plant community demonstrated resilience to

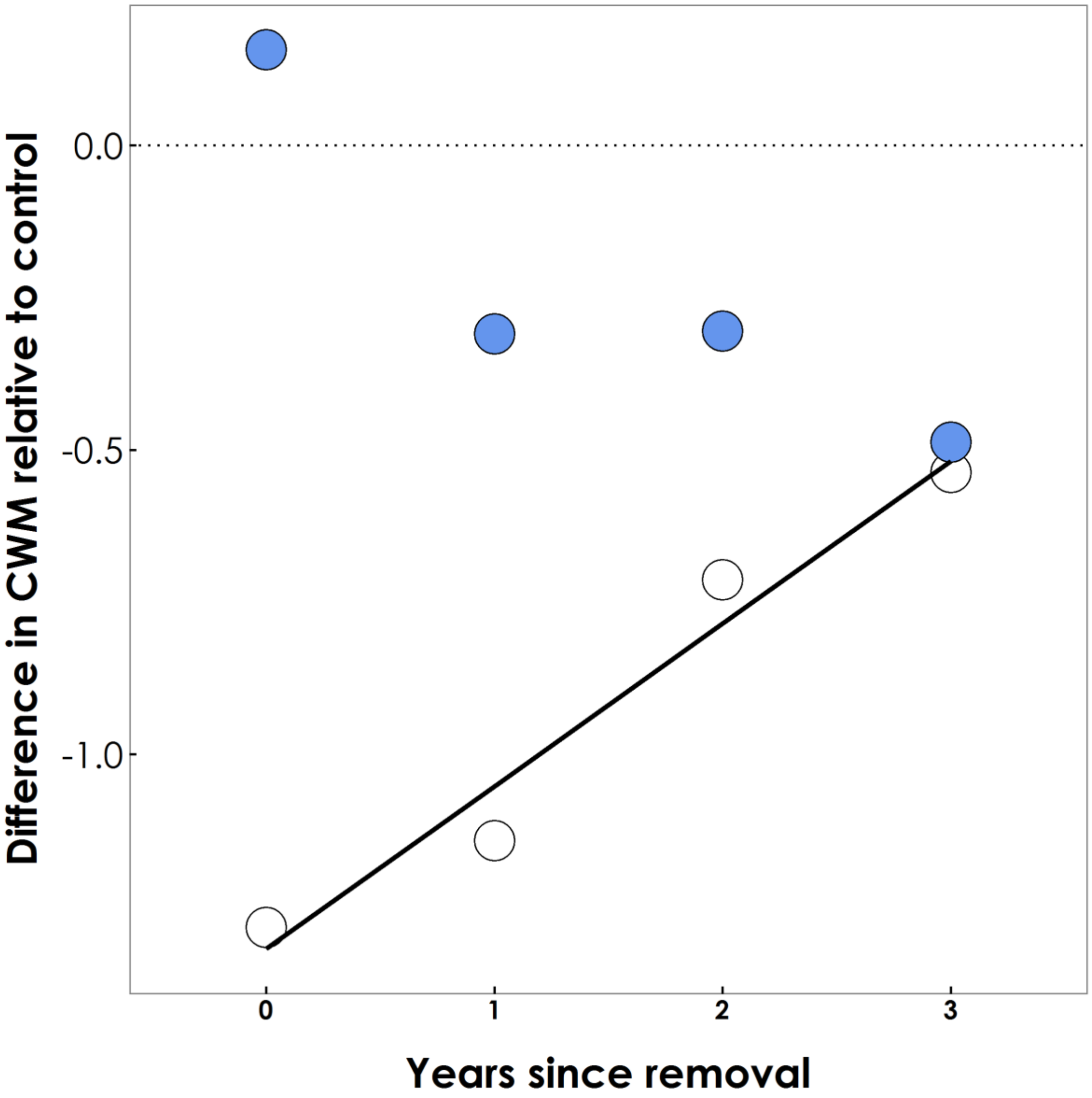

Figure 31. Trait convergence of dominant-species removal plots and control plots since removal. The first principal components axis, encompassing 37% of trait variation, is shown. The difference in median value, relative to the control, of plot-level community-weighted trait mean for the fescue removal (open circles) and random removal treatments (filled circles) is plotted versus time. The trend line shows a significant convergent trend in the fescue removal treatment, in that the difference between these plots and the control plots approached zero over time. The random removal plots show no significant change over time.

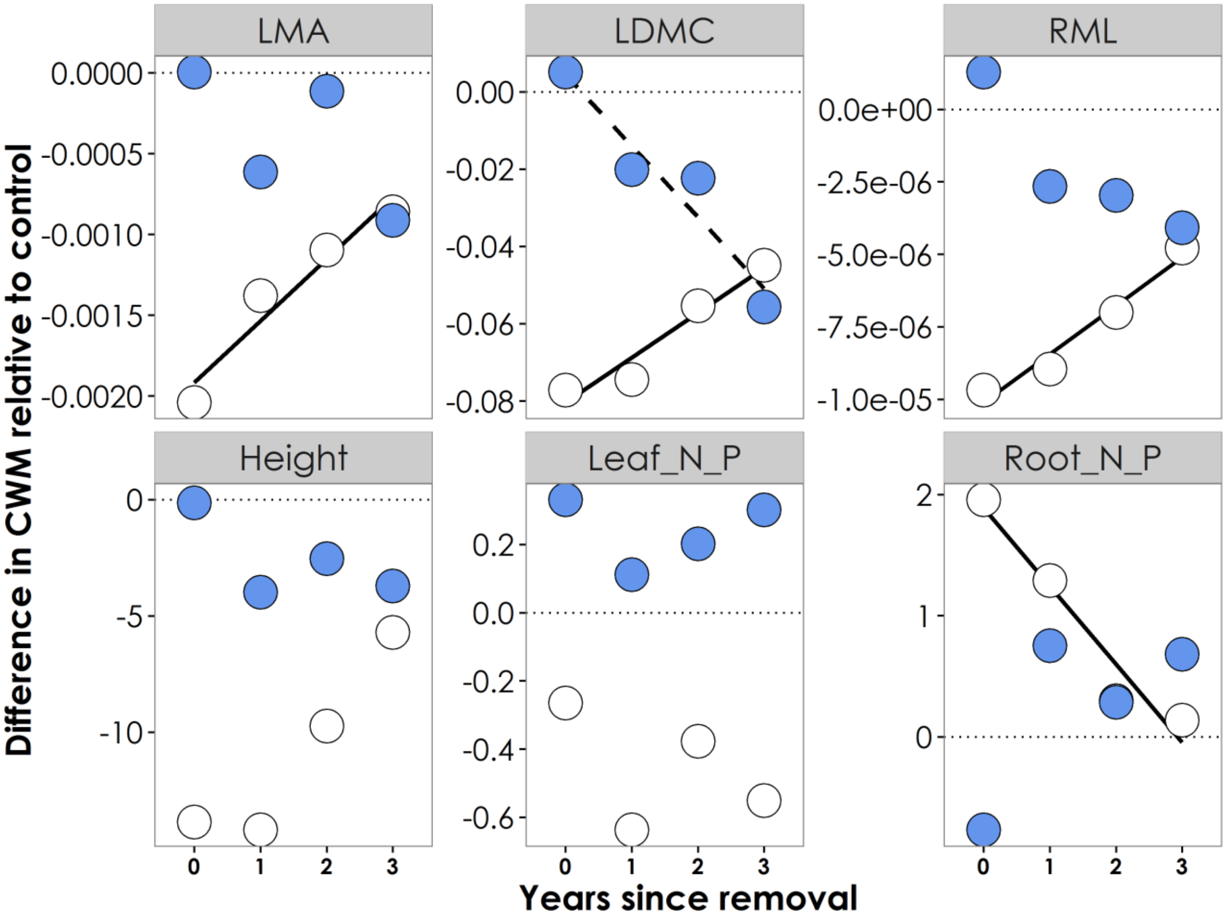

Figure 32. Convergence of each individual trait mean between control plots and dominant-species removal plots since removal. The difference in median value, relative to the control, of plot-level community-weighted trait mean for the fescue removal (open circles) and random removal treatments (filled circles) is plotted versus time. Solid trend lines represent significant changes in the mean of the fescue removal plots relative to the control, and the dashed trend line represents a significant change in the mean of the random biomass removal plots relative to the control. Nonsignificant trends are not shown.

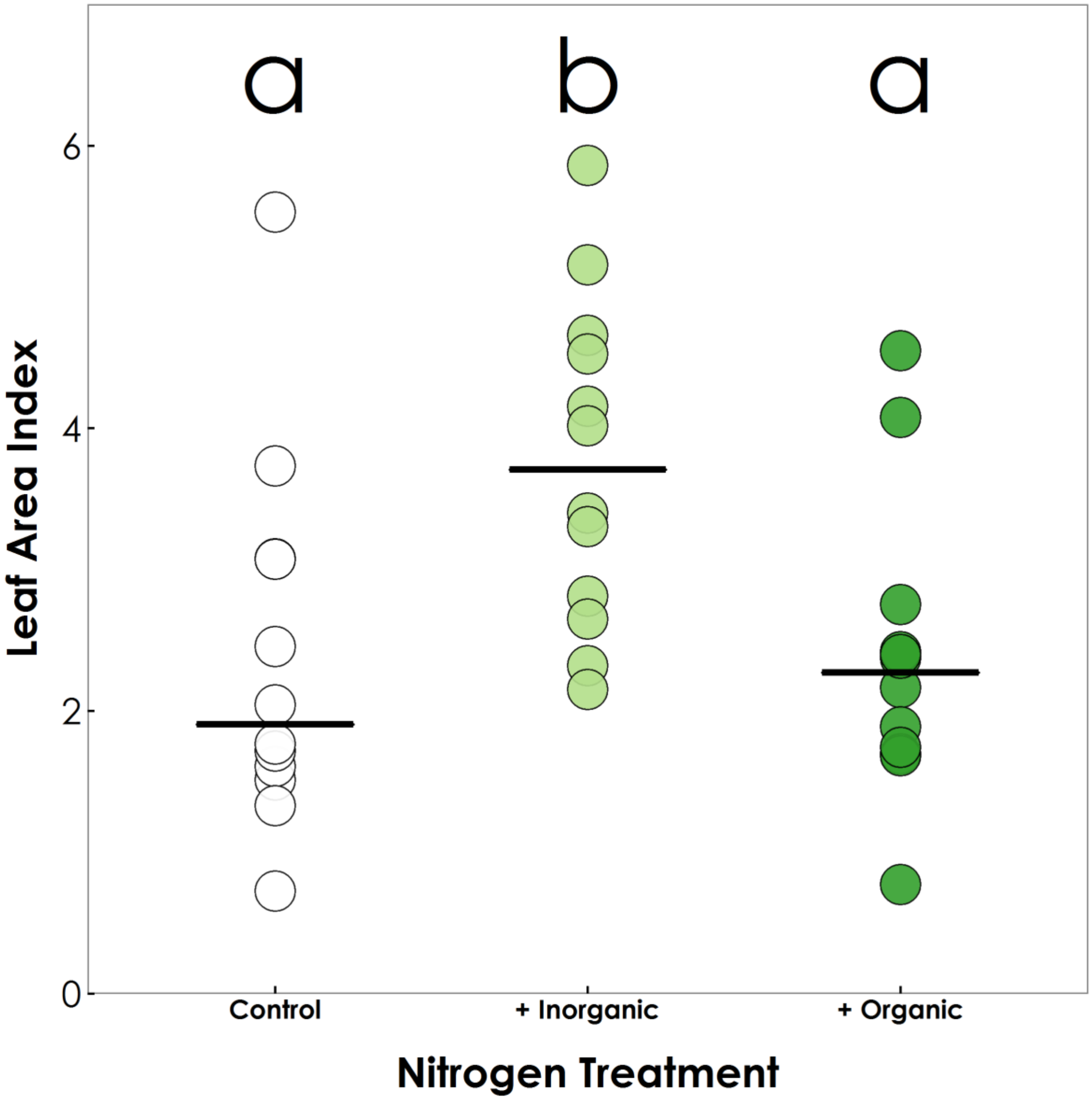

Figure 33. Leaf area index (y-axis) by fertilization treatment (x-axis) in July 2015. Leaf area index is an easily measured variable closely correlated with standing aboveground plant biomass. The unfertilized control is shown in white, inorganic N fertilization in light green, and organic N fertilization in dark green. Each point represents the value from a single experimental plot, and the horizontal bars are the median value from each fertilization treatment group, across all three removal treatment groups. Here, different letters indicate significant differences among fertilization treatments (*post hoc* comparison of least-square means across all levels of removal treatment).

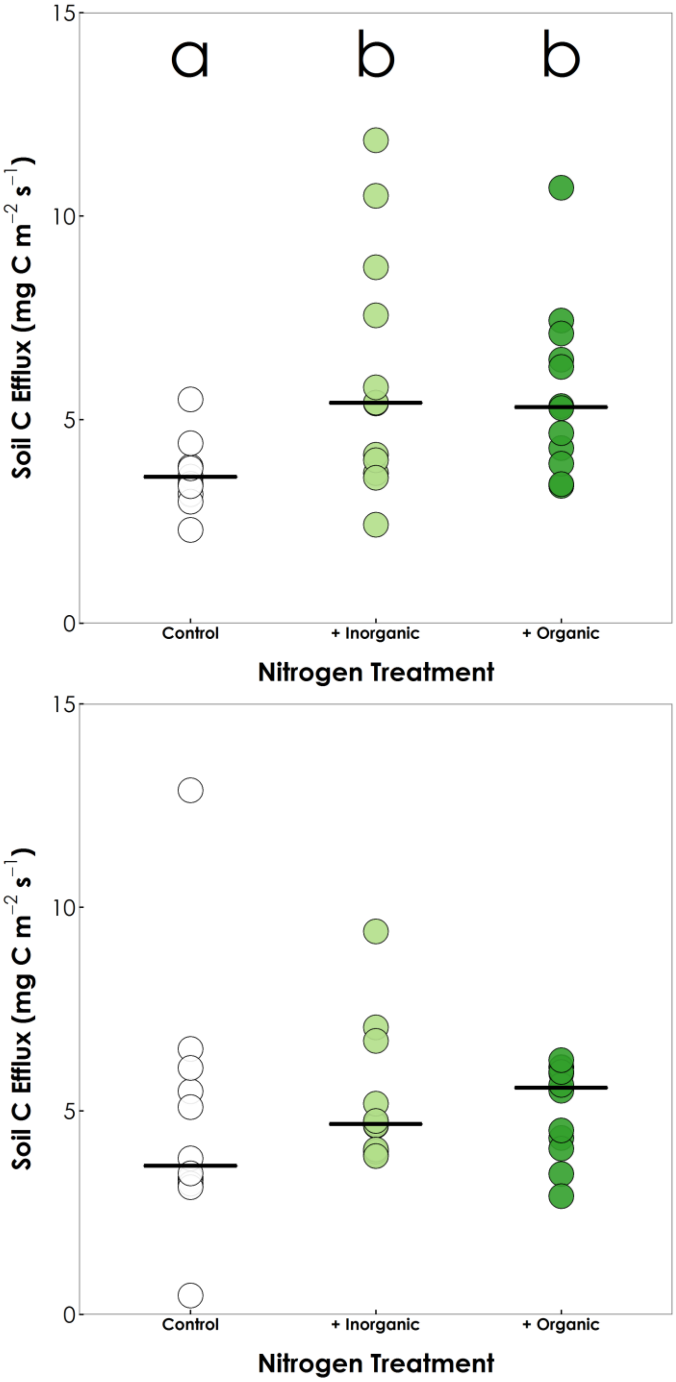

Figure 34. Soil C efflux (y-axis) by fertilization treatment (x-axis) in June 2015 (top panel) and July 2015 (bottom panel). Each point represents the value from a single experimental plot, and the horizontal bars are the median value from each fertilization treatment group, across all three removal treatment groups. Here, different letters indicate significant differences among fertilization treatments.

change—the increase in relative abundance of some species compensated for the loss of *Festuca* by increasing the relative dominance of species with higher LMA, RML, LDMC, and height and lower root N:P ratio, as evidenced by the significant change in the community-weighted mean of these traits over time since the initial removal event in 2012. While nitrogen fertilization did not affect the composition of the plant community, inorganic nitrogen fertilization promoted increases in aboveground biomass, and both forms of nitrogen increased soil respiration during the early part of the growing season.

Changes in plant traits with* Festuca *removal show compensation

Neither community-weighted mean nor variance of any trait differed among treatments in 2015, other than the obvious change resulting from physically removing *Festuca*. The relatively high LMA, RML, leaf N:P ratio, and height of *Festuca* suggests that its high abundance is associated with high investment into structural tissue that is durable and can withstand stress (Wright *et al.* 2004), at the expense of achieving high short-term rates of carbon (C) gain in leaves (Poorter *et al.* 2009) or soil resource uptake in roots (Freschet *et al.* 2010; Bardgett *et al.* 2014). However, plant species with relatively high RML, LDMC, and height, and low root N:P ratio became more abundant in response to removal of *Festuca*, as shown by the increases in community-weighted means for those traits relative to the control. This likely illustrates a compensatory response by those subordinate species whose leaf and root traits were most similar to *Festuca* (McLaren & Turkington 2010). The response I observed suggests that it may be possible to predict which species might compensate for species loss based on trait similarity. The plant community that remained after *Festuca* removal demonstrated resilience by replacing both its aboveground biomass and its functional role (Smith & Knapp 2003; Craine *et al.* 2011).

High leaf mass:area ratio and high root mass:length ratio indicate, for leaves and roots respectively, a tradeoff in favour of stress tolerance and durability over resource acquisition potential (Lind *et al.* 2013). This compensatory response may explain the lack of response in ecosystem process rates related to soil C cycling, as both the biomass and function of *Festuca* were replaced by other species. It is likely that there is a combination of traits that optimizes competitive ability within this plant community; one would expect the most abundant plant species to exhibit traits closest to that optimum (Gilman *et al.* 2010). When that species is lost from the community, the remaining species that are closer to the optimum value will increase in relative abundance. However, full convergence will probably not occur if *Festuca* is prevented from regeneration, since there are no species with such extreme values of LMA and RML in the community. The timeframe of this experiment was short relative to the lifespan of the plants in this system. For example, many *Helianthella* individuals have been censused in this meadow since 1973 and are still living (Inouye 2008). Therefore, this study only simulates the short-term phase of change by vegetative growth, which may not capture the magnitude or direction of long-term change (Sandel *et al.* 2010). Over the long term, recruitment from other locations might cause the mean trait value of the community to return closer to the optimum value.

Nitrogen addition had moderate effects on carbon cycling processes belowground

I found that inorganic N addition, but not organic N addition, led to increased aboveground plant biomass as measured by LAI, indicating that the plant community is at least partially limited by lack of soil N. This pattern is unsurprising given that N fertilization tends to increase shoot:root biomass allocation ratio in natural communities (Li *et al.* 2010). Additionally, rates of soil respiration increased after adding both organic and inorganic forms of N, but this effect dissipated after several weeks. Neither form of N addition had any significant impact on the species composition of the plant community nor on functional trait composition. This striking lack of community response, especially in a region where the rate of anthropogenic N deposition is among the lowest in North America (Schwede & Lear 2014), may arise because there is no significant variation among the plant species in their ability to take up N. Therefore, no individual species was able to benefit disproportionately from N addition.

I had initially hypothesized that organic and inorganic N would have positive effects on different plant species because plant community diversity may be maintained by partitioning of different sources of soil N (McKane *et al.* 2002; Chesson *et al.* 2004). However, the only difference observed in different forms of N fertilization was a greater blanket increase in aboveground biomass when inorganic N was added. One plausible set of explanations is that most plants in this system can take up inorganic N from the soil more readily, and that partitioning of soil N pools is not an important mechanism helping to maintain diversity in this plant community. Another possible explanation for the superior fertilization effect of ammonium nitrate relative to urea is that the urea tends to be rapidly nitrified and leached from the soil as nitrate ions. Therefore, the fertilization effect may primarily be due to ammonium fertilization.

Lack of interaction may be due to legacy effects

The effect of *Festuca* removal did not depend on N addition, or vice versa; that is, there were no interaction effects retained as predictors for any of the variables I measured. Perhaps there has been insufficient time for the loss of *Festuca* to have changed abiotic conditions in the plots or to have changed the microbial community. Results from a related project conducted in Summer 2013 show that both living *Festuca* individuals and belowground legacy effects (Kostenko *et al.* 2012) from dead *Festuca* individuals affect mycorrhizal communities on neighbouring *Helianthella* individuals similarly (Jeremiah Henning, unpublished data). Because the mycorrhizal community associated with a plant's roots is an important determinant of the rate at which that plant can take up soil resources such as N (Read & Pérez-Moreno 2003), the persistence of *Festuca*-associated mycorrhizae may explain the lack of interaction between removal and fertilization. As I plan to continue maintaining the experimental treatments and collecting data over the next several years, we hypothesize that such interactions may manifest themselves once the legacy effects of *Festuca* become more attenuated.

Shifting dominance in time

The study system is dominated by long-lived species that are adapted to deal with fluctuations in temperature, precipitation, herbivory, physical damage, and resource availability both within and among growing seasons. As a result of these fluctuations, grasses including *Festuca* are more dominant in drier years, while asters such as *Helianthella* have much greater cover and live aboveground biomass in wetter years. This has large impacts on year-to-year C storage in the system, because *Festuca* litter is much more recalcitrant and ties up C and N in undecomposed tissue for many years, as confirmed by a decomposition experiment at an adjacent site (Shaw & Harte 2001). However, in the wetter years, light becomes limiting, and the asters like *Helianthella* that are superior competitors for light are able to suppress *Festuca* and prevent it from accumulating biomass. This plant community may maintain stability in the face of among-year changes because the bulk of the biomass shifts between two or more dominant species that achieve relative abundance peaks at different moisture levels (Allan *et al.* 2011; Dangles, Carpio & Woodward 2012). This storage effect arises from functional tradeoffs and may explain the coexistence of multiple dominant plant species within a community (Angert *et al.* 2009). Furthermore, the effects of yearly climate fluctuations are dampened by the large storehouse of biomass and nutrients belowground, especially in tap-rooted forbs such as *Helianthella.* Due to the resilience conferred by this storage effect, it is possible that only repeated extreme events would cause a regime shift (Biggs, Carpenter & Brock 2009) that would significantly change properties such as C cycling.

In addition to temporal scale, spatial scale may obscure the effect of dominant species removal: it is not clear across what distance plant individuals interact with one another in this community. While 2-5 tussocks of *Festuca* were removed from each removal plot, it is possible that only a subset of plants within the 1 m^2 plot were affected by these removals. Aggregating response variables at the plot scale may have obscured any effects. Finally, factors not manipulated here may be stronger drivers of structure and function relative to *Festuca* presence and N levels, namely temperature, snowmelt date, herbivory by cattle and pocket gophers (Cantor & Whitham 1989), and frequent avalanches in winter.

Conclusion

I experimentally manipulated multiple global change drivers, tested ecological niche theory, and united global-change ecology with a trait-based approach. This innovative approach indicated that shifts over time in relative abundance of plant species partly compensated for the loss of a dominant grass species. In addition, I found that chronic addition of soil N only had moderate effects on C cycling processes and that plant community structure was entirely unchanged in response to N addition. These results may indicate that the storage effect is of paramount importance in conferring resilience and maintaining diversity in montane meadow communities. Approaches such as this one, replicated across sites, would greatly improve global vegetation models by enabling them to incorporate the storage effect that confers resilience to plant communities by the rapidly shifting dominance of different plant species from year to year.

Appendices

Table 4. Summary of statistical test results for models fit to each response variable, including coefficients of determination, information criteria, p-values, and least-square means (with 95% confidence intervals given in parentheses).

				p-values			
Response variable	*Best model*	R^2	$\Delta AICc$	*ammonium nitrate*	*urea*	*fescue removal*	*random removal*
Soil carbon efflux (June)	Fertilization + Removal	0.369	6.1	0.0048	0.016	0.989	0.024
Soil carbon efflux (July)	null	—	—	—	—	—	—
Leaf area index	Fertilization	0.247	5.3	0.0076	0.993	—	—
Effective species number	null	—	—	—	—	—	—
Functional dispersion	Removal	0.467	17.73	—	—	7e-6	0.0035
LMA mean	null	—	—	—	—	—	—
RML mean	Removal	0.431	15.34	—	—	0.00002	0.0059
Height mean	Removal	0.187	2.54	—	—	0.022	0.023
LDMC mean	Removal	0.321	9.03	—	—	0.00095	0.0032
Leaf N:P mean	Removal	0.225	4.23	—	—	0.067	0.25
Root N:P mean	null	—	—	—	—	—	—
PCA axis 1 mean	Removal	0.388	12.77	—	—	0.0003	0.0005
LMA variance	null	—	—	—	—	—	—
RML variance	Removal	0.455	16.91	—	—	9e-6	0.023
Height variance	null	—	—	—	—	—	—
LDMC variance	Removal	0.349	10.51	—	—	0.00023	0.17
Leaf N:P variance	null	—	—	—	—	—	—
Root N:P variance	Removal	0.304	8.13	—	—	0.0007	0.017
PCA axis 1 variance	Removal	0.363	11.32	—	—	0.0001	0.095

Table 4. Continued.

	least-square means					
Response variable	*ammonium nitrate*	*urea*	*no fertilization*	*fescue removal*	*random removal*	*no removal*
Soil carbon efflux (June)	6.09 (4.92, 7.26)	5.69 (4.52, 6.86)	3.63 (2.46, 4.80)	—	—	—
Soil carbon efflux (July)	—	—	—	—	—	—
Leaf area index	3.75 (3.05, 4.44)	2.38 (1.68, 3.07)	2.38 (1.69, 3.07)	—	—	—
Effective species number	—	—	—	—	—	—
Functional dispersion	—	—	—	0.177 (0.153, 0.201)	0.214 (0.190, 0.238)	0.267 (0.243, 0.291)
LMA mean	—	—	—	—	—	—
RML mean	—	—	—	1.72e-5 (1.58e-5, 1.86e-5)	1.91e-5 (1.78e-5, 2.05e-5)	2.19e-5 (2.05e-5, 2.33e-5)
Height mean	—	—	—	34.97 (31.27, 38.66)	35.00 (31.31, 38.69)	41.12 (37.42, 44.81)
LDMC mean	—	—	—	0.178 (0.160, 0.195)	0.183 (0.166, 0.201)	0.222 (0.205, 0.240)
Leaf N:P mean	—	—	—	10.985 (10.629, 11.341)	11.743 (11.387, 12.099)	11.454 (11.098, 11.810)
Root N:P mean	—	—	—	—	—	—
PCA axis 1 mean	—	—	—	-0.142 (-0.354, -0.070)	-0.111 (-0.323, 0.101)	0.457 (0.245, 0.669)
LMA variance	—	—	—	—	—	—
RML variance	—	—	—	4.3e-11 (1.5e-11, 7.0e-11)	9.8e-11 (7.0e-11, 1.25e-10)	1.43e-10 (1.16e-10, 1.71e-10)
Height variance	—	—	—	—	—	—
LDMC variance	—	—	—	0.0065 (0.0035, 0.0094)	0.0120 (0.0091, 0.0150)	0.0149 (0.0120, 0.0178)
Leaf N:P variance	—	—	—	—	—	—
Root N:P variance	—	—	—	34.753 (30.702, 38.804)	31.375 (27.324, 35.426)	24.275 (20.224, 28.325)
PCA axis 1 variance	—	—	—	1.535 (0.984, 2.086)	2.527 (1.976, 3.077)	3.185 (2.634, 3.736)

CHAPTER V

DOMINANT SPECIES LOSS AFFECTS CARBON FLUXES IN WARMED MONTANE MEADOWS VIA CHANGES TO PLANT SPECIES TRAITS

Abstract

Global climate change and loss of dominant species threaten many of the world's ecosystems. It is difficult to predict the indirect effects of environmental change that are mediated by changes in the functional trait composition of plant communities. To address this, I crossed experimental warming treatments with removal of dominant plant species. In contrast to many similar experiments conducted at single sites, the experiment was replicated at a high and a low elevation site that differ in their climate and the functional composition of their plant communities. After the third season of warming and species removal, neither warming nor dominant-species loss changed the trait composition of the remaining community. However, because the dominant species differed functionally from the remaining community, the loss of dominant species affected community-level functional trait means and variances and associated ecosystem processes. Warming increased soil carbon efflux only at the low-elevation site, while dominant-species removal increased efflux at the low site and decreased it at the high site. A structural equation model revealed that the functional trait composition of the plant community was the most important determinant of net ecosystem carbon exchange. In addition, there was a positive relationship between functional diversity and net ecosystem exchange, and loss of the dominant species tended to reduce the magnitude of net ecosystem exchange. Taken together, my results demonstrate that the indirect effects of climate change and species loss may be both considerable and context-dependent.

Introduction

Global climate change and loss of dominant species threaten many of the world's ecosystems, putting mountain ecosystems especially at risk (IPCC 2014). While the direct effects of human activity on the global climate system and nutrient cycles are well known, the indirect effects of climate change, through effects on plant communities and traits, can have a larger impact on ecosystem carbon (C) dynamics than the direct effects of climate (McMahon *et al.* 2011; Niu *et al.* 2013). Mountains are an ideal testbed for disentangling the direct and indirect effects of climate change and species loss on communities and ecosystems for three key reasons. First, mountain regions harbor high levels of biodiversity: temperature and other environmental factors vary widely with elevation over small spatial scales such that plant communities, plant trait compositions, and dominant plant species also differ greatly (Spehn & Körner 2005). Second, montane ecosystems may be under especially severe threat from global warming (Engler *et al.* 2011; Gottfried *et al.* 2012). Third, spatial patterns along temperature gradients are similar to patterns observed with historical climate change through time (Blois *et al.* 2013; Elmendorf *et al.* 2015). For these three reasons, mountains can act as powerful study systems to help us understand longer-term, larger-scale, community and ecosystem responses to environmental changes, especially when coupled with experimental manipulations (Fukami & Wardle 2005; Sundqvist *et al.* 2013).

Because dominant plant species determine ecosystem properties, small relative changes in their abundance can have dramatic absolute effects on ecosystem function (Ellison *et al.* 2005; Gaston and Fuller 2008), often greater in magnitude than the vaunted relationship between biodiversity and ecosystem function (Winfree *et al.* 2015). In different environments, different traits confer competitive dominance on different species (Hillebrand *et al.* 2008; Gilman *et al.*

2010). If the dominant species is lost from a system, the consequence of that loss for community structure and ecosystem function depends on the traits of the lost species, the traits of the remaining species, and the baseline environmental conditions at the site (Emery & Gross 2007). While many hypotheses about the outcome of species loss from plant communities have been tested (Díaz *et al.* 2003; Selmants *et al.* 2012), few studies have tested how the effects of species loss vary among sites that differ in their environmental conditions and in the traits of the dominant species (Wardle & Zackrisson 2005). Fewer studies still have tested how these effects may be mediated by temperature (Alexander *et al.* 2015), although we expect climate warming to disrupt, or at least modify, interactions among species and the functions they mediate (Tylianakis *et al.* 2008; Michalet *et al.* 2014). To address this gap in our knowledge, I have experimentally increased temperature and removed the dominant plant species at a low- and high-elevation site. In particular, I asked three related questions:

- How does simulated anthropogenic warming alter the species composition, functional trait composition, community-level thermal affinity, and carbon fluxes of montane meadow ecosystems?
- How does the loss of dominant plant species affect the species composition and functional composition of the remaining community, and its associated carbon fluxes? Is this effect mediated by warming?
- Are the magnitudes and directions of warming and dominant-species effects contingent on the baseline compositions and environmental conditions of meadow communities in warm low-elevation sites and cold high-elevation sites?

I predicted that increased temperatures would have a greater effect on carbon flux at the high-elevation site, causing both increased biomass and faster rates of carbon uptake, because of the preexisting temperature limitation at that site and the strong filtering over time for species that can respond rapidly to take advantage of short-lived warm temperatures and pulses of resource availability. I also predicted that warmed temperatures would cause an increase in community thermal index of the plant community at both sites due to increases in relative abundance of species with warmer thermal affinities. Finally, I predicted that the loss of dominant plant species would have a greater effect on the species composition and carbon fluxes at the low-elevation site, where competition for resources may be more intense, but that the loss of the dominant species would be relatively unimportant for the remaining species at high elevation.

Methods

The experiment described here consists of a replicated warming × dominant-species removal treatment at both high and low elevations. This approach enabled me to determine whether the relative strengths of abiotic climate change and biotic species loss for determining community structure and function show the same pattern in both warm and cold environments.

Site description

The two sites at which I established the replicated warming × removal treatment are in the Gunnison Valley near Gothic, Colorado, USA. The low-elevation site, in the Almont Triangle land parcel just northeast of Almont, Colorado, is 2740 m above sea level in sagebrush steppe habitat. At the low-elevation site the most abundant species is *Wyethia amplexicaulis*, a perennial aster with large leaves and wide rosettes. The high-elevation site is on Cinnamon Mountain at 3460 m above sea level. *Juncus drummondii*, a perennial rush that grows in dense clumps intermixed with large amounts of standing dead biomass, dominates the high-elevation site. Both sites were selected to be relatively flat or on a shallow slope with a west-facing aspect to minimize confounding variation in topography and hydrology.

Treatments

The experiment is a 2 × 2 factorial design with two levels of warming (warmed and ambient) crossed with two levels of species removal (dominant species present and dominant species removed). There are 8 replicates of each treatment combination at each site, yielding 32 plots at each site for a total of 64 plots.

Warming treatment: The warming treatment is achieved using an open-top warming chamber (OTC). The design of the OTCs is based on the chambers used by the International Tundra Experiment specifications (Molau & Molgaard 1996), with slightly adjusted dimensions. Each chamber consists of six translucent trapezoidal fiberglass panels of approximately 1 mm thickness attached to one another with cable ties. Together the panels form a hexagonal cone with sloping sides, about 50 cm high in profile, with a basal diameter of roughly 170 cm and an opening at the top of roughly 100 cm diameter. All plot-level response variables are recorded within a circle of 140 cm diameter at the center of the plot, which leaves a roughly 15 cm buffer between the measured area and the chamber base. The OTCs for warmed plots are installed in early spring and kept in the field for the duration of the growing season; I put them in the field in early June (later for the high-elevation site) and disassembled them in late September as plants senesced.

To ensure that the warming treatment was effective, I placed iButton temperature loggers (Maxim Integrated Corp., San Jose, CA, USA) on pin flags 5 cm above the soil surface at the center of each plot. In order to prevent the temperature loggers from receiving direct sunlight, I made packets out of a layer of Mylar film around a layer of window screening material and sealed the loggers inside. Temperatures were recorded at 30 minute intervals. A subset of the air temperature loggers also had the capacity to record relative humidity at 30 minute intervals (n = 4 per treatment combination per site). In addition, I buried temperature loggers 5 cm below the soil surface sealed inside plastic vials in a subset of plots (n = 2 per treatment combination per site). Soil temperatures were recorded at 60 minute intervals to minimize the need to disturb soil by digging up the loggers to download temperature values.

The OTCs warmed the air temperature of experimental plots 5 cm above the soil at the low-elevation site by 1.7° C, and at the high-elevation site by 1.8°C (Figure 35). The magnitude of daytime warming, between the hours of 6:00 and 21:00, was greater than nighttime warming (2.2° C versus 0.8° C at the low-elevation site, and 2.4° C versus 0.7° C at the high-elevation site). Soil temperature 5 cm below the surface was also warmed effectively by the OTCs (1.8° C at low elevation and 1.2° C at high elevation; Figure 36); this warming was greater during the day than at night (2.6° C versus 0.5° C at low elevation, and 1.3° C versus 1.0° C at high elevation). In addition to warming effects, it has been commonly noted that OTCs tend to alter the relative humidity as an artifact of the warming treatment. However, I did not find any such effect. I fit a linear mixed-effects model to the relative humidity data with site and warming treatment as fixed effects, and plot and measurement time as random effects. After controlling

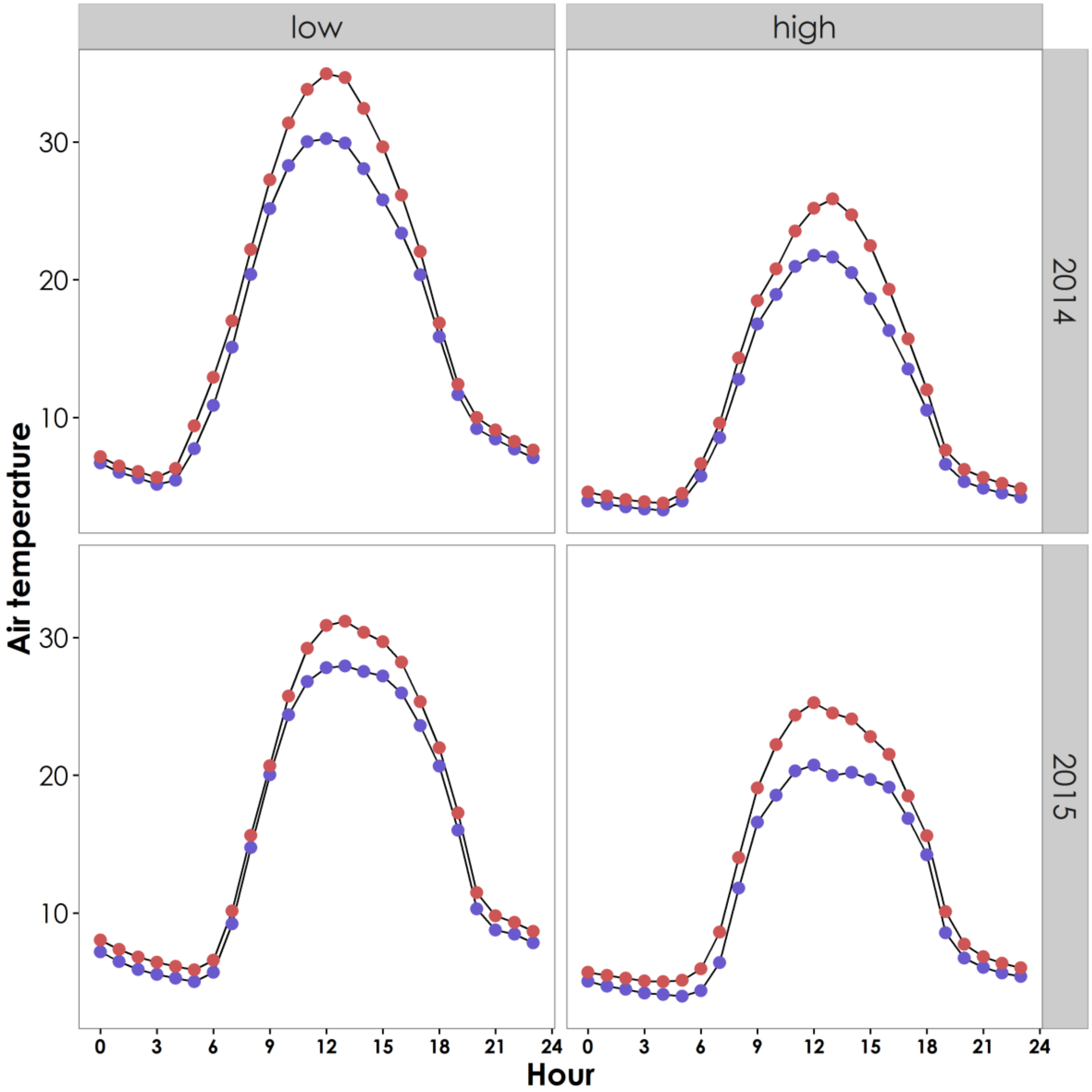

Figure 35. Air temperature 5 cm above ground at low and high elevation sites in 2014 and 2015, averaged by hour. For each hour, red points represent the mean of warmed plots at each site in each year, and blue points represent the mean of ambient plots at each site in each year.

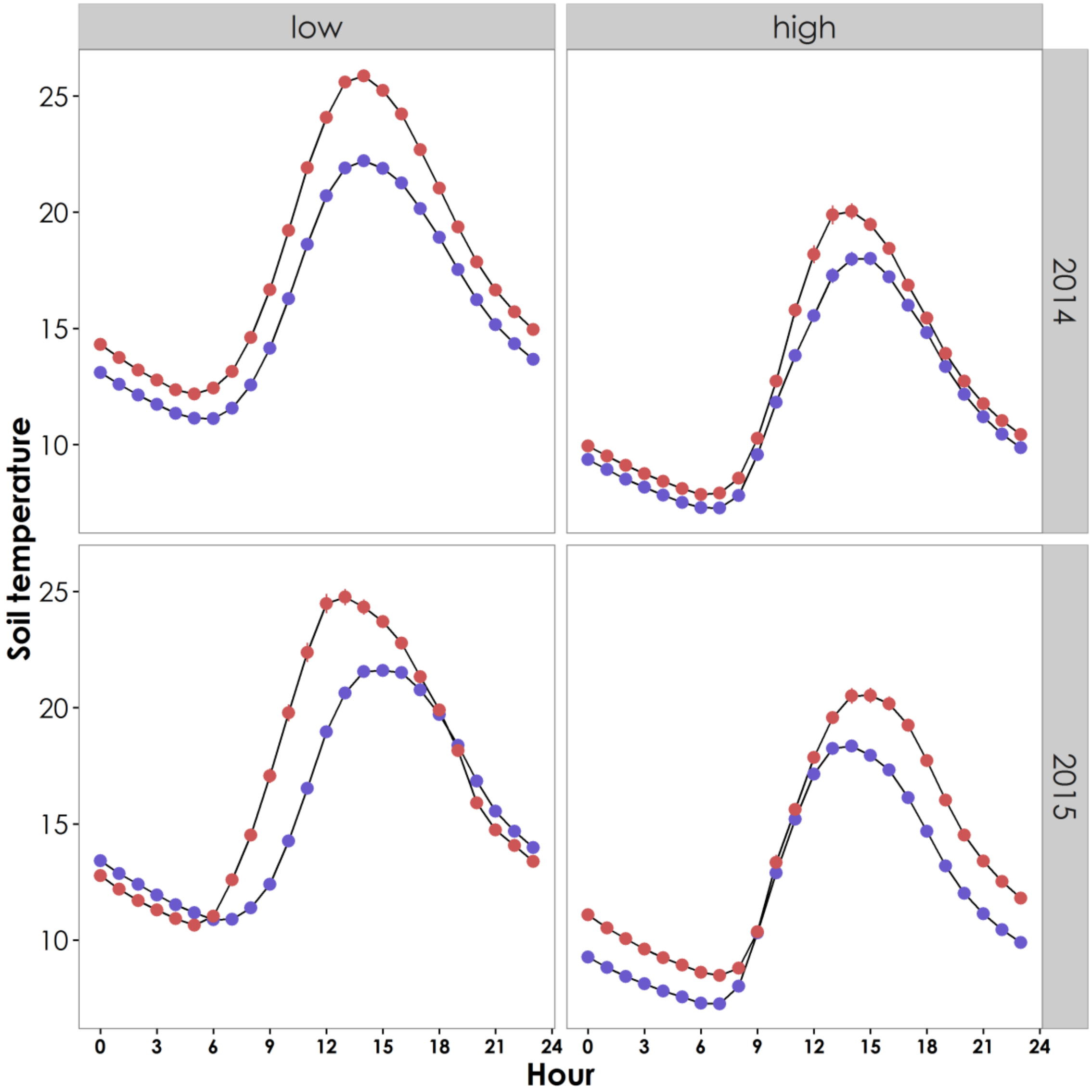

Figure 36. Soil temperature 5 cm above ground at low and high elevation sites in 2014 and 2015, averaged by hour. For each hour, red points represent the mean of warmed plots at each site in each year, and blue points represent the mean of ambient plots at each site in each year.

for plot and measurement time, I found no significant difference in relative humidity between plots with and without OTCs at each site.

Removal treatment: Crossed with the warming treatment, I established a dominant-species removal treatment. I removed dominant species over the entire 4 m^2. Dominant species is defined here as the species that maintains the highest abundance over the longest time during the growing season, averaged over an entire site. This contrasts with other definitions of dominant species that explicitly refer to competitive interactions (Clements 1936). The individual plants were removed by first clipping all aboveground biomass at the base. The biomass was saved and weighed. Next, if necessary, I painted a small amount of diluted glyphosate herbicide onto the clipped stem bases to kill belowground biomass. All removed biomass was dried and weighed. I removed any regenerating stems of the dominant species twice during each growing season. In contrast to the removal experiment in the previous chapter, I did not implement a random biomass removal treatment.

Across all removal events from 2013-2015, the total amount of dry aboveground biomass removed at the low-elevation site was 3.5 times greater than the amount removed at the high-elevation site (unpaired t-test; $p = 5 \times 10^{-5}$). Therefore, there is the possibility of confounding the removal-by-site interaction effect with an effect of the amount of aboveground biomass removed. In addition, the removal treatment caused approximately a 10% decrease in aboveground plant cover in July 2015 as documented in the plant community measurements (ANOVA; $p = 0.036$).

Species composition and functional composition

To measure plant community composition, I estimated plant cover percentages as a proxy for abundance. Cover was estimated visually to the nearest 1% if ≤10%, and to the nearest 5% if >10%. I recorded plant community composition twice during each growing season.

I measured functional traits during the growing seasons of 2014 and 2015. I collected leaf tissue from 10-20 individuals of each of the most common species at each site, from individuals that were not within the experimental plots but were within 20 m of at least one experimental plot. I measured heights of all individuals from which I collected leaf tissue, but I collected root cores from only a subset (5-10 individuals). I collected 20 leaf tissue samples and 10 root tissue samples from the most abundant species, and 10 leaf and 5 root samples from less abundant species. In total, I collected trait data for the species that make up at least 70% of the plant cover in all plots at peak biomass, and at least 80% of the plant cover in 95% of the plots.

For each individual, I collected at least three mature and fully expanded leaves. I transported the leaves on moist paper towels and scanned them on an Epson flatbed scanner. I weighed the scanned leaf material, dried it for 48 hours at 60° C, and weighed it again. I saved the dried leaf material and pooled it with additional leaves that were collected for chemical analysis.

For each individual, I also took two to four 3-cm diameter, 15-cm long soil cores from directly below the plant's base. I extracted as much fine root material as possible from the soil cores, washing the soil over a 2-mm sieve if necessary. I scanned 10 to 20 intact pieces of fine root from each individual on an Epson flatbed scanner after floating the root pieces in a transparent tray of water. I dried the root material for 48 hours at 60° C and weighed it. I saved the dried root material and pooled it with additional collected roots for chemical analysis.

I measured the area of the scanned leaf images, and the total length of the scanned root images, with ImageJ software (Schneider *et al.* 2012), using the IJRhizo macro (Pierret *et al.* 2013) to automatically trace all the roots in each image. I calculated LMA for each individual plant by summing the mass and area of each individual dividing the dry mass by the scanned area. LDMC was calculated as the dry leaf mass divided by the leaf mass before drying. I calculated RML for each individual plant by dividing dry root mass by the total root length.

Carbon fluxes

I measured soil respiration twice per growing season at each plot using a LI-6400 gas analyzer (Li-Cor Corp., Lincoln, NE, USA). Soil respiration measurements entailed measuring the net CO_2 efflux from the soil surface within the area of a PVC collar 10 cm in diameter permanently installed at the same location as the moisture rods. Additionally, I measured leaf area index at each plot using an Apogee MQ-200 PAR sensor (Apogee Instruments, Logan, UT, USA). I followed the same measurement protocols given in Chapter III, above.

In addition, I measured net ecosystem carbon exchange (NEE) at all the experimental plots during July 2015. To do so, I temporarily removed the OTC if necessary. Next, I placed a LI-7500 gas analyzer (Li-Cor Corp., Lincoln, NE, USA) on a tripod in the center of the plot, with the analyzer approximately 75 cm off the ground. The gas analyzer measures infrared absorption and converts it to CO_2 concentration. I placed a cubical chamber made of translucent greenhouse plastic and PVC pipe measuring 1.73 m^3, or 1.2 m on a side, over the plot. I measured CO_2 drawdown for 120 seconds, keeping air circulating inside the cube with small fans. I repeated this procedure with four light levels: (1) translucent plastic only, allowing approximately 80% of ambient light to penetrate; (2) a single layer of shade cloth, allowing approximately 50% light penetration; (3) two layers of shade cloth, allowing 20% light penetration; and (4) a black tarpaulin which effectively blocked all incoming light. I measured photosynthetically active radiation inside the chamber with an Apogee MQ-200 PAR sensor after each carbon flux measurement. Between successive measurements, I removed the chamber from the plot and allowed the air inside the cube to mix well. The data processing procedure is described below.

Data processing and analysis

All analyses were conducted in R 3.2.3 (R Core Team 2015), with packages listed where appropriate. I excluded the dominant species at each site (*Wyethia* and *Juncus*) from the diversity metric calculation because they were physically removed from some of the plots, and I was interested in assessing the diversity of the remaining community. However, for the functional diversity, community-weighted trait mean, and community-weighted trait variance calculations, I calculated the values for each plot both with and without the dominant species, to disentangle the contribution to community-level properties by the removal itself from the contribution by the response of the remaining species. Although there is a relatively large amount of turnover in plant species between the early growing season (early June) and the peak growing season (mid-July), I based all the following analyses on peak-season measurements (July); this follows standard practice. Finally, all analyses are based on data collected in 2015, except for the community thermal index calculations over time.

Calculation of diversity metrics. As in the previous chapter, I calculated species diversity metrics for each treatment combination (Jost 2007). I converted Shannon's alpha diversity (H') to first-order effective species number (d_1) using the formula $d_1 = e^{H'}$. For a particular community, the effective species number is the number of species in a community of maximum evenness that would be required to attain an equivalent value of Shannon's diversity. I excluded the dominant species from the calculations of effective species number.

I also calculated functional diversity by taking the mean trait value for all species in each plot for which I have trait data (I have trait data for 25 species across both sites, comprising a median of 87% of the plant cover in each plot, and for over 50% in all plots). For each of these species, I used the trait values of LMA, RML, LDMC, and plant height. I calculated the pairwise Gower distance between each pair of species and used these distances to estimate an index of functional dispersion (FDisp) for each plot. For this calculation, I used the *gowdis* and *fdisp* functions in the *FD* package in R (Laliberté *et al.* 2014).

Community-weighted trait values. I calculated community-weighted means (CWM) and community-weighted variances (CWV) for LMA, RML, LDMC, and plant height for each of the 64 experimental plots. The calculations are identical to those described in the previous chapter. Community-weighted means are calculated by taking the mean trait value for the 10 species for which I have trait data and calculating a mean trait value for each of the 36 plots weighted by the relative cover of each plant species; variance is calculated in a similar way. After calculating community-weighted trait means for each plot, I ran a principal components analysis on the matrix of trait means (64 plots by 4 trait means) to determine whether there was a single axis of trait variation that explained most of the variation among plots, and whether that variation was correlated with the treatments. This analysis, done using the *prcomp* function in R, revealed that approximately 43% of the variation among plots was explained by a single axis which was associated with increased LMA, LDMC, and height, and decreased RML.

Calculation of community thermal index. I calculated an abundance-weighted thermal index for each of the experimental plots. To do so, I followed the procedure of Stuart-Smith *et al.* (2015). I downloaded global species range data for all available species across all experimental plots from the Global Biodiversity Information Facility database (http://www.gbif.org). Adequate records were available for most of the species; I had data for species comprising more than 80% of total aboveground cover in all but 3 of the 64 plots. I took the spatial location of each species occurrence and extracted the mean annual temperature for that site from the Worldclim database (Hijmans *et al.* 2005). I used the mean of the annual temperatures across all of the coordinates where a species occurred globally as its thermal index. For each experimental plot, I calculated the community-weighted thermal index (CTI) by taking the mean thermal index weighted by the relative aboveground cover of each species. This calculation was repeated for the community at peak biomass in 2013, 2014, and 2015.

Processing of ecosystem carbon exchange data. I visually examined plots of CO_2 concentration versus time and removed poor-quality data points, i.e., those recorded when air in the chamber was not mixing properly. After this quality control procedure, I calculated the slope of concentration versus time for each of the four light levels for each plot. I then fit a linear regression to these four points and used the parameters of this regression to calculate NEE, as well as to partition NEE into two components: gross primary productivity (GPP) and ecosystem respiration (R_e). Both NEE and GPP were estimated at a standardized photosynthetically active radiation level of 800 μmol m^{-2} s^{-1}. Ecosystem respiration is defined as the net flux at 0 μmol m^{-2}

s^{-1}, while gross primary productivity is defined as GPP = NEE – R_e; all fluxes are presented in mg C m^{-2} s^{-1}.

Statistical analysis. I fit linear models (ANOVAs) to each response variable separately, using study site, warming treatment, removal treatment, and their interactions as binary categorical variables. I examined plots of the residuals of the models to visually check that this parametric analysis was appropriate (Ghasemi & Zahediasl 2012). I used the *dredge* function in the *MuMIn* package in R (Bartoń 2016) to compare all possible submodels of the full model, and selected the model with the lowest AICc score as most parsimonious. Where necessary, I calculated partial R^2 values for individual predictors by taking the difference in R^2 values between the models with and without that predictor.

In addition to these models that analyzed each response variable separately, I also fit a structural equation model (SEM) to examine the interactions among the experimental treatments, community-weighted trait means, functional diversity as measured by FDisp, aboveground plant biomass as measured by LAI, and net ecosystem carbon exchange. I fit an initial model based on my hypotheses about interactions among these variables, then used the Bayesian Information Criterion to remove parameters that contributed to model overfitting. By sequentially removing parameters, I found the most parsimonious model, then estimated the standardized path coefficients from that model as well as the percentages of variation explained for each response variable by all of the exogenous and endogenous variables. I fit the SEMs using the *lavaan* package in R (Rosseel 2012).

Results

Species composition and functional composition

I found no significant effect of treatments on species diversity at either site. The effective species number was approximately 1 lower in high-elevation, warmed, intact-community plots relative to other treatment combinations, but this predictor was not retained in the most parsimonious model (Figure 37). In addition, there was no effect of warming or dominant species on functional diversity, although the FDisp values were 31% higher at the low-elevation site ($p = 1 \times 10^{-12}$; Figure 38).

Removal of the dominant species caused significant changes to all trait values in both communities. However, these changes can be largely attributed to the difference in trait values between the dominant species and the remainder of the communities. For example, removal of *Wyethia* caused the CWM of leaf mass:area ratio to be 18% lower at the low-elevation site, but removal of *Juncus* caused the CWM to be 18% higher at the high-elevation site (Figure 39). The first principal components axis, which takes the CWMs of all four traits at the plot level into account, decreased significantly in warmed plots with the dominant species present, but increased in warmed plots without dominant species at both sites ($p = 0.01$; Figure 40). This interaction effect indicates that warming tends to decrease LMA and plant height but increase RML of intact communities, but when the dominant species is absent, the opposite traits are favored.

Figure 37. Effective species number (exponential transformation of Shannon's diversity) across all treatment combinations. Higher values represent higher richness and evenness in the plant community; effective species number, analogous to effective population size, is defined as the richness of an ideal community with evenness 1 that would have the same Shannon's diversity as the experimental community. All data presented in this and in the following figures were collected during the peak growing season in the third year since treatments were established (July 2015). In all figures, the filled circles are plot-level values from intact plots, and the empty circles are plot-level values from removal plots. Blue circles are ambient plots, and red points are warmed plots. Results from the low-elevation and high-elevation sites are shown side-by-side. The median value across the eight plots within a particular treatment combination at each site is represented with a horizontal bar. Median values within the same removal treatment across warming treatments are connected with a line to highlight any interactions between the treatments. The dominant species at each site was not included in these calculations.

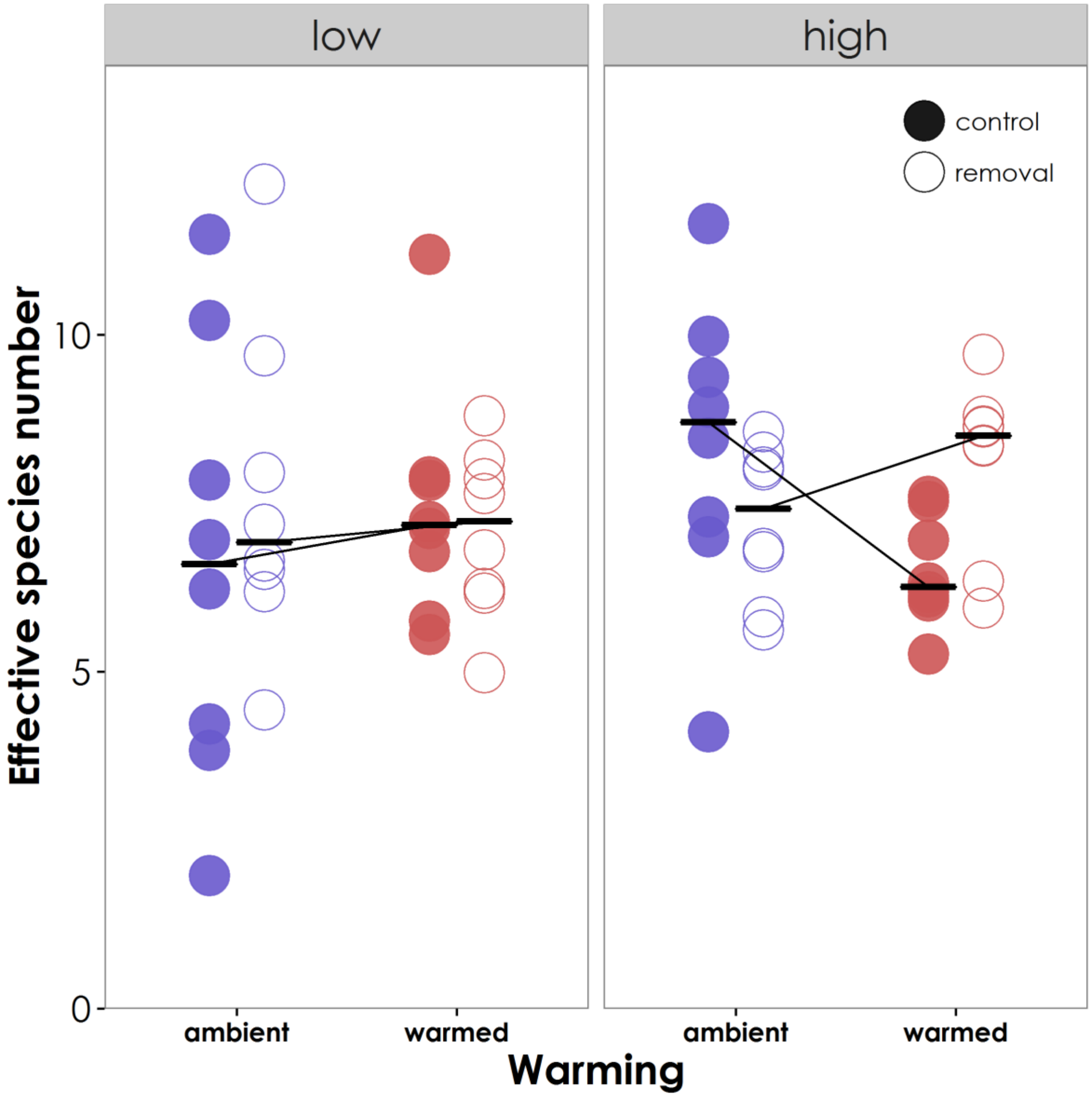

Figure 37. Continued.

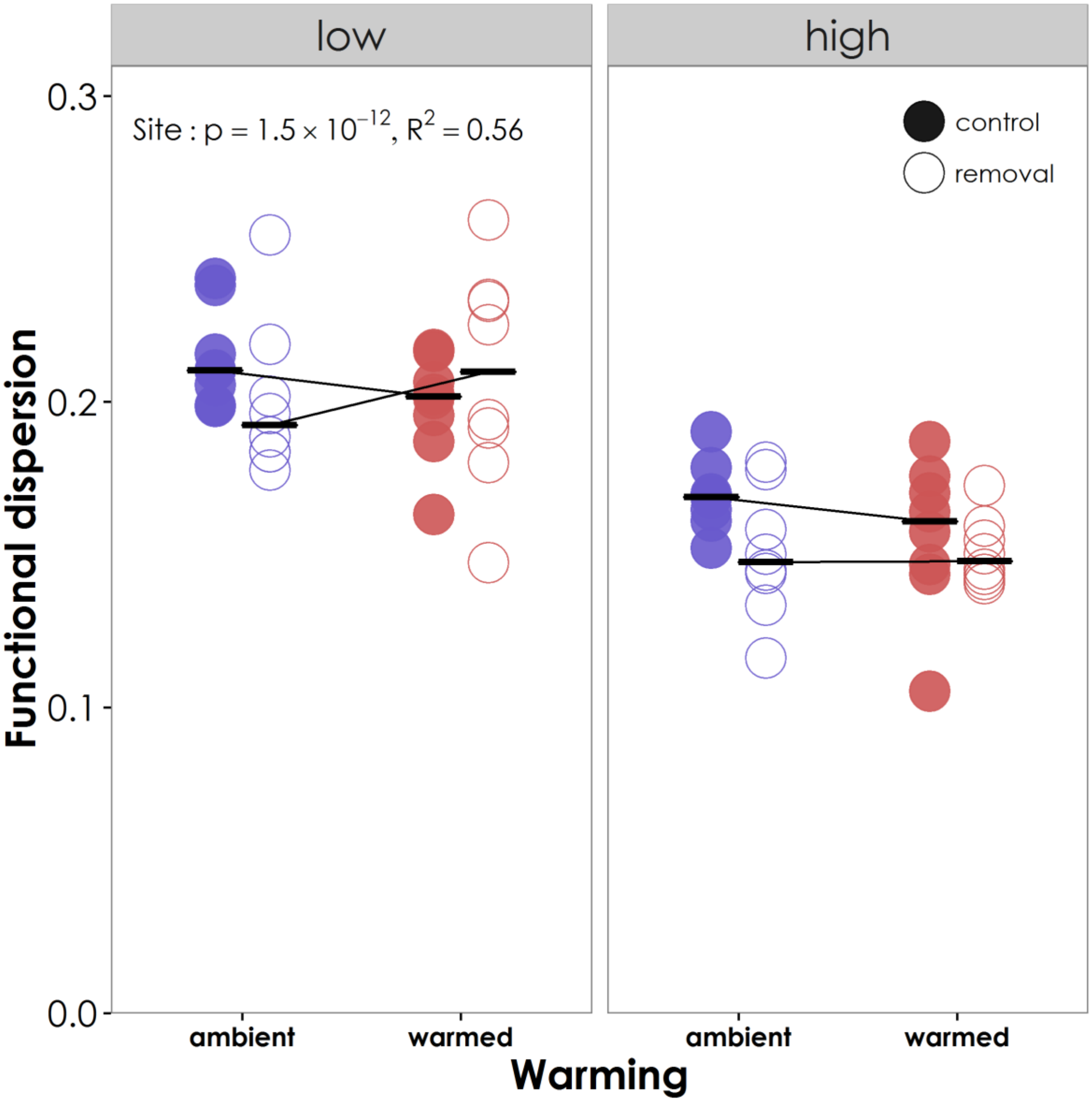

Figure 38. Functional diversity across all treatment combinations, calculated using an abundance-weighted trait distance metric. Higher values indicate higher dispersion in trait space among the individuals in the plant community in a particular plot. See Figure 37 legend. The dominant species at each site was included in these calculations.

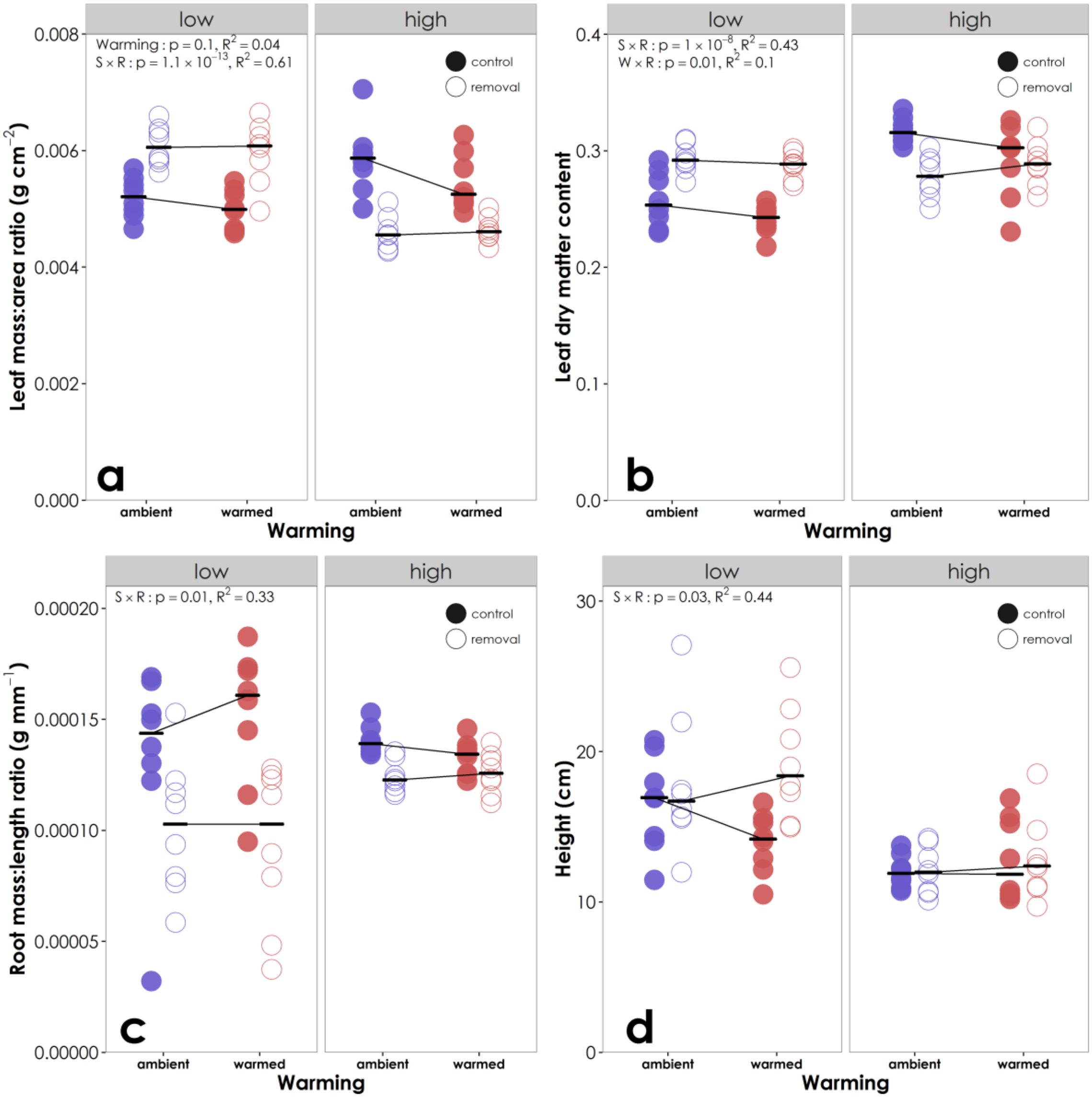

Figure 39. Plot-level community-weighted mean values for leaf mass:area ratio (a), leaf dry matter content (b), root mass:length ratio (c), and plant height (d) across all treatment combinations at both sites in July 2015. Any differences among treatments account for both the removal of the dominant species and the response of the subordinate species. See Figure 37 legend.

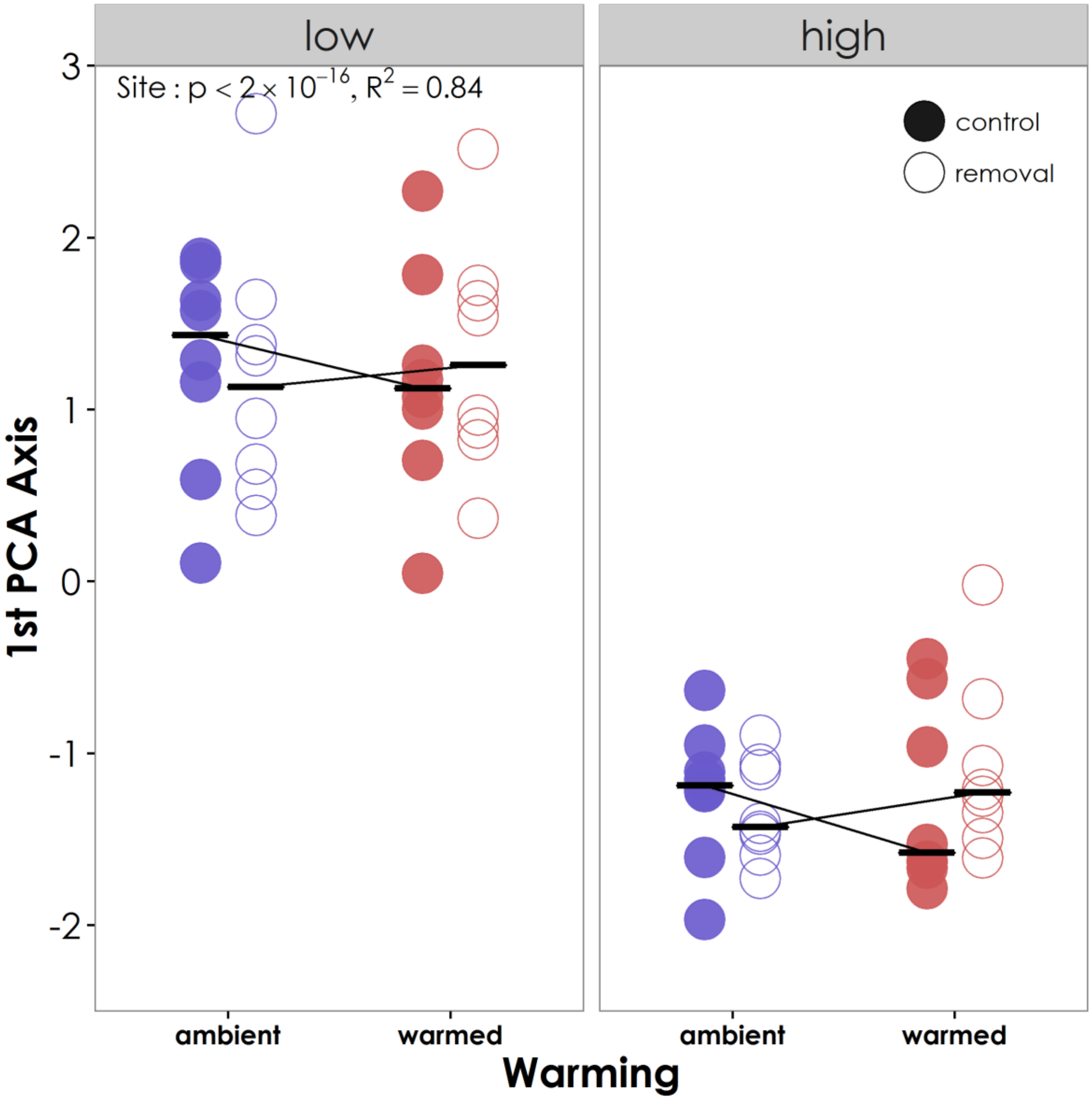

Figure 40. The first principal components axis of the plot-level community-weighted trait means (including LMA, RML, LDMC, and height) for all treatment combinations at both sites, including the dominant species at each site. The axis explains 43% of the variation across the four traits. See Figure 37 legend.

The patterns in community-weighted means were typically not driven by responses of the subordinate community, as I showed by recalculating the means considering only the subordinate community (Figure 41). Neither mean LMA, mean height, mean LDMC, nor the mean value of the first principal components axis of the subordinate species responded to warming or removal. Removal was retained as a predictor in the model for RML ($p = 0.13$), indicating a non-significant 8% decrease in RML when the dominant species is absent.

Community-weighted variances (CWV) of traits were higher for LMA, RML, and plant height at the low-elevation site compared to the high elevation site ($R^2 > 0.5$ in all cases; Figure 42), but variance was higher at the high-elevation site for LDMC ($R^2 = 0.71$). When including the dominant species in calculations, removal had significant effects on trait variance (Figure 42). However, since no significant effects among treatment groups were observed when the dominant species was excluded from calculations (Figure 43), it appears that the subordinate community is not increasing or decreasing in trait variance in response to dominant species loss. This parallels the lack of treatment response in functional dispersion values.

Community thermal index did not differ across treatment combinations, despite the hypothesis that increased temperatures would cause increases in the relative abundance of warm-adapted species, increasing CTI (Figure 44). However, CTI has changed from year to year at each site, regardless of treatment (Figure 45). In particular, CTI decreased by almost 1° C from 2013 to 2015 at the high-elevation site, while remaining relatively unchanged at the low-elevation site ($p = 2 \times 10^{-5}$).

Carbon fluxes

Warming caused leaf area index at both sites to be higher relative to ambient plots ($p = 0.02$; Figure 46), indicating that aboveground plant biomass was also greater (for confirmation of this relationship, see Chapter IV). While leaf area index was 24% higher in warmed plots at the low-elevation site, it was 227% higher in warmed plots at the high-elevation site. Plots without dominant species had 41% lower LAI at low elevation and 34% lower LAI at high elevation ($p = 0.0002$), indicating that subordinate species have yet to fully compensate for the biomass lost from the system.

Soil carbon efflux was 23% lower in warmed plots at the low-elevation site, but was unaffected by warming treatment at the high-elevation site (site × warming $p = 0.005$; Figure 47). In addition, the removal of the dominant plant was associated with efflux that was higher by about 1 μmol C m^{-2} s^{-1} within each warming treatment at the low-elevation site, but lower by the same amount at the high-elevation site (site × removal $p = 0.01$; Figure 47).

Net ecosystem exchange was significantly closer to zero when the dominant species was absent ($p = 0.04$; Figure 48), decreasing by 1.9 mg C m^{-2} s^{-1} at the low-elevation site, and by 0.3 mg m^{-2} s^{-1} at the high-elevation site. Warmed plots did not differ in net ecosystem exchange from ambient-temperature plots, nor was there an effect of warming on the flux components gross primary production (Figure 49) and ecosystem respiration (Figure 50). Both component fluxes were lower in the absence of dominant species at both sites. In addition, ecosystem respiration was lower in warmed plots at both sites ($p = 0.10$; Figure 50).

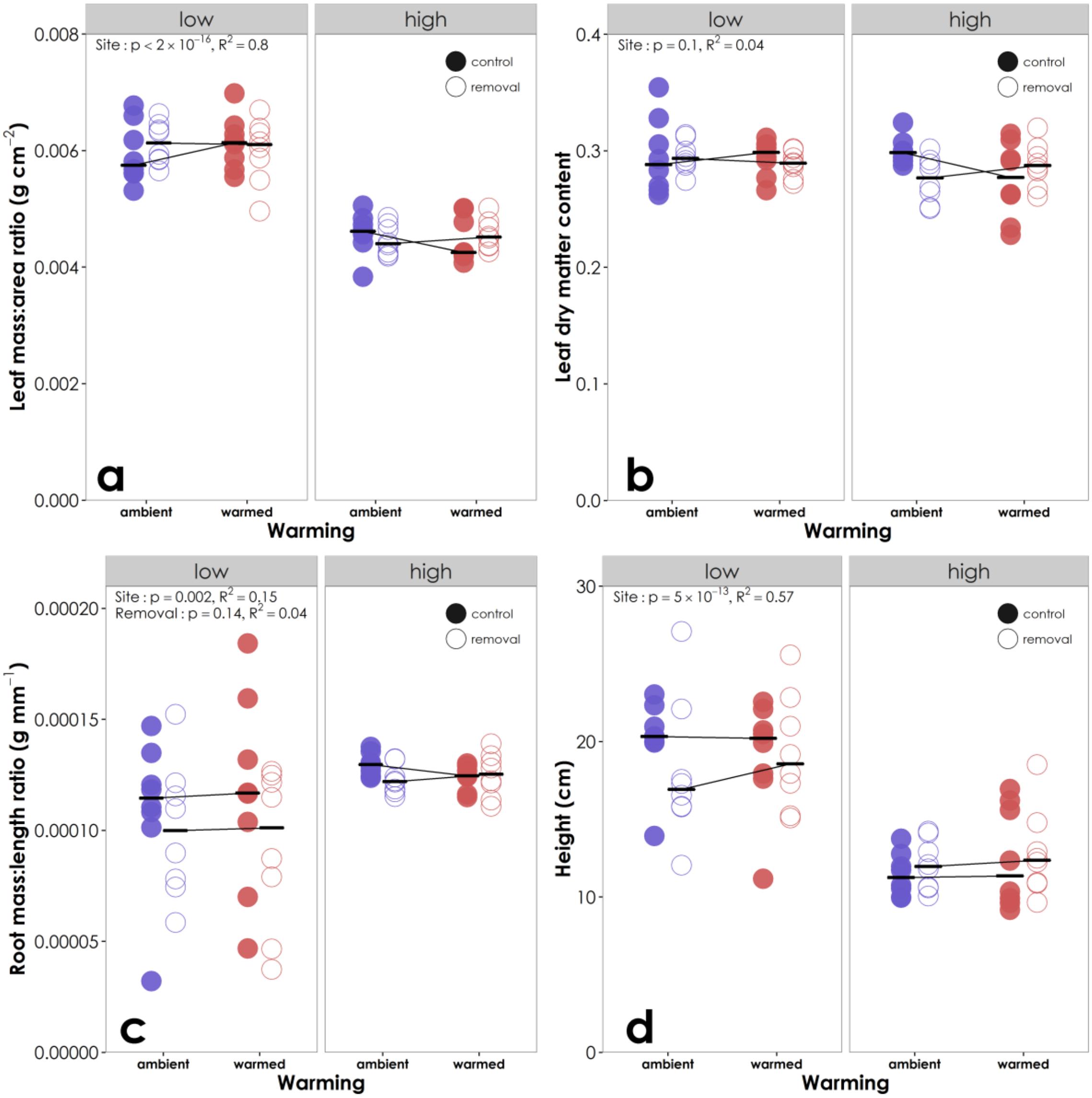

Figure 41. Differences in subordinate-community trait means for leaf mass:area ratio (a), leaf dry matter content (b), root mass:length ratio (c), and plant height (d) across all treatment combinations. In contrast to the previous figures, these trait means only account for the response of the remaining plant community after removal of the dominant species; the dominant species was excluded from these trait mean calculations. See Figure 37 legend.

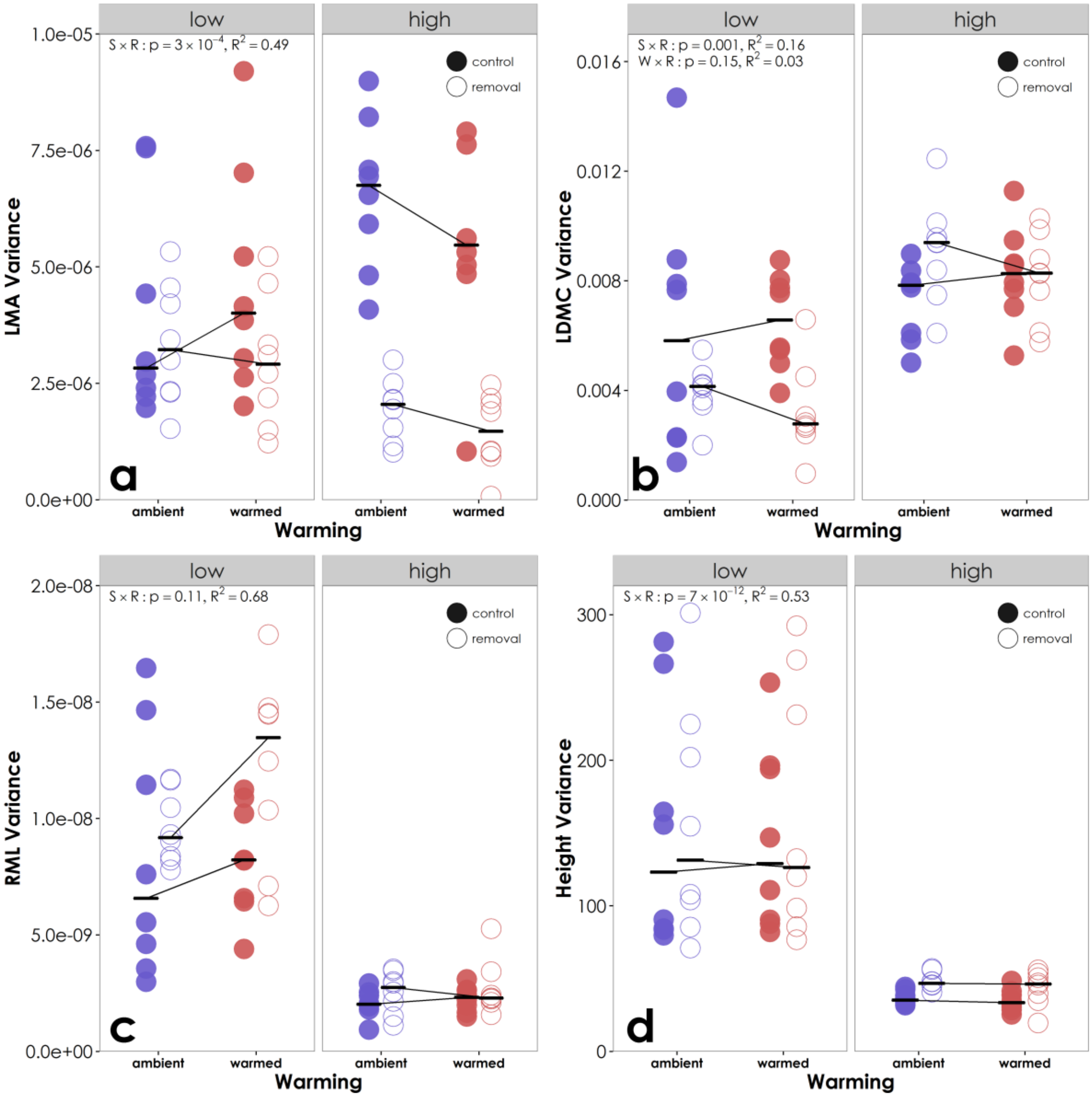

Figure 42. Plot-level community-weighted variance values for leaf mass:area ratio (a), leaf dry matter content (b), root mass:length ratio (c), and plant height (d) across all treatment combinations at both sites in July 2015. Any differences in variance among treatments account for both the removal of the dominant species and the response of the subordinate species. See Figure 37 legend.

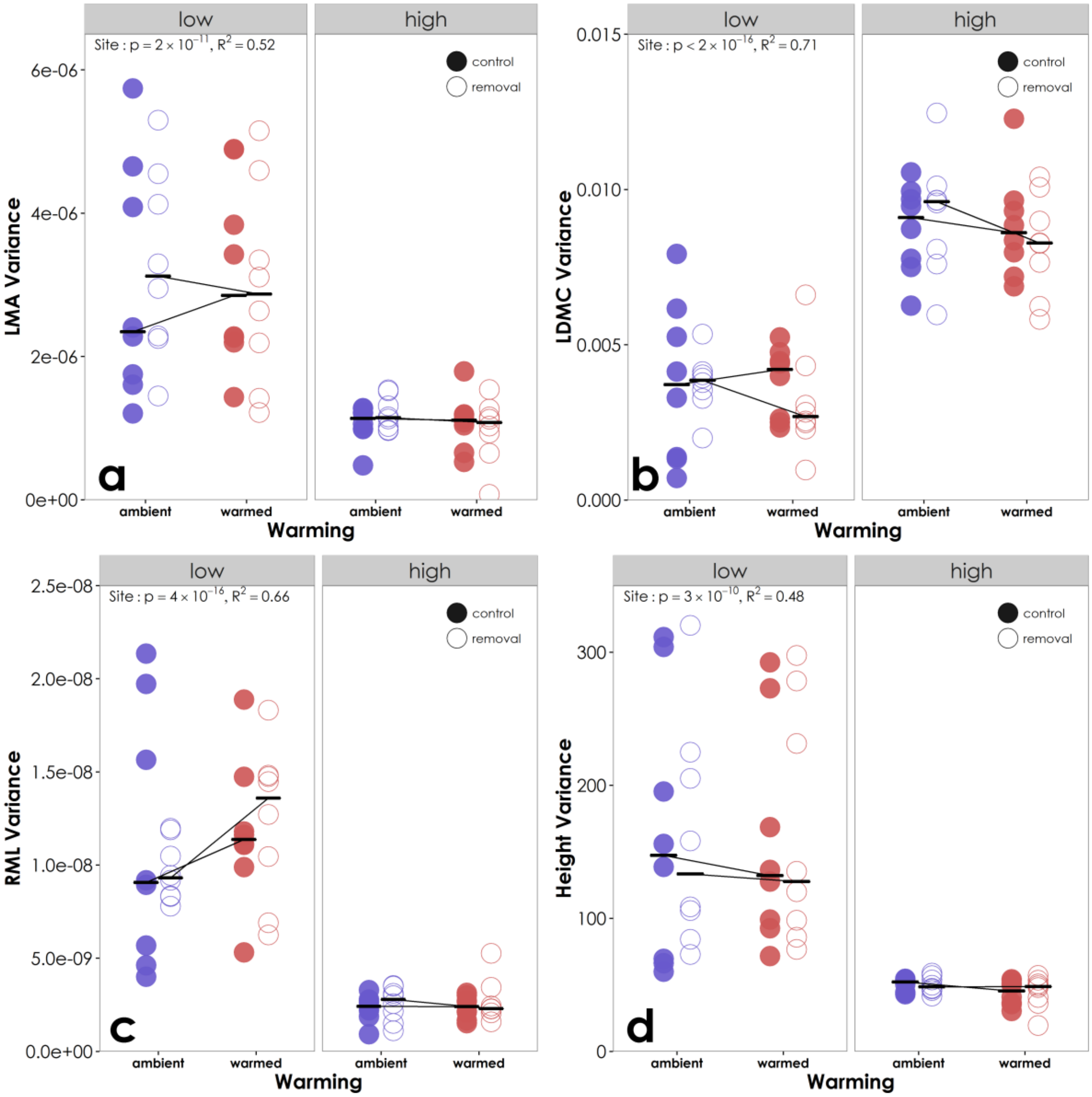

Figure 43. Plot-level subordinate community-weighted variance values for leaf mass:area ratio (a), leaf dry matter content (b), root mass:length ratio (c), and plant height (d) across all treatment combinations at both sites in July 2015. Any differences among treatments only account for the response of the remaining community when the dominant species is removed; the dominant species was not included in these variance calculations. See Figure 37 legend.

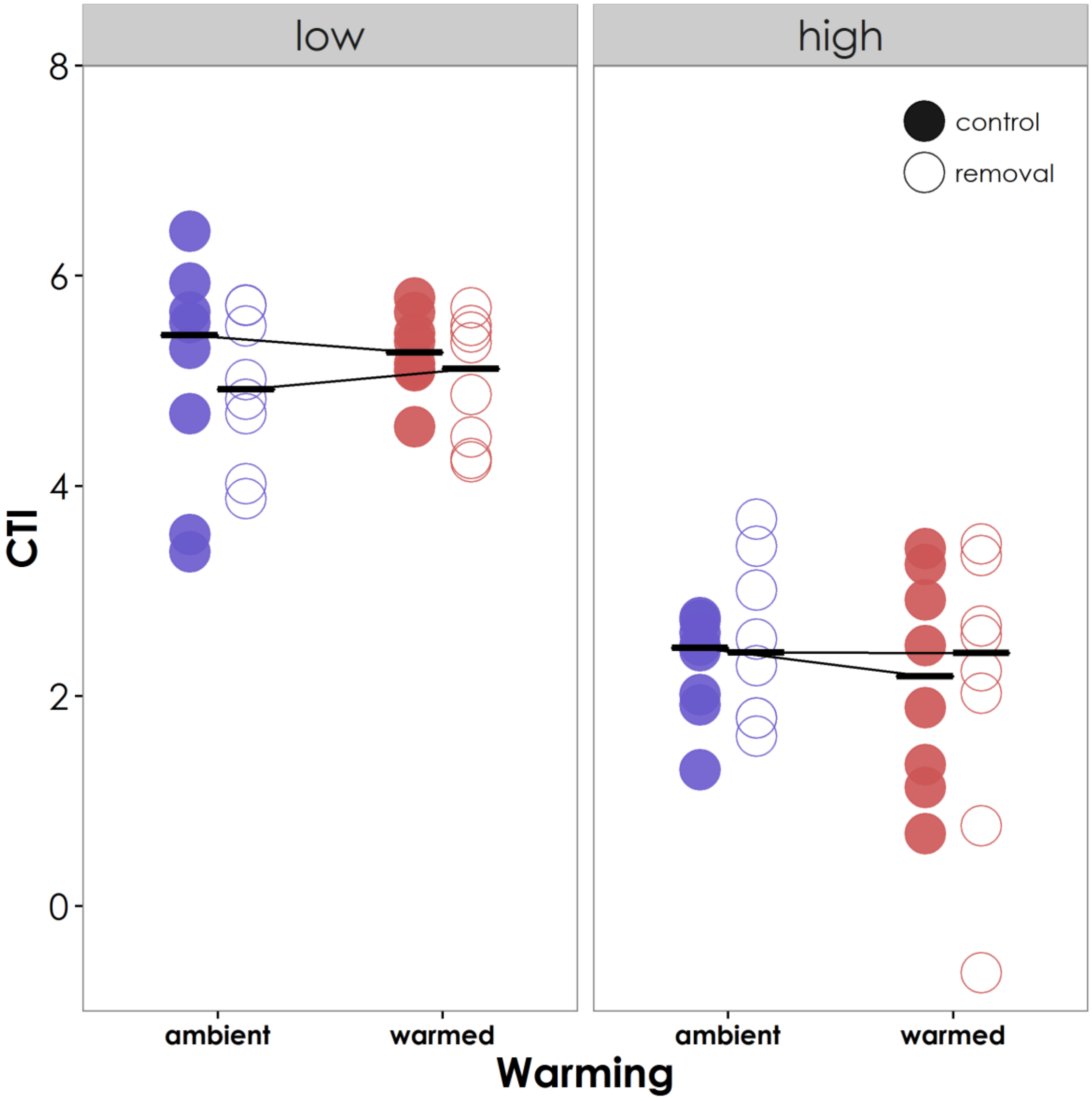

Figure 44. Community thermal index (CTI) across all treatment combinations. The CTI value of a plot represents the abundance-weighted peak value of thermal niche across all the individual plants in the plot, as inferred from their global distribution data—higher CTIs characterize more warm-adapted communities. See Figure 37 legend. The dominant species at each site was included in these calculations.

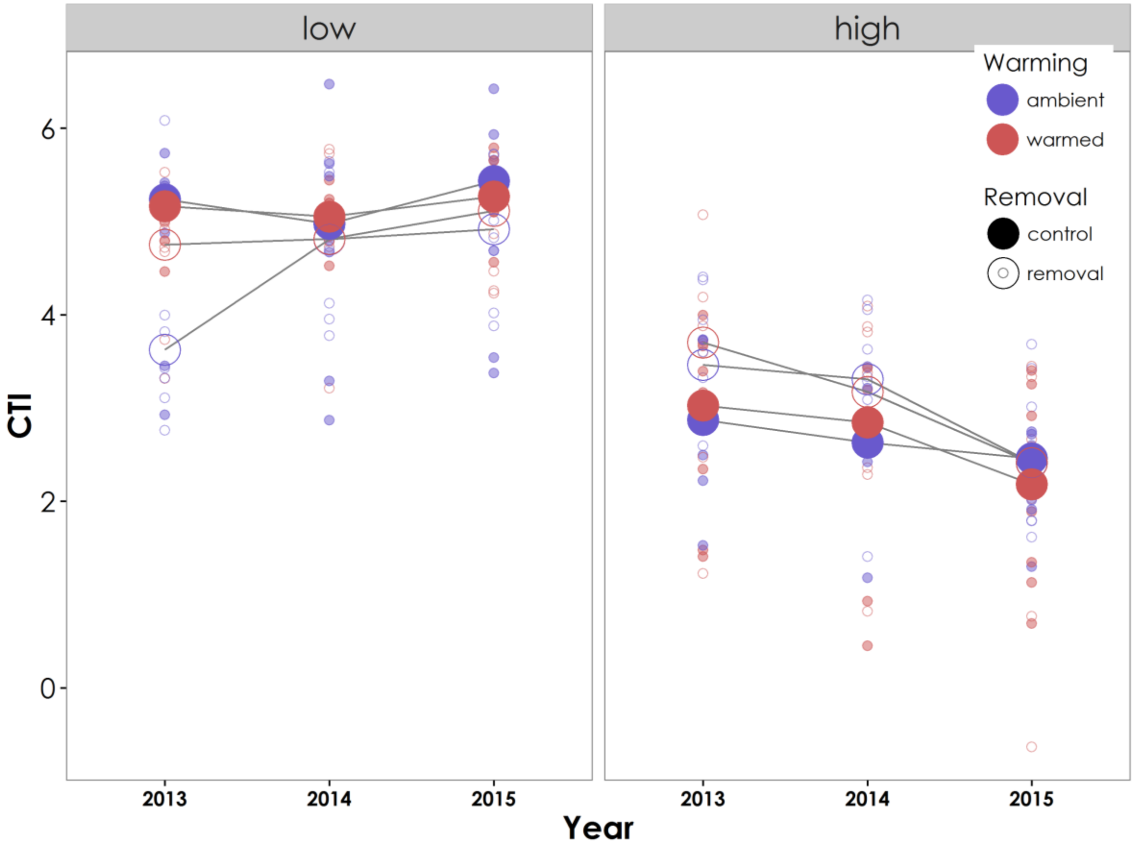

Figure 45. Community-weighted thermal index (CTI) for all plots across both sites, 2013-2015. Median values within a treatment combination at a site are displayed as larger circles. Medians from the same treatment combination in multiple years are connected by lines. As in other figures, filled circles represent intact control plots, while empty circles represent dominant-species removal plots. Blue circles represent ambient-temperature plots, and red circles represent warmed plots.

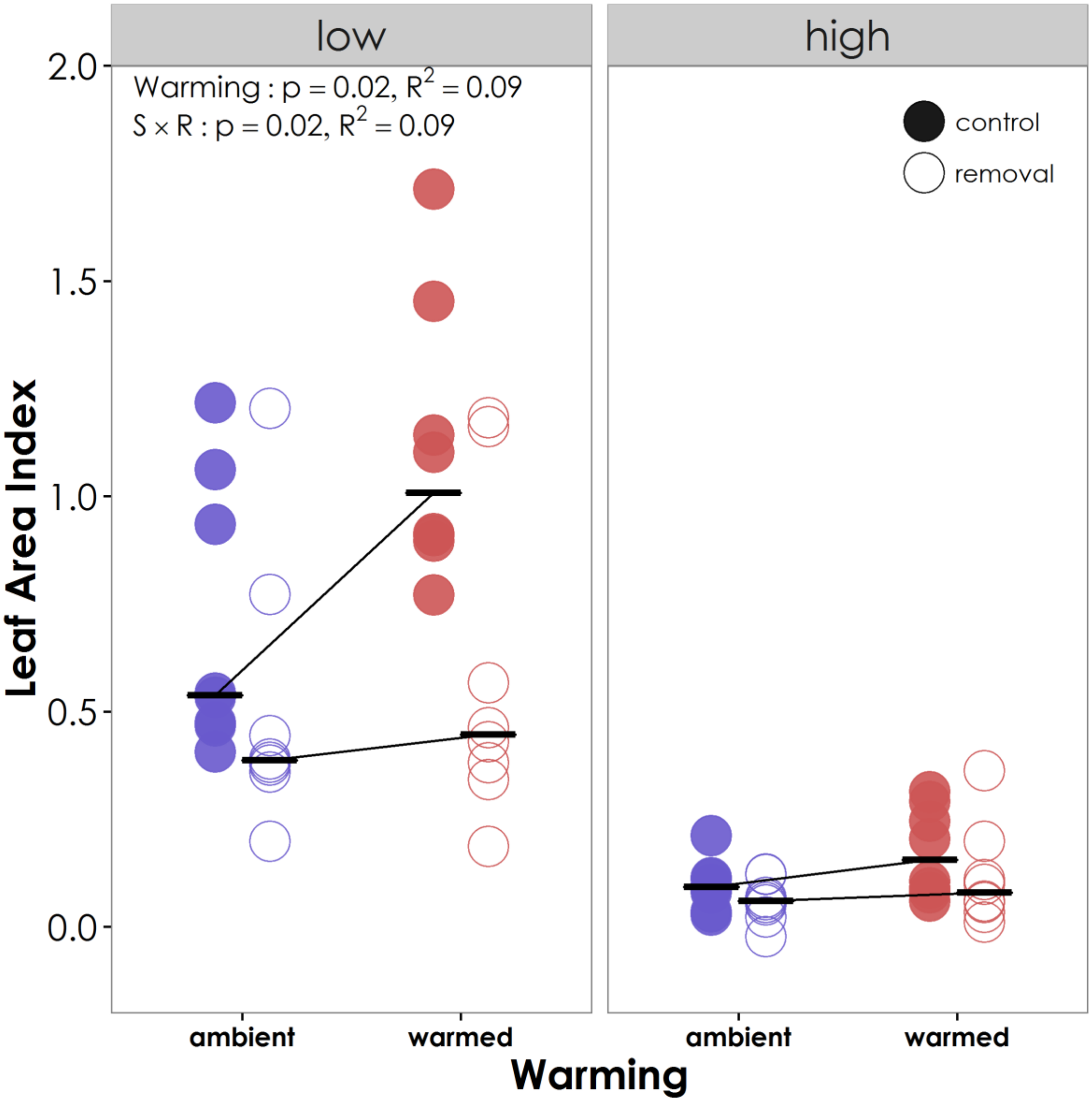

Figure 46. Leaf area index (LAI) at peak biomass in July 2015 for all treatment combinations. See Figure 37 legend.

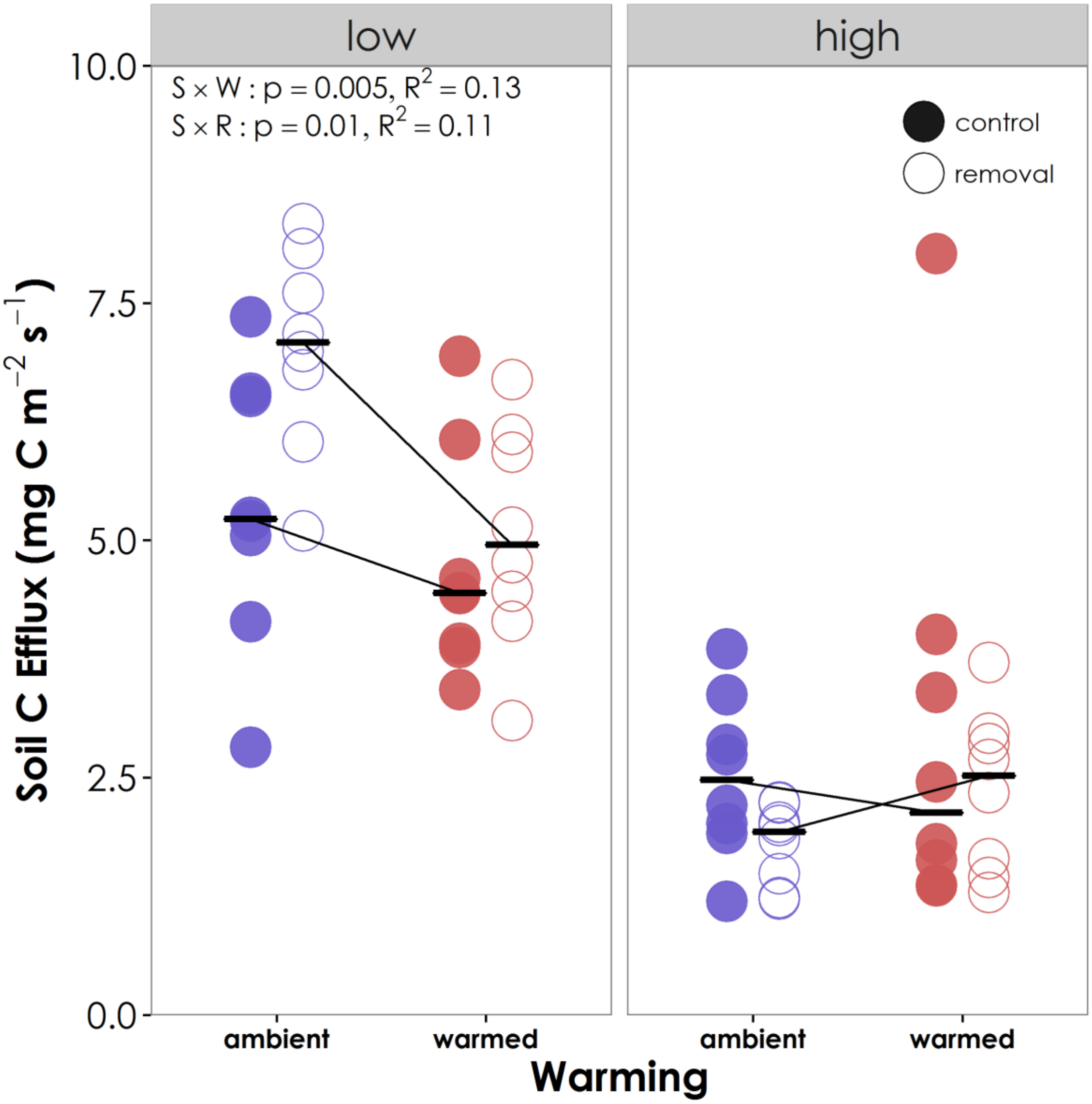

Figure 47. Soil C efflux at peak biomass (July 2015) across all treatment combinations. See Figure 37 legend.

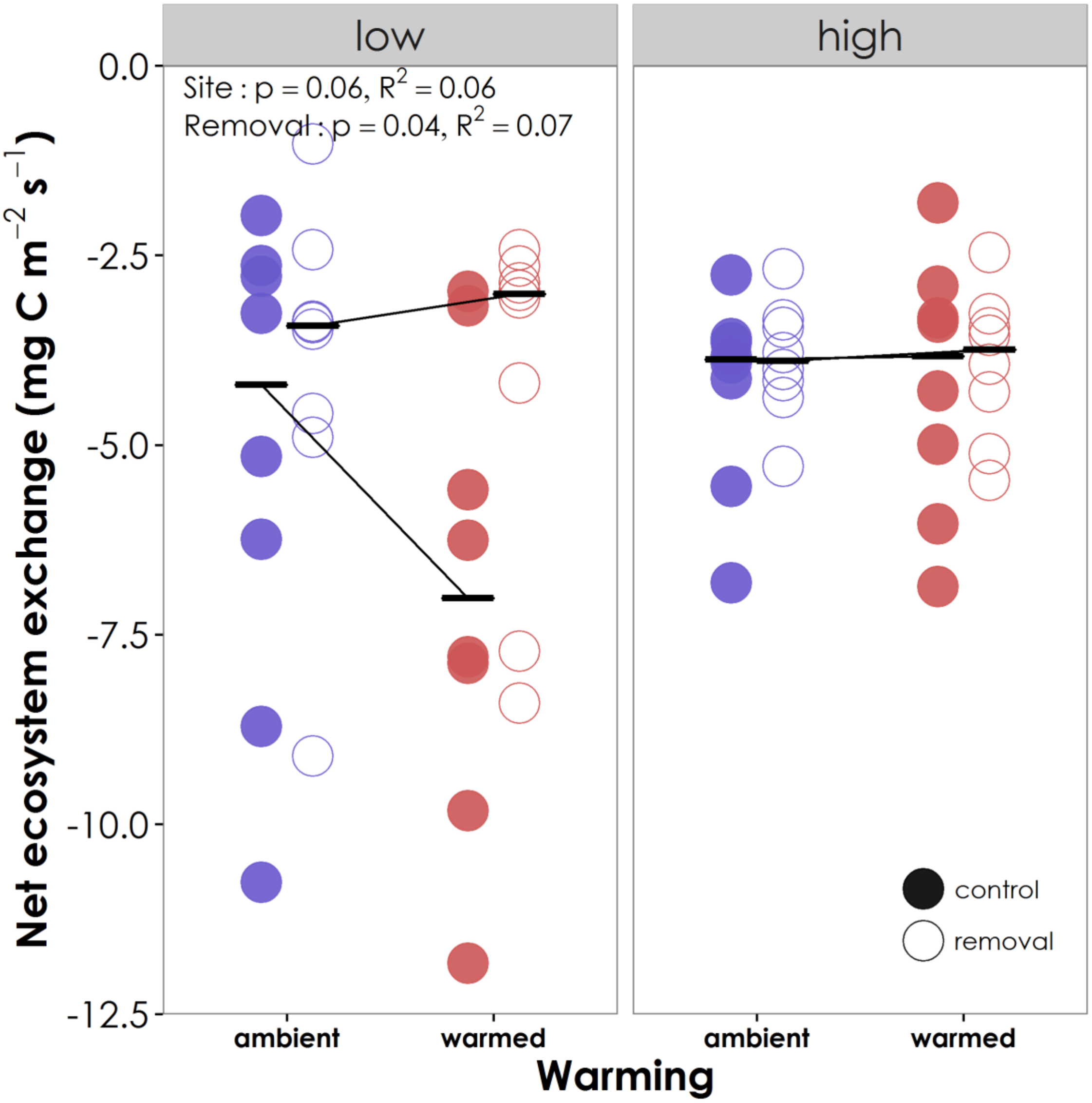

Figure 48. Net ecosystem exchange at peak biomass (July 2015) for all treatment combinations. See Figure 37 legend.

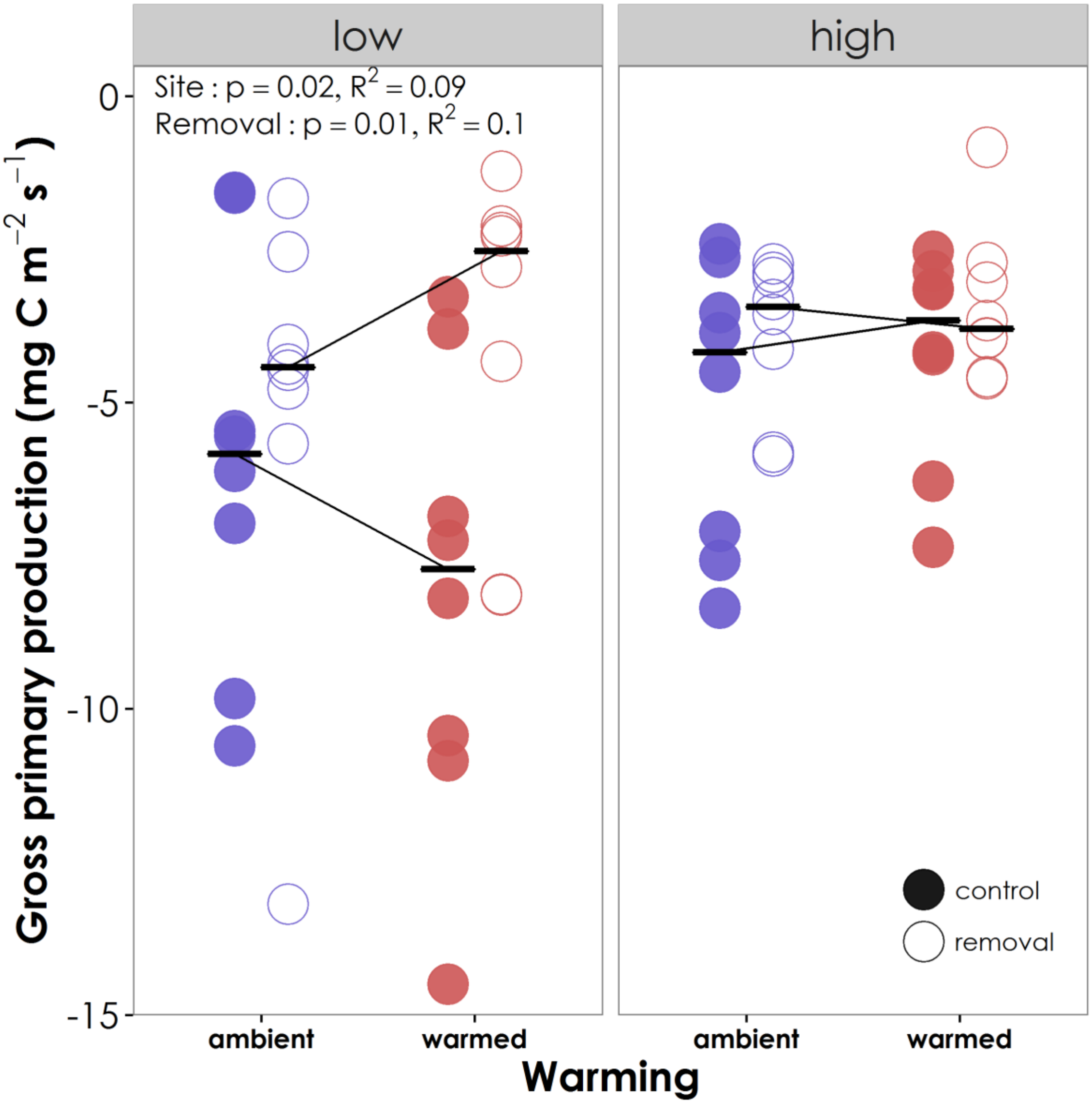

Figure 49. Gross primary production measured during July 2015 for all plots. See Figure 37 legend.

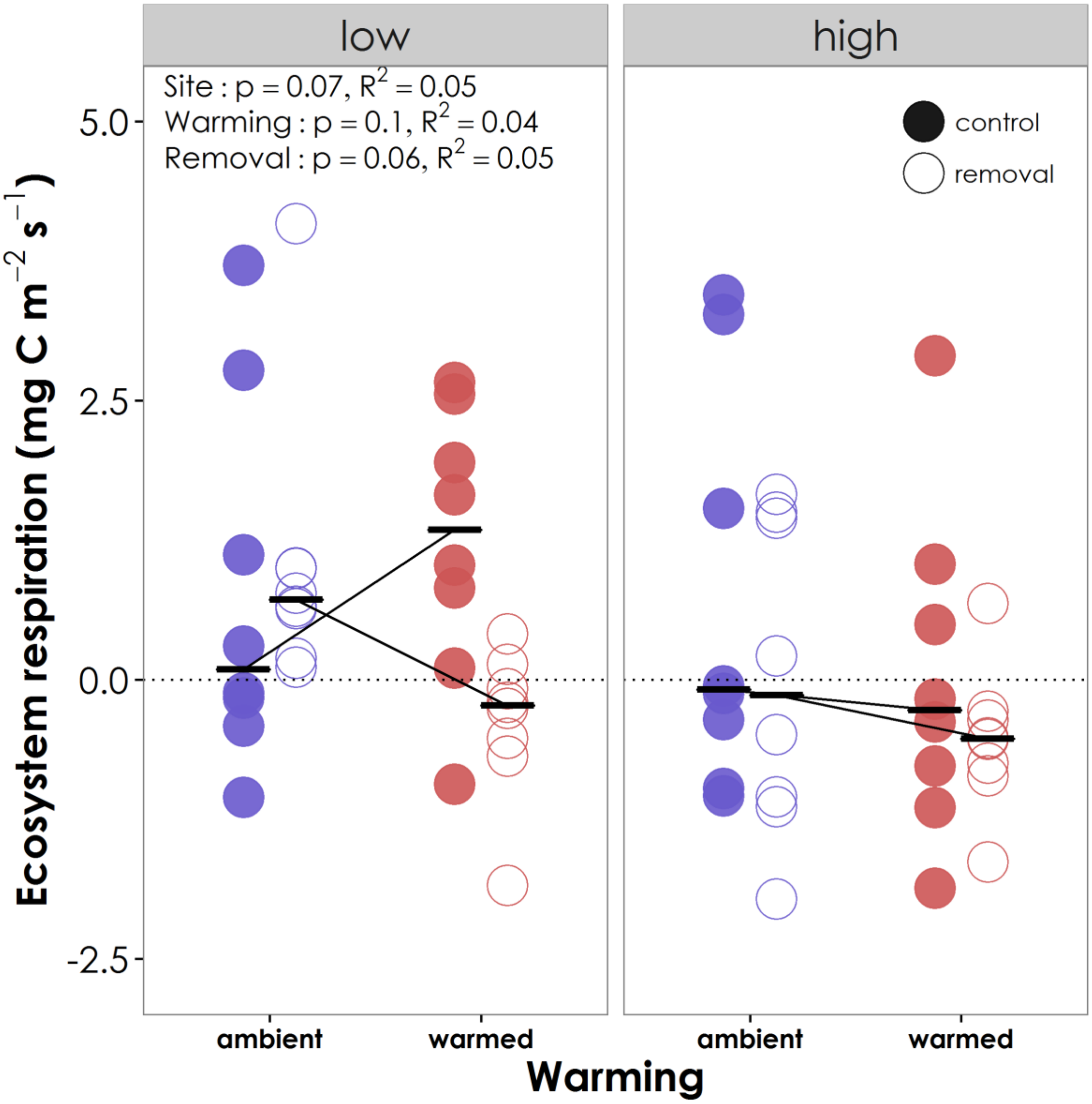

Figure 50. Ecosystem respiration measured during July 2015 for all plots. See Figure 37 legend.

Structural equation model

The path diagram for the structural equation model that was selected by the model selection procedure is given in Figure 51. In the model, 41% of the variation among plots in net ecosystem carbon exchange was explained by a combination of treatment variables, interactions among treatments, and endogenous variables including trait means and functional trait diversity. As more negative NEE values indicate a greater carbon sink, the model indicates that the largest effect on NEE is mediated by the functional composition of the plant community. In particular, plots with lower CWMs for LMA and RML were greater carbon sinks, as were plots with higher functional diversity. In addition, intact control plots were greater carbon sinks than removal plots; surprisingly, this effect does not appear to be mediated by differences in LAI among plots. Warming treatments and their interactions only had slight influence on NEE. In addition, although the low-elevation site had a higher CWM for leaf mass:area ratio when comparing the sites in isolation, once variation due to treatments is accounted for in the SEM, the relationship becomes negative.

Discussion

After the third season of experimental warming and dominant species removal at low and high elevation, I observed that both treatments have effects that are somewhat contingent on elevation. Neither warming nor dominant-species loss changed the trait composition of the remaining community. However, because the dominant species differed functionally from the remaining community, the loss of dominant species affected community-level functional trait means and variances and their associated ecosystem processes. In addition, warming increased soil carbon efflux only at the low-elevation site, while dominant-species removal increased efflux at the low site and decreased it at the high site; in general, species removal had a greater effect on carbon fluxes than experimental warming. The structural equation model revealed that the functional trait composition of the plant community was the most important determinant of net ecosystem carbon exchange. In addition, there was a positive relationship between functional diversity and net ecosystem exchange, and loss of the dominant species tended to reduce the magnitude of net ecosystem exchange.

Site-level plant community differences drive global change responses

The different effects of global change which I observed at each site may be due to differences in plant community characteristics between the two sites: first, the plant community at the low elevation site is composed of taller species with lower leaf mass:area ratio and lower leaf dry matter content than the high elevation site. Moreover, the trait diversity and the variance in trait values is generally higher at the low-elevation site; functional diversity has been shown to predict ecosystem responses better than trait means (Pakeman *et al.* 2011), especially at small spatial scales like that of this study (de Bello *et al.* 2013). In particular, the very low diversity in trait values at the high-elevation site indicates that the response of that community to both biotic and abiotic change may be more predictable, but the rapid growth favored at the high site may mean that those species are more responsive to change. This may indicate that there are more viable life strategies for plants at the low-elevation site (Adler *et al.* 2014).

Environmental conditions at the two sites have shaped the traits of the dominant plants. The low-elevation site typically has no persistent snowpack in winter and a longer midsummer

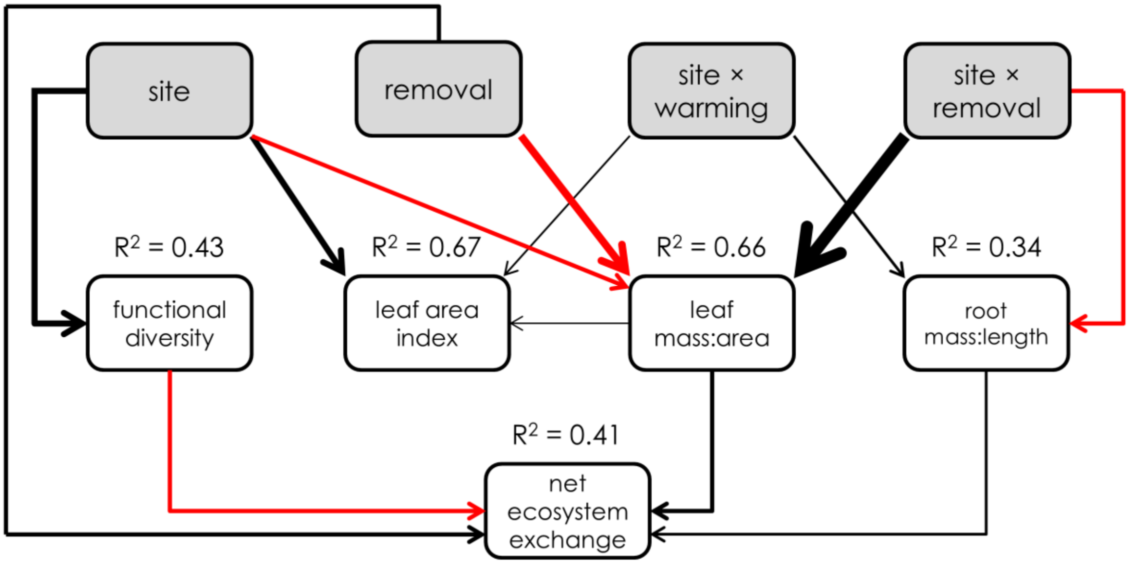

Figure 51. Structural equation model path diagram showing the relationships between site, treatments and their interactions, community-weighted trait means of LMA and RML, functional diversity, leaf area index, and net ecosystem exchange. Thickness and color of arrows represent standardized path coefficients (red = negative effect causing greater carbon sink, black = positive effect causing smaller carbon sink). Treatments and sites are coded as 0 or 1: the high-temperature, low-elevation site is coded as 1; warming treatment and removal treatment are coded as 1 while the corresponding controls are coded as 0. Treatment variables are shaded in gray.

As more negative NEE values indicate a greater carbon sink, the model indicates that the predominant effects on NEE are that plots with lower CWMs for LMA and RML were greater carbon sinks, as were plots with higher functional diversity. In addition, intact control plots were greater carbon sinks than removal plots. Warming treatments and their interactions only had slight influence on NEE. In addition, although the low-elevation site had a higher CWM for leaf mass:area ratio when comparing the sites in isolation, once other variation is accounted for in the SEM, the relationship becomes negative.

drought than the high-elevation site. Precipitation variability has been shown to increase resilience in plant communities due to the number of different strategies for coping with water stress (Gherardi & Sala 2015). To cope with this stress, plant species may do one or more of three things: grow rapidly and photosynthesize more before the drought, build durable leaf tissue that can continue to gain carbon through the drought, or grow deep roots to tap into otherwise inaccessible water resources. The dominant species, *Wyethia*, seems to fall into the last category, and has higher RML, lower LMA, and is taller than most of the other plants at the low site.

In contrast to the low site, the high-elevation site has a very short growing season whose length is governed by snowmelt date (Baptist & Choler 2008). Therefore, most plants there must have fast-growing, less durable leaves that provide a rapid return on carbon investment. The notable exception to this pattern is *Juncus*, the dominant plant species, which maintains some leaf tissue from year to year and has higher RML and LMA than most other species at the site. In addition, because the speed of leafing out is more responsive at the high site relative to the low, the effect of warming on leaf area index manifested itself more dramatically. I measured leaf area index in early July, which may record an increase in peak LAI at the low site, but a speeding up of leaf-out phenology at the high site (Inouye 2008). Therefore, because the plant communities are so different functionally between the two sites, and because the dominant species achieves its high abundance through very different mechanisms, the two sites respond differently to global change drivers.

Changes in community thermal index driven by annual temperature fluctuations

While the relative abundance of species with different functional traits in the plots did not change dramatically in response to the treatments, the relative abundance of the species year-to-year is flexible, as shown by the changes in community thermal index driven by changes in species relative abundance. The CTI at the low-elevation site stayed stable, while the CTI at the high-elevation site decreased, paralleling a decrease in the mean annual temperature during three seasons of manipulation. The annual changes in mean annual temperature are greater than the chronic changes imposed by the experimental treatment (Lawson *et al.* 2015). This may indicate that we need very long-term experiments, spanning several decades, to determine the effects of chronic warming on these communities (Walker *et al.* 2006; Elmendorf *et al.* 2015). The high-elevation site may have responded more to annual fluctuations because long-term environmental filtering has selected for species able to respond to a greater magnitude of temperature change from year to year (Adler *et al.* 2006).

Trait-related properties drive size of carbon sink

The structural equation model revealed that among plots, the most important measured drivers of carbon flux were the leaf and root characteristics of the plant community. Both within sites and among sites, the plots with lower LMA and lower RML were greater carbon sinks. In addition, plots where the dominant species was absent had a smaller net ecosystem exchange, although this was not mediated by any decrease in LAI in removal plots. This is perhaps because the plant species that dominates access to limiting resources in a community can attain the highest total rate of photosynthesis across all its biomass (Fauset *et al.* 2015). Furthermore, plots with higher functional diversity were greater carbon sinks, lending support to the body of work relating functional diversity to productivity (Díaz & Cabido 2001; Flynn *et al.* 2011). Finally, the identity of the dominant species that was removed from each site was the most important driver of trait change (removal × site interaction), which in turn affected NEE, indicating that the ecosystem-level consequences of species loss are highly context-dependent (Wardle *et al.* 2011). In addition, this model shows that warming and interactions between warming and species loss had relatively small effects on community structure and ecosystem function, echoing the results I observed when examining response variables in isolation.

Conclusion

Our understanding of how species respond to changes in their environment is limited by the contingency inherent in ecological systems—an environmental driver may have opposite effects in systems occupied by species with different ways of coping with environmental change (Saavedra *et al.* 2013). For example, species in harsh environments tend to be more adaptable to abiotic change, while species in benign environments tend to withstand biotic stress from competitors more readily (Callaway *et al.* 2002). My factorial manipulation of warming and species loss at both low and high elevations allowed me to explore the relative importance of biotic and abiotic factors at different elevations, in addition to realistically simulating the multiple simultaneous drivers of global change that human-influenced ecosystems face.

These results should be interpreted with some caution. I cannot currently say that the patterns among sites are driven by elevation (in the sense of mean annual temperature) because there are so many confounding variables across sites. In particular, the amount and quality of biomass removed was very different across sites. I also caution that although there may be significant intraspecific trait variation in these systems, in particular plasticity induced by the treatments, I do not currently have data to assess how much those processes are contributing to the observed patterns. Finally, it is possible that effect sizes may be somewhat underpredicted in ecological field experiments such as this because of the temporal and spatial scale of manipulation (Wolkovich *et al.* 2012).

This study, conducted across multiple sites and simultaneously manipulating two global change factors, contributes to the newest generation of field experiments in global change ecology. I demonstrate that the response of montane meadow communities to increased temperature and to species loss is context-dependent. Both at the scale of experimental plots separated by a few dozen meters and of study sites separated by over 700 meters of elevation with disparate climates, the functional composition of the plant community was the key determinant of ecosystem function and of the magnitude of response to both abiotic and biotic change. These communities are remarkably resilient due to long-term community assembly processes that have taken place on the backdrop of harsh, fluctuating environmental conditions (Walker *et al.* 1999; Gherardi & Sala 2015). However, the fact that the responses are so dependent upon species with particular traits may mean that a longer period of change during which species change more dramatically in abundance or are replaced may cause a regime shift (Biggs *et al.* 2009). It remains to be seen, from future work in this experimental system and in others, whether these long-term shifts will compromise the provision of ecosystem services from mountain ecosystems.

CONCLUSION

The research presented here connects community and ecosystem ecology, explores theory and applications dealing with variation among organisms and its consequences for how natural systems function, and predicts how anthropogenic global change will affect communities of species and the ecosystem processes they mediate. It is clear that processes of climatic filtering, biotic interactions, and random fluctuation have different importance in different climatic environments. This heterogeneity creates a landscape of diverse organisms forming diverse communities that in turn make disparate contributions to global cycles of matter and energy and have disparate responses to anthropogenic change.

In Chapter I, I scoured existing literature and demonstrated that leaf traits related to plants' ability to tolerate environmental stress and to take up resources from their environment vary along temperature gradients, and that this variation is largely at the level of the individual organism. In Chapter II, I used simulations and case studies from experiments to show that this high level of individual variation must be accounted for in ecological studies. Chapter III uses community composition and functional trait data from a large number of field sites to demonstrate that individual variation in functional traits is a stumbling block that must be overcome in order to incorporate species traits into a process-based predictive model of species abundances. Finally, in Chapters IV and V, I use experimental field manipulations grounded in a trait ecology approach to explore the forces structuring natural communities and make predictions about their responses to global change. A factorial manipulation of dominant species presence and soil nitrogen level (Chapter IV) showed that a mountain meadow community has functional redundancy, allowing it to maintain functional stability when the dominant species is lost or when soil nitrogen levels increase. In Chapter V, I use an experiment manipulating both temperature and dominant species presence across two elevational sites to show that soil carbon dynamics are dependent on long-term ecological filters that have led to species with different functional roles dominating in different communities. In fact, these long-term filters have a greater impact on the plant community and associated function than the temporary perturbation of warming.

In the future, I will continue working on these and related questions. I have begun to use large datasets that span space and time to explore relationships between environmental conditions, human activity, species traits, and ecosystem function. The data sources I am using include long-term national forest inventories, species composition inferred from historical records, species identity and trait data from the National Ecological Observatory Network (NEON), and results from long-term ecological field experiments. I am collaborating with researchers from the University of Notre Dame, the University of Copenhagen, and the National Forest Inventory of Norway on these projects, and I am beginning postdoctoral research at Michigan State University in Summer 2016. As a postdoctoral researcher, I will develop statistical and mathematical techniques to assimilate NEON data into predictive models. With these models, I will generate hypotheses about the forces structuring communities and ecosystems, as well as predictions of how communities and ecosystems will respond to ongoing global change.

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