

**THE STRUCTURE AND FUNCTION OF SUBALPINE ECOSYSTEMS IN THE
FACE OF CLIMATE CHANGE**

By

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A Dissertation Submitted to the Faculty of the
DEPARTMENT OF ECOLOGY AND EVOLUTIONARY BIOLOGY

In Partial Fulfillment of the Requirements
For the Degree of

DOCTOR OF PHILOSOPHY

In the Graduate College
THE UNIVERSITY OF ARIZONA

2012

**THE UNIVERSITY OF ARIZONA
GRADUATE COLLEGE**

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ACKNOWLEDGEMENTS

I would like to thank my advisor, Brian Enquist, who took a chance on a graduate student with no background in biology, and helped me to become the ecologist that I am today. Brian showed me how to take a love of natural history and transform it into a research science and conservation career. I am also indebted to all of the members of the Enquist lab, past and present, who supported me as mentors, colleagues and friends throughout my time at the University of Arizona.

I would also like to thank my committee members for their feedback and support. Scott Saleska taught me how to do ecophysiological measurements and introduced me to the Rocky Mountain Biological Lab, which became the stage for my dissertation. He also showed me how to bring together science and environmental activism, and is my role model for what science activism can look like. Judie Bronstein was particularly helpful in designing my field experiments, her expertise in species interactions, and her wisdom in dealing with personal struggles. Lara Kueppers provided expertise in ecosystem functioning and sagebrush encroachment, and Steve Archer provided expertise in the physiology and ecology of sagebrush encroachment.

Special thanks goes to the entire staff of Rocky Mountain Biological Laboratory who enabled me to do research there. Ian Billick, Jennie Reithel, Dan Jones and billy barr helped with permitting, site selection, data depositing and climate data. Jennie Reithel, John Harte, Nick Waser, Mary Price and David Inouye provided invaluable guidance on my research and showed me how important a love and knowledge of place is for an ecologist. My heartfelt thanks goes to the researchers and graduate students of the RMBL, who were my “other” department during my dissertation, and provided support, guidance and many wonderful memories. Rocky Mountain Biological Laboratory, founded 1928. We do science over there.

DEDICATION

This work is dedicated to my parents, Richard and Nancy Lamanna, who became the first in their families to go to college, and enabled me to become the first person in our family to earn a Ph.D.

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ABSTRACT

Subalpine ecosystems are experiencing rapid changes in snow pack, temperature, and precipitation regime as a result of anthropogenic climate forcing. These changes in climate can have a profound effect on subalpine ecosystem structure and functioning, which may ultimately feed back to climate change. In this study, I examined the response of the subalpine meadow plant communities at the Rocky Mountain Biological Laboratory to natural and simulated climate change. First, I looked at whether changes in growing season precipitation or temperature regime would have the larger effect on subalpine ecosystem carbon flux. In a simulated warming experiment, changes in growing season precipitation had a tenfold larger effect on cumulative carbon flux than did the warming treatment. Along a natural climatic and elevational gradient, precipitation stimulates carbon uptake, particularly at higher elevations. Given projected decreases in summer precipitation in the high elevation Rockies, we predict a 20% decrease in carbon uptake from subalpine meadows. Second, I compared the taxonomic, phylogenetic and functional structure of plant communities along an elevational gradient to infer which climatic and biotic factors influence community assembly at each elevation. Floral and phenology traits become overdispersed at high elevation, mirroring phylogenetic relatedness, and suggesting pressure to diversify to attract pollinators during the abbreviated growing season. At the same time, leaf functional traits become clustered at high elevation, indicating multiple opposing assembly mechanisms in subalpine communities. Finally, I studied the natural history of sagebrush, *Artemisia tridentata* ssp. *vaseyana*, at its elevational range limit in subalpine meadows. In particular, I focused on

the importance of warming and species interactions in elevational advance of the species. I found that facilitation by neighboring forbs was critical for sagebrush seedling survival, decreasing mortality by 75%. Seedling mortality was overwhelmingly due to desiccation of seedlings; therefore, neighboring forbs moderate temperature and water stress for seedlings. Despite the extremely limited growing season at high elevation caused by subfreezing temperatures, subalpine ecosystem structure and function are closely tied to water availability during the growing season. Therefore, improved predictions of future precipitation regimes over the Rocky Mountains will be our best tool for conservation of these fragile habitats.

INTRODUCTION

An explanation of the problem and a review of the literature

Fossil fuel combustion and land use practices have caused global atmospheric carbon dioxide (CO₂) concentrations to rise 35% since pre-industrial times (IPCC 2007). The additional greenhouse forcing from this and other greenhouse gases has caused the mean global temperature to increase by 0.8°C, and it is predicted to increase a further 1-5°C in the next century (IPCC 2007). These changes in temperature will be accompanied by changes in precipitation regimes, relative humidity, stream flow, snow and ice cover, and other climate factors. Such dramatic changes in climate are predicted to have significant effects on the earth's biota, particularly in the distribution of vegetation types around the world (Walther et al. 2002). Changes in vegetation distribution can significantly impact not only the distribution of other dependent species, but can also feed back to climate change through alteration of albedo, evapotranspiration, disturbance regimes and storage of carbon dioxide itself (Cox et al. 2000; Friedlingstein et al. 2006).

Nowhere is climate change progressing more rapidly than in high elevation and high latitude ecosystems. As warming reduces snow cover in these cold ecosystems, the albedo of the land surface decreases, resulting in further warming (Serreze et al., 2000; IPCC 2007). This warming, and concomitant changes to hydrologic cycles from snow and permafrost melt, have caused a suite of changes to ecosystem structure and function (Callaghan *et al.*, 2011). Shifts in vegetation structure, particularly the increase of shrub or tree cover in sites with sufficient moisture, is a common response (Grabherr et al.

1994; Sturm et al. 2001; Forbes et al. 2010; Callaghan et al. 2011; Elmendorf et al. 2012;). Similarly, losses of non-vascular plants and cryptograms and subsequent declines in biodiversity are also well documented across many sites (Klanderud & Totland 2005; Elmendorf et al. 2012). Changes to biogeochemical cycling are also prevalent, although the net effect of warming stimulated production and respiration varies by site and over time (Shaver et al. 2000).

Heterogeneous responses of high elevation and high latitude ecosystems to climate change appear to be commonplace. A recent meta-analysis of 61 experimental warming studies found that the effects of warming varied over space and time and depended on other environmental conditions (Elmendorf *et al.* 2012). Indeed, within climate change experiments, species-specific responses to treatment are common (de Valpine & Harte 2001; Klanderud 2008; Suding et al. 2008). Given the diversity of responses of high latitude and high elevation ecosystems to climate change, it becomes even more pressing to try to understand the effects on these rapidly changing ecosystems, in order to conserve biodiversity and ecosystem services.

The goal of this dissertation was to understand how subalpine meadows in the Colorado Rockies respond to climate factors and to predict how these ecosystems are likely to respond to future changes in climate. In the face of seemingly idiosyncratic responses of high elevation systems to climate change, I chose to take a multidisciplinary approach and investigate how climate affected subalpine community structure and function across multiple spatial scales. I focused on how climate affects species interactions for a key species, *Artemisia tridentata*, that in turn structures communities

and alters ecosystem functioning. I also looked at how species interactions and variation in individual species functional traits structure communities with changes in climate. Finally, I examined how whole ecosystem functioning is affected by changes in climate.

At its heart, this work is a study in the “ecology of place”, relying on field studies across multiple years and spatial scales in order to understand mechanistically how climate influences ecology (Billick & Price 2010). A mechanistic understanding of how climate change alters an ecosystem is critical for developing adaptation strategies to conserve biodiversity and ecosystem services. Furthermore, it allows the insights from one small place to extend to other ecosystems, and contribute to our understanding of ecology in general.

The goal of this dissertation was to understand how subalpine meadows in the Colorado Rockies respond to climate factors and to predict how these ecosystems are likely to respond to future changes in climate. At its heart, this work is a study in the “ecology of place”, relying on field studies across multiple years and spatial scales in order to understand mechanistically how climate influences ecology (Billick & Price 2010). I utilized techniques from multiple disciplines to investigate the effects of climate on subalpine meadow structure and function at multiple temporal and spatial scales. This type of detailed place-based ecological research is critical for conservation of threatened habitats, but also allows for insights that extend to ecology as a field.

An explanation of the dissertation format

In this dissertation, I present the results of a broad study using techniques from multiple disciplines to understand how anthropogenic climate change will impact the structure and functioning of subalpine ecosystems in the Colorado Rockies. This work was done along an elevational gradient, representing natural climate change, encompassing the entire subalpine life zone near the Rocky Mountain Biological Laboratory in Gothic, Gunnison Co., CO, USA. I also utilized several types of experimental climate manipulation to determine the effects of climate change on multiple biological scales, from one species' interactions, to the structure of the plant community, to the functioning of the entire ecosystem.

Appendix A, "Assessing the differential effects of temperature and precipitation on subalpine ecosystem functioning," examines the effect of changing temperature and precipitation on whole ecosystem functioning. In particular, we examine net ecosystem exchange and cumulative carbon flux, both along an elevational gradient and within a simulated warming experiment. In Appendix B, "Microbes on mountainsides: Contrasting elevational patterns of bacterial and plant diversity", we look at the effects of climate on community structure in subalpine meadows, for two different communities: plants and soil bacteria. We use taxonomic and phylogenetic diversity metrics to understand how changes in climate effect diversity in these two very different ecological communities. Then, in Appendix C, "Disentangling differential trait drivers of phylogenetic community assembly across an elevational gradient", we use the functional traits of the plants in subalpine meadows to investigate why we observe the diversity

patterns across the elevational gradient that we do in Appendix B. Finally, in Appendix D, “Facilitation is critical for the survival of sagebrush seedlings at their elevational range limit,” I go to the smallest scale, to investigate the effects of climate change on one of the species interactions that structure subalpine communities. I use a factorial experiment of warming and neighbor removal to look at the effect of warming on sagebrush seedling survival and on interactions between sagebrush seedlings and the meadow forb community.

These chapters together address the depth of climate change effects on subalpine ecosystems. Climate change may affect the interactions between species, which in turn affect the structure of communities. Community structure is the sum of evolutionary history and ecological interactions between species and their biotic and abiotic environments. Ultimately, ecosystem structure determines ecosystem functioning, and resiliency of ecosystem functioning to changes in climate. By examining the effects of climate change on each of these biological scales, we are able to generate a more comprehensive understanding of how climate change is likely to affect the structure and functioning of vulnerable subalpine meadows.

PRESENT STUDY

I present the methods, data, analyses, and conclusions of this study in the four appended manuscripts. Below, I briefly summarize the main conclusions from each of these studies.

In Appendix A, I examined the effects of temperature and precipitation on growing season carbon flux in subalpine meadows, both in a long term study along a natural climate gradient and in a warming experiment. I found that along the elevational gradient, soil respiration is stimulated by both temperature and available soil moisture, and leaf level respiration is stimulated by soil moisture. In the warming experiment, the effect of inter-annual variation in precipitation was much greater than the effect of warming; cumulative carbon uptake was 10 times higher in the wet year relative to the dry year, while heating had no effect on carbon uptake. Inter-annual variation in precipitation also influenced peak carbon uptake along the elevational gradient. Wet summers resulted in higher peak uptake rates, and the strength of this effect increased with elevation. These results suggest that carbon uptake from subalpine meadows in the Colorado Rockies is likely to decrease as summers become increasingly drier in the area.

In Appendix B, I looked at how changes in climate along an elevational gradient affect community assembly in two groups of organisms critical to ecosystem functioning: angiosperm plants and the *Acidobacteria* soil bacteria. We used both traditional taxonomic diversity metrics and phylogenetic diversity metrics, to try understand not only how diversity changes across an elevational gradient, but why. Interestingly, the taxonomic richness of angiosperms (the number of species) and *Acidobacteria* (the

number of operational taxonomic units, or OTUs) did not show the same pattern across elevation: while the richness of *Acidobacteria* declines with elevation, richness in angiosperms peaks at middle elevations. Phylogenetic richness, which we defined as the branch length of the phylogenetic tree encompassing all the species in a community, mirrored the taxonomic richness for both *Acidobacteria* and angiosperms. For angiosperms, taxonomic and phylogenetic turnover with elevation were identical. However, *Acidobacteria* had more phylogenetic turnover than expected given the amount of taxonomic turnover, suggesting that environmental filtering was more important in *Acidobacteria* communities at this spatial scale than it was in the angiosperm communities. Finally, we examined the phylogenetic dispersion, or relatedness, among species in the angiosperm and *Acidobacteria* communities across elevation. *Acidobacteria* communities were clustered at all elevations, while angiosperm communities became increasingly overdispersed at higher elevations. These results suggest that *Acidobacteria* communities are subject to strong environmental filtering at all elevations along our gradient. However, despite the increasing climatic harshness at higher elevations, angiosperm communities become more overdispersed. For plant communities, species interactions may be more important for community assembly than environmental filtering at higher elevations.

Next, in Appendix C, I use functional traits of subalpine angiosperms to investigate the ecological mechanisms that create the phylogenetic overdispersion we saw in plant communities at the highest elevations in Appendix B. We chose a suite of functional traits that encompasses several dimensions of ecological variation in plants,

including physiological, phenological and floral traits. Functional richness, or the volume of trait space occupied by each elevational community peaked at middle elevations, just as taxonomic and phylogenetic diversity did. Phylogenetic conservatism of traits varied, with aggregate traits derived from principle component analysis of independent axes of trait variation showing the highest degree of conservatism. While phylogenetic relatedness within angiosperm communities was increasingly overdispersed with elevation, trait dispersion showed many different patterns, depending on the trait. Floral and phenological traits paralleled the phylogenetic pattern and were increasingly overdispersed at higher elevations. Conversely, foliar %C was clustered at high elevation, suggesting multiple assembly mechanisms working on angiosperm communities. We hypothesize that at higher elevations, there is a stronger environmental constraint on leaf physiology, while also stronger competition for pollinators during the abbreviated growing season.

Finally, in Appendix D, I report on a field study of sagebrush, *Artemisia tridentata* ssp. *vaseyana*, at its elevational range limit in the Gunnison Valley, Colorado. Sagebrush abundance affects carbon flux and storage in subalpine meadows, and is projected to increase with warming and drying. Here I use a factorial experiment involving heating and neighbor removal to investigate the effects of warming on sagebrush seedling recruitment, as well as the effects of warming on interactions between sagebrush and the meadow forb community. I found that while heating had no effect on growth or survival of sagebrush seedlings, neighbor removal decreased relative growth rates and quadrupled mortality relative to seedlings with neighbors intact. Seedlings that

eventually died in the experiment experienced higher high temperatures and lower low temperatures than those that survived. This suggests that facilitation by neighboring forbs acts by moderating ambient temperatures and is critical for the survival of sagebrush seedlings near their elevational range limit.

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**APPENDIX A- ASSESSING THE DIFFERENTIAL EFFECTS OF
TEMPERATURE AND PRECIPITATION ON SUBALPINE ECOSYSTEM
FUNCTIONING**

Assessing the differential effects of temperature and precipitation on subalpine ecosystem functioning

Running Title: Abiotic controls on subalpine NEE

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Keywords: NEE, carbon cycle, subalpine, temperature, precipitation, path analysis

Primary Research Article

****In review at *Global Change Biology*****

Abstract

Productivity in high elevation and high latitude ecosystems is thought to be primarily temperature limited. However, a key challenge is to assess how changes temperature *and* precipitation will together influence ecosystems. Here, we quantify their relative importance on subalpine meadow carbon dynamics at instantaneous, daily, and growing season scales across an elevational gradient and within a long-term warming experiment. Across the gradient, instantaneous rates of heterotrophic and root respiration showed equal and positive relationships with variation in both temperature and water. However, on diurnal time scales net ecosystem carbon exchange was differentially affected by water and temperature. On seasonal time scales the cumulative autotrophic uptake of carbon dioxide is primarily controlled by available soil moisture indicating that short-term temperature effects were negated by increased water availability. As a result, wet years increased (i) instantaneous rates of aboveground plant and soil respiration and (ii) annual plant growth and carbon uptake. We find that these effects are more pronounced and *increase* with increasing elevation. Within the long-term experiment, warming did have a slight negative impact on soil moisture and instantaneous NEE and seasonal carbon uptake in a dry year but no effect in a wet year. In contrast, a wet year dramatically increased instantaneous rates and seasonal uptake by 26-fold. Together, these results suggest that annual production in subalpine meadows is primarily limited by available soil moisture during the snow-free growing season. The implication is that the projected 20%, decrease in summer rainfall in the Colorado Rockies would result in a 37% decrease in growing season carbon uptake from subalpine meadows. These findings

contrast with the expectation that temperature is a primarily limiting factor in these systems. Thus, accurate predictions of precipitation change in the subalpine zone are necessary in order to better forecast future production in high elevation ecosystems.

Introduction

Variation in net ecosystem exchange (NEE), the net balance of photosynthetic uptake and auto- and heterotrophic respiration, is primarily controlled by daily and seasonal climate factors, namely temperature and available soil moisture (Lieth 1973; Law et al. 2002). The standard expectation in ecosystem ecology is that NEE in mid- to high-latitude sites is primarily limited by low temperature, while NEE at mid- to low-latitudes, except for the wettest tropical forests, is limited by low precipitation, with a zone of co-limitation observed in latitudes between (Yi et al. 2010; Zhao & Running 2010). This model, or the ‘SE model’ for short, has some empirical support. Indeed, increasing temperature in high-elevation and high-latitude ecosystems results in increased rates of soil respiration (Oechel et al. 1993; Hobbie & Chapin III 1998; Huxman et al. 2003; Saito et al. 2009), and increased plant productivity (Hobbie & Chapin III 1998; Huxman et al. 2003; Epstein et al. 2008; Blanken et al. 2009; Saito et al. 2009). In addition, within a mid-latitude low elevation annual California grassland, where the effects of precipitation should be more pronounced, both experimental heating *and* increasing precipitation increased net plant productivity, while experimentally increasing *both* temperature and precipitation produced an additive effect on productivity, (Shaw et al. 2002). Together these results have supported the SE model in that temperature has an increasingly dominant effect on ecosystem carbon balance at higher elevations and latitudes.

While the SE model appears to apply broadly (Nemani et al. 2003; Yi et al. 2010) and may aid in predicting ecosystem-level responses to climate change, the mechanistic

linkage between climate and organismal physiology, which ultimately controls variation in NEE is still unclear. For example, in high elevation plants, while higher temperatures can result in higher rates of photosynthesis and respiration via temperature effects on biochemical kinetics (Körner & Larcher 1988; Day et al. 1991; Huxman et al. 2003), warming may not influence instantaneous physiological processes directly, but instead result in a longer growing season and thus more time for uptake of carbon dioxide (White et al. 1999; Lund et al. 2010; Berdanier & Klein 2011; Enquist 2011). This is particularly the case in high elevation ecosystems where plant phenology is closely tied to snowmelt date (Galen 1995; Dunne et al. 2003; Inouye et al. 2002). Also, plant physiological acclimation can alter patterns of NEE (Shaver et al. 1992; Zeng 2004), and thus accelerate (or decelerate) climate change (Friedlingstein et al. 2006). Therefore, in order to utilize the SE model in order to predict how ecosystems will respond to climate change it is important to consider both short-term physiological responses of organisms and longer-term cumulative effects on ecosystem NEE (Shaver et al. 2000; Kerkhoff et al. 2005; Kikuzawa & Lechowicz 2006).

In this study, we assessed the SE model in order to predict how future changes in temperature and precipitation will affect the carbon balance of subalpine and alpine ecosystems. We take the powerful approach of integrating experimental climate manipulations with natural climate variation along an elevational gradient and across years (Dunne *et al.* 2004). In order to disentangle the short-term effects of climate on organismal physiology we use path analysis to mechanistically assess the drivers of variation in components of NEE along a natural climate gradient of temperature and

precipitation. Additionally, to disentangle the relative influence of changes in water availability and changes in temperature on ecosystem NEE we compare daily and seasonal patterns of NEE in an experimentally heated subalpine meadow within and across years that vary with precipitation. Finally, by comparing results on short- and long-term time scales, within a temperature manipulation and across a natural gradient we can make predictions of how subalpine carbon flux is likely to respond and feedback to climate change in the future.

Materials and Methods

Study Area: This study was conducted within meadows in the subalpine life zone of the Colorado Rockies near the Rocky Mountain Biological Laboratory (hereafter RMBL, elevation 2900 m, Gunnison Co., CO, USA latitude 38°53 N, longitude 107°02 W). These meadows support a large diversity of plants with over 100 herbaceous species, which exist in a mosaic between aspen forest, mixed conifer forest and sagebrush steppe (Langenheim 1962). They exist from lower elevations of approximately 2500 m, at the border of Great Basin desert scrub, to treeline (~3500 m) where alpine vegetation is dominant.

Elevational Gradient: We sampled plant communities along an elevational gradient that ranged between 2,460 to 3,380 m above sea level and spanned a geographic distance of 39 km. The gradient consists of five long-term study sites that were established by B.J. Enquist in 2003 and has been sampled every year since. The gradient spans the Washington Gulch and East River valleys near RMBL, Crested Butte, and Almont, CO (see Table 1). Each study site is approximately 50 m² and is characterized by similar local slope, aspect, and vegetation physiognomy. All sites consist of a mixture of shrubs, grasses, and forbs. There is substantial turnover of plant species between sites with very few of the 120 species sampled occurring in more than two of the sites (Bryant et al. 2008). Additionally, shrub cover across the gradient decreases from a high of 33% at the lowest elevation site to 0% at the highest.

Within each site, and in each year, five 1.3 m² plots were randomly chosen to span the local topography of each of the site. The mean growing season temperature

changes along the gradient by approximately 8°C at 10 cm soil depth, from 21.6°C at the lowest elevation to 13.7°C at the highest. Mean annual precipitation is 750 mm, of which over 80% falls as snow. Snowmelt varies from mid-March at our lowest elevation site to the end of June or early July at our highest elevation site.

Experimental Temperature Manipulation: We paired our measures across the elevational gradient with a long-term temperature manipulation experiment at RMBL, which is the midpoint of our elevational gradient. The “warming meadow” vegetation community consists of more than 85 species of long-lived perennial herbaceous plants and two woody shrubs. All vegetation in the meadow senesces completely in the winter, except sagebrush (*Artemisia tridentata ssp. vaseyana*), which maintains evergreen, overwintering leaves.

The experimental warming meadow consists of five control and five heated plots (3m x 10 m), containing vegetation characteristic of the meadow. Since 1991, overhead infrared lamps have constantly warmed the experimental plots. Additional details of this experimental design have been described previously (Harte & Shaw 1995; Saleska et al. 1999).

Measurement of NEE: Along the elevational gradient, we measured diurnal whole-system CO₂ and H₂O flux on each plot using an open path IRGA, Li-Cor Li-7500 (Li-Cor Biosciences, Lincoln, NE) mounted inside a collapsible 2.2m³ polyethylene tent (Arnold & Obrist 2003; Huxman et al. 2004; Cable & Huxman 2004). The tent (Shelter Systems, Menlo Park, CA) is designed to admit 75% of photosynthetically active radiation (PAR) without heat build-up and to allow CO₂ and water vapor concentrations

to change in response to leaf and soil processes. We measured NEE twice per growing season: early (approximately four weeks after local snow melt) and at peak biomass (approximately eight week after snowmelt). Each NEE measurement consisted of daytime peak uptake (at 10:00) and nighttime respiration (at 22:00). Following the method of Jasoni *et al.*, ambient CO₂ was measured for 30 seconds, and then the tent was lowered and the CO₂ concentration within the tent was measured for 90s, under clear sky and low wind conditions (2005). Air inside the tent was well mixed by fans, and the chamber was sealed to the ground using a heavy chain.

In the warming meadow, daytime and nighttime NEE were measured biweekly throughout the growing season in 1994 and 2006. NEE was measured in 1994 by S.R. Saleska and published (Saleska et al. 1999). In 2006, we repeated NEE measures throughout the growing season using the same methodology as in 1994 (with a 75 cm x 75 cm clear Plexiglas chamber), but using the Li-Cor 7500 rather than the Li-Cor 6200 to quantify CO₂ concentrations inside the chamber. In total five measurements were made throughout the growing season between May 31 and July 31. To match the more frequent 1994 measurements to the 2006 measurements, we used only the subset of 1994 measurements that most closely matched the 2006 measurement times (daytime (8:00) and nighttime (22:00)).

Measurements of Soil, Biomass and Climate: In both the elevational gradient as well as the warming meadow we measured soil respiration the same way. Soil efflux was measured concurrently with NEE using a Li-Cor 6400 on two soil collars per plot. Soil temperature was measured using the Li-Cor external temperature probe inserted 10 cm

into the soil near the soil collar. Soil moisture was measured gravimetrically from four 10 cm sample cores taken across each plot. During each flux measurement, the percent cover by functional group (forb, shrub, grass) was recorded in a 10 cm² grid on each plot, and maximum height was measured in each corner and the center of each plot.

Within each site, we harvested biomass from several plots to assess the relationship between cover, height, and biomass. Biomass, height and % cover were sampled in 2006, from each of our five NEE sites twice in the season - on representative plots during the early NEE measurement and on the actual plots after the peak season NEE measurement. Above ground biomass from each plot was harvested with pruning clippers and was then dried in a drying oven at 60°C and weighed. Using these data, and allowing for differences between sites, we built multivariate model to predict biomass from a measured height and cover using stepwise regression and choosing the model with the lowest AIC (Burnham & Anderson 2004). The final model resulted in predicting plot biomass with plot cover and height data ($r^2 = 0.78$, and $p < 0.0001$).

Calculation of NEE: All carbon flux data were analyzed in the same way. To determine NEE, we first, assumed that the tent was not perfectly sealed to the ground, so that the flux of carbon dioxide was dependant both on the activity of the plants and microbes, and on diffusion of CO₂ through the “leaky” tent. We fit the exponential decay model of Saleska et al. (1999) to the relationship between tent CO₂ concentration and time as measured by the Li-7500. Second, if this model failed to converge, we then used a linear fit over the middle 70s of the measurement, correcting for water vapor pressure (following Jasoni *et al.* 2005). Next, we partitioned NEE into above- and below-ground

(soil) components by multiplying the measured soil efflux by the percentage of exposed bare soil in each plot (Potts et al. 2006). All statistical analyses were done in R (www.r-project.org).

Calculation of Cumulative Carbon Flux: To calculate cumulative NEE for the warming meadow, we first averaged daytime and nighttime NEE measurements to get an estimate of mean daily NEE for each measurement day, and truncated the 1994 time series to match the 2006 series in length. We then fit quadratic regressions to each time series of mean daily NEE and integrated this daily rate across the growing season to estimate cumulative carbon flux (in grams of C per m²). Quadratic regressions were chosen because mean NEE across the growing season at high elevation typically reaches a peak value in the middle of the growing season. Errors on each integral were estimated as the standard deviation of the integrals for 1000 new times series generated with the same mean and standard deviation as the original data. For each new data set we fit a quadratic regression and integrated the resulting function.

Path Analysis: Due to the correlation between temperature and soil moisture, we used path analysis to test the relative importance of the direct effect of each environmental factor versus its indirect effect on subalpine carbon flux (Li 1981; Huxman et al. 2003). We decomposed each NEE measurement into its aboveground (i.e. leaf level photosynthesis and respiration) and belowground (auto- and heterotrophic respiration) components. We created several models using combinations of environmental variables, including soil temperature, air temperature, soil moisture, soil carbon, biomass, and year effects. The model presented here is the best-fit model, chosen

by lowest AIC score, where all explanatory variables act on the same, instantaneous timescale. This model was tested separately for nighttime and daytime NEE measurements, using the SEM package in R. We calculated standardized partial regression coefficients for each path in the model, and overall model fit using the χ^2 lack of fit test (Shipley 2000).

Results

Elevational Gradient

Bivariate Linear Regressions: Across the elevational gradient, the relationship between NEE and *in situ* soil temperature and moisture varied with time of day. While daytime NEE shows no relationship to *in situ* soil moisture (Fig 1b; $r^2 = 0.03$, $p = 0.29$), but is negatively correlated to temperature (Fig 1a; $r^2 = 0.176$, $p = 0.0056$), nighttime NEE shows the opposite pattern. Nighttime ecosystem respiration shows a positive relationship to soil moisture (Fig. 1d; $r^2 = 0.198$, $p = 0.008$), but, surprisingly, no relationship with temperature (Fig 1c; $r^2 = 0.06$, $p = 0.29$). Because covariation between moisture and temperature could confound the true relations with carbon fluxes, we used a more sophisticated path analysis to account for such interactions.

Path Analysis: The path model that best fits the data is shown in Figure 2, and incorporates both the direct and indirect effect of temperature on the components of NEE. This model had strong support for both daytime and nighttime fluxes (lack-of-fit test $X^2_1 = 0.241$, $p = 0.624$ and $X^2_1 = 0.122$, $p = 0.727$, respectively). *In situ* temperature and moisture affected the below ground components of daytime (Fig 2a) and nighttime (Fig 2b) similarly. In both cases, temperature and water had equal, positive direct impacts on heterotrophic and root respiration. However, daytime and nighttime aboveground flux was differentially affected by climate. During the day, temperature has a strong, direct and negative impact on net photosynthesis, while water has no direct impact (Fig 2a). At night, temperature has no direct impact on plant respiration, while water has a positive direct impact (Fig 2b). In both cases, though, the interaction between water and

temperature is strongly negative. Therefore, during the day, water's impact on net photosynthesis is indirect, by modifying the temperature. At night, however, temperature's impact on respiration is indirect via modifying water availability. The strongest effect in our model is the stimulation of nighttime leaf respiration by available soil moisture.

Warming Meadow

Growing season NEE patterns for the experimental warming meadow in 1994 and 2006 are shown in Figure 3. In general, warmed plots have higher soil temperature (Figure 3c) and lower soil moisture (Figure 3b) than control plots throughout the growing season. In both 1994 and 2006, the heated plots had higher early season uptake while control plots have higher uptake later in the growing season (Figure 3a). This is due to an earlier snowmelt in the warmed plots (Harte & Shaw 1995; Saleska et al. 1999). In both years, the date at which the control plots achieved higher NEE values than heated plots was the same: day 165, or June 14. However, the major difference in seasonal patterns of NEE between these two years is the CO₂ uptake rates later in the season. In 1994, uptake peaks in early June and decreases throughout the rest of the growing season. Conversely, in 2006, uptake continues to increase on both heated and control plots throughout the growing season. As a result, the cumulative net carbon assimilated at the end of the growing season in 2006 exceeded the net carbon assimilated during 1994 (Figure 4). As we discuss below, we hypothesize that this difference is due to the importance of soil moisture controls on growing season length.

The two sampling years in the Warming Meadow corresponded to an exceptionally dry and exceptionally wet growing season. Due to variability in the North American monsoon, 1994 was an unusually dry summer, with 19.8 mm of rain falling in June but only 6.6 mm in July (Figure 5). This amount is 38.5% and 90.2% *less* than the average of 32.2 mm and 67.5 mm that normally falls in June and July, respectively. Conversely, 2006 was unusually wet, with 33 mm falling in June and 133 mm in July (Figure 5). This corresponds to 4% and 97% *more* than the average monthly values, respectively. This variance in rainfall is evident in the measured soil moisture in the heated and control plots, in both 1994 and 2006 (Figure 3b). In both years, soil moisture shows a strong early season decline following snowmelt. However, in 2006 there was an increase in soil moisture beginning near day 180 (June 29), and no corresponding increase in 1994.

Comparing variation in growing season NEE patterns between 1994 and 2006 enabled us to disentangle the relative impact of temperature and precipitation on short-term and longer-term variation in NEE. For example, in 2006, the increase in soil moisture near day 180 is mirrored by an increase in net photosynthesis and ecosystem respiration in 2006, while in 1994, carbon flux and soil moisture continued to decrease concurrently. In both years there is a tendency, after day 150 for the warmed plots to have lower NEE and less respiration than the control plots (Figure 3a) although the difference in any one comparison date may not be statistically significant.

The effect of difference in rainfall on the cumulative NEE between 1994 and 2006 is much larger than the cumulative effect of the heating treatment in the warming

meadow experiment on NEE (Figure 4). While heating has no effect on cumulative carbon uptake across the growing season ($t_{3393}=-0.804$, $p = 0.4211$), the difference between years is significant ($t_{3106}=119.26$, $p<0.0001$). In 2006, plots took up an average 278.4 g C/m^2 , 10 times more than plots in 1994, which took up 27.8 g C/m^2 on average. There is a small but opposite within-year effect of heating on cumulative growing season NEE in both 1994 and 2006. In 1994 there was a negative decrease in the cumulative NEE (-18.6 g C/m^2) and in 2006 an increase in cumulative NEE ($+11.33 \text{ g C/m}^2$). However this difference between years is quite small when compared to the order of magnitude difference in cumulative NEE between wet and dry years.

Discussion

In the subalpine meadows of the Colorado Rockies, we find that variation in growing season NEE is primarily controlled by variation in soil moisture. In a long-term heating experiment, the effect of growing season precipitation on growing season NEE is almost two orders of magnitude more than the effect of artificial warming. These findings imply that any future changes in the precipitation regime will have a much larger effect on subalpine NEE than changes in temperature.

Our results also suggest that the interaction between soil moisture and soil temperature may have implications for understanding the importance of changes in climate on variation in instantaneous physiological rates. For example, several studies on the effects of water stress on species-level plant respiration have shown increases in respiration with increasing water availability, while other studies have suggested that plant respiration may show negative or no relationship with plant water status (Hsiao 1973; Hanson & Hitz 1982; Amthor & McCree 1990). These individual-level discrepancies between water availability and respiration have been explained by species-level differences in tolerance to water stress (Flexas et al. 2005). Our results are unique as our methodology integrates across species-level differences and assesses how broad plant-level responses influences ecosystem rates. In short, our results suggest that these past discrepancies between species-level respiration studies may be resolved by incorporating the interaction between soil moisture and soil temperature (see Figure 1) at the community and ecosystem level.

Our results also suggest an apparent contradiction. While the results from the warming experiment show that increased precipitation results in *increased* carbon uptake (higher NEE) at longer temporal scales, the path analysis results show a uniform stimulation of plant respiration by increased soil moisture (i.e. lower net carbon uptake) on shorter temporal scales as measured by instantaneous rates. This apparent contradiction is resolved if the higher rates of respiration are also indicative of increased net carbon assimilation and plant growth rates (Lambers et al. 1983) or reflect the stimulation of photosynthesis through photosynthetic overcompensation (Wan et al. 2009). Indeed, we observe that along the elevational gradient, the peak instantaneous NEE was higher in years with more growing season precipitation (Figure 6a). Interestingly, the effect of growing season precipitation on instantaneous NEE increases with elevation (Figure 6b). These findings are in direct contrast to the SE model, as we do not observe an increasing importance of precipitation at lower, and thus warmer elevations. Instead we find an increasing importance of growing season precipitation with elevation. We speculate that in lower elevation ecosystems, where the growing season is significantly longer than at higher elevations, the role of winter precipitation may be more important.

That precipitation should have an increasingly stronger control over growing season NEE with increasing elevation is perhaps not surprising. While growing season initiation is controlled by snowmelt date (Harte et al. 1995), soil moisture during the growing season can vary substantially. Further, once the snow has melted, there is comparatively little variation in ambient temperatures between years (Figure 5). In

contrast, variation in summer precipitation is driven by the North American Monsoon (Bowling et al. 2010). The intensity of the Monsoon throughout the southwestern United States as well as Colorado is notably variable across years (Adams & Comrie 1997). Locally at RMBL, July precipitation can vary more than 4 fold between years, depending on the strength of the monsoon. Thus, given the amount of variability in precipitation, we might expect plants in this ecosystem to be adapted to take advantage of the seasons with available soil moisture.

Unexpected sensitivity is common in variable environments. For example, in the Sonoran Desert ecosystem, winter annual plants are adapted to perform best under the cloudy, cool conditions that follow rain events, rather than the warm, sunny conditions that are typical of this environment (Huxman et al. 2008). Similarly, subalpine conifers are adapted to take advantage of early season moisture availability following snowmelt, and have optimal photosynthetic performance at low temperatures (Huxman et al. 2003). When determining how ecosystem NEE is likely to respond to changes in climate, it is critical to know what factors are most limiting when growing conditions are optimal, not necessarily what is most limiting over the entire year.

While the central Rockies are topographically complex, recent advances in regional climate modeling have made it possible to predict changes in precipitation in the Rockies on relatively fine scales. Most RCM/GCM combinations agree that the Colorado Rockies will see reduced precipitation during the growing season of June through August (NARCCAP – North American Regional Climate Change Assessment Project), and generally more precipitation during the rest of the year. If summer precipitation were

reduced by 20% as predicted in the models, then we would expect a 37% reduction in carbon uptake throughout the growing season, assuming a linear response of carbon uptake to seasonal precipitation based on the cumulative carbon uptake data presented in Figure 4. This reduction would not be compensated for by earlier snowmelt and later snowfall; rather, the longer growing season would simply result in a longer period of water-limitation.

Increasingly, evidence indicates that longer growing seasons in high elevation and high latitude ecosystems caused by earlier snowmelt will not result in increased carbon uptake by those systems. While growing season length sets an upper limit on the total amount of production in subalpine ecosystems, soil moisture controls actual ANPP within that limit (Berdanier & Klein 2011). Across the high latitude northern hemisphere, increased uptake in the spring caused by early snowmelt is essentially cancelled out by decreased production caused by water limitation later in the summer (Angert et al. 2005), or by equal increases in ecosystem respiration (Parmentier et al. 2011). These studies support our findings that precipitation has a larger influence on carbon uptake in the subalpine Rockies than temperature.

Given our results there are several caveats and questions that stem from our findings. First, within the long-term warming experiment, our data are limited to two years of carbon flux measurements. Clearly, future work comparing the effects of precipitation variability either via experimental altering of precipitation and/or via comparing additional dry and wet years is needed. Second, within the warming experiment and the elevational gradient we are unable to control for the effect of previous

weather patterns, such as rainfall in the previous summer, or preceding snowfall, which may be important to controlling uptake (Patrick *et al.* 2009, Shim *et al.* 2009) and phenology patterns (Inouye *et al.* 2003) in high elevation ecosystems. Future work should assess the role of antecedent conditions as well as multiple climatic effects on differing temporal scales. Third, since 1994 the species composition in the warming meadow experiment has changed somewhat over the time between measurements. For example, herbaceous cover has decreased in heated plots by 38.4% and shrub cover has increased by 70.9%, despite no loss or gain of species (Harte & Shaw 1995). Nevertheless, despite these limitations, because we observe consistent results in both the warming experiment and across the elevational gradient we feel confident that we can attribute the differences in carbon uptake between 1994 and 2006 to differences in rainfall. Most notably, the change in species composition in the warming plots is not as great as the species composition change we observe over our elevational gradient (Bryant *et al.* 2008) where we still observe significant effects of precipitation between wet and dry years.

In this paper, we have shown that the effect of precipitation and water availability expresses differential responses and is influenced by the temporal scale of measurement. The effect of experimental warming on growing season NEE is relatively minor compared to the much larger influence of water availability. Our results indicate that subalpine NEE is primarily controlled by precipitation and available soil moisture during the growing season. In a longer term warming experiment, the effect of increased precipitation is two orders of magnitude larger than the effect of increased temperature on total carbon uptake. We observe a 6-fold change in growing season precipitation between

years, which leads to a disproportionate 26-fold change cumulative NEE while heating only increases cumulative carbon uptake by 6%. Therefore, we conclude that changes in summer precipitation patterns over the central Rockies will have a greater impact on NEE than will growing season warming.

Acknowledgements

We thank B. Blonder, B. Chaszar, M.Colner (Wilson), L. Crumbacher, P. Gaube, A. Henderson, A. Kerkhoff, M. McCarthy, J. Pither, R.E. Poore, N. Prohaska, R. Sleith, L. Sloat, and J. Stegen for assistance with fieldwork. We thank the staff of the RMBL for support and Mr. Curtis Allen and family, Mr. and Mrs. Pfeiler and Mr. Jed Frame, of Crested Butte, CO for allowing us access to their land. J. Vano and the Graduate Climate Conference assisted with climate predictions. This work was supported in part by the National Science Foundation (CAREER and ATB awards to BJE, and grants DBI 0753774 for REU support and DBI 0821369 for instrumentation to RMBL).

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Tables

Table 1: Description of sites along elevational gradient near the Rocky Mountain

Biological Laboratory, Gothic, CO.

Site Name	Lat/Long	Elevation
Almont	38°39'16" N, 106°51'40" W	2465 m
Crested Butte Land Trust	38°52'55" N, 106°58'46" W	2700 m
Allen Ranch	38°53'50" N, 106°58'43" W	2815 m
RMBL	38°53' N, 107°02' W	2920 m
Pfeiler	38°57'38" N, 107°01'53" W	3165 m
Painter Boy Mine	38°58'10" N, 107°02'28" W	3380 m

Figure Captions

Figure 1: Linear regression of instantaneous daytime net ecosystem exchange from 2004 through 2009 vs. soil temperature (a) and soil moisture (b), showing a negative effect of soil temperature on carbon uptake ($r^2 = 0.176$, $p = 0.0056$). Nighttime ecosystem respiration (i.e. negative uptake) vs. soil temperature (c) shows no effect, while nighttime ecosystem respiration increases with increasing soil moisture (d) ($r^2=0.198$, $p = 0.008$). Measurements from different elevations are shown in different colors.

Figure 2: Resulting path diagrams for daytime (a) and nighttime (b) NEE along a subalpine elevational gradient measured twice yearly from 2004-2009. Positive effects are shown with solid lines, while negative effects are shown with dashed lines. Each model is significant (lack-of-fit test daytime $X^2_1 = 0.241$, $p = 0.624$ and nighttime $X^2_1 = 0.122$, $p = 0.727$). Below ground respiration is equally and positively affected by soil moisture and soil temperature, i.e. warmer and wetter soils result in more respiration. Daytime net photosynthesis is negatively impacted by temperature, and only indirectly through soil moisture, while nighttime plant respiration shows the opposite pattern. Nighttime plant respiration is affected positively by soil moisture and only indirectly by temperature. In both cases, soil moisture and temperature are strongly inversely related through latent heat flux. * $p < 0.1$, ** $p < 0.05$, *** $p < 0.01$

Figure 3: Comparison of growing season patterns of carbon flux in 1994 and 2006 (a). For both years, dashed lines represent control plots, while solid lines represent heated plots. Daytime measurements were conducted at approximately 8 a.m. in both years, and

are represented by the dark lines. Nighttime measurements were conducted at approximately 10 p.m. in both years and are represented by the light lines. Growing season soil moisture shows a decline after snowmelt in both years (b), with no recovery from growing season rainfall in 1994, and recharge in 2006 with summer rain. Soil temperature patterns in 1994 are shown in panel (c).

Figure 4: Cumulative carbon uptake in 1994 (dry year with 26.4 mm growing season precipitation, or 26.5% of average) and 2006 (wet year with 166 mm growing season precipitation, or 166.5% of average) on heated and control plots. 2006 uptake is significantly greater than 1994 ($t_{3106}=119.26$, $p<0.0001$), while heating had no effect on uptake between years ($t_{3393}=-0.804$, $p = 0.4211$).

Figure 5: Mean monthly temperature (black bars) and precipitation (gray bars) data, recorded at the RMBL from 1990 – 2009, EPA CASTNET Station “GTH161”. It is important to note that the variance in precipitation, represented by standard deviation of the mean, is highest in July, which is also the peak of the growing season. Conversely, variance in temperature reaches its annual low during the growing season.

Figure 6: (a) Peak CO₂ uptake for each year, 2004 – 2009, versus growing season (June and July) precipitation for each elevation along the gradient. In general, peak uptake is higher in years with more growing season rainfall. This trend is strongest at higher elevations where the growing season is shorter than at lower elevations, as seen in (b)

increasing slope and R^2 of a linear regression of peak uptake vs. growing season precipitation.

Figures

Figure 1:

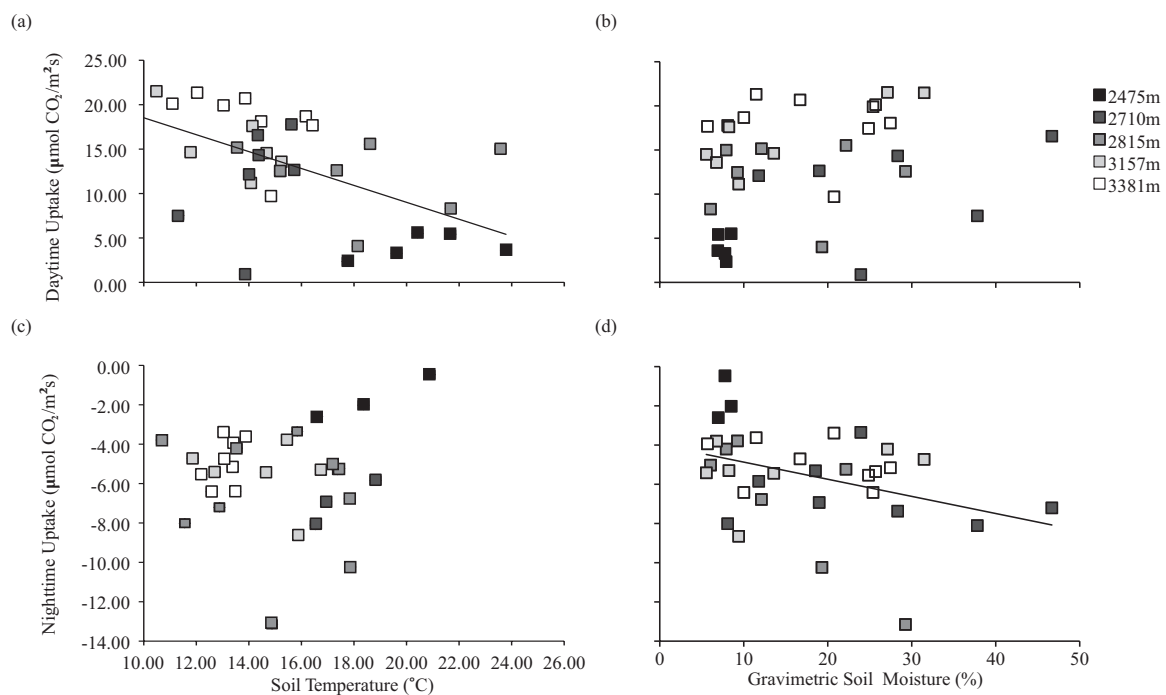
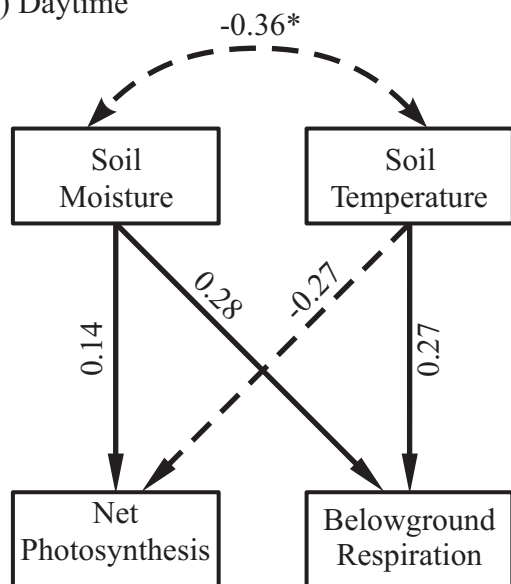


Figure 2:

(a) Daytime



(b) Nighttime

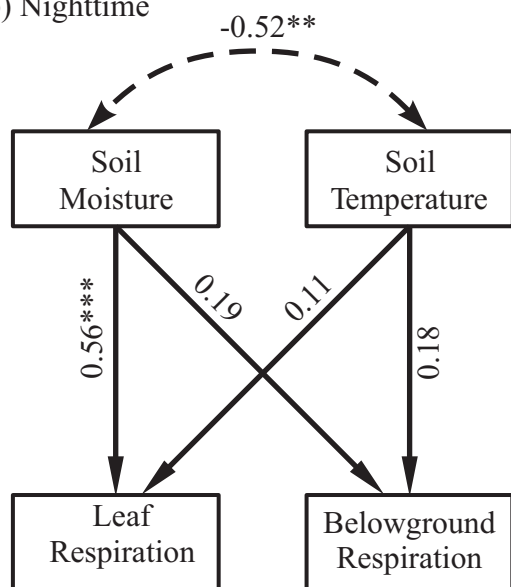


Figure 3:

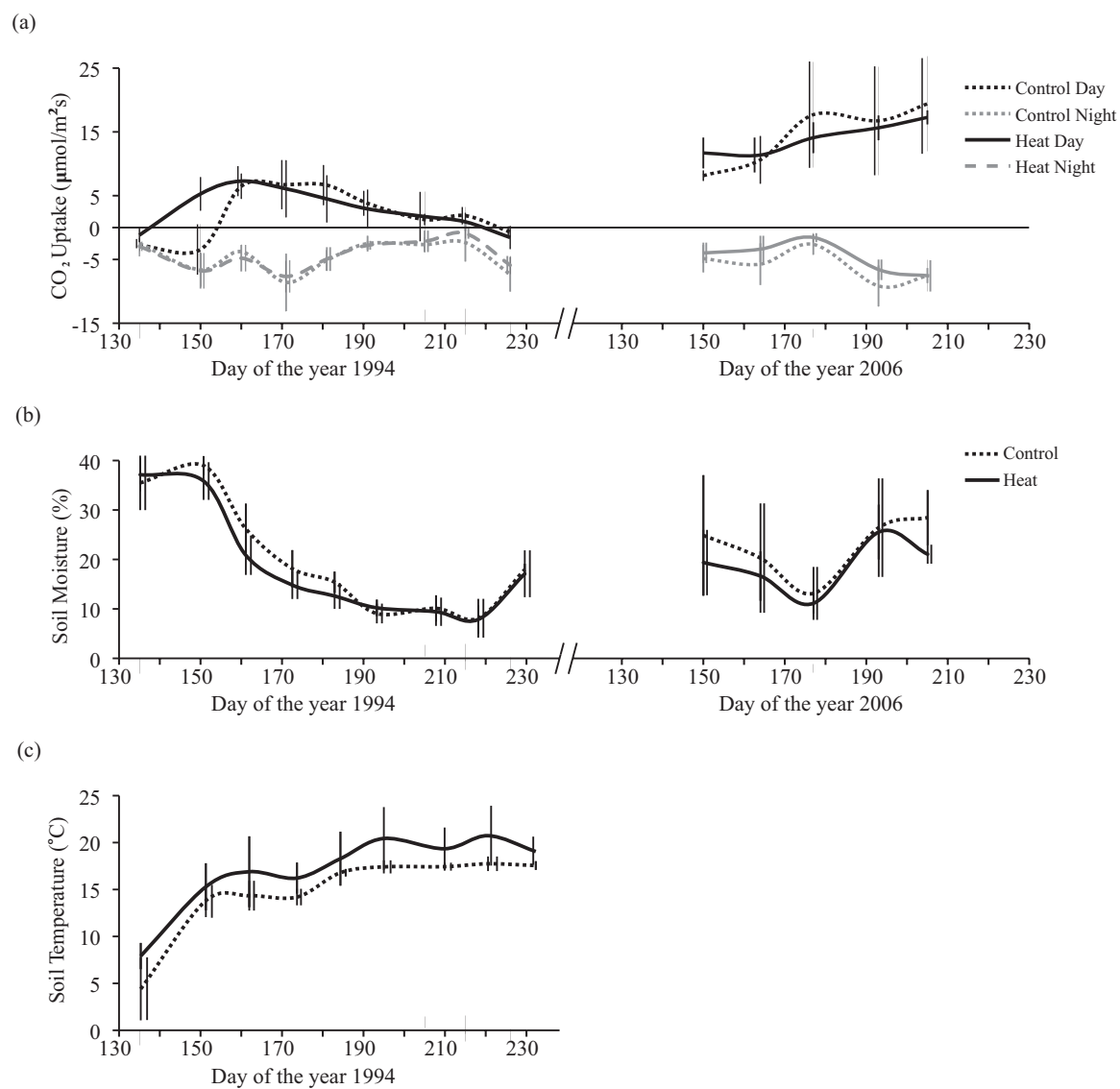


Figure 4:

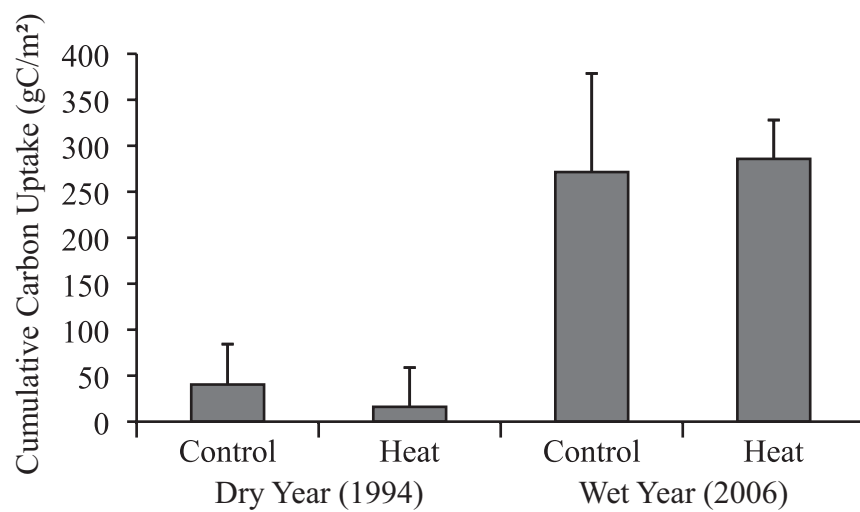


Figure 5:

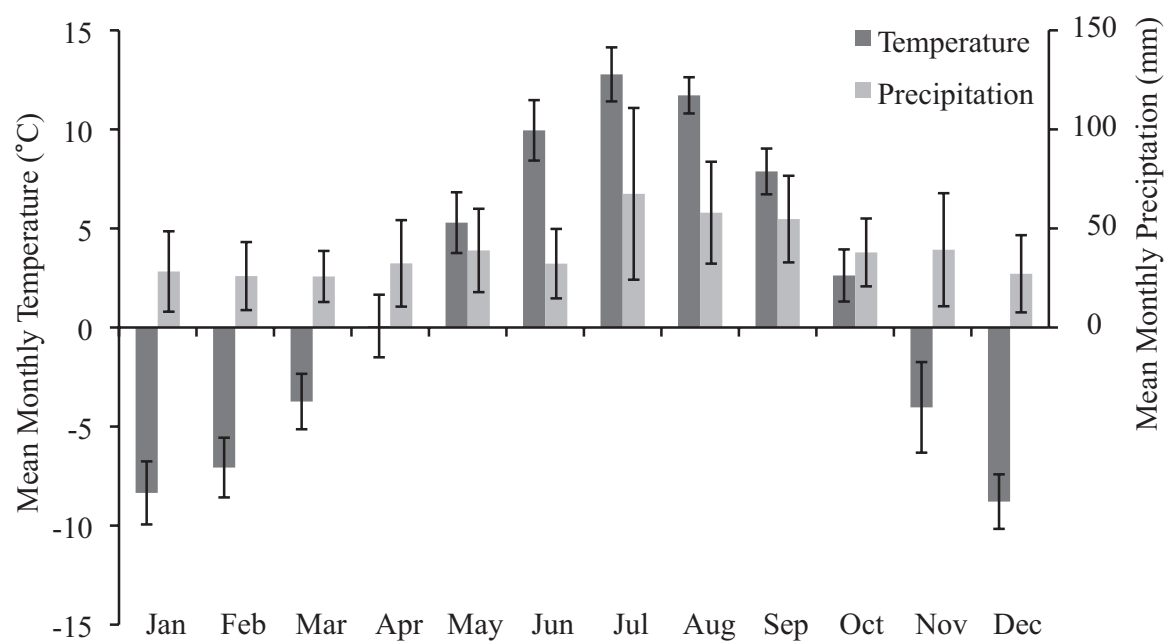
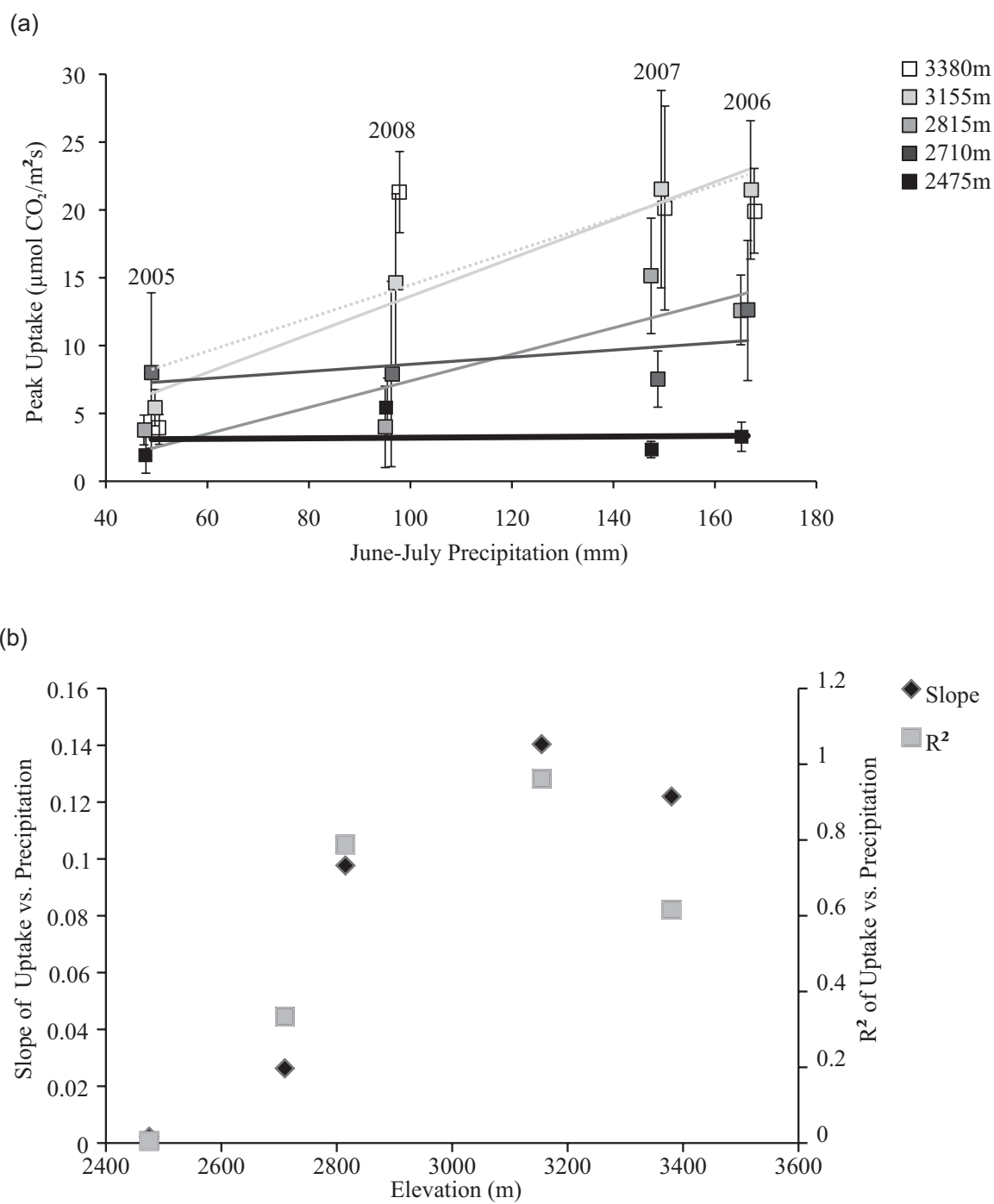


Figure 6:



**APPENDIX B- MICROBES ON MOUNTAINSIDES: CONTRASTING
ELEVATIONAL PATTERNS OF BACTERIAL AND PLANT DIVERSITY**

Microbes on mountainsides: Contrasting elevational patterns of bacterial and plant diversity

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Keywords: elevation gradient; microbial ecology; phylogenetic diversity; macroecology; biogeography

Published Aug 12, 2008 as:

Bryant JA, Lamanna C, Morlon H, Kerkhoff AJ, Enquist B, Green JL (2008) Microbes on mountainsides: Contrasting elevational patterns of bacterial and plant diversity. *Proceedings Of The National Academy Of Sciences Of The United States Of America* **105**: 11505-11511

DOI: 10.1073/pnas.0801920105

Abstract

The study of elevational diversity gradients dates back to the foundation of biogeography. Although elevational patterns of plant and animal diversity have been studied for centuries, such patterns have not been reported for microorganisms and remain poorly understood. Here, in an effort to assess the generality of elevational diversity patterns, we examined soil bacterial and plant diversity along an elevation gradient. To gain insight into the forces that structure these patterns, we adopted a multi-faceted approach to incorporate information about the structure, diversity, and spatial turnover of montane communities in a phylogenetic context. We found that all observed patterns of plant and bacterial diversity were fundamentally different. While bacterial taxon richness and phylogenetic diversity decreased monotonically from the lowest to highest elevations, plants followed a unimodal pattern, with a peak in richness and phylogenetic diversity at mid-elevations. At all elevations bacterial communities had a tendency to be phylogenetically clustered, containing closely related taxa. In contrast, plant communities did not exhibit a uniform phylogenetic structure across the gradient: they became more overdispersed with increasing elevation, containing distantly related taxa. Finally, a novel metric of phylogenetic beta-diversity showed that bacterial lineages were not randomly distributed, but rather exhibited significant spatial structure across the gradient, whereas plant lineages did not exhibit a significant phylogenetic signal. Quantifying the influence of sample scale in intertaxonomic comparisons remains a challenge. Nevertheless, our findings suggest that the forces structuring micro- and macroorganism communities along elevational gradients differ.

Introduction

Roughly 250 years ago, Carolus Linnaeus documented how distinct plant and animal communities characterized the succession of climatic zones along the slopes of mountains (Linnaeus 1781). Such elevational gradients are characterized by dramatic changes in climate and biotic turnover over short geographic distances. Indeed, the patterns observed by Linnaeus and his contemporaries played a foundational role in the development of ecology and biogeography (Briggs & Humphries 2004). Studies of how individual taxa and community composition respond to elevational gradients have led to a search for generalized elevational patterns of biodiversity (Brown 2001; Lomolino 2001; McCain 2005). These studies have documented elevational patterns of diversity across a wide variety of taxonomic groups, including trees, mammals, birds, reptiles, insects, and amphibians. In sum, this work has shown that taxa generally exhibit either monotonically decreasing or hump-shaped richness patterns with elevation (Stevens 1992; Rahbek 2005). However, despite a large number of proposed hypotheses to explain elevation patterns of diversity, their causes remain poorly understood. Improved knowledge of elevation gradients is fundamental to advancing basic ecology and for predicting the potential consequences of climate change. Species in montane regions are often cited as being particularly sensitive to warming (McDonald & Brown 1992; McCain 2005; Parmesan 2006; Thuiller 2007).

While elevational patterns of diversity for plants and animals are well established, we know very little about how microbial diversity varies across elevational gradients.

This is a serious gap in our general understanding of biodiversity, given that microbes are abundant, diverse, play a central role in ecosystem functioning, and will likely be an important component of ecosystem response to global warming (Rillig *et al.* 2002; Wardle 2002; Monson *et al.* 2006; Carney *et al.* 2007). Elevational diversity studies that consider empirical patterns of macro and microorganisms in parallel are needed to provide a more unified framework for understanding diversity patterns in earth's major environmental gradients, and for predicting system-wide ecological responses to climatic change.

Traditional elevational diversity studies have focused on how patterns of species richness, abundance, and range size change with altitude. These analyses have used a nomenclatural approach by focusing on species identities. However, the increasing availability of molecular phylogenies has renewed interest in using phylogenetic approaches to study the forces that influence patterns of biodiversity and biogeography. Because many species traits are conserved during the evolution of a lineage, one would expect, in general, a positive relationship between a measure of the phylogenetic relatedness of two species and a measure of their overall ecological similarity (phylogenetic niche conservatism) (Harvey & Pagel 1991; Webb 2000; Brooks *et al.* 2006). As a result, analysis of the degree of phylogenetic relatedness of taxa found within and across communities should provide insight into the ecological and evolutionary processes that organize these communities.

Here, in an effort to assess the generality of elevational diversity patterns and the forces that structure these patterns, we quantified both plant and soil bacterial diversity

patterns along an elevational gradient in the Colorado Rocky Mountains. A parsimonious hypothesis is that if the forces structuring biodiversity across the gradient are the same for bacteria and plants, then the resulting taxon and phylogenetic biogeographic patterns will be similar for both groups. Alternatively, if ecological and evolutionary processes along elevational gradients differ between the two groups (e.g., the taxa differ in their dispersal ability, response to environmental heterogeneity, interspecific interactions, or speciation rates), then we would expect them to be characterized by distinct patterns of diversity. To test these hypotheses, we adopted a multifaceted approach that examines diversity in the context of both ecological and evolutionary patterns. Therefore, in addition to the established convention of quantifying patterns of taxon richness and taxon turnover along the gradient (e.g. (Whittaker 1960; Whittaker 1967)), we examined several biodiversity measures that incorporate information about the phylogenetic structure, phylogenetic diversity and phylogenetic turnover of plant and bacterial communities.

Elevational diversity in a phylogenetic context

While the sampling methods and taxonomy used to quantify plant diversity are well established and standardized, microbial surveys vary greatly in their approach to characterizing diversity (Eisen 2007). We determined the bacterial community composition of our soil samples by analyzing a PCR-amplified region of 16S ribosomal DNA (rDNA), the most commonly used indicator of microbial biodiversity. Because bacteria are overwhelmingly diverse in soils we chose PCR primers that narrowed our focal group to the phylum *Acidobacteria*. This subgroup of bacteria is diverse and

ubiquitous in soils (Janssen 2006) and thought to play an important role in biogeochemical cycling (Eichorst *et al.* 2007).

We followed the classic approach to intertaxonomic diversity analysis by comparing patterns of species richness and phylotype richness of plants and bacteria, respectively, along the gradient. We also calculated the phylogenetic diversity of every sampled community, which measures the sum of the total branch length in the community phylogeny (Faith 1992). Phylogenetic diversity is more inclusive than a simple count of species or types, in that it quantifies the evolutionary history of a group of taxa (Vane-Wright *et al.* 1991). Conservation biologists are interested in preserving phylogenetic diversity, as this is fundamental to maximizing evolutionary options for the future (Faith 1994; Myers & Knoll 2001; Sechrest *et al.* 2002; Forest *et al.* 2007). Phylogenetic diversity is also believed to correspond to ‘feature diversity’, meaning the number of evolutionarily derived traits within a biological community (Faith 1992).

In addition to measuring phylogenetic diversity, we quantified community phylogenetic structure along the gradient using two commonly used metrics: a mean pairwise distance metric sensitive to phylogeny-wide patterns (net relatedness index [NRI]) and a nearest taxon- based measure sensitive to patterns at the “tips” of the phylogeny (nearest taxon index [NTI]) (Webb *et al.* 2002). The degree of phylogenetic relatedness quantified by these metrics provides insight into drivers of community assembly. Assuming phylogenetic niche conservatism, phylogenetic clustering within a local assemblage is considered consistent with the hypothesis that selective filters (e.g. environmental conditions) cause local assemblages to comprise closely related taxa

(Webb *et al.* 2002). Phylogenetic overdispersion, on the other hand, can be explained by two possible biotic interactions: competition (Webb *et al.* 2002) or facilitation (Lortie 2007; Valiente-Banuet & Verdu 2007). In the case of competition, more closely related species are hypothesized to compete more strongly with one another. This results in competitive exclusion, which leads to a community of distantly related species. In the case of facilitation, facilitator species are hypothesized to create microhabitats that permit distantly related species adapted to different environments to persist within a local assemblage.

In addition to considering patterns in the diversity and phylogenetic structure within communities along the elevation gradient (so-called alpha-diversity), we investigated how community composition changes across a landscape (beta-diversity). Ecologists have long recognized that beta-diversity is important for understanding the biodiversity of montane ecosystems (Jaccard 1912; Whittaker 1960; Beals 1969; Huey 1978; Hart *et al.* 1999; Brehm *et al.* 2003; Mena & Vazquez-Dominguez 2005). We examined beta-diversity in terms of compositional similarity, defined as the fraction of taxa shared between two samples (Sørensen index), and phylogenetic similarity, defined as the fraction of branch lengths shared between two samples. By analogy with the well-established distance-decay relationship, which describes the decrease in compositional similarity between two communities with increasing geographic distance (or equivalently elevational separation) between them (Soininen *et al.* 2007), we described the decrease in phylogenetic similarity with distance (phylogenetic distance-decay). Our objective in exploring both measures of beta-diversity is to understand not only if there are shifts in

compositional similarity with increasing elevational distance, as expected along an environmental gradient, but to quantify the phylogenetic nature of the shifts.

Phylogenetic similarity reflects the combined additive influence of: 1) lineages that are shared between two communities that lead to shared taxa, and 2) lineages that are shared but ultimately lead to unshared taxa. One can test if the phylogenetic similarity between two communities is solely a consequence of compositional similarity, or if it is also due to a nonrandom structure of shared lineages. A significant phylogenetic distance-decay pattern (i.e. one that differs from that expected by taxa turnover alone: see *Materials and Methods*) reflects significant spatial variability in lineage composition across a landscape. Based on the assumption of phylogenetic niche conservatism described above, changes in lineage composition should correspond to changes in the traits of species. Under this model, a significant phylogenetic distance-decay relationship should reflect strong variability in the ecologically relevant traits of biological communities across a landscape.

Results and Discussion

While bacterial richness decreased monotonically from the lowest to highest elevations, plant richness followed a unimodal pattern with a peak in species richness at mid-elevations (Figure 1A). These contrasting diversity patterns emerged when richness values were calculated for bacterial and plant samples individually, and also when the samples for each respective group were pooled together at every elevational band (following the protocol suggested by (Whittaker 1960)). An altitudinal richness pattern has never been reported for microorganisms. The patterns observed here for microbes and plants are consistent, respectively, with the classical monotonically decreasing and hump-shaped patterns observed across most macroorganism groups (Stevens 1992; McCain 2005; Rahbek 2005). It has been argued that these two contrasting richness patterns may emerge as a result of inconsistent sampling approaches among different studies, rather than an underlying ecological mechanism (Lomolino 2001; Rahbek 2005). By implementing a parallel sample design for the bacteria and plants, we controlled for two potential biases: variation in sampling extent (i.e. the geographic distance between the furthest sampled elevations) which often occurs among studies, and sampling intensity (or effort) along the gradient, which often occurs within studies. As a result, the disparity in elevational richness patterns observed between bacteria and plants is likely due to differences in how ecological and evolutionary processes have operated across the gradient (although see below for a discussion on the potential influence of scaling effects).

As expected, for both bacteria and plants (Brooks *et al.* 2006; Forest *et al.* 2007)

we found that the patterns of phylogenetic diversity mirrored those of taxon richness (Figure 1B). However, a more detailed look at the phylogenetic structure of the bacterial and plant communities revealed another significant difference. At all elevations bacterial communities had a tendency to be more phylogenetically clustered than expected by chance (Figure 2). This observation is consistent with results reported by Horner-Devine and Bohannan (Horner-Devine & Bohannan 2006) who found that bacterial communities in a wide range of environments tended to be phylogenetically more closely related than expected by chance. Given the parsimonious hypothesis that closely related taxa are more ecologically similar (i.e. phylogenetic niche conservatism), our results suggest that abiotic filtering tends to be a more prominent force in the structuring of bacterial communities along the gradient. Several studies have suggested that for most macroorganisms, ecological traits are phylogenetically conserved (Prinzing *et al.* 2001; Blomberg *et al.* 2003; Cavender-Bares *et al.* 2004). It is important to emphasize that while this statement may be correct for macroorganisms, the generality of niche conservatism for microorganisms is unknown. Phylogenetic clustering in microbial communities could also be the result of a radiation event combined with dispersal limitation (Horner-Devine & Bohannan 2006). As we discuss below, alternative explanations for the patterns we observe relate to the phylogenetic and spatial scale of our analyses (Swenson *et al.* 2006; Swenson *et al.* 2007). Scaling issues are relevant to all of the biodiversity patterns we examined.

In contrast to bacteria, plant communities did not show a uniform phylogenetic structure across the gradient. Plant communities tended to exhibit either random

phylogenetic structure or phylogenetic overdispersion. Surprisingly, our analyses indicated that plant communities also tended to become increasingly overdispersed at higher elevations. As discussed above, given niche conservatism, phylogenetic overdispersion is consistent with the importance of biotic forces (competitive exclusion or facilitation) structuring community diversity. Recent experimental evidence suggests that both of these forces are important drivers in alpine plant community assembly, with a shift from competition at lower elevations, where conditions are less physically stressful, to facilitation at higher elevations where abiotic stress is high (Callaway *et al.* 2002). Increased overdispersion at high elevations suggests that the influence of facilitation on high elevation communities is stronger than the influence of competition at low elevations. An alternative explanation is that the evolution of traits necessary to cope with environmental conditions at high elevations has occurred independently in distantly related lineages (i.e. convergent evolution in high alpine plants). This explanation goes against the assumption of niche conservatism (Webb 2002).

We observed that both plant and bacterial compositional similarity significantly decreased with elevational distance (Figure 3). Plant and bacterial communities differed, however, in their phylogenetic distance-decay patterns. The bacterial phylogenetic distance-decay curve was significantly steeper than expected from the observed bacterial taxa turnover alone (Figure 3a). In contrast, the plant phylogenetic distance-decay curve was not significantly different than expected from the observed decay in plant compositional similarity. These results are consistent with those reported above for the NRI and NTI measures of community phylogenetic structure, indicating that, unlike

plants, bacteria lineages were not randomly distributed across the elevation gradient. Rather, bacterial lineages exhibited a spatially structured pattern across the gradient. Given the parsimonious hypothesis that closely related taxa are more ecologically (or functionally) similar, our observations suggest that bacterial lineages harbor increasingly disparate ecological features (or functions) at increased elevational distances as a probable consequence of abiotic filtering. These findings highlight the utility of gathering information on phylogenetic relationships between communities in montane regions as a means to quantify the potential consequences of selectively trimming evolutionary lineages under the scenario of mountaintop extinctions in response to global warming.

Although our study was not designed to directly examine the environmental drivers of elevational diversity patterns, our results do illuminate their potential role in shaping biodiversity patterns across the gradient. The contrasting phylogenetic diversity patterns we observed in plants and microbes suggest a differing role in how abiotic forces structure communities across the gradient. Soil temperature and pH were consistently correlated with diversity in both plants and bacteria, and bacterial diversity was also strongly correlated with slope in a univariate analysis (Table 1). A multivariate analysis suggested that soil temperature was the major explanatory variable of taxon richness and phylogenetic diversity for both plants and bacteria ($P < 0.001$ in six out of eight multivariate models). Turnover in taxon and phylogenetic composition of plant and bacterial communities was significantly correlated with changes in the majority of our measured environmental parameters (Table S3); however, the combined influence of soil

temperature, pH and total nitrogen was the most important predictor for both groups. After controlling for these environmental parameters, geographic distance between samples significantly correlated with all turnover patterns (partial Mantel test, $P < 0.001$). These results imply that dispersal limitation could be occurring, but given the small geographic range of our gradient, they are more likely due to effects of environmental heterogeneity that we did not characterize. The correlation of richness and turnover with temperature and pH is consistent with the findings of other studies of plant (Allen *et al.* 2002; Hawkins *et al.* 2003; Currie *et al.* 2004) and microbial (Fierer & Jackson 2006) diversity.

It is well documented that the scale over which biodiversity is sampled will strongly influence observed patterns. For example, recent empirical studies have shown that decreasing the spatial grain at which organisms are sampled shifts their diversity patterns (Rahbek 2005; Cavender-Bares *et al.* 2006; Slingsby & Verboom 2006; Swenson *et al.* 2007). Although the spatial extent of our study was the same for bacteria and plants, the grain of our sample observations was different between these two groups. The spatial scales over which bacteria interact with each other are likely to be several orders of magnitude smaller than the scale at which they were sampled. This suggests that relative to plants, bacteria were sampled at a coarser grain, and thus we may have included a greater amount of environmental heterogeneity within a sample. Sampling bacteria at a spatial scale that more closely approaches the ‘ecologically equivalent’ grain of plants may result in convergent biodiversity patterns between these two groups.

Taxonomic scale also influences biodiversity patterns. For example taxonomic

breadth, which defines how broadly or narrowly a target community is defined from a phylogenetic perspective (e.g. bacteria versus *Acidobacteria*), can shift the degree of observed overdispersion, or clustering in that community. Although species are a natural taxonomic unit by which to measure plants (Mayr 1942), such an intuitive unit does not exist for prokaryotes. In this study we classified *Acidobacteria* 16S rDNA sequences into taxonomic units based on the commonly used 99% sequence similarity designation (see *Materials and Methods*). It is unknown how taxonomic resolution, which determines the threshold at which individuals are binned into taxonomic units, should influence phylogenetic patterns, although it has been shown to impact taxonomic patterns such as the taxa-area relationship (Horner-Devine *et al.* 2004a). We found that binning bacteria into increasingly broader taxonomic units (i.e. 97%, 94% and 90% sequence similarity) tended to dampen the strength of all observed elevational diversity patterns. The general trends did not qualitatively change (Figures S2-4), suggesting that taxonomic resolution is not the cause of disparate bacterial and plant biodiversity patterns in this study. Alternative approaches to defining bacterial taxonomic units such as ‘ecotypes’ (Cohan & Perry 2007) could significantly change the results, and lead to plant and microbial diversity patterns that more resemble one another.

Differences in the approach to building the *Acidobacteria* and Angiosperm phylogenetic trees should also be considered when comparing phylogenetic patterns between these two groups. The *Acidobacteria* phylogeny was estimated solely from molecular data identified in this study, whereas the Angiosperm phylogenetic tree topology was constructed using the widely accepted super tree approach (Webb & Donoghue

2005) and branch lengths were assigned based on estimates of the minimum age of internal nodes (see *Materials and Methods*). Comparative analyses using molecular approaches alone for both plants and microbes would improve our confidence in such phylogenetic comparisons. Such approaches will be facilitated in the future by increased accessibility to molecular data.

Microorganisms (especially prokaryotes) are very diverse in soils (Torsvik *et al.* 1990; Janssen 2006). On par with most microbial diversity studies, it is likely that we sampled the most abundant taxa in each soil core along the elevational gradient. While sampling effort (i.e. the proportion of a community that is sampled) is known to significantly influence taxonomic biodiversity patterns (Plotkin & Muller-Landau 2002; Woodcock *et al.* 2006; Green & Plotkin 2007), to our knowledge the influence of sampling effort on phylogenetic biodiversity patterns has not been explored. For example, estimators are available to predict the taxon richness (Hughes *et al.* 2001) and taxon similarity (Chao *et al.* 2005) of ecological communities from sample data, but there are currently no estimators to predict phylogenetic richness, phylogenetic structure or phylogenetic turnover from sample data. A new generation of estimators is needed for future comprehensive studies that examine taxonomic and phylogenetic diversity patterns in parallel.

As discussed by others, a promising approach to understanding elevational diversity patterns (Rahbek 2005), and more generally biodiversity patterns (Green & Bohannan 2006), is to conduct intertaxonomic comparisons to elucidate the spatial and taxonomic scales and degree of sampling effort over which microbial biodiversity

relationships approach those of macroorganisms. Such an approach is ambitious, but increasingly tractable as molecular approaches advance our ability to comprehensively characterize biodiversity. Here, we have shown that across an elevation gradient, plant and microbial communities exhibit very different patterns of diversity. Phylogenetic-based analyses suggest that the evolutionary and ecological processes driving the biogeographic patterns may differ significantly between these two domains of life. Further work is needed to link the phylogenetic patterns to functional differences among plant and bacterial taxa. Such comparative analyses are needed to provide the empirical foundation for a truly inclusive and predictive theory linking patterns of biodiversity to ecosystem function.

Materials and Methods

Study site and Sampling.

We sampled angiosperm and *Acidobacteria* communities at five sites along an elevational transect located near the Rocky Mountain Biological Laboratory, Gunnison County, Colorado. The sites extend from 2460 m to 3380 m above sea level and spanned a geographic distance of 39 km. Within each study site, we placed five 1 m by 1 m quadrats in a sub-transect running down the slope. Three soil samples, separated by 1 cm were collected adjacent to the middle, highest, and lowest quadrats at each site (9 total). All soil samples were collected from the B-horizon using sterile glass collection jars. After collection, the soil samples were homogenized and stored at -80°C until analysis.

Average soil temperature at each site was measured by placing Hobo Temperature Data Loggers (OnSet Corporation, Bourne, MA) at 10 cm depth in relatively open patches and recording soil temperature every hour for the month of July in 2007. Total carbon and nitrogen in the soil samples were measured using a Costech ECS 4010 CHNS-O system (Costech Instruments, Milan, Italy). Soil pH was measured after shaking a soil water (1:3 weight/volume) suspension for 30 minutes. Soil moisture was measured gravimetrically. The ArcGIS data and area photos used to calculate slope and aspect of the sites had a 6 inch resolution per pixel and a horizontal accuracy of 2-3 feet. The environmental data along with a description of how environmental parameters correlate with one another is provided in Table S1 and S2 respectively.

Characterization of Acidobacteria Communities with 16s Clone Libraries.

At each site, the bacterial communities within the three soil samples collected adjacent to the middle quadrat and one soil sample adjacent to the lower and upper quadrats were characterized using sequence analysis of clone libraries (5 total). DNA was extracted using Mobio Power Soil DNA Isolation kits (MoBio Laboratories, Carlsbad, CA). Triplicate PCR reactions were carried out on each soil extraction using the Acidobacteria-specific PCR primer set Acid31/Eub518 (Barns *et al.* 1999). The 25 μ l PCR mixtures were composed of 10 μ l of 5 Prime MasterMix (5 Prime, Inc. Gaithersburg, MD), 14 μ l of water, 1 μ mole of each primer and 1 μ l of DNA extract. The PCR conditions used were as follows: 3 minutes at 94°C 25 cycles of 30 seconds at 94°C, 30 seconds at 50°C (Fierer *et al.* 2005), 60 seconds at 72°C and a final extension for 5 minutes at 72°C.

Triplicate PCR reactions were pooled and then gel-purified using a MinElute PCR Purification kit (Qiagen, Valencia, CA). Amplicons were ligated into pCR4-TOPO vectors and cloned using a TOPO-TA cloning kit (Invitrogen). 96 clones from each soil sample were selected for sequencing. Plasmid purification and sequencing of cloned PCR products was done at the Qiagen Genomic Services/Sequencing facility with an ABI 377 or 377xl sequencer (Applied Biosystems, Foster City, CA). Sequences have been deposited into GenBank under the accession numbers EU424348-EU426543.

A total of 2239 cloned 16s rRNA genes were aligned with the NAST alignment tool (DeSantis *et al.* 2006a) and the alignments were manually edited based on conserved primary sequence and secondary structure information in the ARB software package

(Ludwig *et al.* 2004). The phylum affiliation of each sequence was checked using the classify tool available at the green genes database (DeSantis *et al.* 2006b) and the blast tool within NCBI (Altschul *et al.* 1990). Potentially chimeric sequences were identified using the Bellerophon server (Huber *et al.* 2004) and then manually assessed by building trees in ARB that contained a set of reference sequences and the 5'- and 3'- sides of the putative chimeras. Sequences were removed from the analysis, that had 5'- and 3'- ends affiliating with different groups of reference sequences in the tree (Horner-Devine *et al.* 2004b).

There is no standard definition of microbial species. Therefore we grouped our 2196 non-chimeric *Acidobacteria* sequences into phylotypes with a <99% sequence similarity cutoff using the programs Phylip (Felsenstein 1989) and DOTUR (Schloss & Handelsman 2005) This is a commonly used phylotype designation (Kroes *et al.* 1999), which provides high phylogenetic resolution. One sequence was randomly chosen to represent each phylotype. The representative sequences were used to build a phylogenetic tree by maximum likelihood methods using the program phyML (Guindon & Gascuel 2003). We used Jukes-Cantor and gamma substitution models where the gamma distribution parameter was estimated from the data. Only informative base positions were used to bin sequences into phylotypes and to build the microbial phylogenetic tree. The newick format and image of the *Acidobacteria* phylogenetic tree along with a list of OTUs identified in each soil core are available in the supplementary material (Figure S5, Figure S7 and Table S1 respectively). All diversity analyses were later repeated using 97%, 94% and 90% sequence similarity cutoffs.

Characterization of Plant Communities.

Angiosperms within each quadrat were identified to species level and checked against RMBL Herbarium specimens jointly by Brian Enquist, Andrew Kerkhoff and Christine Lamanna. Vouchers are currently being prepared for deposition in the RMBL Herbarium and the University of Arizona Herbarium. All plants were identified in 2005 except for the lowest site, which was identified in 2006. Plots were sampled at one point during the growing season, and thus some individuals with later phenologies could not be identified to species. We staggered the plant sampling dates with the aim of sampling each community at the same relative phenological time point. Any individuals that could not be identified to species or differentiated from known species were excluded from the analysis. This affected between 10 and 15% of the possible species at each site. We used version R20031202 of phylomatic to construct a tree topology consisting of all the angiosperms identified in all our quadrats, based on the Angiosperm Phylogeny Group (APG) II backbone (II 2003; Webb & Donoghue 2005). We used results from recent plant cladistics studies to resolve polytomies (Worberg *et al.* 2007; Winkworth *et al.* in press). The final tree we used for our analyses was resolved to the family level. We assigned branch lengths to the tree by using the phylocom module BLADJ to constrain the internal nodes with available age estimates (Wikstrom *et al.* 2001) and interpolated the other nodes for which direct age estimates are not available. The newick format and image of the angiosperm phylogenetic tree along with a list of plants identified in each quadrat are available in the supplementary material (Figure S5, Figure S6 and Table S1

respectively).

Diversity Analyses

Taxon richness and phylogenetic diversity. We define the term community as all phylotypes originating from a single soil core (bacteria) or species identified in a single quadrat (plants). Taxon richness within each community was quantified as the total number of species or phylotypes within that community. Phylogenetic diversity within each community was quantified as the minimum total branch length connecting all species within the community to the root of the phylogenetic tree (Faith 1992).

Phylogenetic diversity was calculated using the pd module within phylocom-3.40 (Webb *et al.* 2007). We used a rarefaction sampling approach to account for the unequal sample sizes of each microbial community (number of clones) by calculating the mean of the taxon richness and phylogenetic diversity of 1000 randomized subsamples of each community. Each community was subsampled by the number of clones in the smallest library (75 clones).

Phylogenetic community structure. Using the classical net relatedness index (NRI) and nearest taxon index (NTI) (Webb 2000; Webb *et al.* 2002), we measured the extent to which co-occurring species in a community are phylogenetically related compared to what is expected by chance. With both indices, the phylogenetic structure of the observed community was compared to a null expectation obtained by randomly sampling the pool of all the species identified in the study 1000 times, while constraining both the number

of taxa in the community and species occurrence across communities (Kembel & Hubbell 2006). Observed values smaller or larger than 975 of the randomizations were considered significantly structured ($P < 0.05$).

Compositional and phylogenetic similarity. Compositional similarity between all pairwise comparisons of communities was quantified with the Sørensen Index:

$$Sor_{ij} = \frac{S_{ij}}{(S_i + S_j)^{1/2}}$$

where S_{ij} is the number of taxa common to both communities i and j , while S_i and S_j are the total number of species found in community i and j , respectively (Krebs 1998). By analogy, phylogenetic similarity between two communities was quantified using a newly derived index, coined PhyloSor:

$$PhyloSor_{ij} = \frac{BL_{ij}}{(BL_i + BL_j)^{1/2}}$$

Here, BL_{ij} is the branch length common to both communities i and j , while BL_i and BL_j are the total branch lengths of community i and j , respectively.

The PhyloSor index ranges from indefinably close to 0, (two communities only share a very small root) to 1 (both communities are composed of the same taxa). Similar approaches have been carried out by Lozupone and Knight (Lozupone & Knight 2005) and Ferrier et al. (Ferrier *et al.* 2007) when considering the closely related Jaccard and Bray-Curtis similarity indices. Using PhyloSor, one can test if two communities are

phylogenetically more or less similar than what is expected given their taxa similarity. This is done by comparing the phylogenetic similarity of the observed communities to a null expectation obtained by randomly sampling the pool of all the species identified in the study while constraining the number of taxa in each community and the number of taxa shared by the two communities.

By analogy with the well-established distance-decay relationship, which describes the decrease in compositional similarity between two communities with increasing geographic distance (or equivalently elevational separation) between them (Soininen *et al.* 2007), we used PhyloSor to quantify the decrease in phylogenetic similarity with distance (phylogenetic distance-decay). We tested whether the slope in the decay of phylogenetic similarity was greater or less than what was expected given the taxonomic decay in similarity by comparing the observed slope to a distribution of distance-decay slopes obtained by randomizing the location of taxa at the tips of the community phylogenetic tree 1000 times. This is equivalent to randomly sampling the taxa while constraining the number of taxa in each community, the number of taxa shared by any two communities, and taxa occurrence across all communities. The observed phylogenetic slope was assumed significantly different from the null if it was greater than or less than 975 of the slopes of the randomizations (2-tailed test, $P < 0.05$). To address the unequal sampling of microbial communities, we repeatedly calculated and tested the significance of distance-decay slope on subsampled communities, where communities were subsampled by the number of clones in the smallest library. The significance of results did not differ between repeated subsamples.

Linking Diversity Measurements to Environmental Parameters. To determine the correlation between potential environmental drivers and the observed diversity patterns, we used a polynomial regression analysis. For each environmental variable, we fit a linear and a quadratic regression model. Model selection was based on Akaike's Information Criterion (AIC) differences (Burnham & Anderson 2002). Using a stepwise regression to select variables and interactions, a multivariate model was constructed for each diversity type in JMP.

Mantel tests (999 permutations) were used to determine if compositional and phylogenetic similarity decayed significantly with elevational distance (Legendre & Legendre 1998). Similarity values between pairwise comparisons of microbial communities were the averages of 1000 rarefaction samples, as described above (*Taxon richness and phylogenetic diversity*). The best fit and the most homoscedastic residuals were found in models that used the log transformation of similarity against elevational distance, with the exception of angiosperm taxa similarity, which was best described by a linear-linear distance-decay model. We used Mantel tests to examine correlations between community similarity and environmental similarity (for a discussion on these methods see Legendre *et al.* (2005); Toumisto & Ruokolainen (2006)). We chose the combination of environmental variables that best explained the changes in angiosperm and *Acidobacteria* community composition between samples with BIO-ENV (Clark & Ainsworth 1993) and tested the importance of these variables after controlling for geographic distance and vice versa using partial mantel tests. For all analyses, Moisture,

Carbon and Nitrogen were $\arcsin(\sqrt{y})$ transformed and aspect was $1/y$ transformed (Legendre & Legendre 1998).

Supplementary Information:

Table S1: Excel spreadsheet of all biotic and abiotic data (available online)

Table S2: Pearson correlation coefficient for all measured environmental parameters

Table S3: Mantel statistics between community and environmental similarity

Figure S1: Within-site portion of Figure 3.

Figure S2: Microbial elevational richness patterns at broader taxonomic resolutions

Figure S3: Microbial NRI/NTI at broader taxonomic resolutions

Figure S4: Microbial turnover at broader taxonomic resolutions

Figure S5: Newick format of phylogenetic trees

Figure S6: Angiosperm phylogenetic tree

Figure S7: *Acidobacteria* phylogenetic tree

Acknowledgements

J.L.G. acknowledges support from the National Science Foundation (MCB 0500124).

B.J.E., A.J.K. and C. A. L acknowledge funding support from an NSF CAREER award, the University of Arizona and a Los Alamos (DOE) LDRD award to BJE. This work could not have been completed without the support of the University of California, Merced. We also especially thank our field crew from the University of Arizona - Laura Crumbacher, Peter Gaube, Melissa Wilson, James Stegen, Jason Pither, and Robin Sleith for help collecting the plant data and soil cores. In addition, we wish to thank the staff and researches of the Rocky Mountain Biological Station for their support of this project, in particular J. Harte and I. Billick. We thank Brendan Bohannon, Michael Donoghue, Ian Wright, James O'Dwyer, Kathryn Docherty, Rebecca Mueller and an anonymous reviewer for useful comments. We thank John Avise, Francisco Ayala and Steve Hubbell for organizing this Sackler Colloquium.

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Tables

Table 1: Pearson correlation coefficients from univariate models predicting diversity as a function of environmental parameters.

Diversity Type	Elev	Temp	pH	Slope	Aspect	% N	% C	% M
Microbial NRI	0.056	-0.063	-0.025	<i>0.227</i>	0.018	0.037	0.0001	0.002
Plant NRI	-0.696***	0.699***	0.536***	-0.411**	0.132	<i>0.094</i>	0.056	0.001
Microbial NTI	0.101	-0.120	-0.054	0.048	-0.008	0.0004	-0.233	-0.026
Plant NTI	-0.109	0.109	-0.015	-0.015	0.002	-0.059	-0.037	-0.004
Microbial Phylo Richness	-0.228*	0.216*	0.132	<i>-0.311*</i>	0.030	-0.0001	<i>0.116</i>	-0.0009
Plant Phylo Richness	<i>0.473**</i>	<i>-0.443**</i>	-0.283*	0.069	0.002	<i>0.229</i>	0.022	0.056
# OTUs	-0.222*	0.174*	0.205*	-0.239*	0.044	-0.002	0.0002	-0.148
# Plant Species	<i>0.533***</i>	<i>-0.502***</i>	-0.189*	0.004	0.039	<i>0.271</i>	0.077	0.045

For each environmental parameter both a linear and a quadratic model were fit. The best fit model was chosen using the lowest Akaike Information Criterion (AIC) value, where we required the AIC to be drop at least three points to consider the quadratic model the best fit. Quadratic models are indicated with *italics*, and a minus sign (-) is used to indicate the direction of the linear correlation if it is negative. *: $p \leq 0$

Figure Captions

Figure 1. Variation in (A) taxon richness and (B) phylogenetic diversity across the elevation gradient. Data are presented as the fraction of total richness and phylogenetic diversity across the gradient. Solid symbols indicate sample richness (core or quadrat) while open symbols indicate the pooled richness at each elevational site ($n = 5$ per site). *Acidobacteria* richness and phylogenetic diversity linearly decrease with elevation at the sample level, whereas angiosperm diversity patterns are hump-shaped (Table 1). Model choice was based on Akaike information criteria (see *Materials and Methods*).

Figure 2. Variation in community phylogenetic relatedness along the elevation gradient as measured with the (A) NRI and (B) NTI. Positive index values indicate phylogenetic clustering and negative values indicate phylogenetic overdispersion. Observed community phylogenetic structures unlikely to arise by chance ($P < 0.05$) are depicted with solid symbols. All microbial communities are clustered with more than 50% being significantly clustered. Angiosperm communities are not uniformly clustered or dispersed across the gradient, but rather become increasingly overdispersed with increasing elevation. This trend in increased overdispersion with elevation is significant when measuring relatedness with the NRI (solid line, $r_2 = 0.70$, $P < 0.001$).

Figure 3. Compositional and phylogenetic similarity of A) *Acidobacteria* communities and B) angiosperm communities, as a function of the elevation separating the communities. The compositional (blue) and phylogenetic (black)

similarity for both angiosperm and *Acidobacteria* communities significantly decrease with increasing elevational separation (Mantel test, $P < 0.001$). Lines represent best-fit regressions of similarity versus change in elevation (see *Materials and Methods*). The slope of the decay of phylogenetic similarity between *Acidobacteria* communities is significantly steeper than predicted by a null model constrained by the decrease in taxon turnover (red) ($P < 0.05$). The slope of the decay in phylogenetic similarity across the angiosperm communities is not significantly different from the null prediction given species turnover. Figure S1 illustrates within-site similarity as a function of elevational distance.

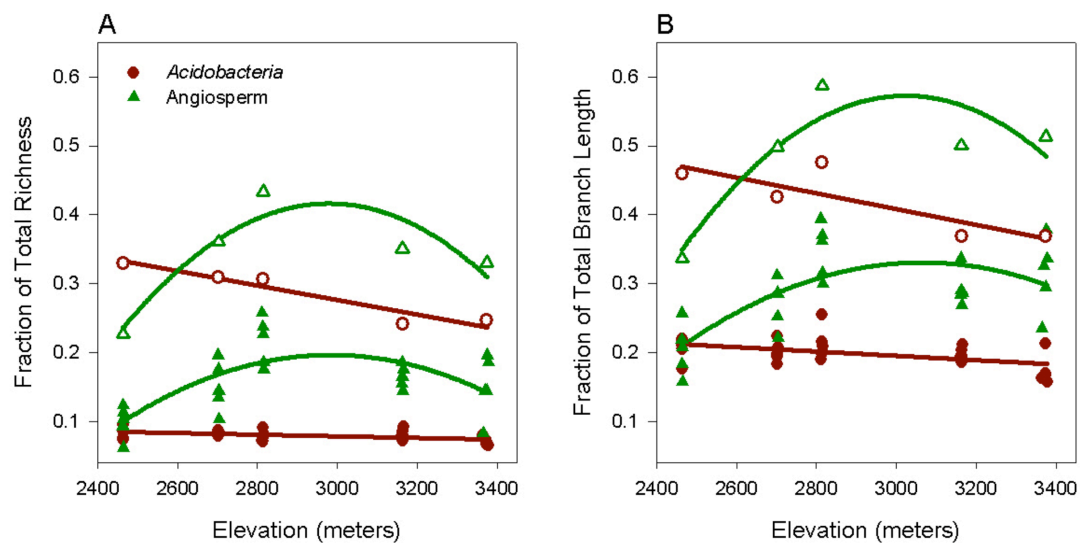
Figure 1:

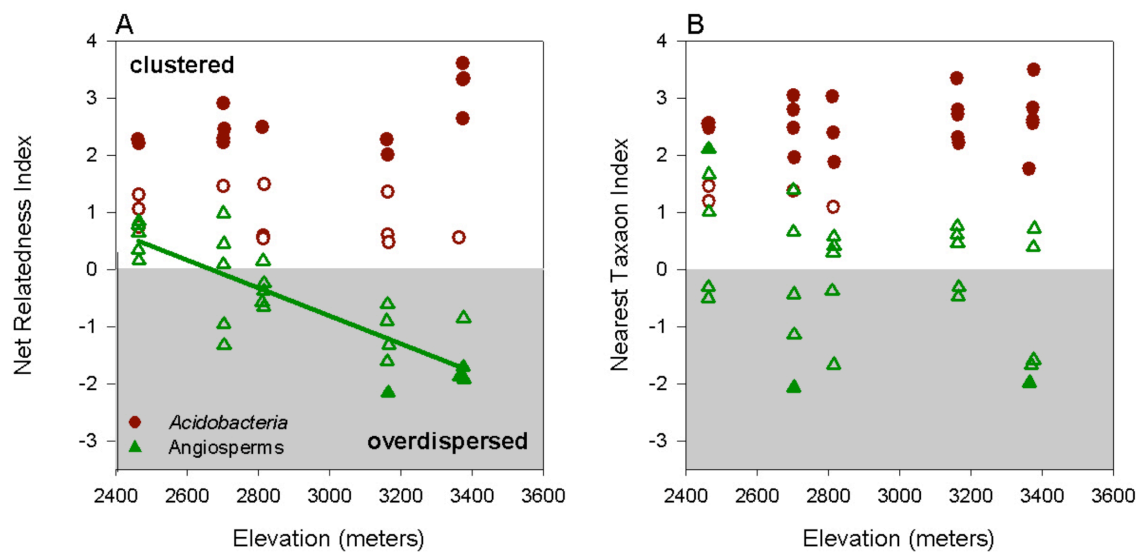
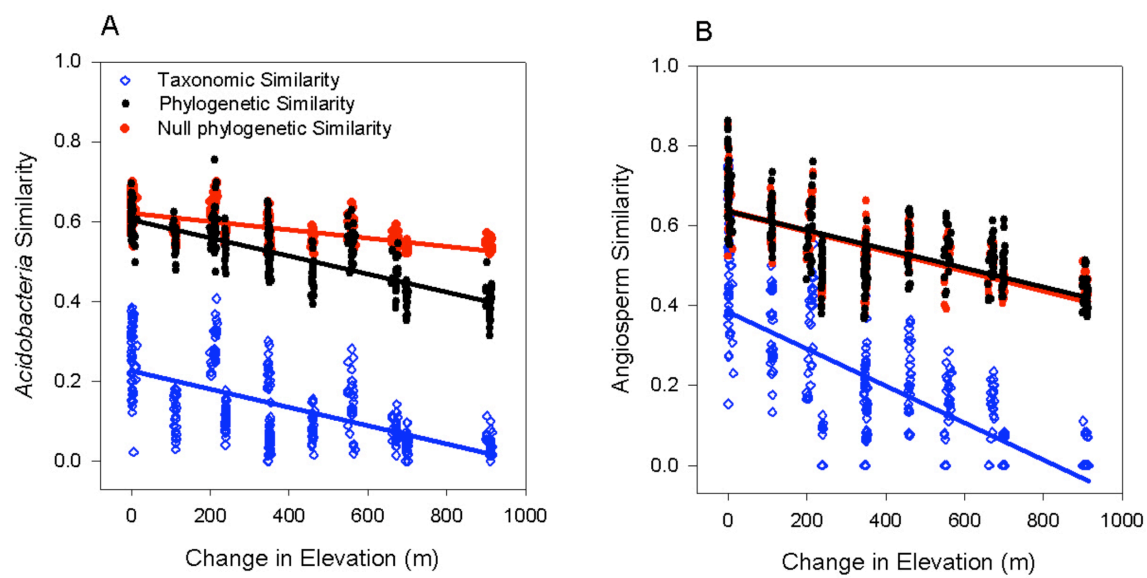
Figure 2:

Figure 3:

SUPPLEMENTARY MATERIAL

Table S2: Pearson correlation coefficients between environmental parameters.

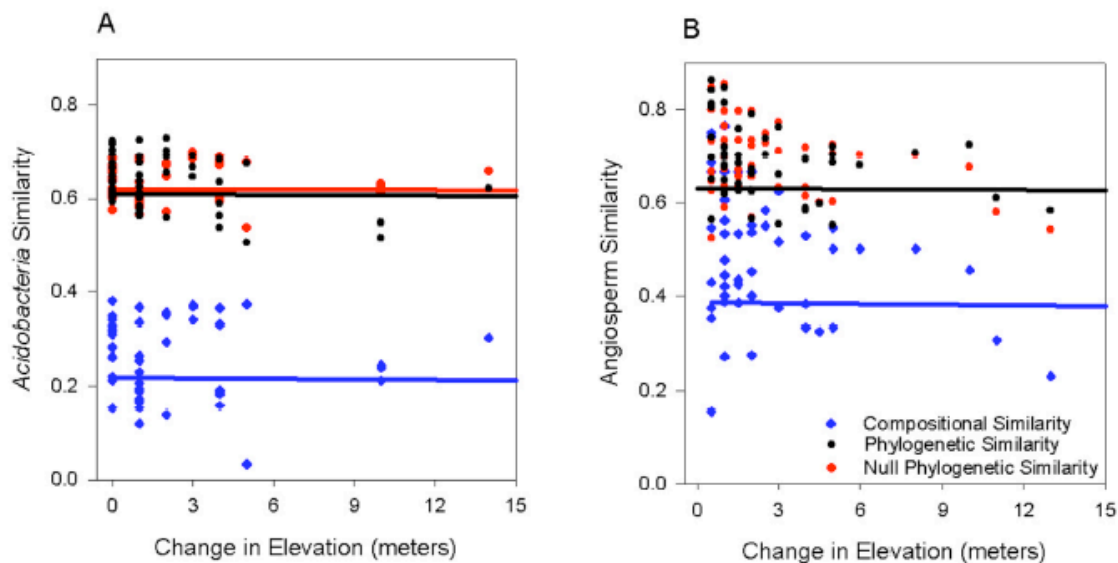
	Elev	Temp	pH	Slope	Aspect	% N	% C	% M
Elev	1.000	-0.989***	-0.882***	0.795***	-0.415*	-0.117	-0.327	0.001
Temp	-0.989***	1.000	0.858***	-0.721***	0.327	0.071	0.297	0.036
Ph	-0.882***	0.858***	1.000	-0.758***	0.416	0.072	0.245	-0.097
Slope	0.795***	-0.721***	-0.758***	1.000	0.801***	-0.491*	-0.582**	-0.085
Aspect	-0.415*	0.327	0.416	-0.801***	1.000	0.683***	0.624***	0.381*
% N	-0.117	0.071	0.072	-0.491*	0.683***	1.000	0.944***	0.641**
% C	-0.327	0.297	0.245	-0.582**	0.624***	0.944***	1.000	0.585**
% M	0.001	0.036	-0.097	-0.085	0.641*	0.641**	0.585**	1.000

*: $p \leq 0.05$, **: $p \leq 0.005$, *** : $p \leq 0.0005$

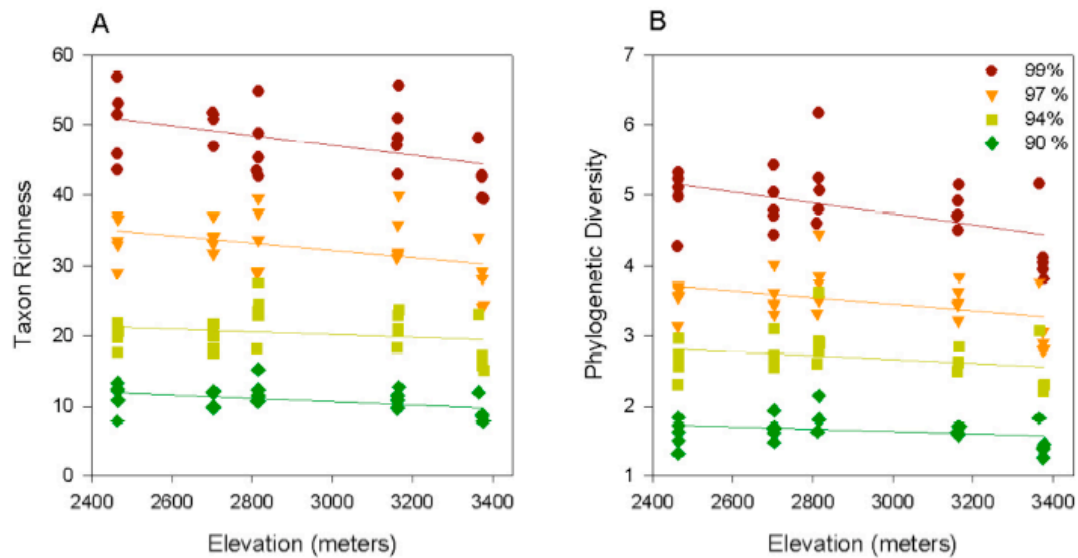
Table S3: Mantel correlations between community and environmental similarities.

	Elevation	Temp	pH	Slope	Aspect	Total Nitrogen	Total Carbon	Moisture
Acidobacteria compositional similarity†	-0.57**	-0.57**	-0.64***	-0.19*	-0.10	-0.32**	-0.17*	0.17*
Acidobacteria phylogenetic similarity††	-0.78	-0.76**	0.76**	-0.40**	-0.24*	-0.18*	-0.06	-0.04
Angiosperm compositional similarity†	-0.69**	-0.72**	-0.44**	-0.24*	-0.26*	-0.33*	-0.25*	-0.07
Angiosperm phylogenetic similarity††	-0.65**	-0.65**	-0.37**	-0.16	-0.19	-0.38*	-0.27*	-0.23*

†Sørensen index, ††PhyloSor (*see Materials and methods*). *: $P \leq 0.05$, **: $P \leq 0.005$

Figure S1

Within-site (0 to 15 meter abscissa range) portion of Figure 3, showing the compositional and phylogenetic similarity of A) *Acidobacteria* communities and B) angiosperm communities, as a function of elevational separation. Lines represent the best-fit regressions of similarity versus change in elevation across cores within and between sites.

Fig. S2:

Variation in *Acidobacteria* (A) taxon richness and (B) phylogenetic diversity

across the elevation gradient at four different taxonomic resolutions. Taxonomic

richness is the total number of taxa (operational taxonomic units) and phylogenetic

diversity is the minimum total branch length connecting all taxa in the community and

the root. Richness and phylogenetic diversity are best modeled as linearly decreasing

with elevation at all taxonomic resolutions (Akaike information criteria). Linear

regression slopes of the taxon richness patterns: 99%: -0.006982*; 97%: -0.005158*;

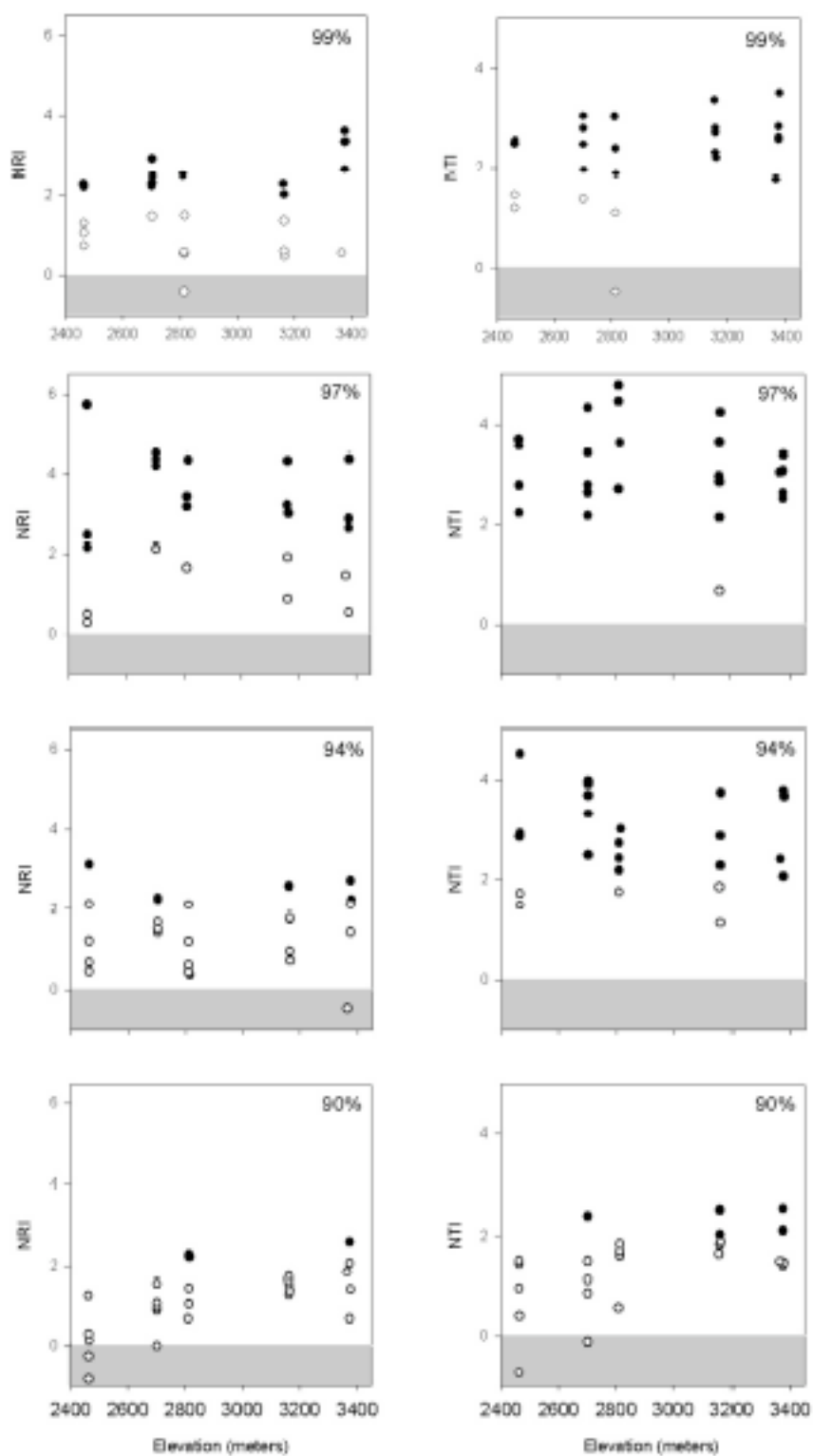
94%: -0.001939; 90%: -0.002364*. Slopes of the phylogenetic diversity pattern: 99%: -

0.000168; 97%: -0.000276; 94%: -0.000460; 90%: -0.0007653. Asterisks indicate that

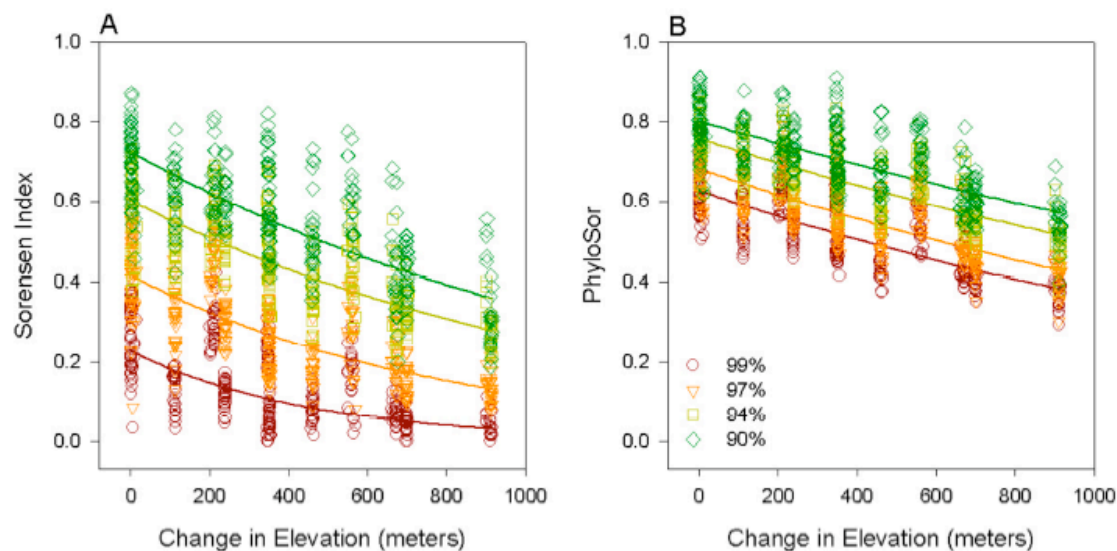
the decrease in diversity with elevation is significant ($P < 0.05$) based on linear

regression analysis.

Fig S3:



Microbial NRI and NTI values across the elevational gradient at four different taxonomic resolutions. Observed community phylogenetic structures unlikely to arise by chance ($P < 0.05$) are depicted with solid circles.

Fig S4:

A) Compositional and B) phylogenetic similarity of *Acidobacteria* communities at four taxonomic resolutions. The compositional and phylogenetic similarity between *Acidobacteria* communities significantly decreases with increasing elevational separation (Mantel test, $P < 0.001$) at all taxonomic resolutions presented. The slope of the decay of phylogenetic similarity between *Acidobacteria* communities is significantly steeper than predicted by a null model constrained by the decrease in taxon similarity ($P < 0.05$). As in Figure 3, lines represent an exponential model fit to the observed data.

Figure S5: Newick formatted phylogenetic trees used in this study:

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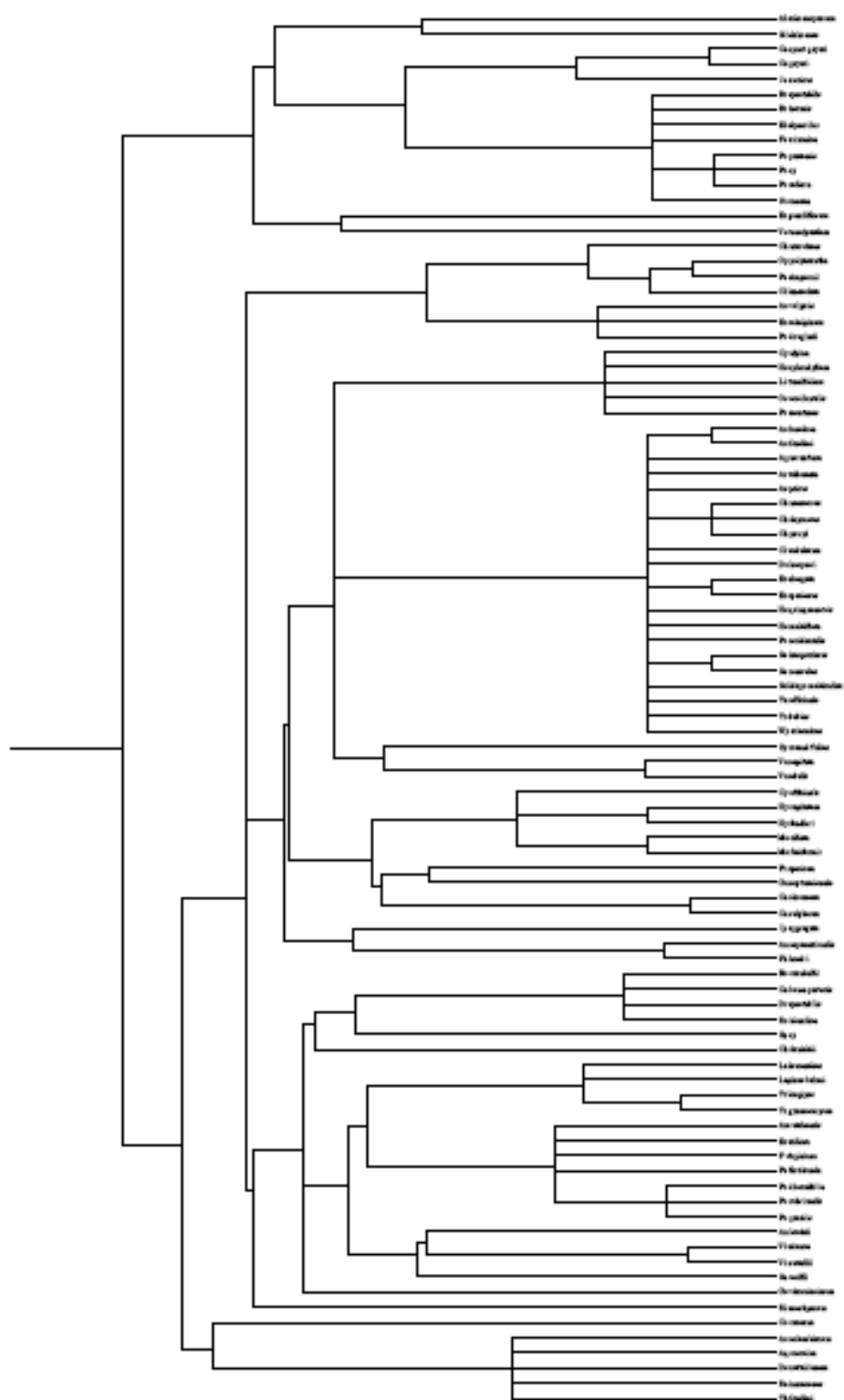
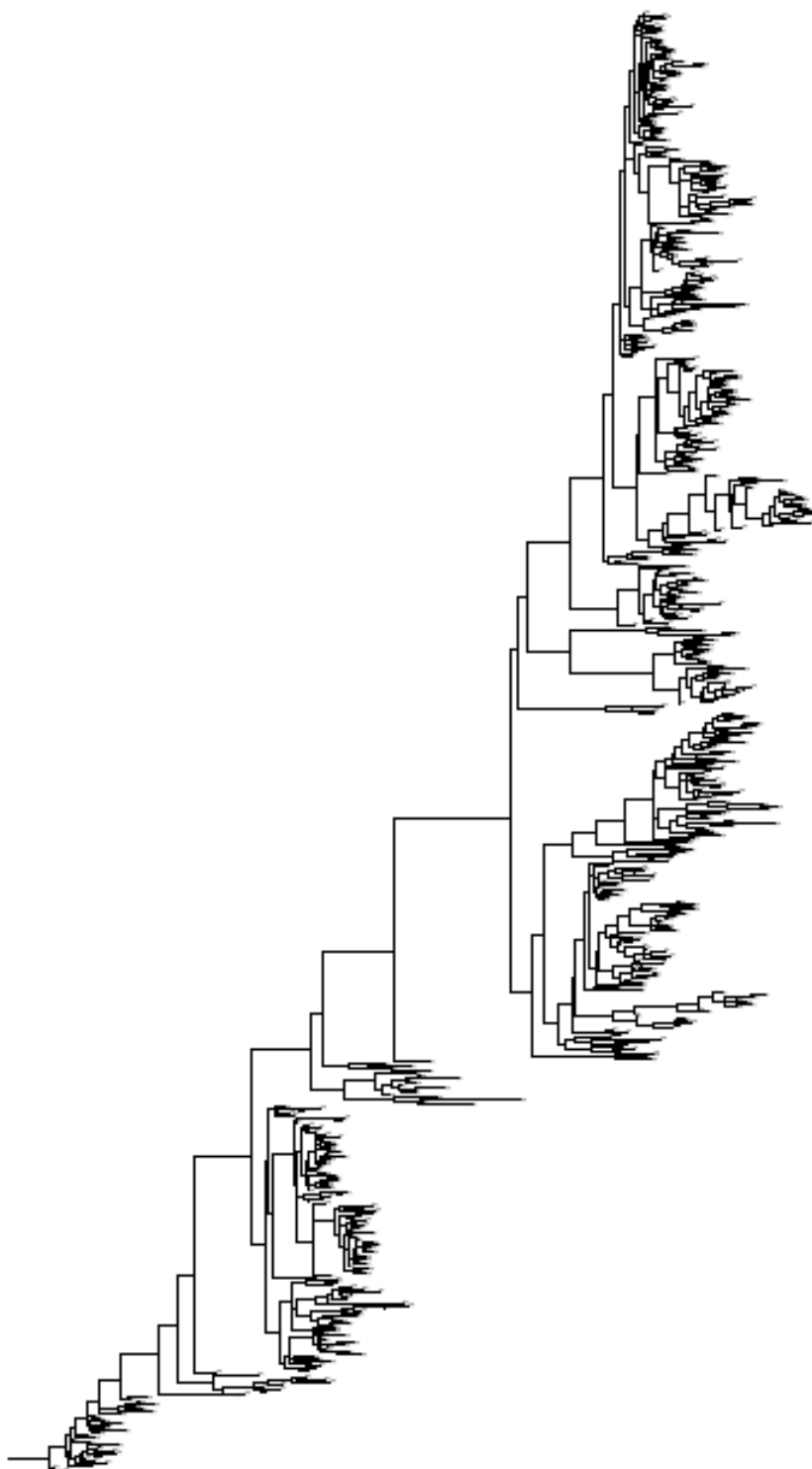


Figure S7:

**APPENDIX C- DISENTANGLING DIFFERENTIAL TRAIT DRIVERS OF
PHYLOGENETIC COMMUNITY ASSEMBLY ACROSS AN ELEVATIONAL
GRADIENT**

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Keywords: functional traits, phylogenetics, beta diversity, functional beta diversity, functional richness, subalpine, elevational gradient

Abstract

Patterns of species diversity have long been used to ask questions about ecological assembly of communities. Recently, phylogenetic and functional diversity have been added to traditional species or taxonomic diversity metrics to infer how particular environmental or biotic factors shape communities. Here we compare patterns of taxonomic, phylogenetic and functional diversity of angiosperm communities along an elevational gradient in subalpine meadows of the Rocky Mountains, and test the hypothesis that functional diversity can be inferred from phylogenetic diversity. We find that individual traits are variably conserved in the phylogeny, but suites of traits are highly conserved. Richness or alpha diversity patterns are similar across elevation for all three diversity types. Functional turnover or beta diversity patterns depend on the type of metric used to quantify functional similarity, with a metric based on the proportion of shared multidimensional trait volume giving the least functional similarity between communities. Phylogenetic and functional trait dispersion patterns also differ across elevation. We find that floral and phenology traits become overdispersed at higher elevations, mirroring the phylogenetic pattern, while foliar %C becomes clustered. This suggests that communities face multiple selective pressures, in particular, pressure to diversify floral traits while maintaining fast growing leaves.

Introduction

A central goal of community ecology is to understand how the ecological attributes of species interact to form community assemblages, despite the opposing assembly mechanisms of environmental filtering (Weiher and Keddy 1995) and limiting similarity (Dayan and Simberloff 2005), which should drive species to be more ecologically similar or different, respectively. Recent advances in phylogenetic (Webb et al. 2002) and functional community ecology (McGill et al. 2006) have brought new tools and insights into the study of community assembly mechanisms. In particular, the analysis of the phylogenetic and functional trait diversity of communities can yield insights into the differing processes that shape ecological communities (Swenson 2011a). The promise of these new approaches is that by the study of both phylogenetic and functional trait diversity, ecology can more readily assess the mechanisms influencing community structure as well as more quickly identify vulnerable species (Cavender-Bares et al. 2009).

A key concept that links phylogenies and trait-based ecology is evolutionary trait or niche conservatism: species that are more closely related should be more functionally similar (Prinzing et al. 2001; Ackerly 2003). Trait conservatism is a key assumption that links patterns of phylogenetic diversity to trait diversity, within and between communities, which in turn allows researchers to make inferences on the mechanisms that structure these communities. Recently, a few studies have begun to integrate both phylogenetic and trait-based approaches in the same study system (Cavender-Bares et al. 2004; Kraft et al. 2008; Swenson and Enquist 2009; Swenson et al. 2011; Swenson et al.

in press Ecology). These studies have increasingly questioned the findings raised from the analysis of phylogenetic diversity alone. Together, they highlight an important challenge to phylogenetic and trait-based ecology. Specifically, there appear to be four central issues:

First, even if there are strong patterns of niche-conservatism, patterns of phylogenetic and functional diversity may not necessarily match. Just as species richness does not necessarily predict phylogenetic richness (Faith 1992), phylogenetic richness does not necessarily predict functional richness (Swenson 2011a). Indeed, phylogenetic and functional dispersion metrics can be opposite or even independent of each other. For example, in a neotropical dry-forest, while tree communities were phylogenetically overdispersed at small spatial scales, most functional traits were clustered at small spatial scales (Swenson et al. 2007; Swenson and Enquist 2009). Finally, phylogenetic turnover does not necessarily predict functional turnover, and this relationship may depend strongly on the degree of trait conservatism (Swenson 2011b). Such discrepancies between phylogenetic and functional diversity can help to reveal the specific mechanisms and drivers of community composition. Thus, the assumption that functional dispersion will mirror phylogenetic dispersion, even in cases where niche-conservatism has been demonstrated, may not be correct.

Second, the analysis of dispersion patterns of multiple traits has revealed that traits may respond differentially to changes across space and environment. For example, within a community, some traits may be overdispersed, some underdispersed and others randomly dispersed (Grime et al. 2006). In short, there may be a decoupling of patterns of

phylogenetic dispersion and functional dispersion for some traits, but not others. Indeed, several recent studies have found opposing patterns of trait dispersion in the same community (Kraft et al. 2008; Swenson and Enquist 2009) but also over larger more regional scales (Swenson et al. *in press GEB*). Most trait-based approaches rarely include more than a few traits in their study, and may therefore miss key trait assembly mechanisms. For example, if community assembly is based on reproductive traits (Sargent and Ackerly 2008), the analysis of vegetative traits alone may not necessarily reveal the mechanisms responsible for assembly.

Third, trait-based ecology is still grappling with how to go from an ‘atomized’ view of the organism, characterized by single traits, to a more multivariate or multi-trait view of integrated phenotypes (Findley 1973; Ricklefs and O’Rourke 1975; Petchey and Gaston 2002; Villéger et al. 2008). Further, plant trait-based ecology is still very much focused on the suite of functional traits defined by standard protocols (Cornelissen et al. 2003) which includes mainly leaf, stem, and root traits closely linked to physiology and allocation differences. Reproductive traits, however, have been surprisingly minimized and mainly consist of measures of seed size (e.g. Moles et al. 2005). However, a long line of literature on pollination biology and dispersal/pollination syndromes all point to the potential importance of additional pollination and dispersal traits on community structure (Sargent and Ackerly 2008). In short, the challenge is how to link multiple traits (physiological traits, allocation traits, life history traits, and reproductive traits) of taxa and communities so as to assess how communities are assembled via functional diversity of phenotypes rather than a single-trait approach.

Fourth, the analysis of phylogenetic, and trait diversity can be altered or even negated by considering the relative abundance or dominance of the taxa within and between communities (Vamosi et al. 2009; Cadotte et al. 2010). Abundance varies considerably within communities and reflects the outcomes of biotic and abiotic interactions (Norberg et al. 2001). Thus, using a species list and assigning trait and phylogenetic measures to that list places rare species on equal footing with abundant species and can potentially distort measures of community dispersion.

Here we address each of these challenges to the development of phylogenetic and trait-based approaches. We build upon a previous study of phylogenetic community structure of meadow plants along an elevational gradient in the subalpine Colorado Rockies. Bryant et al. (2008) found that angiosperm communities were phylogenetically random at low elevation and phylogenetically overdispersed at high elevation. Using the common assumption that of evolutionary niche conservatism, (Prinzing et al. 2001; Ackerly 2003), Bryant et al. inferred that the effects of species interactions are stronger than environmental filtering at higher elevations. However, given the potential issues with linking patterns of phylogenetic and trait dispersion with the mechanisms assembling communities, we now ask how phylogenetic and functional dispersion are related in this community.

In order to address these questions we compiled an extensive trait database of numerous vegetative, reproductive, and life history traits for the plants in and around the Rocky Mountain Biological Lab (RMBL). The database was assembled by many RMBL researchers over several years. It covers 23 vegetative, reproductive, and phonological

traits for approximately 150 species of the angiosperms that occur along our elevational gradient. This extensive trait database, phylogeny, and community sampling allows us to assess the hypothesis that measures of trait dispersion and turnover are a better proxy for disentangling the forces structuring communities rather than phylogenetic dispersion and turnover.

Specifically, we addressed the following questions: (i) For numerous vegetative, phenological, and reproductive traits, which ones are more phylogenetically conserved in subalpine meadow communities?; (ii) Do patterns of functional richness (Alpha diversity) follow patterns of taxonomic and phylogenetic richness along the elevational gradient?; (iii) Do patterns of functional trait dispersion coincide with patterns of phylogenetic dispersion along the gradient?; (iv) How does the functional similarity of communities change across the gradient? ; Lastly, (v) between communities, does functional diversity change or decay (Beta diversity) more or less rapidly than taxonomic and phylogenetic diversity? Addressing these questions enables us to assess various ecological and abiotic mechanisms responsible for community assembly and the implications for climate change for these communities.

Methods

Study Site

Our study was conducted along an elevational gradient spanning the entire subalpine zone in the Elk Mountains, near the Rocky Mountain Biological Laboratory (RMBL) in Gunnison County, CO, USA. The elevational gradient was set up in 2003 and ranges from approximately 8,000 ft. to over 11,000ft. It has been sampled every year and consists of 25 1.2m² plots within five sites (see Table 1). The aspect of each site ranges from S to SE facing or S to SW facing. Within each site, five 1m² plots are arranged within approximately 50m² so as to capture site heterogeneity in slope (ranging from ~5° to ~20°).

Community Sampling

Within each of the 25 1.2m² plots, all individuals were harvested, counted, and identified to species. In total we sampled 30,266 individuals across the gradient. Individuals that could not be identified to species, genus, or family were excluded from the analysis. This affected approximately 10-15% of morpho-species per site and less than 1% of individuals. In total, 356 voucher specimens were deposited in the RMBL and UAZ herbaria¹ and we identified 96 angiosperm species across the elevational gradient. All collected individuals were dried and weighed by species. Thus, for each site we also have abundance and percent total biomass.

¹ Using 'Enquist' as the keyword in the primary collector field, and 'Colorado' under the state field, specimens can be searched and collection numbers accessed at http://ag.arizona.edu/herbarium/search_specimens

Within each 1.2m² plot we characterized the abiotic environment by sampling soil moisture, temperature, and carbon twice during the growing season. In addition, soil nitrogen was also sampled. See Bryant et al. supplemental information for data on environmental factors across elevation and correlation between environmental factors (2008).

Assembling the Angiosperm Phylogeny

We generated a phylogenetic supertree for the 96 Angiosperm species found along the elevational gradient using phylomatic (citation). We used the APG III supertree as the basis for the backbone of the phylogeny (III, 2009). Because this supertree consisted of several polytomies we further resolved the phylogeny by using recently published molecular studies reporting more detailed phylogenies (Worberg et al. 2007). The end result was a phylogeny for the 96 angiosperm species, resolved to the generic level for many taxa and at minimum to the family level for all taxa.

Assembling the Trait Database

The RMBL plant trait database is the result of over 30 years of data collection by many researchers at RMBL. The database consists of observations of plant traits for over 100 herbaceous and shrubby species within the Gunnison Valley, CO, USA. Our trait database spans many independent axes of trait variation (Sloat et al. *in prep*) that may structure angiosperm communities at high elevation, including: physiological functioning and stoichiometry, which influences plant growth and sensitivity of growth to climate;

floral morphology, which influences pollinator visitation and competition for pollinators (Sargent and Ackerly 2008); phenology, which influences growth and flowering and frost sensitivity. The database will be made publically available via the RMBL.

For this analysis, we used a subset of 11 traits representing independent axes of plant variation. These traits were chosen on the basis of their coverage across species and their ecological significance.

Phenology traits – We compiled the mean date of first flowering and phenophase (mean number of days flowering). These traits appear to be important for survival and reproduction (van Schaik et al. 1993) and indicative of sensitivity to climate change (Walther et al. 2002). These traits were measured by D. Inouye and are averages of yearly observations of many individuals per species at three elevations between 2800m and 3000m since 1975.

Reproductive traits – We assembled data for flower size, floral display volume, height above the ground of floral display, due to their importance in pollination (Wyatt 1982; Fenster et al. 2004). These data were collected by K. Whitney in 2009 from observations of all species blooming throughout the summer at 5 elevations within the East River Valley from 2700m to 3300m. In addition, seed size data were compiled from the Kew Botanical Gardens seed information database or SID² (Kew 2008). Variation in seed size is associated with variation in reproductive allocation and reflects variation in reproductive life histories and regeneration strategies (Moles and Westoby 2006;

² <http://data.kew.org/sid/>

Westoby et al. 2002). We used mean generic seed size for species that did not overlap with the SID.

Vegetative traits - We assembled species mean values for numerous traits. These included foliar % Carbon, foliar % N, maximum photosynthetic rate ($\mu\text{mol s}^{-1} \text{m}^{-2}$) and specific leaf area (SLA, $\text{cm}^2 \text{g}^{-1}$). Together, these leaf traits characterize how species solve the tradeoff between leaf life span and photosynthetic capacity (Reich et al. 1999). These data were collected between 2004 and 2010 by B. Enquist's lab along an elevational gradient from 2400 to 3300 m. Finally, the average species size was measured based on the individual biomass values collected in 2005.

Phylogenetic Analyses

Phylogenetic analyses were done using the Picante package in R³ (Kembel et al. 2010). Mean pairwise distance (MPD) and mean nearest taxon distance (MNTD) (equivalent to -1x Net Relatedness Index and -1 x Nearest Taxon Index, respectively) (Webb 2000; Webb et al. 2002) were calculated using biomass weighted abundances, where abundance = species biomass/ total biomass in each community. Niche conservatism was calculated with Blomberg's K statistic (Blomberg et al. 2003), using partial phylogenies, excluding species for which there was no data on a particular trait. Additionally, using principle components techniques, we separately analyzed the three independent trait groups: phenology, reproductive, and physiology. Secondly, we then combined these trait groups to analyze all traits together. Suites of traits, or ecological

³ <http://picante.r-forge.r-project.org/>

phenotypes, tend to be more conserved within phylogenies than individual traits (Webb et al. 2002). Using the first principle component of variation for each trait group, we repeated the analysis of trait conservatism. The loading value for each species was then used as a multivariate measure of ‘trait’ value for each axis. In all analyses, null phylogenies were generated by shuffling taxa or traits at the tips of the phylogeny. Patterns of trait dispersion were analyzed using Phylocom (Webb et al. 2008).

Multivariate measures of functional diversity

Functional diversity metrics were calculated on the standardized trait values, weighted by biomass, at each elevation. We used the convex hull method to estimate functional richness in the R package FD (Cornwell et al. 2006; Villéger et al. 2008).

Phylogenetic and Functional Beta Diversity

We calculated Beta Diversity in three ways. We first calculated the taxonomic turnover between sites using Sørensen’s index (Krebs 1998). Second, we calculated phylogenetic turnover or beta diversity by using the *PhyloSor* index (Bryant et al. 2008). *PhyloSor* calculated the turnover between sites as the proportion of total branch lengths that are shared between sites. Lastly, we compared functional trait beta diversity using two metrics: (i) functional trait dendrograms following recent protocol in Swenson et al. 2010; and (ii) a multi-dimensional trait analog of Sørensen’s index, *TraitSor*. We calculated *TraitSor* as the proportion of multi-dimensional trait space shared between two communities, or the intersection of two multi-dimensional hypervolumes representing the

trait space of the communities (Hutchinson 1957). To calculate this, we first generated biomass weighted trait distributions for each of the 11 traits in our analysis at each elevation.

Due to the analytical difficulty of calculating the overlap of 11 dimensional surfaces, we used a Monte Carlo method to approximate the proportion of overlapping trait space between two sites. For each site, we generated 100,000 data points with 11 trait values sampled from normal distributions with the weighted mean and standard deviation for each trait, as seen in Table 2. We then asked whether the generated data point also fell within the allowed trait ranges for each trait in each of the other sites. The allowed trait range was defined as the range of trait values for a normal distribution with the mean and standard deviation measured in the data that were within the 0.05 to 0.95 limits. These limits were then corrected with the Bonferroni correction for 11 independent comparisons per site. The fraction of the 100,000 generated points falling within is the portion of that site's trait space shared with the other site. *TraitSor* is then the amount of shared trait space between two sites divided by the average trait volume in those sites.

Results

(i) Which functional traits are phylogenetically conserved in subalpine meadow communities?

Not all traits in our analysis show phylogenetic conservatism. We find significant niche conservatism in foliar stoichiometry (Leaf %C, $K = 0.441$, $p = 0.006$; Leaf %N, $K = 0.490$, $p = 0.003$), mean date of first flowering ($K = 0.594$, $p = 0.046$), and height of inflorescence ($K = 0.605$, $p = 0.011$). Thus, we see some evidence for conservation in each of our three independent trait axes of physiology, phenology and floral morphology. The other traits analyzed were not significantly conserved in our phylogeny. When traits were combined into three independent trait groups (phenology, floral morphology and physiology) the first principle component explained 59.5%, 63.4% and 45.8% of the variance in each group, respectively. Each of these first principle components showed significant phylogenetic conservatism, and a higher degree of conservatism than the individual traits. As multivariate groups, phenology traits were conserved ($K = 0.682$, $p = 0.01$), floral morphology traits were conserved ($K = 0.591$, $p = 0.023$) and functional traits were conserved ($K = 0.86$, $p = 0.005$). Trait loadings for each PCA trait are shown in Table 2.

(ii) Do patterns of functional richness follow patterns of taxonomic and phylogenetic richness along the elevational gradient?

Biomass weighted trait means and standard deviations for each elevational site are shown in Table 3. Date of first flowering becomes increasingly earlier with elevation, and

the height of floral displays becomes higher. Foliar Nitrogen increases with elevation, as does specific leaf area. Other traits show peak values at mid elevations. Functional richness (Villegger et al. 2008) parallels patterns of taxonomic and phylogenetic diversity, and peaks at middle elevations (Figure 1).

(iii) Do patterns of functional trait dispersion coincide with patterns of phylogenetic dispersion along the gradient?

As we reported previously, based on presence/absence of species in each community, the phylogenetic structure of communities changes across elevation (Bryant *et al.* 2008). When the community composition is weighted by proportion of site level biomass, the reported pattern becomes more robust (Figure 2). Both biomass weighted mean pairwise distance (MPD = -NRI) and mean nearest taxon distance (MNTD = -NTI) show significant overdispersion at the higher elevations (MPD $z = 1.799$, $p = 0.996$ at 3380m). Additionally, there is significant phylogenetic clustering of species at the lowest elevation (MPD $z = -2.661$, $p = 0.016$ at 2475m). Additionally, both MPD and MNTD increase significantly with elevation, i.e. the linear trend of MPD vs. elevation is significant.

When we look at trait assembly in these communities, we see some traits that parallel the above pattern for phylogenetic relatedness, as we would expect given niche conservatism, but also many that do not (Figure 3). Phenophase is significantly overdispersed at the highest elevation ($z = 1.54$), but there is no significant trend with elevation for either phenophase or date of first flowering (Figure 3a). Floral morphology traits flower volume and height of display both show significant trends towards

increasing overdispersion with elevation (Figure 3b, $p = 0.0203$ and $p = 0.1077$, respectively). However, foliar traits showed no trend (%N) or the opposite trend (%C) to the phylogenetic data ($p=0.0189$) (Figure 3c). Foliar %C is significantly overdispersed at lower elevations ($z= 1.29$ and 1.55) and clustered at the highest elevation ($z = -2.65$). Physiological traits SLA and Amax showed no significant trends with elevation (Figure 2d), nor did seed mass (Figure 3e). While biomass is not significantly clustered or overdispersed at any elevation, there is a marginally significant trend towards increasing overdispersion at high elevation ($p = 0.0526$, Figure 3e). There is no consistent pattern between trait dispersion and trait conservatism in our system (Table 4).

(iv) How does the functional similarity of communities change across the gradient? Does functional diversity decay more or less rapidly than taxonomic and phylogenetic diversity? (Beta diversity)

The amount of trait space shared between communities decreases with elevational separation (Figure 4). The degree of functional similarity, as defined by overlap in trait space between two communities, is much lower than either taxonomic or phylogenetic similarity for the same community (Bryant et al 2008, Figure 3). Furthermore, the degree of functional similarity calculated by trait space overlap is much lower than the degree of functional similarity calculated as fraction of shared branch lengths on the functional trait dendrogram. Sites that are most similar in elevation share only approximately 10% of their functional trait space, but as much as 91% of their functional dendrograms. Decay of functional similarity with elevational distance was similar for floral, phenological and functional traits using the dendrogram method.

Discussion

In this study, we have combined taxonomic, phylogenetic and functional data on meadow angiosperm communities in the subalpine Rockies in order to assess potential community assembly mechanisms without making the assumptions of niche conservatism. Within the angiosperm communities in the subalpine zone of the Colorado Rockies we find both convergence and divergence of diversity metrics for each of three principle diversity types: taxonomic, phylogenetic and functional (see Table 5). Taxonomic, phylogenetic, and functional richness all peak at middle elevations along our elevational gradient. Communities become increasingly phylogenetically overdispersed with elevation, but patterns of functional dispersion with elevation depend on the particular trait in question. Phylogenetic turnover is no more than expected given the amount of taxonomic turnover with elevation, while functional turnover is higher than expected.

Floral and phenological traits parallel the pattern of phylogenetic dispersion and become increasingly overdispersed with elevation. This means that there is more variation in floral morphology and phenology at high elevation than expected given the species diversity present at those elevations. Floral morphology is one important cue that animals use to choose appropriate flowers to visit (Chittka et al. 1999), and floral phenology can impact the number and species of pollinators available (Mommott et al. 2007). Pollinator availability affects plant reproductive success via increased pollen delivery, higher seed set and higher outcrossing (Vamosi et al. 2006). Furthermore, it has long been thought that variation in floral morphology due to pollinator attraction can

drive speciation in flowering plants (Sargent 2004, Fenster et al. 2004), although this issue is under debate (Waser et al. 1996).

Plants compete with one another for pollinators. This may occur when either number of pollinator visits is limiting, or when accurate pollen delivery (placement of pollen, or pollen type) by pollinators is limiting (Mitchell et al. 2009). Where effective pollination increases plant fitness, and those pollinators are attracted on the basis of floral morphology and phenology, we would expect to see limiting similarity in floral traits. This suggests that with increasing elevation, the effect of species interactions mediated by floral and phenological traits increasingly determines which species can coexist locally. In particular, this suggests that in the highest elevation subalpine communities, just below treeline, there is pressure to diversify floral displays and timing.

Contrastingly, we see that foliar nutrients are strongly phylogenetically conserved, but show no pattern, or the opposite pattern of dispersion to the phylogeny. Foliar %C is overdispersed at low elevations and clustered at high elevation. Leaf carbon is indicative of a leaf's production capacity and a plant's allocation to leaves' structural components relative to nitrogen rich photosynthetic components such as Rubisco, or other functional components (Shipley et al. 2006). Therefore, clustering in foliar %C suggests that plants at higher elevation are employing a similar carbon allocation strategy in leaves.

This pattern of foliar carbon dispersion with elevation is in contrast to the observed pattern of phylogenetic dispersion with elevation, despite the fact that we find foliar carbon is conserved within the phylogeny. Overdispersion of traits, even when

those traits are phylogenetically conserved, may occur in communities assembled from fewer lineages, when the traits examined do not represent the fundamental ecological strategy of the species, but instead represent the axes along which those species diversified in order to coexist (Prinzing et al. 2008). Indeed, our lowest elevation site shows phylogenetic clustering and the lowest species diversity, but also the highest diversity of foliar %C.

Community assembly is the result of multiple selective pressures acting simultaneously on plant diversity (Swenson and Enquist 2009). Plants must maximize fitness, which is a combination of both survival and reproduction. Thus, given the wide range of traits we sampled, including traits important in both survival and reproduction, it should not be surprising that we see such varied dispersion of traits with elevation. While there may be pressure from limited numbers of pollinators to diversify in flowering strategy at high elevation, the abbreviated growing season and colder climate may force species to be similar along other trait axes, such as carbon allocation. At the highest elevations and shortest growing seasons, we may see convergence towards a strategy favoring leaves with a shorter life span (Reich et al. 1997), with higher specific leaf area and conserved %C, at the same time as there is pressure to diversify for pollinator attraction.

Functional similarity of communities decreases with elevational separation along the gradient. However, the degree of functional turnover depends greatly on the metric used to measure it. Studies comparing measures of functional beta diversity have shown that different metrics yield different results, as they are sensitive to different components

of turnover (Anderson et al. 2011). In particular, methods of functional beta diversity that rely on constructing functional trait dendrograms are variably sensitive to how deep particular functional splits are in the dendrogram (Swenson et al. 2011), and to the degree of conservatism of particular traits (Swenson 2011). Instead, relying on the traditional ecological concept of the niche as a hypervolume defined by multiple trait axes (Hutchinson 1957; Webb et al. 2002), relies solely on the definition of a community's trait spaces along each trait axis. This method yielded much lower functional similarity with elevational separation than functional dendrogram metrics. In our method, only fractions of trait space that were shared all of the 11 traits were considered overlapping between two communities. Therefore, this method is likely a conservative estimate of functional similarity.

In our study, we necessarily assigned each species a mean trait values based on all observations within the RMBL plant trait database. In doing so, we neglected intraspecific trait variation, which is known to be important in many communities, particularly across broad environmental gradients (Hulshof and Swenson 2010; Messier et al. 2010). Local adaptation of species to specific environments would increase the amount of functional turnover that we measured in our study. The effect of local adaptation on trait dispersion could make communities more overdispersed, if clustering is the result of applying similar species level trait means to the species in the community, or could result in more clustering, if overdispersion is the result of applying species level trait means that are gathered from populations with different elevational mean trait values. Furthermore, we only sampled our communities at peak biomass. Therefore, we

may be missing some diversity of species that are out earlier and later in the season, and our estimates of functional diversity are likely underestimates of functional diversity in these communities.

In this study we present a comparison across an elevational gradient of patterns of taxonomic, phylogenetic and functional diversity. We find that phylogenetic dispersion and turnover are not necessarily good predictors of functional dispersion and turnover, independent of the degree to which individual traits are conserved. However this variation in dispersion and turnover in functional traits may reveal the multiple selective pressures that operate in plant community assembly across broad environmental gradients.

Acknowledgements

We would like to thank M. Wilson Colner, L. Crumbacher, R. Sleith and J. Stegen for help with field sampling of angiosperm communities. The Rocky Mountain Biological Lab provided support and access to field sites, as well as funding for the development of the RMBL plant trait database. Many thanks go to the numerous researchers at RMBL who contributed data over the years to the plant trait database, including George Aldridge and Markus Stansberry. Ian Billick and Dan Jones at RMBL designed the database. CL would like to thank Stuart Evans for helpful comments on the analysis.

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Tables

Table 1:

Description of sites along elevational gradient near the Rocky Mountain Biological Laboratory, Gothic, CO.

Site Name	Lat/Long	Elevation
Almont	38°39'16" N, 106°51'40" W	2465 m
Crested Butte Land Trust	38°52'55" N, 106°58'46" W	2700 m
Allen Ranch	38°53'50" N, 106°58'43" W	2815 m
RMBL	38°53'N, 107°02' W	2920 m
Pfeiler	38°57'38" N, 107°01'53" W	3165 m
Painter Boy Mine	38°58'10" N, 107°02'28" W	3380 m

Table 2:

PCA loadings used to create multivariate traits for each of three trait groups:

phenological, floral and physiological. All traits were standardized prior to PC analysis.

Trait	PC1Pheno	PC1Floral	PC1Physio
First Flowering Date	-0.707		
Phenophase	-0.707		
Height of Floral Display		-0.559	
Floral Volume		-0.498	
Display Volume		-0.664	
Foliar %C			0.372
Foliar %N			0.507
Specific Leaf Area			0.397
Photosynthetic Rate			-0.239
Seed Mass			0.479
Biomass			0.401

Table 4:

Trait conservation vs. trait dispersion for each of the 11 tested traits in our dataset. Floral traits tend to show a trend towards increasing overdispersion at higher elevation regardless of whether or not they were significantly conserved. Only foliar %C is both conserved but shows the opposite pattern from phylogenetic relatedness across elevation; foliar %C becomes more clustered at higher elevation.

Trend of Trait Dispersion with Elevation				
		Clustering	Random / N.S.	Overdispersion
Phylogenetic Niche Conservatism	Conserved	<i>Foliar % C</i>	<i>Foliar % N</i> <i>Phenophase</i>	<i>Flowering Date</i> <i>Flower Height</i>
	Not Conserved		<i>SLA</i> <i>Amax</i> <i>Seed Mass</i>	<i>Display Volume</i> <i>Flower Volume</i> <i>Biomass</i>

Table 5:

Summary of diversity patterns vs. elevation for each of three diversity types, taxonomic, phylogenetic and functional, in each of three diversity metrics, richness or alpha diversity, dispersion and turnover or beta diversity. For alpha diversity, all three diversity types show a peak of richness at middle elevations. While we see increasing phylogenetic overdispersion with elevation, functional dispersion patterns depended on the trait in question. Finally, phylogenetic turnover was no more or less than that expected from the observed taxonomic turnover.

Trends with increasing elevation:		Diversity Metric		
		Richness (α)	Dispersion	Turnover (β)
Diversity Type	Taxonomic	<i>Humped</i>	-----	-----
	Phylogenetic	<i>Humped</i>	<i>Overdispersion</i>	<i>Same</i>
	Functional	<i>Humped</i>	<i>Overdispersion and clustering</i>	<i>Steeper</i>

Figure Captions

Figure 1: Functional richness and the log of functional richness vs. elevation for each site along the gradient. Functional richness peaks at middle elevations, and is lowest at low elevations.

Figure 2: Phylogenetic relatedness across elevation, for biomass weighted community abundances. Closed diamonds indicate mean pairwise distance (equivalent to the negative of the net relatedness index) and open diamonds are mean nearest taxon distance (equivalent to the negative of nearest taxon index). Both indexes show significant linear trends towards overdispersion at higher elevations. Both MPD and MNTD are significantly overdispersed at high elevation and MPD is significantly clustered at low elevation.

Figure 3: Community trait clustering across elevation, measured as MPD for (a) phenology, (b) floral morphology, (c) foliar stoichiometry, (d) physiological and (e) size traits. Flower volume, display volume, biomass and foliar % C all show significant trends of MPD with elevation.

Figure 4: Comparison of elevational distance decay of trait similarity between sites, calculated as proportion of shared 11-dimensional trait space (dark diamonds) and as proportion of shared branch lengths on a functional trait dendrogram (dark circles). Shared trait space has a much lower degree of similarity than shared dendrogram length. Functional dendrograms constructed for phenological, floral and physiological traits

(white, light grey and dark grey squares, respectively) all show similar degrees of similarity and decay of similarity with elevational distance.

Figures

Figure 1:

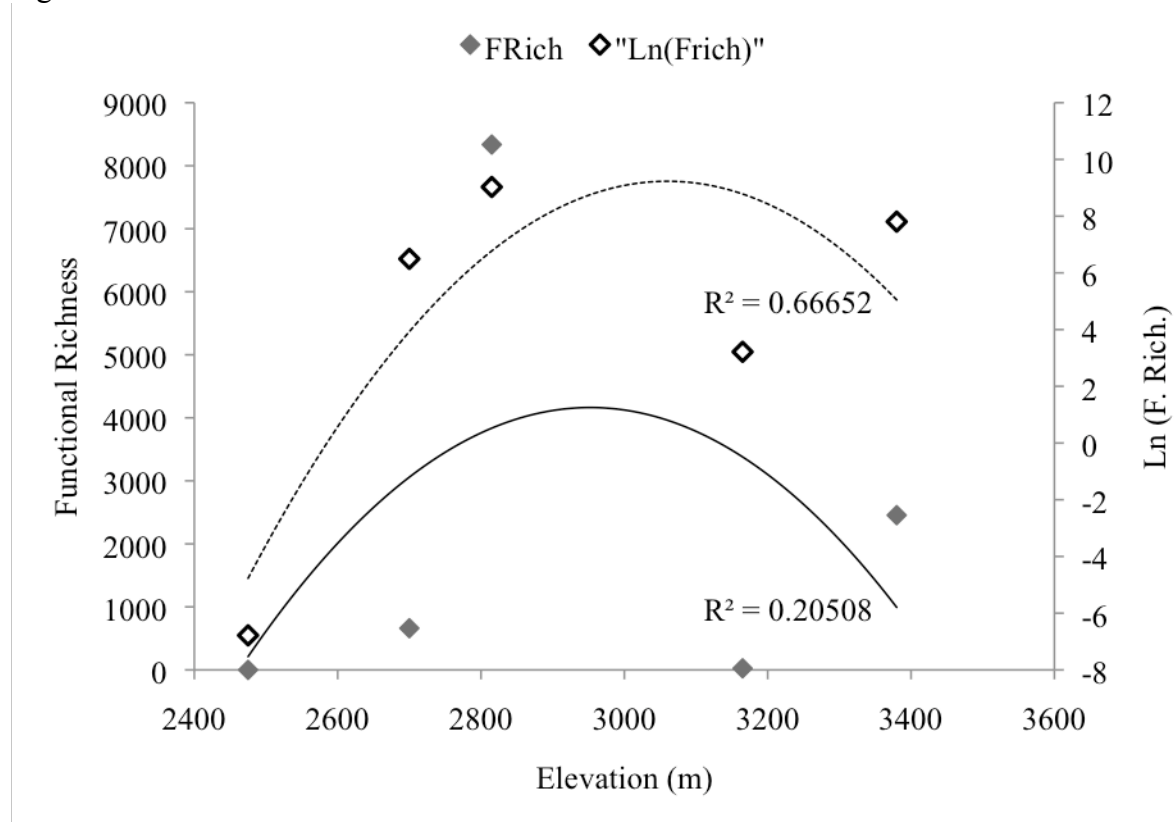


Figure 2:

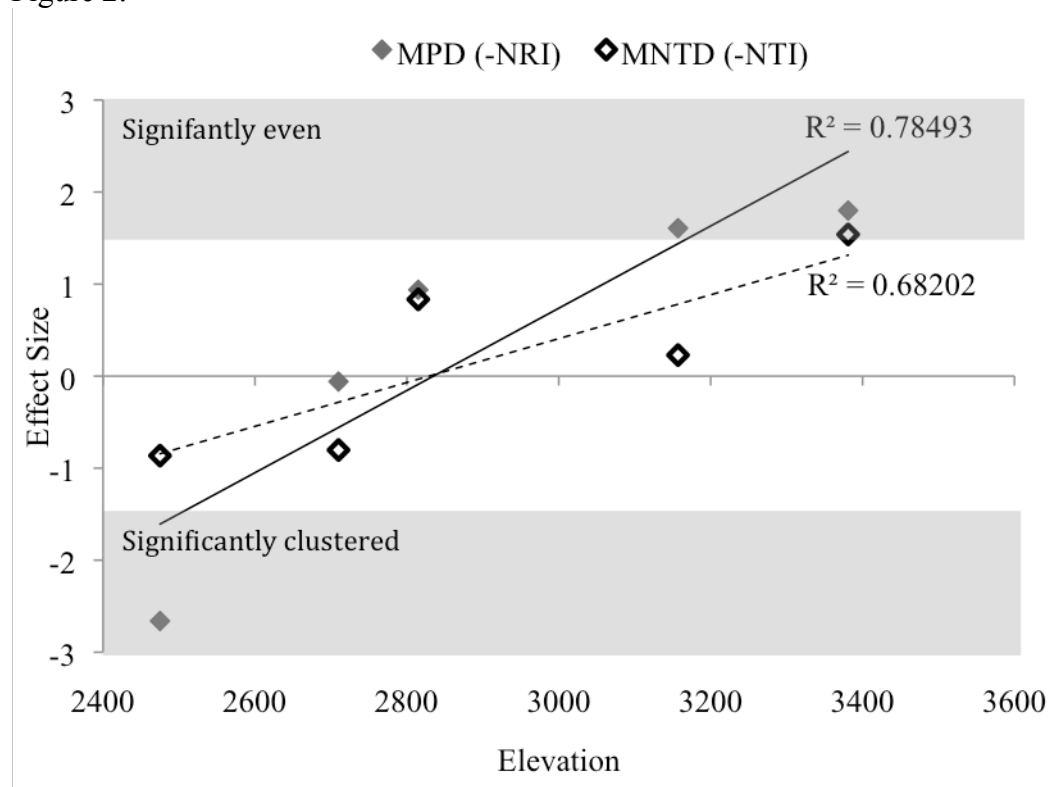


Figure 3:

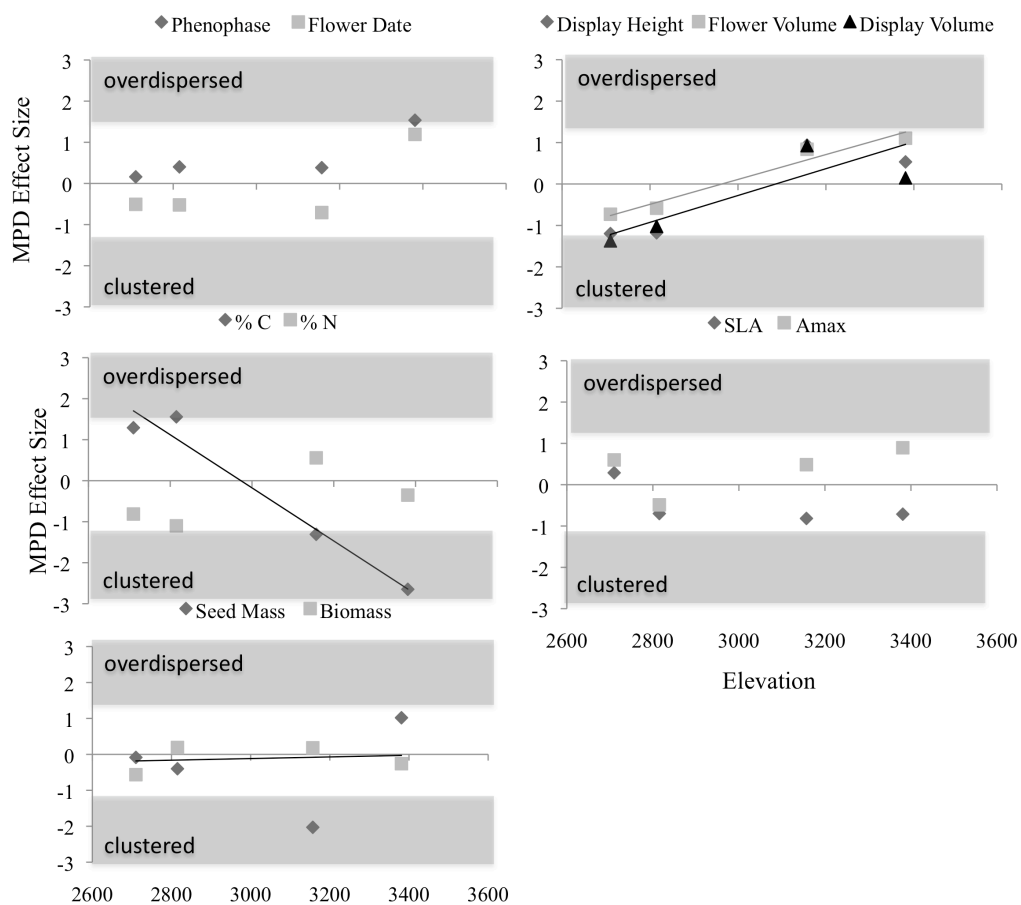
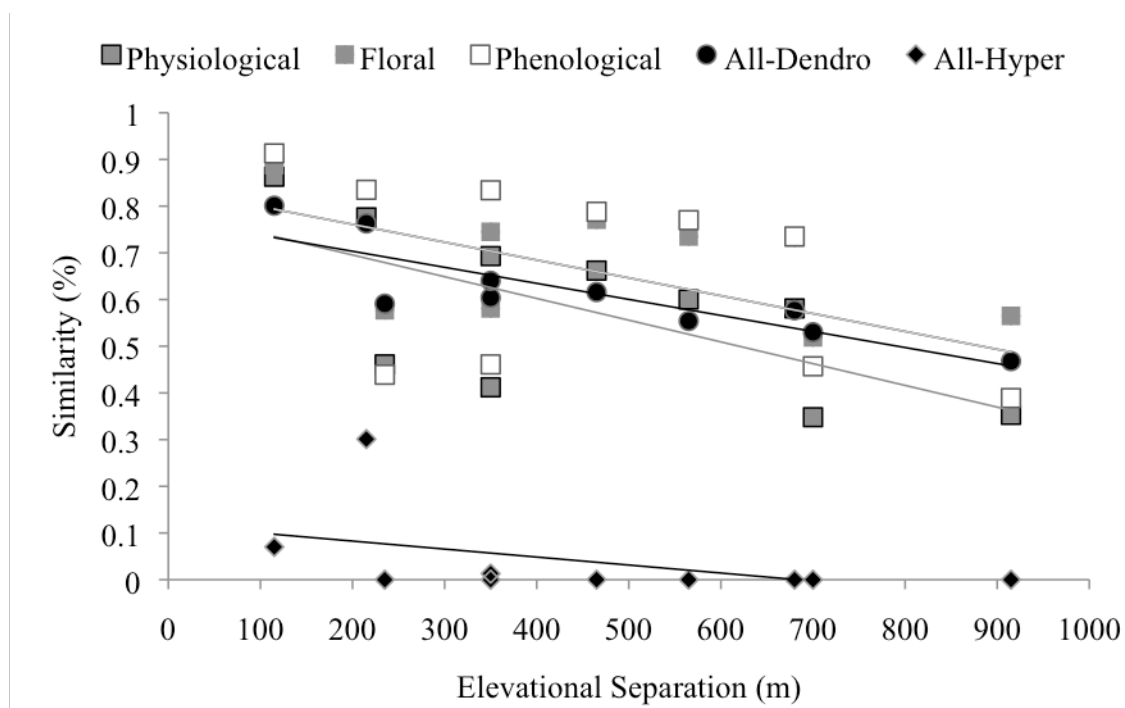


Figure 4:



**APPENDIX D- FACILITATION IS CRITICAL TO THE SURVIVAL OF
SAGEBRUSH SEEDLINGS AT THEIR ELEVATIONAL RANGE LIMIT**

**Facilitation is critical for survival of sagebrush seedlings at their
elevational limit**

Running Title: Facilitation and Seedling Recruitment

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Keywords: Facilitation, elevational range limit, *Artemisia tridentata*, subalpine, climate change, neighbor removal, OTC, species interactions

Abstract

Vegetation shifts are a dominant response of many ecosystems to climate change. Indeed, experimental warming in the subalpine meadows of the Colorado Rockies has shown that sagebrush (*Artemisia tridentata* ssp. *vaseyana*) responds positively with increased rates of growth and recruitment. It has been hypothesized that sagebrush's elevational range is likely to expand upwards with future climate warming and drying due to increased tolerance of hotter and dryer conditions. However, warming may also enhance competitive interactions of other plants so as to potentially lead to increase competition from non-sagebrush plants. To test these differing hypotheses, I conducted a factorial experiment of heating and neighbor removal on sagebrush seedlings near their elevational range limit. Heating did not directly influence growth or survival of sagebrush seedlings, however, neighbor removal did. Mortality was four times higher with removal than without and seedlings were smaller. Seedlings with neighbors removed were exposed to *higher* daytime highs and *lower* nighttime lows than control and heated seedlings. Thus, heating did not directly impact sagebrush establishment but instead the presence of neighboring plants did. Rather than limiting growth and survivorship, neighbors moderate the extremes of the thermal environment so as to influence potential elevational shifts in sagebrush distribution. Together, these results suggest that any observed shifts in the distribution of sagebrush is mediated by neighbor interactions as the temperature moderation provided by neighboring forbs is critical to the survival of sagebrush seedlings near their elevational range limit.

Introduction

Shifting plant community composition is one of the dominant responses of ecosystems to changes in climate (Walther *et al.* 2002). Changes in plant community composition have been documented over long time periods as a result of glacial cycles (Whitlock & Bartlein 1997; Jackson *et al.* 1997), as a result of anthropogenic climate change over recent decades (Sturm *et al.* 2001; Kullman 2001; Gottfried *et al.* 2012), and in response to experimental climate manipulations (Chapin *et al.* 1995; Harte & Shaw 1995; Klein *et al.* 2004; Klanderud & Totland 2005). Shifts in vegetation can potentially impact global climate via alterations to albedo and carbon cycling (Cox *et al.* 2000; Friedlingstein *et al.* 2006), over both long and short timescales (Shaver *et al.* 2000).

Shifts in plant community composition have been generally attributed to differences in physiological tolerance between species, which results in species' specific responses to climate change (de Valpine & Harte 2001; Klanderud 2008; Enquist & Enquist 2011). However, other biotic processes, such as plant-plant interactions, dispersal limitation, and trophic interactions may also be important in influencing the geographic distribution of plants across climatic gradients (Davis *et al.* 1998; Brooker 2006; Sutherst *et al.* 2007). Several studies have shown how these non-climatic factors can shape the current distributions of species and composition of communities (Grime 1977; Maestre *et al.* 2009). Further, the intensity, importance, and nature of biotic interactions are often correlated with climate (Brooker *et al.* 2005; Kikvidze *et al.* 2005).

Links between species interactions and variation in climate have a long theoretical and empirical history in ecology. One prominent idea is the “Stress-Gradient” hypothesis,

which states that biotic interactions should become more positive (facilitative) as the environment becomes more stressful, and more negative (competitive) when the environment is less stressful (Callaway 2007). In a study of neighbor-removals in mountain ranges around the globe, there is a strong trend towards increasingly positive interactions between plants at high altitude compared to interactions at lower altitude (Callaway *et al.* 2002) and that interaction strength is significantly correlated with annual temperature extremes (January minimum, July maximum) (Kikvidze *et al.* 2011).

As a result, a prediction from the ‘stress-gradient’ hypothesis is that species interactions will themselves be affected by changes in climate (Tylianakis *et al.* 2008). For example, in the Alaskan subarctic tundra, earlier snowmelt results in an increasing intensity of facilitation between the shrub *Empetrum nigrum* and its neighbors (Wipf *et al.* 2006). In the Austrian Alps, germination and growth of alpine plants was hampered by competition from grassland species, but the strength of this competition varied with snowmelt date (Hübler *et al.* 2011). Finally, in the Norwegian alpine zone, warming and nitrogen deposition resulted in an increased competitive advantage for graminoids and forbs at the expense of the once-dominant shrub *Dryas octopetala* (Klanderud & Totland 2005). Therefore, in the context of climate change, it is important to not only understand how species interactions may shape community structure, but also how those interactions are likely to be themselves affected by climate change.

At the Rocky Mountain Biological Laboratory (RMBL, elevation 2900m, Gunnison Co., CO, USA) a global warming experiment has heated plots of subalpine meadow by an effective 2°C continuously since 1991 (Harte *et al.* 1995). Results from

the experiment show a positive feedback to climate change via loss of soil carbon on heated plots (Saleska *et al.* 2002). This loss is attributed to increased growth of a woody shrub, sagebrush (*Artemisia tridentata* ssp. *vaseyana* [Rydberg] Beetle) (Harte & Shaw 1995), and a concomitant decline in forb biomass (de Valpine & Harte 2001), which reduces litter inputs to the soil. Furthermore, the enhanced growth of sagebrush shifts seasonal patterns of net ecosystem exchange of carbon dioxide to earlier and lower peak uptake (Lamanna *et al.* in review). Thus, the response of this ecosystem to warming in the short term is driven by changing dominance of sagebrush within the community.

The evidence of increased sagebrush growth in the heating treatment at RMBL has lead to the suggestion that sagebrush will increase its range upwards in elevation with climate change (Perfors *et al.* 2003). However, *in situ* measurements of physiological responses of sagebrush and declining forbs showed little difference in freezing tolerance, PSII efficiency, or gas exchange in heated vs. unheated plots (e.g. Loik & Harte 1996; Loik *et al.* 2004, Henderson *et al.* in prep). This suggests that more than simple differences in physiological tolerance are responsible for the shifts in vegetation seen at RMBL. Increased dominance of sagebrush on heated plots may alternatively be due to changes in competitive interactions between sagebrush and forbs. The observed decrease in forb biomass may release sagebrush from competition with forbs and allow an increase in sagebrush growth, or warming may increase the competitive advantage of sagebrush over forbs, resulting in increased dominance of sagebrush.

Ultimately, in order to see an elevational advance of sagebrush in the subalpine Rockies with climate warming, there must be increased successful recruitment of

sagebrush seedlings above the present elevational limit. In this study, I investigated the effects of warming and the forb community on sagebrush seedling recruitment in a subalpine population of sagebrush. Specifically, I address three questions: (1) How does warming affect sagebrush seedling survival? (2) How does the presence of neighboring forbs affect sagebrush seedling survival? and (3) How does climate change, via warming, alter the interaction between sagebrush seedlings and neighboring forbs? If sagebrush seedling survival depends solely on its physiological tolerance to the environment, then we would predict warming to increase survival at the upper elevational limit of its range, and neighbors to have no effect on survival (Table 1). Alternatively, if sagebrush survival is limited by the presence of neighboring forbs, as suggested by the decline of forbs at the RMBL warming meadow, then neighbors should have a negative effect on survival and this negative effect should be lessened under warming. Finally, the stress gradient hypothesis would predict that sagebrush, at the upper elevational limit of its range, should have higher survival with warming, and that neighbors should have a facilitative effect on survival. In this scenario, warming should make the environment more similar to the core of sagebrush's range, and thus make interactions between sagebrush and neighbors more negative (i.e. less facilitative).

Methods

Study Site - This study was conducted in the subalpine research meadow at the Rocky Mountain Biological Laboratory (hereafter RMBL, elevation 2900 m, Gunnison Co., CO, USA latitude 38°53 N, longitude 107°02 W). The meadow supports over 100 plant species in a mosaic of shrubs, grasses and forbs (Langenheim 1962). The vast majority of meadow forbs are long-lived perennials, with variable rooting profiles (Cross & Harte 2007). The study was conducted on a NW facing slope in the meadow, where abundant forbs included *Potentilla gracilis* (Rosaceae), *Lathyrus leucanthus* and *Lupinus* sp. (Fabaceae), *Galium septentrionale* (Rubiaceae), *Dugaldia hoopesii* and *Helianthella quinquenervis* (Asteraceae), *Linum lewisii* (Linaceae), *Delphinium nuttallianum*, *Aquilegia coerula* (Ranunculaceae), and *Thalictrum fendleri* (Thalictraceae), among others.

Sagebrush Natural History - Sagebrush, *Artemisia tridentata*, is a woody shrub that is dominant in much of western North America, and is typical of the Great Basin Desert, ranging from 300 to 2800m in elevation. At the highest elevations and in the most mesic habitat, the dominant subspecies is *vaseyana*, which shows strong local adaptation (Wang *et al.* 1997) (but not in photosynthetic allocation, Loik & Redar 2003) and episodic recruitment (Perryman *et al.* 2001; Maier *et al.* 2001). In the Gunnison Valley, CO, sagebrush reaches unusually high elevations, typically up to approximately 2900m and in small, isolated populations as high as 3300m (personal observation). At the RMBL, sagebrush exists on well-drained areas of the research meadow, in well established populations that average about 50 years in age (Poore *et al.* 2009). Aerial

repeat photography indicates little to no movement of sagebrush populations over the last 50 years (Miller-Rushing *et al.* unpublished). Sagebrush is one of the last species to flower in meadows, although flowering time is nonetheless correlated with snowmelt date (Dunne *et al.* 2003). Seeds vary in size with elevation, ranging from 0.27 mg at 8,000' and increasing linearly with elevation to 0.46 mg at 11,000'. Seeds are dispersed from the plant by wind from August to October, with seeds from the highest elevations maturing and dispersing first, and seeds from the lowest elevations dispersing last. Sagebrush germinates with spring snowmelt at RMBL, in contrast to fall germination at lower elevations within its range (Maier *et al.* 2001).

Factorial Manipulations - Sagebrush (*Artemisia tridentata* ssp. *vaseyana*)

seedlings that had germinated with snowmelt in 2009 in the research meadow were identified in May by their persistent cotyledons. A total of 56 seedlings were identified and assigned to one of four treatment combinations at random, for n=14 per treatment combination. Treatments were a factorial combination of heating and neighbor removal. To determine the effects of treatments on microclimate, I monitored near-surface soil temperatures hourly with iButtons (Maxim Integrated Products, Sunnyvale, CA) buried 2 cm in the soil near the edge of each plot and soil moisture weekly using a Campbell Scientific Hydrosense probe with 12 cm tines. Heating was achieved with conical, fiberglass, open top chambers (Marion *et al.* 1997; Hollister & Webber 2000) which had a basal diameter of 40 cm, top diameter of 20 cm and height of 18 cm. Each seedling in a heating treatment had its own open top chamber. These chambers passively heated the enclosed vegetation by approximately 3.1°C during the day and 1.2°C at night ($p <$

0.0001). They do not affect gas exchange or light infiltration and did not significantly alter humidity or soil moisture inside the chambers.

Neighbor removal was achieved by clipping the aboveground biomass of all individuals within 20 cm of the focal sagebrush seedling. The diversity of removed individuals was high and included grasses, forbs and some overhanging portions of shrubs. Clipping was repeated several times during the growing season to ensure lack of aboveground interaction. I chose clipping in order to reduce interaction between the focal seedling and neighboring forbs while minimizing disturbance to the shallowly rooted seedlings.

Weekly throughout the growing season, I measured focal seedlings' height and number of leaves. At the end of the growing season, in September, I harvested all seedlings with intact rootballs. Seedlings were separated into above- and belowground portions at the base of the cotyledons, dried and weighed for biomass. Mortality was assessed visually throughout the growing season. Although actual seedling mortality can be very difficult to distinguish in the field (Reinhardt *et al.* 2011), I considered seedlings "dead" when all leaves were brittle to the touch and growth ceased for at least two weeks.

Statistical analyses – Survivorship analysis was conducted using the survival package in R (Therneau 2002). I also calculated the Relative Interaction Index (RII, Armas *et al.* 2004). The $RII = (C-T)/(C+T)$, where C= height of seedlings in control plots and T= height of seedlings in removal plots. Positive RII values indicate facilitative interactions, while negative RII values indicate competitive interactions between sagebrush seedlings and neighbors.

Results

Thermal environment – Heating resulted in warmer soil subsurface temperatures at night and in the morning compared to non-heated plots over the course of the growing season. Heated plots were on average 0.41 to 1.15°C warmer than non-heated plots (two-tailed t test, $p = 0.039$ and $p < 0.0001$ respectively), with largest warming between 8 and 10 a.m. Temperatures at other times were not significantly different from non-heated plots. Removal plots were on average colder at night and warmer during the afternoon than non-removal plots. Removal plots averaged 0.44°C colder in the pre-dawn hours than non-removal plots (two-tailed t-test $p = 0.0495$). However, during the day, removal plots were 0.61 to 3.07°C warmer (two-tailed t-test, $p = 0.017$ and $p < 0.0001$, respectively). Individual treatment combinations also differed in their growing season mean ($F_{3,5368} = 12.68$, $p < 0.0001$) and diurnal temperature profiles (Figure 1). There were no significant differences in soil moisture between treatments ($F_{3,52} = 0.46$, $p = 0.71$).

Seedling growth rate and biomass - In contrast to expectation that neighbor interactions are competitive, seedlings that did not have neighbors removed were taller than those with neighbors removed at the end of the growing season ($p = 0.021$, Figure 2). This result was the same whether all seedlings that were alive at a given sampling time were included in the analysis, or only the seedlings that survived until the end of the experiment and were present in all sampling periods were included. Heating did not affect seedling growth ($p = 0.633$), nor did we see evidence of an interactive effect of heating x neighbor removal ($p = 0.194$). Seedlings did not differ in their total dry mass, above-ground biomass or below ground biomass, or their ratio at the end of the growing

season ($F = 0.729$, $p = 0.542$). Seedlings also did not differ in the net number of leaves added during the growing season ($F = 1.16$, $p = 0.337$). Based on changes in height, the relative interaction index for the removal treatment was 0.65, indicating a facilitative interaction between sagebrush seedlings and neighbors.

Seedling survivorship - Mortality was not affected by the heating treatment (ANOVA, $p = 0.545$). However, neighbor removal significantly increased seedling mortality (ANOVA, $p = 0.0198$, Figure 3). Seedlings that had neighbors removed were almost four times more likely to die during the course of the experiment than those with neighbors intact (mortality with removal = 39.3%, mortality without removal = 10.7%). Mortality occurred first on plots with both heating and neighbor removal. 86% of seedlings that were considered dead became brittle and ceased growth, but remained in the plots for the duration of the experiment. Only two seedlings in the experiment disappeared, and may have succumbed to herbivory or rodent activity.

Discussion

These results indicate that in the subalpine meadows of the Colorado Rockies, the forb community facilitates the growth and survival of sagebrush (*Artemisia tridentata* ssp. *vaseyana*) seedlings. In particular, I found that neighboring forbs moderate the microclimate around seedlings, reducing daytime maximum temperatures and increasing nighttime minimum temperatures, and thus increase the survival of seedlings by 50% and the height of seedlings by 96%. This facilitation by neighboring forbs had a larger effect on survival of seedlings than direct temperature modification via passive heating chambers. Therefore, I reject the hypothesis that recruitment of sagebrush seedlings is dependant on the physiological tolerance of sagebrush alone (Table 1). I also reject the hypothesis that forbs have a competitive interaction with sagebrush seedlings and prevent their establishment. The stress gradient hypothesis is variably supported in this study. While forbs facilitate sagebrush seedling recruitment at their upper elevational limit, the warming treatment had no direct affect on seedling growth or survival.

However, temperature appears to play a crucial role in the establishment of sagebrush seedlings. Maximum daytime temperatures in removal treatments were on average 3.07°C warmer than in non-removal treatments ($p < 0.0001$), but there was no detectable difference in soil moisture between treatments ($p = 0.711$). This is likely because soil moisture was integrated over 12 cm in soil depth and temperature was recorded in the top 2 cm, just below the soil surface. Furthermore, seedlings in removal treatments were unshaded by neighboring vegetation, and likely received more radiation, although this was not quantified. This suggests that seedlings in removal treatments likely

experienced higher leaf temperatures and thus stronger water potential gradients than those with intact neighboring forbs. Thus, seedlings in removal treatments may have experienced higher water stress.

Water availability is critical for the successful germination and recruitment of sagebrush across much of its range. In many populations, germination of *Artemisia* seedlings is episodic and tied to inter-annual variation in soil moisture. For example, in British Columbia, at the northern range limit of *Artemisia tridentata*, recruitment is correlated with June and July rainfall (Cawker 1980), while in Wyoming, recruitment of all sagebrush subspecies is tied to precipitation (Maier *et al.* 2001). In ssp. *wyomingensis*, seedling survival is reduced under water stress conditions (Young *et al.* 1991). In the Sierra Nevada of California, a related species, *Artemisia rothrockii*, colonizes herbaceous habitats at high elevation following wet years (Bauer *et al.* 2002). These results broadly indicate that sagebrush germination, survival and recruitment is strongly limited by water-stress, across the entire range of the species. Microclimate modification by shading may be a mechanism for facilitation of sagebrush seedlings by neighboring vegetation.

The frequency and strength of plant-plant facilitation is usually highest at the climatic extreme of species ranges (Choler *et al.* 2001; Callaway *et al.* 2002). Alpine treeline appears to be controlled not only by a physiological inability for trees to survive at higher altitudes, but also by facilitative plant-plant interactions, which promote establishment (Bansal *et al.* 2011). Our study was conducted near the elevational range limit for sagebrush in the Gunnison Basin and in one of the highest populations of sagebrush in North America. I conclude that facilitation is critical for the survival of

sagebrush seedlings and thus recruitment of sagebrush in subalpine meadows, at their elevational range limit.

Our results suggest that it is critical to understand how the ecology of each life stage of a species responds to change in climate when predicting range shifts.

Additionally, species interactions that have a significant impact on species fitness must be addressed in the context of climate change. Elevational advance of sagebrush is unlikely to occur in the subalpine Colorado Rockies unless there is adequate relief from water stress for seedlings from facilitation by neighboring forbs.

Acknowledgements

The author would like to acknowledge the field crew from the University of Arizona and RMBL for assistance with seed collection, chamber assembly and field work: B. Blonder, K. Buffington, V. Buzzard, B. Chaszar, A. Henderson, N. Pierce, T. Potter, N. Prohaska, J. Prohaska and L. Sloat. I thank the Rocky Mountain Biological Laboratory for support. BJ Enquist, L. Kueppers, J. Harte, S. Saleska, J. Reithel, D. Inouye, N. Waser all provided invaluable guidance on this project. This work was supported by an EPA STAR fellowship FP 91699101 to the author.

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Tables

Table 1: Summary of predictions of the effect of warming and neighbors on sagebrush seedling recruitment for three different hypotheses.

Hypothesis	Effect on sagebrush recruitment		
	Warming	Neighbors	Warming x Neighbors
<i>Physiological Tolerance</i>	+	0	0
<i>Forb Decline</i>	0	-	Less negative
<i>Stress Gradient</i>	+	+	More negative

Figure Captions

Figure 1: Mean diurnal growing season temperature differences from control by treatment. Subsurface soil temperatures were logged hourly in all plots and averaged by treatment and time of day from June 20, 2009 to August 20, 2009. Heated plots (dark squares) were not significantly different than control plots for most of the day. However, removal plots (open circles) were significantly warmer than controls from about noon to 7 p.m. and colder than controls from about 11 p.m. to 9 a.m. The combination of heat and removal were not significantly different from controls at night, but were warmer during the afternoon. Error bars are standard deviation.

Figure 2: Weekly mean cumulative change in height for focal seedlings throughout the growing season for all seedlings in the study. Seedlings without neighbor removal (solid markers) had consistently larger growth than those with neighbors removed (open markers). Weeks with significant differences among groups are indicated by asterisks (*). Results were similar when only seedlings that survived the entire course of the experiment were included.

Figure 3: Mortality versus time during the growing season in 2009 for each of 4 treatment types: control, heating, neighbor removal and heating x neighbor removal. Mortality was 4 times higher among seedlings in either of the removal treatments relative to those in either of the non-removal treatments.

Figure 4: Mean soil temperature difference between plots of seedlings that died during the experiment and those that survived to the end of the experiment. All data points are significantly different from zero ($p < 0.0001$) except for where indicated. Seedlings that died experienced temperatures that were on average more than 2°C warmer in the afternoon, and as much as 1°C colder in the morning than those that survived, regardless of treatment.

Figures

Figure 1:

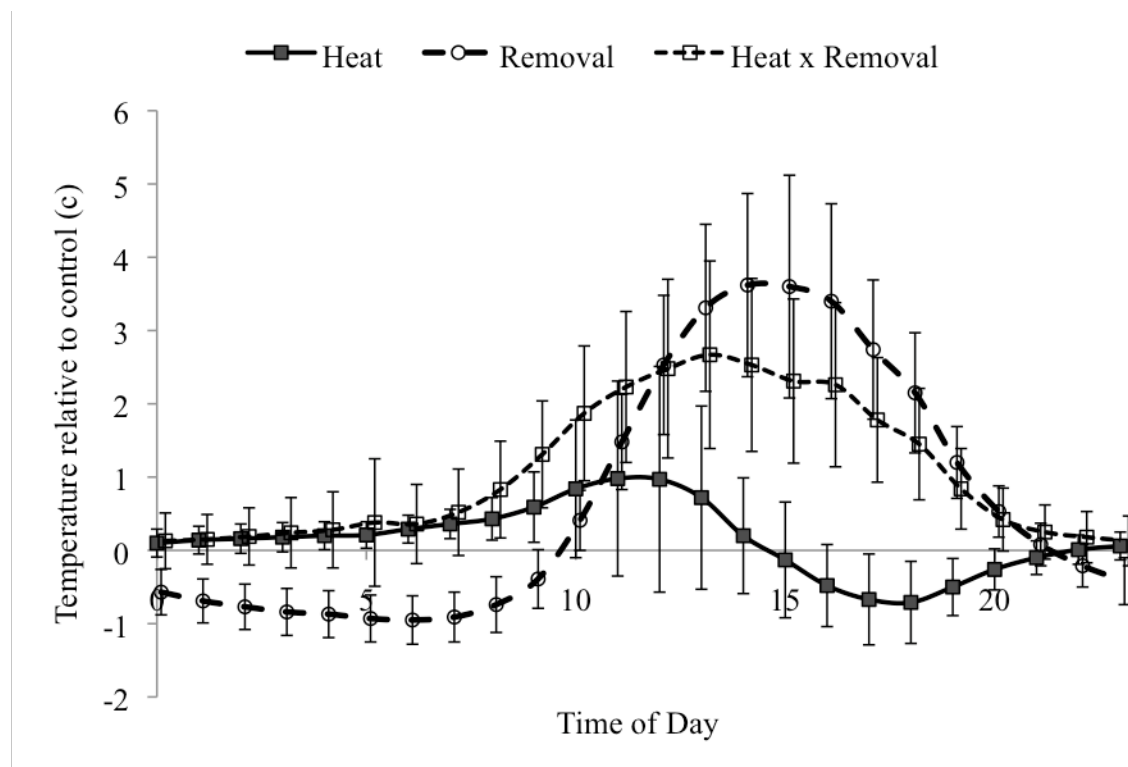
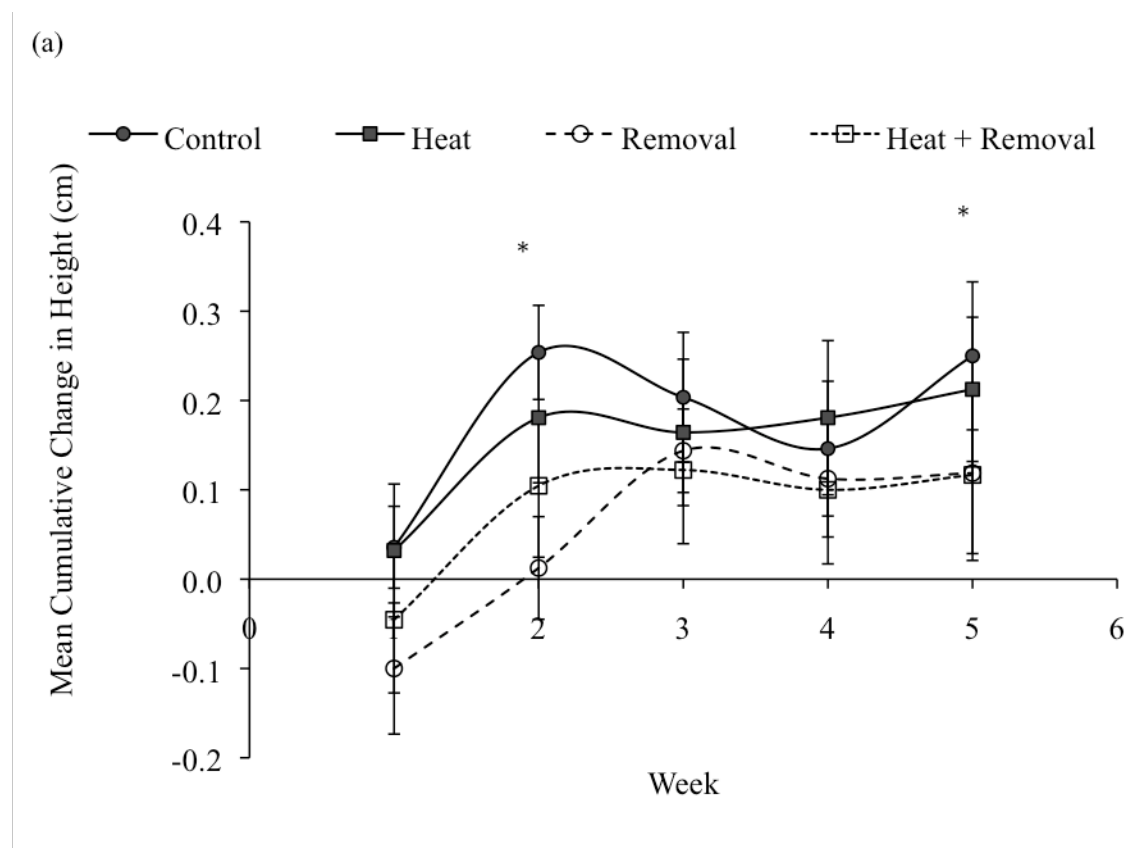


Figure 2:



Figure

3:

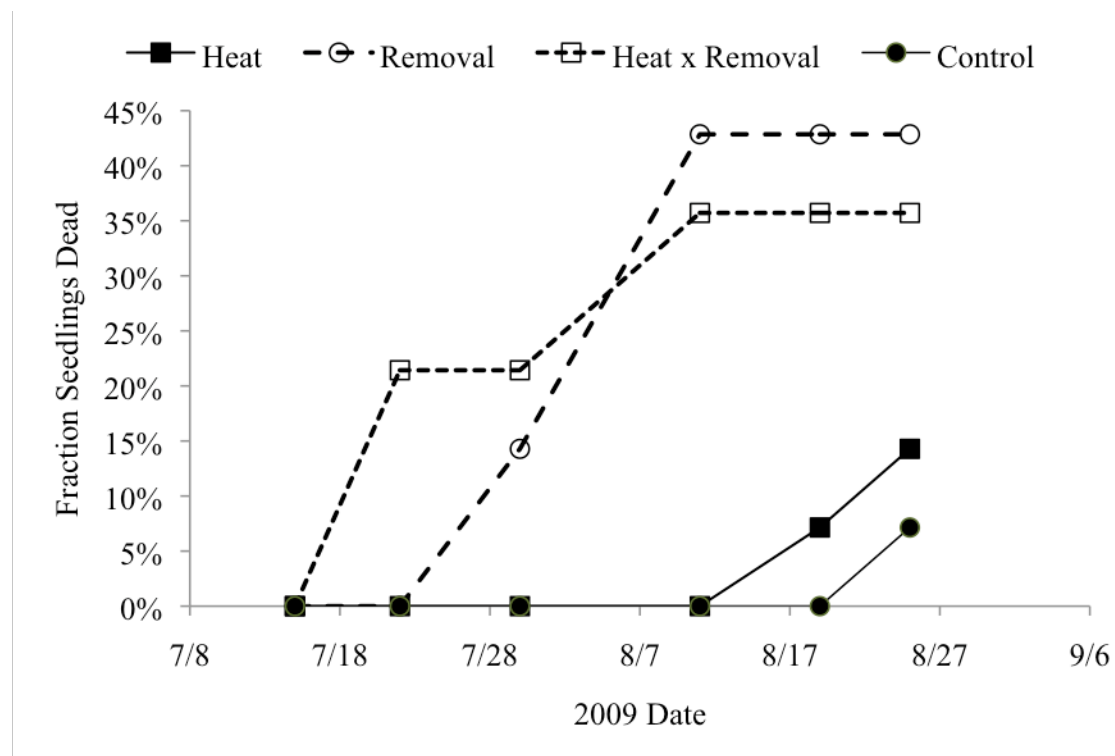


Figure 4:

