CLIMATE CHANGE EFFECTS ON ARBUSCULAR MYCORRHIZAL FUNGI AND PRAIRIE PLANTS ALONG A MEDITERRANEAN CLIMATE GRADIENT

by

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A THESIS

Presented to the Department of Biology
and the Graduate School of the University of Oregon
in partial fulfillment of the requirements
for the degree of
Master of Science

December 2012

THESIS APPROVAL PAGE

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Title: Climate Change Effects on Arbuscular Mycorrhizal Fungi and Prairie Plants Along a Mediterranean Climate Gradient

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Degree awarded December 2012

THESIS ABSTRACT

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Title: Climate Change Effects on Arbuscular Mycorrhizal Fungi and Prairie Plants Along a Mediterranean Climate Gradient

Arbuscular mycorrhizal fungi (AMF) provide numerous services to their plant symbionts. Understanding the effects of climate change on AMF, and the resulting plant responses, is a crucial factor in predicting ecosystem responses on a global scale. We used a manipulative climate change experiment embedded within a natural climate gradient in Oregon and Washington to examine how the effects of future climate change on AMF-plant symbioses are mediated by soil water availability, soil nutrient availability, and vegetation dynamics.

Using structural equation modeling, we found that the direct effect of increasing temperatures was to decrease AMF colonization. Indirect effects of temperature, mediated through other variables, canceled each other out. However, future shifts in these relationships could either exacerbate or mitigate the negative direct effect of temperature. As ecosystems in Mediterranean climates experience more intense droughts and heavier rains, decreases in AMF colonization could have substantial consequences for plant communities and ecosystem function.

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ACKNOWLEDGMENTS

I wish to express sincere appreciation to Dr. Scott Bridgham for his thoughtful and insightful ideas that helped improved the experimental design and analysis of this research, for his useful advice and careful edits to the manuscript, and for the many words of encouragement along the way. I am also grateful for the thorough review of the manuscript by Dr. Bart Johnson, who never misses a grammatical error or slip in logic, and also for his guidance throughout the project. They have both contributed immensely to my growth as a researcher, critical thinker, and writer. I would also like to thank Dr. Brendan Bohannan for his contributions of knowledge and views from a different perspective, and for allowing me access to his lab and to be a graduate student at the University of Oregon.

Special thanks goes out to Dr. Rebecca Mueller for her time, patience and assistance in the lab and analysis of the molecular data. Also special thanks to Dr. Laurel Pfeifer-Meister for her thoughtful suggestions to the analysis, and guidance in the field. Maya Goklany deserves special recognition for providing direction and resources for the analysis. I would like to thank Dr. Tim Tomaszewski and Lorien Reynolds for providing me with essential data and Roo Vandegrift for assistance with laboratory techniques.

I am indebted to Dr. Alan Kelly, Mark Carrier, Carl Steifbold, and Holly Lynn for acting as friends and mentors in my teaching endeavors. They have inspired me to continue a career in science education and have opened countless doors that have lead me to where I am today. I would also like to thank my undergraduate advisors, Bitty Roy and George Carroll, as they sparked my interested in mycology and botany, and initiated my foundation in research science and writing. I could not have finished without the love and support of

my fellow graduate students, friends outside the department, and the unwavering optimism of my parents.

Much appreciation goes out to The Nature Conservancy, The Center for Natural Lands Management, and The Siskiyou Field Institute for providing the location of the field sites. This research was funded by the Office of Biological and Environmental Research, Department of Energy (DE-FG02-09ER604719).

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CHAPTER I

INTRODUCTION

Research Overview

Arbuscular mycorrhizal fungi (AMF) provide numerous services to their plant symbionts and are especially important in regard to nutrient and carbon cycling and plant productivity (Perry et al., 1990; Drigo et al., 2008). Understanding the effects of climate change on AMF, and the resulting plant responses, is a crucial factor in predicting ecosystem responses on a global scale (Drigo et al., 2008; Compant et al., 2010). Though mycorrhizas and other soil microbes have been increasingly studied over the past decade, there have been no clear conclusions about how this group of mutualists will respond to a changing climate (Compant et al., 2010). My study looks specifically at how plant-mycorrhizal interactions will be affected by climate change in prairies of the Pacific Northwest (PNW).

Originally making up the largest vegetative province in North America (66 million ha across the Great Plains), prairies have suffered a larger percent decrease than any other ecosystem, including old growth forests (Samson and Knopf 1994). A dramatic example of this loss are the prairies of the PNW, which now cover less than 1% of their original area (Noss et al 1995). The western part of the PNW, including its prairies, has a Mediterranean climate (Kottek et al., 2006), which is defined as having temperate, rainy winters with dry, hot summers (Kottek et al., 2006). Mediterranean ecosystems occupy less than 5% of the earths land surface, but harbor over 20% of known vascular plant species (Cowling et al., 1996). The biodiversity of Mediterranean and grassland ecosystems may be among the most sensitive of the world biomes to global climate

change, as these biomes may experience synergistic responses to changes in climate, nutrient deposition, invasive species, and land use changes (Sala et al., 2000).

Prairie plant communities are closely connected with their AMF symbionts, with the plant community affecting AMF community, and vice versa (Hartnett and Wilson, 1999; Eom et al., 2000). Studies have shown that AMF can help aid the establishment of native prairie species (Smith et al., 1998), native prairie species may interact more favorably with their local AMF symbionts (Johnson et al., 2010), and that AMF are not randomly distributed throughout tall grass prairies but show some degree of host specificity (Eom et al., 2000). Consequently, it is crucial to develop a firm understanding of the interactions between AMF and prairie plants to accurately predict climate change effects on these diverse and sensitive ecosystems (Araújo and Luoto, 2007).

My study is a part of a multi-year, manipulative climate change experiment of upland prairies along a Mediterranean climate gradient in the PNW. The experiment consists of treatments that mimic the climatic conditions projected for the PNW in the coming century. Global climate change models for the PNW project an increase in average annual temperatures of +3.0°C by 2080 (Mote and Salathe, 2010). While average annual precipitation projections are highly variable among different emission scenarios, across models there is a consistent projection of warmer, wetter winters and hotter, dryer summers (Mote and Salathe, 2010). This trend is also predicted for Mediterranean climates worldwide (Solomon et al., 2007; Ruffault et al., 2012). Our sites exist along a gradient of increasing severity of Mediterranean climate from north to south. The climate change manipulations of the experiment include a 3.0°C increase in temperature applied

with infrared heaters, a 20% increase in precipitation, and the combination of both heat and precipitation.

This thesis consists of three chapters. The current chapter contains the research overview and a general description of arbuscular mycorrhizal fungi. Chapter two is the main body of the thesis and is written in the format of a stand-alone journal article with introduction, methods, results and discussion. Chapter three discusses the conclusions, possible applications, and future research questions generated from this study.

Description of Arbuscular Mycorrhizal Fungi

Arbuscular mycorrhizal fungi (AMF) form symbiotic relationships with the roots of the majority of terrestrial plant species, where the fungi provide enhanced nutrient and water uptake to the host plant in exchange for carbon (Smith and Read, 2008). The name arbuscular comes from the Latin term ‘arbusculum’, which was coined for the characteristic structure the fungi make resembling a little tree or bush (Smith and Read, 2008). This structure, the arbuscule, forms within the cortical root cells of plants (Figure 1) and is thought to be the main site of nutrient and carbon exchange (Smith and Read, 2008).

AMF belong to the phylum Glomeromycota, which consists solely of arbuscular mycorrhizal-type fungi. Other types of mycorrhizal fungi (e.g., ectomycorrhizas, and ericoid and orchid mycorrhizas) have evolved from multiple lineages of higher order fungi (Brundrett, 1991). AMF are also morphologically distinct in that they have aseptate hyphae, arbuscules, hyphal coils, vesicles, large multinucleate spores, do not form a fruiting body, and live entirely as microscopic organisms within the roots of plants

(Brundrett, 1991). While other types of mycorrhizas are more limited in the families of plants they associate with, AMF are ubiquitous, and are estimated to make associations with over 200,000 species of terrestrial plants (Young, 2012). Whereas other types of mycorrhizas can make either obligate or facultative associations with their host plant, depending on the fungal symbiont (Brundrett, 1991), all known AMF are considered obligate biotrophs, and have never been successfully cultured in the complete absence of a plant host (with one exception using a co-culture of bacteria) (Hildebrandt et al., 2005).

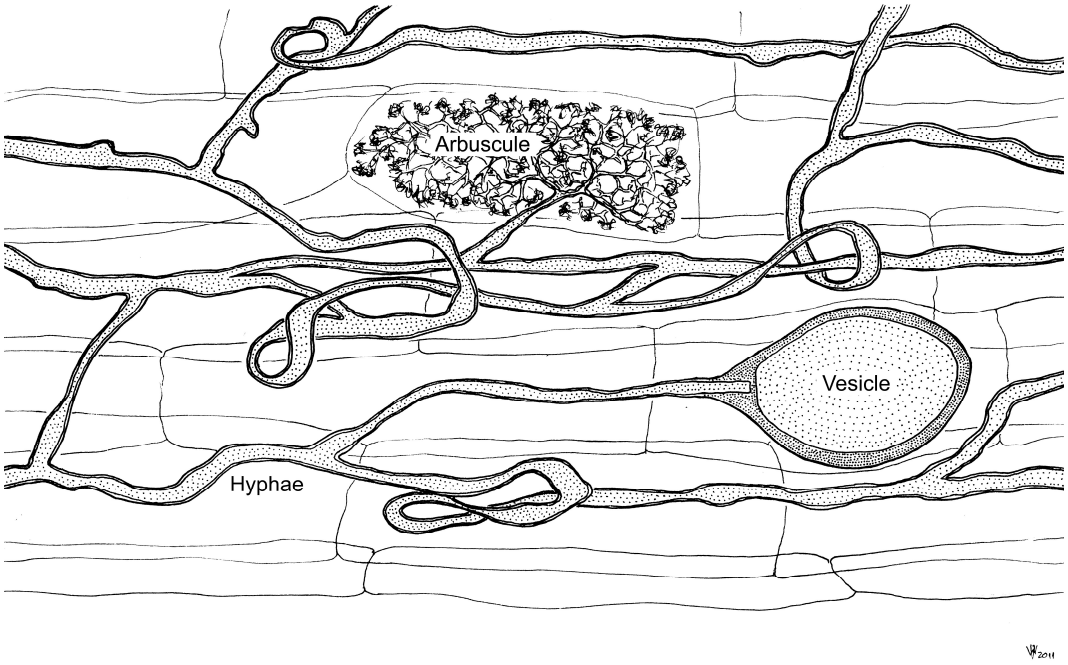

Figure 1. Illustration of AMF. Image courtesy of Roo Vandegrift, 2011**.**

AMF have been closely studied for over a century, but it is only recently that scientist have discovered how diverse the phylum is, both functionally and phylogenetically. Less than a decade ago, AMF were thought to consist of six genera and approximately 200 species, identified morphologically by spore cell wall characteristics

(Redecker et al., 2003; Smith and Read, 2008). However, rDNA sequence information is uncovering vast numbers of unidentified phylotypes, indicating that cryptic species diversity is much higher than previously assumed (Krüger et al., 2012). For example, the most recent phylogenic analysis of the Glomeromycota showed that nearly every known species in the genus *Glomus*, historically the most common genus of AMF, now belong to new genera (Krüger et al., 2012). To date, at least 18 genera are now recognized, and a recent study of the global diversity of AMF found almost 700 operational taxonomic units (OTUs) with a 97% sequence similarity cut-off (Kivlin et al., 2011).

AMF are best known for their ability to increase plant nutrient uptake, especially for phosphorus. The hyphal network can extend more than 20 cm away from the plant root system and occupy over 100 m cm^{-3} in total soil volume, thereby greatly increasing the total surface area of the plant root system (Miller et al., 1995). Additionally, AMF mycelium are particularly well equipped for scavenging phosphorus from insoluble soil organic compounds due to specialized phosphate transporters that are expressed in the extraradial mycelium (Harrison and Van Buuren, 1995). Smith et. al. (2003) demonstrated that colonization of AMF can result in the complete inactivation of phosphorus uptake through root hairs and that up to 100% of phosphorus within the plant was delivered via the AMF symbiont.

Nitrogen uptake can also be greatly increased by AMF colonization, especially in soils where nitrogen is limiting. AMF can increase the uptake of available nitrogen, as well as transfer usable forms of nitrogen from one plant to another via the root systems (Ames et al., 1983). Though less well cited in the literature, AMF can also enhance the acquisition of a range of other micronutrients important for plant growth, such as Zn, Cu,

Mn, and Fe, via processes similar to that of phosphorus uptake (Liu et al., 2000). In contrast, plants that thrive in heavy metals soils are often heavily colonized by AMF hyphae, where it is thought that the fungi prevent excess heavy metal accumulation in plant tissue (Hildebrandt et al., 2006).

AMF provide more services to their plant hosts than enhanced nutrient uptake. They can be important in plant-water relations by substantially increasing stomatal conductance and providing enhanced drought tolerance (Ruiz-Lozano et al., 1995; Augé, 2001; Subramanian et al., 2006). Soil hyphae and glomulin secretion can also enhance soil stability (Rillig et al., 2002; Rillig, 2004; Wilson et al., 2009). Root colonization has been shown to increase disease resistance in various economically important crops (Graham et al., 1982b; Sharma et al., 1992). AMF diversity can have a significant impact on plant biodiversity and plant productively by allowing them to more fully utilize limiting resources (Van der Heijden et al., 1998; Klironomos et al., 2000). Likewise, it has been shown that the type of plant community is just as important in influencing AMF diversity and community composition (Johnson et al., 2004; Kivlin et al., 2011).

AMF are not always beneficial to their plant hosts, but exist on a continuum from mutualistic to parasitic (Johnson et al., 1997). Plants that are nutrient stressed often allocate more carbohydrates into root exudates and, thus, are often more heavily colonized by AMF than plants that are not nutrient limited (Sylvia and Neal, 1990). Fertilizing nutrient-poor soils selects for AMF isolates that are inferior mutualists over isolates that benefit plant growth (Johnson 1993). However, the majority of studies that have demonstrated a parasitic effect of AMF have used only a few isolates of AMF and/or a few species of plants (Johnson et al., 1997, 2010). The benefits and costs of

individual AMF species and entire communities within root-hyphal networks are complicated, technically difficult to study, and largely unknown (Klironomos, 2003).

Due to their wide range of functional services, AMF are arguably the most important microbial symbionts to the majority of terrestrial plant species. They are often considered an important link between below- and aboveground ecosystem processes, especially in regard to nutrient and carbon cycling (Perry et al., 1990; Compant et al., 2010). Because of their known importance to plant and ecosystem functions, AMF are an essential component for predicting how terrestrial ecosystems will respond to climate change (Drigo et al., 2008). Recent studies have revealed that AMF and other soil microorganisms are important factors that influence and potentially ameliorate plant responses to climate change (Compant et al., 2010). Thus, the second chapter of this thesis is dedicated to a study of how climate change will affect AMF and their plant symbionts, and what abiotic and biotic factors may be mediating these responses.

CHAPTER II

CLIMATE CHANGE EFFECTS ON ARBUSCULAR MYCORRHIZAL FUNGI AND PRAIRIE PLANTS ALONG A MEDITERRANEAN CLIMATE GRADIENT

Introduction

Arbuscular mycorrhizal fungi (AMF) form symbiotic relationships with the majority of terrestrial plants and provide a wide range of services including enhanced nutrient and water uptake, drought and disease resistance, and increased plant productivity, in exchange for carbon (Smith and Read, 2008). AMF are a major contributor to terrestrial carbon and nutrient cycles (Fitter et al., 2000) and are considered an important link between above- and belowground processes (Leake et al., 2004). They can consume up to 20% of carbon produced by their plant host (Bago et al., 2000), and the hyphal network can occupy over 100 m cm^{-3} in total soil volume (Miller et al., 1995), making up 20-30% of the total microbial biomass in terrestrial systems (Leake et al., 2004).

Given their widespread importance, it is perhaps not surprising that recent studies have shown that AMF may be a major factor in mediating plant and ecosystem responses to climate change (Drigo et al., 2008; Compant et al., 2010). The majority of studies have seen an increase in AMF colonization in response to experimentally increased CO_2 levels and/or temperature (Compant et al., 2010). This could be due to the overall response of increased plant productivity to climate change, resulting in a larger demand for plant nutrients and enhanced production of root exudates (Fitter et al., 2000; Zavalloni et al., 2012). However, the response of AMF to climate change may be mediated by other

factors in addition to increased plant productivity. Enhanced drought severity is a major concern for many regions, and AMF have been shown to increase drought resistance and improve water relations (Augé, 2001). However, numerous studies have found that enhanced drought can have a negative effect on AMF, depending on the species of AMF (Davies et al., 2002), hyphal growth within or outside the roots (Staddon et al., 2003), and the species of plant (Ruiz-Lozano et al., 1995). Moreover, many studies that revealed a positive response of AMF colonization to increased temperature and CO_2 were performed with one or a few species of AMF and plant hosts under laboratory or greenhouse conditions (Graham et al., 1982a; Baon et al., 1994; Staddon et al., 2004; Heinemeyer et al., 2006). Because AMF have recently been shown to have much higher species diversity than previously estimated (Kivlin et al., 2011), and relative contributions of AMF benefits are not equal among plants in natural ecosystems (Leake et al., 2004), more studies are needed before generalizations can be made about the responses of AMF and their plant hosts to climate change.

Field experiments that revealed a positive effect of experimental warming on AMF have noted confounding variables that may be influencing their response to increased temperature. In a long-term climate manipulation, Staddon et al. (2003) demonstrated that increased colonization in response to heat and drought was mediated by the effect of soil moisture content. Furthermore, they speculated that the effect of soil moisture could have been further mediated by changes in plant diversity and cover of various species, which were also highly correlated with mycorrhizal measures (Staddon et al., 2003). Rillig et al. (2002) noted that increased nutrient mineralization in their heated plots may have influenced AMF growth. They also found that although internal

root colonization increased, soil aggregation decreased due to a decrease in glomulin production (a glycoprotein secreted by extraradical hyphae) with experimental heating (Rillig et al., 2002). A later study showed that extraradical colonization was highly correlated with soil aggregation and C and N sequestration, suggesting that a decrease in both root and extraradical colonization in response to climate change could have major impacts on ecosystem functions (Wilson et al., 2009). Interactions between plant vegetation, water availability, and soil characteristics should be considered when analyzing the effects of climate change on AMF.

It has been long assumed that a decrease in soil nutrient levels, especially of phosphorus and nitrogen, results in an increase in AMF colonization, while excess nutrients results in lower colonization (Mosse and Phillips, 1971; Smith and Read, 2008). However, the relative limitation of nitrogen to phosphorus, i.e. ecological stoichiometry, may serve as a more powerful tool for assessing AMF responses to climate change (Treseder and Allen, 2002; Johnson, 2009). In the most recent study of nutrient limitation on AMF, Blanke et al. (2012) found that in N and P co-limited soils, an addition of phosphorus decreased colonization while an addition of nitrogen increased colonization.

To our knowledge, all previous experimental studies on AMF-plant responses to climate change were performed at a single site. However, climate varies regionally, while other important factors such as soil characteristics and plant community composition often have high local variability. To extrapolate site-specific results to a regional scale requires understanding the roles of both regional and local controls on AMF and plant responses. To this end, we examined how increased temperature affects AMF and their host plants along a Mediterranean climate gradient in the Pacific Northwest (PNW). The

PNW and California are part of the world's Mediterranean ecosystems (Kottek et al., 2006), which hold a large percentage of global biodiversity of terrestrial plants (20%) in proportion to their total terrestrial area (5%) (Cowling et al., 1996). They appear to be among the most sensitive biomes to global climate change (Sala et al., 2000), especially in terms of changes in seasonal precipitation, with both increased severity of summer drought and amplified winter rains predicted (Solomon et al., 2007). To predict responses to climate change on a regional scale, variation in factors such as seasonality and biotic interactions between plant and soil processes must be taken into account (Araújo and Luoto, 2007; Ruffault et al., 2012).

We used a manipulative climate change experiment embedded within a 520-km climate gradient in the PNW to determine the underlying direct and indirect effects that increased temperatures have on AMF and their plant hosts in Mediterranean climates. We hypothesized that much of the effect of temperature on AMF colonization, as well as the host plants' nutrient composition and biomass, would be mediated through interactions with vegetation dynamics and soil water and nutrient availability. We were also interested in whether these effects were regionally consistent along a gradient of increasing severity of Mediterranean climate.

Methods

Site Descriptions

We studied three prairie sites along a 520-km latitudinal climate gradient in the inland valleys of the PNW. The most southern site is in the Illinois Valley of southwestern Oregon at the Siskiyou Field Institute's Deer Creek Center near the town of

Selma. The central site is in central-western Oregon at the southern end of the Willamette Valley on The Nature Conservancy's Willow Creek Preserve near the city of Eugene. The northernmost site is in the Puget Trough of central-western Washington on the Center for Natural Lands Management's Tenalqout Prairie near the town of Tenino.

The sites exist along a gradient of increasing severity of Mediterranean climate from north to south (Table 1; Kottek et al., 2006). The southern site has the most extreme seasonal variation (Csa climate zone, "hot summer"), experiencing the wettest, coolest winters and driest, warmest summers. The central and northern sites have comparatively milder winters and summers in terms of rainfall and temperature (Csb climate zone, "moderate summer"), with the central site having warmer average summer and winter temperatures than the northern site. Global climate change models for the PNW predict an increase in average annual temperatures of +3.0°C by 2080 (range +1.5°C to over +5.8°C) (Mote and Salathe, 2010). While average annual precipitation predictions are highly variable among different emission scenarios and models (range -10% to +20% by 2080), across models there is a consistent prediction of warmer, wetter winters (precipitation range +8% to +42%) and hotter, dryer summers (precipitation range -14% to -40%) (Mote and Salathe, 2010).

Typical for a study spanning a large regional area, each site has a different soil type. The soil at the southern site is a loamy Mollisol (coarse-loamy, mixed, superactive, mesic Cumulic Haploxeroll), at the central site is a silty-clay loam Mollisol (very-fine, smetitic, mesic Vertic Haploxeroll), and at the northern site is a gravelly sandy loam Andisol (sandy-skeletal, amorphic-over-isotic, mesic Typic Melanoxerand). The southern site has a circumneutral pH, and the central and northern sites are mildly acidic (Table 1).

These differences in soil characteristics translate into large differences in nutrient availability, with the southern site having much greater nitrogen and phosphorus availability and a greater N:P ratio (Appendix A, Figure S1). The central site had moderately greater nitrogen and phosphorus availability and a lower N:P ratio than the northern site.

Table 1. Site characteristics.

Site	Southern	Central	Northern
Latitude	42°16’41”N	44°01’34”N	46°53’47” N
Longitude	123°38’34” W	123°10’56” W	122°44’06” W
Elevation (m)	394	165	134
Mean Precip. (mm)	1598	1201	1229
Mean Mon. Temp. (°C)	12.2	11.4	9.8
Max. Mon. Temp. (°C)	19.9	17.3	15.3
Min. Mon. Temp. (°C)	4.1	5.3	4.9
%SAND	31.4	36.4	73.9
%CLAY	22.5	11.9	2.4
%SILT	46.0	51.6	23.7
%Total soil nitrogen	0.3	0.5	0.4
%Total soil carbon	3.4	4.2	3.8
pH	6.5	5.8	5.6

Experimental Design

Treatments within each site were organized in a fully factorial design. Five replicates of each of the following treatments were applied to 3-m diameter circular plots: constant +3°C above ambient canopy temperature, 20% increased precipitation, increased temperature and precipitation, and control plots (ambient temperature and precipitation). The precipitation treatment was applied within two-weeks of a rainfall event, and thus increased rainfall intensity during the rainy season (Oct.-June), with little water added during the dry summers (July-Sept.). Heating treatments used infrared heaters (Kalglo

heaters model HS 2420, Kalglo Electronics Co., Inc.) controlled by a dimmer system that modulated heat output to maintain consistent +3°C canopy heating compared to reference control plots (Kimball, 2005; Kimball et al., 2008). Dummy heaters were installed in non-heated plots to account for potential shading effects of the heaters. Belowground processes within the plots were isolated from the surrounding soil by burying an aluminum barrier to 40-cm depth, or to the depth of major obstruction. For the current study, we used only the heated and control treatments to make the project more tractable. Moreover, the precipitation treatments have shown only minor effects on all response variables for which it has been examined, including a wide array of plant responses (unpublished data).

Plots at each site were treated in 2009 with one or two applications of glyphosate (spring and fall) followed by thatch removal and seeding with an identical mix of 33 annual (12 forbs, 1 grass) and perennial (15 forbs, 5 grasses) native prairie species within each plot. For each site, we collected seed from the nearest local population of each species, or purchased seed from a native plant nursery that used first-generation plants from the nearest seed source. During the 2010 growing season, the most aggressive exotic species were weeded, but natural succession was allowed to occur afterwards resulting in a mix of species that were either intentionally seeded, came from the seed bank, or dispersed into the plots.

In the 2011 growing season, we selected four native forbs for assessment of climate effects on AMF associations. Graminoid species were not assessed because no common species grew within all plots across all sites. The selected focal species were: *Achillia millefolium* L., Asteraceae (perennial); *Eriophyllum lanatum* (Pursh) Forbes,

Asteraceae (perennial); *Plectritis congesta* (Lindl.) DC., Valerianaceae (annual); and *Prunella vulgaris* L. ssp. lanceolata (W. Bartram) Hultén., Lamiaceae (perennial).

Plot Measures

Soil temperature and volumetric water content were continuously monitored in the center of each plot with Campbell Scientific, Inc., Model 107 Temperature Probes and Campbell Scientific, Inc., CS616 Water Content Reflectometers, respectively. The average plot values of data for the one-month period prior to harvesting were used for analysis (Figure 2). We considered other time frames, but this time period had the strongest correlation with AMF colonization. To support comparison across sites, volumetric water content was converted to matric potential using site-specific values of soil texture and organic matter (Saxton and Rawls, 2006).

Soil nitrogen and phosphorus availability were determined with anion and cation exchange probes (PNS™Western Ag Innovations Inc., Saskatoon, Canada) that were inserted 15-cm into the ground from April-July, 2011. NH_4^+-N and NO_3^--N were combined into a single value for total inorganic nitrogen, though the value was dominated by NO_3^--N.

Belowground net primary productivity (NPP) was measured using the root in-growth core method (Lauenroth, 2000) with 5 cm-diameter by 20 cm-depth cores. Aboveground NPP was estimated by destructive harvesting at peak standing biomass of a 0.30 m^2 area within each plot. All vegetation was dried to a constant mass at 60°C before weighing. Aboveground biomass was also separated into forb and grass NPP. We calculated the ratios of aboveground:belowground NPP and forb:grass aboveground NPP.

Total cover of all species was averaged per plot by using the point-intercept method (Jonasson, 1983) with two 1-m^2 quadrats of 25 points each. Presence/absence was determined for all species that were not hit by a pin in a plot, and they were assigned a cover of 0.4%. We calculated species diversity using the average of two 1-m^2 quadrats per plot using Simpson's Diversity Index (1/D).

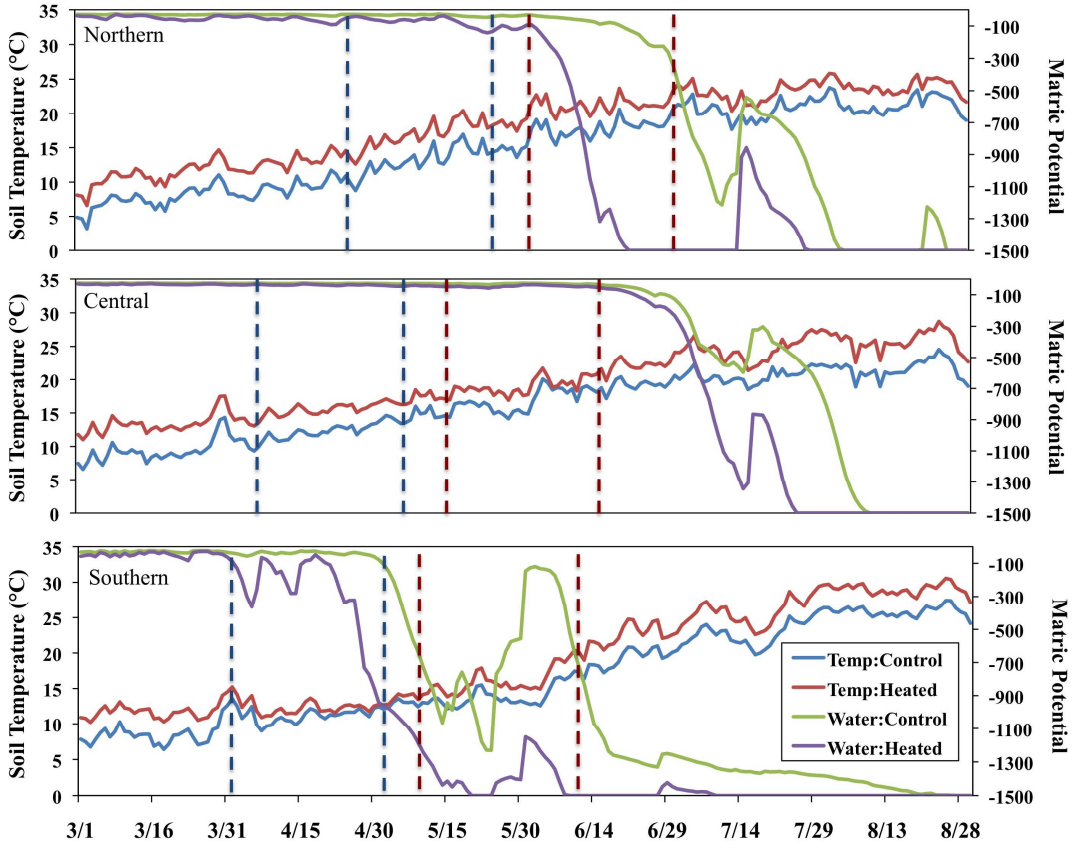

Figure 2. Soil temperature and water availability in the 2011 growing season. Panels correspond with sites, dotted lines indicate the time period used to estimate soil temperature and matric potential one month prior to plant collection. Because of the different phenology, red lines refer to perennial species and blue lines refer to the annual species.

Individual Plant Measures

We harvested three individuals of each of our focal plant species within each heated and control plot. Plants were collected at peak flowering to maintain consistency in phenology across the treatments and sites; thus, the annual species was collected approximated one month before the perennial species (Figure 2). We weighed aboveground plant material after drying at 60°C for 48 hours. Using subsamples of ground and dried material, we determined total phosphorous by performing a hydrogen peroxide-sulfuric acid digest (Haynes, 1980) using a Lachat BD-46 Digester (Hach Company, Loveland, CO) and then measuring phosphate with the vanadate-molybdate colorimetirc method (Motsara and Roy, 2008). Carbon and nitrogen content were measured with a Costech Elemental Analyzer ECS 4010 (Costech Analytical Technologies Inc., Valencia, California, USA).

Due to the small size of the annual species, *Plectritis congesta*, we pooled across plots within a treatment in order to obtain enough plant material to measure phosphorus at all of the sites, and nitrogen at the northern site, resulting in a sample size of one per treatment. Thus, we do not report pair-wise comparisons between treatments on plant phosphorus or nitrogen for these sites for this species.

Mycorrhizal Measures

The percentage of plant root colonized by arbuscular mycorrhizas (i.e., AMF colonization) is a measure of AMF abundance. To quantify AMF colonization, a subsample of roots from each plant was taken and boiled in a 5% Sheaffer® black ink-to-white vinegar solution for 10 minutes after being cleared with 10% KOH (Vierheilig et

al., 1998). Using the grid-intersect method (McGonigle et al., 1990), we calculated the percentage of arbuscule, vesicles, and total root colonization separately by counting the presence or absence of arbuscules and/or vesicles connected by characteristic AM hyphae for each millimeter of root segment.

To compare the community of AMF across treatments and sites, DNA was extracted using the PowerPlant® DNA isolation kit, and purified with Zymo DNA Clean & Concentrator™. AMF DNA extracted from the plant roots was amplified by PCR using the AMF-specific rDNA primers AML2 and NS31 (Lee et al., 2008). We performed a trial run using high-throughput sequencing with an in-house Illumina HiSeq 2000 sequencer (Genomics Core Facility, University of Oregon). Preliminary sequence data was analyzed using MOTHUR (Schloss et al., 2009). Unfortunately, less than 1% (92/14,000) of the sequences were identified as AMF (the remaining was plant DNA) using a BLAST comparison, and further community analyses were not performed. A list of species identified is in the Appendix A: Table S1.

Greenhouse Study

Because of large differences in nutrient availability, pH, and texture among sites (Table 1 and Appendix A: Figure S1), we performed a greenhouse experiment to determine the effect of soil type on AMF colonization. Ten previously germinated seedlings of each species were planted in flats containing soil from each site. Plants grew for eight weeks in a climate-controlled greenhouse at a constant 25°C under natural light (approximately 12-14 hours a day) and were watered as needed to remain above wilting point. After eight weeks, we harvested, measured the dry weight of aboveground

biomass, and used the same protocol described above to quantify the AMF colonization for each plant.

Data Analysis of the Greenhouse and Field Experiment (ANOVAs)

For the greenhouse experiment, we used two-way ANOVAs to test for differences in AMF colonization and aboveground plant biomass by species, soils, and their interaction. For the field experiment, we used three-way ANOVAs to test for differences in AMF colonization, aboveground plant biomass, soil nitrogen availability, soil phosphorus availability, the ratio of soil N:P availability, plant nitrogen content, plant phosphorus content, and the plant N:P ratio, among the species, sites, treatments (heated and control), and their interactions. Though we measured arbuscule, vesicle, and total colonization separately, arbuscule colonization never differed from total colonization, and vesicle colonization was minimal. Thus, we only report total colonization for both greenhouse and field experiments. Soil and plant nutrient analyses can be found in Appendix B.

For all analyses of the greenhouse and field experiments, we performed separate ANOVAs on each species when there was a significant species interaction with any of the other main effects. Post-hoc comparisons were performed using Tukey's HSD. For both greenhouse and field data sets, we used an arcsine-square root transformation to normalize the AMF colonization data, and a logarithm transformation to normalize the plant biomass and nutrient data.

Structural Equation Models of the Field Experiment

We used structural equation modeling (SEM) to examine how the effect of experimental warming on AMF colonization is mediated by soil water availability, soil nutrient availability, and plot vegetation. We also determined how these interactions with AMF colonization affected host plant nutrient content and biomass.

The greenhouse data suggested that soil type had a significant effect on AMF colonization, and we hypothesized this was due to differences in soil nutrient availability of phosphorus and/or nitrogen. However, the ratio of N:P has been suggested to be a more powerful predictor of AM responses then availability (Johnson, 2009). Therefore, we developed three *a priori* models to determine if the availability of nitrogen and phosphorus, or the ratio of N:P, had a larger effect in mediating AMF responses to temperature (Figure 3).

Net Primary Productivity (NPP) and species diversity have been shown to affect AMF (Vandenkoornhuyse et al., 2003; Johnson et al., 2004). We tested our models using above- and belowground NPP, the ratio of above:below NPP, grass and forb NPP, the ratio of grass:forb NPP, and species diversity. Species diversity had the greatest effect on AMF colonization, so we dropped the NPP measures from subsequent analyses to simplify our models.

The maximum likelihood method was used for model evaluation and to estimate the standardized path coefficients. For all analyses, we present only models that had good model fit as estimated by Pearson's chi-square goodness of fit (χ^2) ($P > 0.05$ indicates good model fit), the Bentler Comparative Fit Index (CFI) (< 0.90 indicates good model fit), and the Root Mean Square Error of Approximation (RMSEA) (< 0.05 indicates good

model fit). For models with good fit, we present only path coefficients that were significant at $P < 0.10$. SEM analyses were performed using Amos 20.0 SEM software (SPSS Inc., Chicago IL, USA).

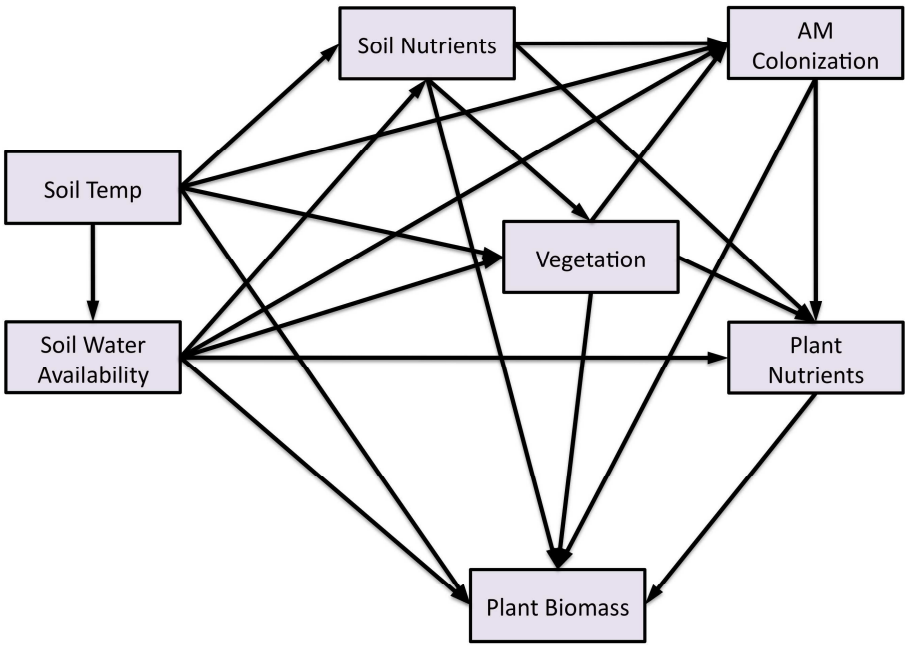

Figure 3. SEM of the effect of temperature on AMF colonization and plant biomass. We tested three *a priori* models that included either soil and plant nitrogen, soil and plant phosphorus, or soil and plant N:P ratios. Each box represents a variable in the model, while each arrow represents a predicted direct effect of one variable on another. A series of connected arrows through multiple variables represent indirect effects.

Results

Greenhouse Experiment

AMF colonization differed in plants grown in the three soils [F (2, 103) = 37.4, $P < 0.0001$], among the four species [F (3, 103) = 11.7, $P < 0.0001$], and the effect of soil

type marginally depended on species [F (6, 103) = 1.9, P = 0.09, Figure 4A)]. For the three perennial species, we consistently found that plants grown in soil from the southern site had the lowest colonization ($P \leq 0.0006$), while plants grown in soil from the central and northern site did not differ. The annual species, *Plectritis*, had the greatest colonization when grown in soil from the central site (P = 0.006).

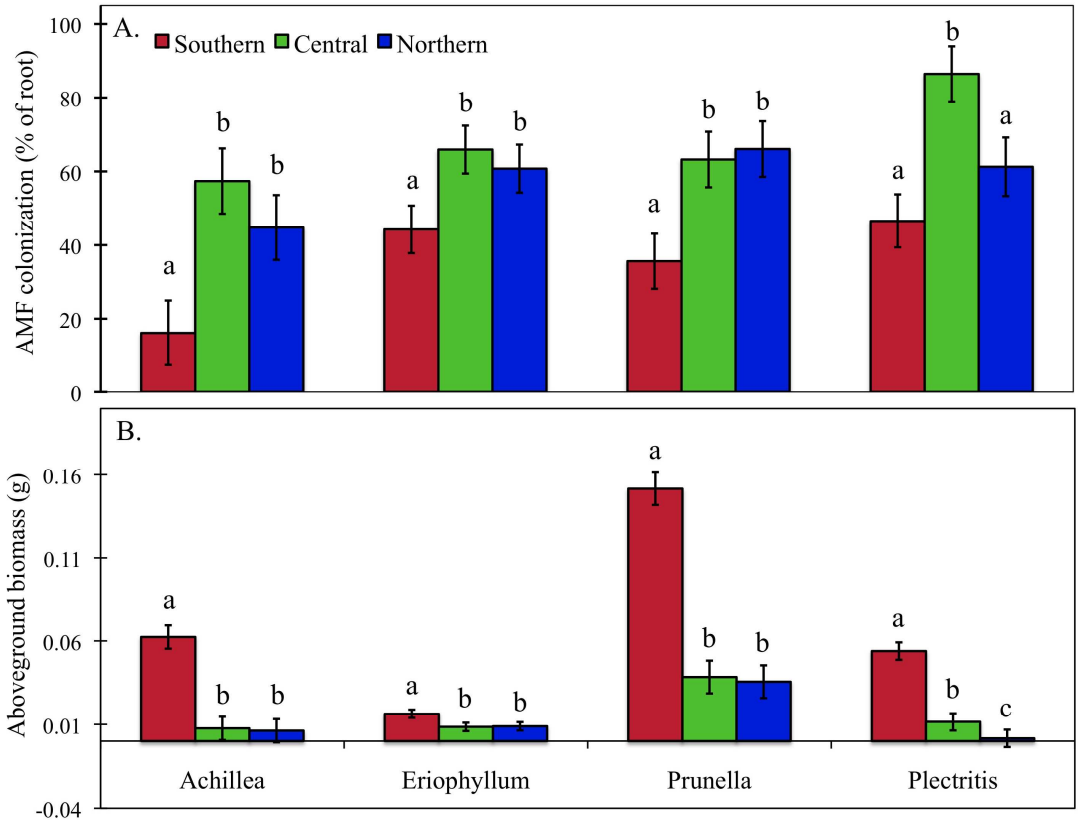

Figure 4. Greenhouse experiment. AMF colonization (A) and aboveground biomass (B) of the four species grown in soil from the three sites (Southern, Central, Northern) in a greenhouse. Different letters indicate significant differences among sites within a species. Error bars represent +/- one SE.

Aboveground plant biomass differed by soil type [F (2, 103) = 187.6, P < 0.0001], among the four species [F (3, 103) = 97.2, P < 0.0001], and the effect of soil type depended on species [F (6, 103) = 23.9, P < 0.0001]. Despite the significant interaction, we found a consistent trend among the three perennial species, which were largest when grown in soil from the southern site (P < 0.0001, Figure 4B), and plants grown in the central and northern site soil did not differ in size. The annual species, *Plectritis*, was largest when grown in southern site soil, intermediate in the central site soil, and smallest in the northern site soil (P < 0.000).

Field Experiment

AMF colonization differed among the three sites [F (2, 281) = 3.8, P = 0.021] and plant species [F (3, 281) = 18.9, P < 0.0001]. The effect of site also depended on species [F (6, 281) = 4.4, P < 0.0001, Appendix A: Table S2]. Colonization did not differ among the sites for *Achillea and Prunella*, but *Eriophyllum* had the greatest colonization in the southern site (P < 0.07), and *Plectritis* had the lowest colonization in the central site (P = 0.01). Across all sites and species, the heating treatment had consistently lower colonization than the control [F (1, 281) = 17.7, P < 0.0001, Figure 5A].

Aboveground plant biomass differed among sites [F (2, 290) = 55.8, P < 0.0001], plant species [F (3, 281) = 457.1, P < 0.0001], and the effect of site depended on species [F (6, 290) = 19.5, P < 0.0001, Appendix A: Table S3]. *Achillea* was largest at the central site (P < 0.0001) and *Eriophyllum* and *Prunella* were smallest at the northern site ($P \leq$ 0.05). *Plectritis* was largest at the southern site (P < 0.0001).

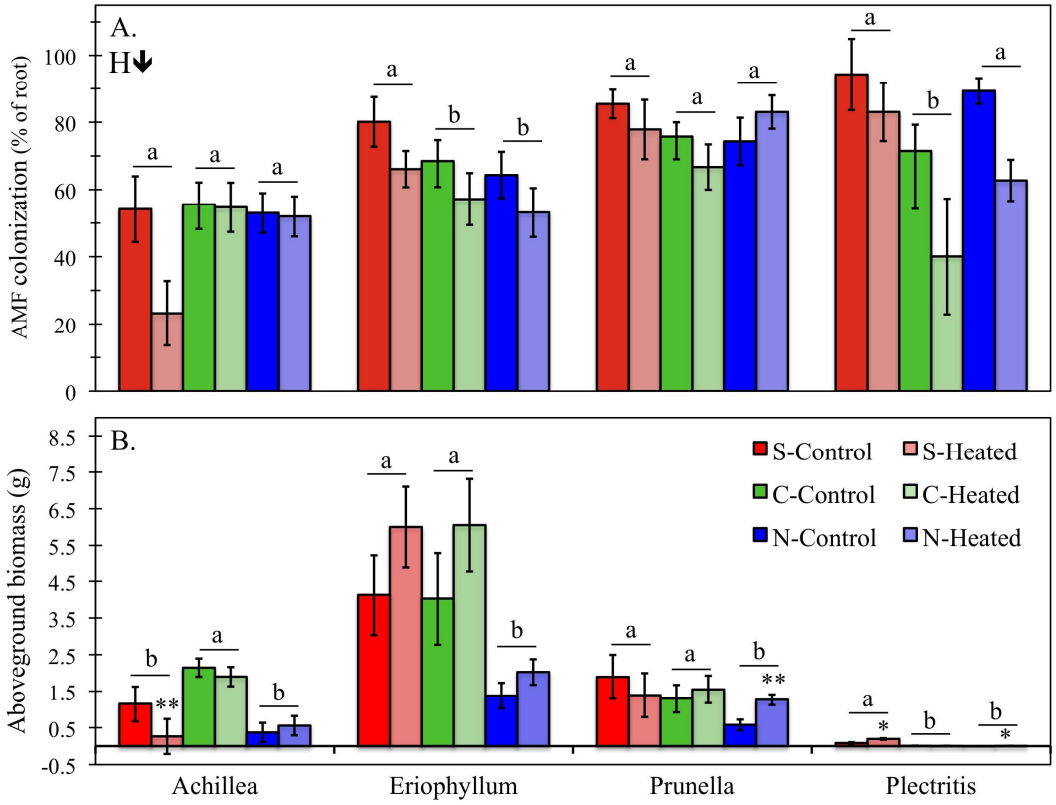

Figure 5. The effect of heating on AMF colonization (A) and aboveground plant biomass (B) of the four plant species collected from the three sites (S=southern, C=central, N=northern). H↓ represents a significant main effect of heating. Darker and light colored bars represent the control and heating treatments, respectively. Different letters indicate significant differences among sites within a species. Asterisks represent significant differences between control and heated treatments (** = $P < 0.01$, * = $P < 0.10$). Error bars represent +/- one SE.

The effect of the heating treatment on plant biomass depended on both site [F (6, 290) = 4.6, P = 0.01] and species [F (6, 290) = 3.5, P = 0.02]. Heating decreased the size of *Achillea* plants at the southern site (P = 0.001), increased the size of *Prunella* plants at

the northern site ($P = 0.001$), and increased the size of *Plectritis* plants in the southern and northern sites ($P \leq 0.042$). *Eriophyllum* size was not affected by heating treatments, though it trended toward larger plants in the heating treatments across sites (Figure 5B).

Structural Equation Models

To test our three *a priori* SEMs (Figure 3), we used data across all sites and species (for a table of means and Pearson's correlations of the data, see Appendix A: Table S4 and S5). Both the phosphorus and the N:P ratio model had good model fit (Phosphorus SEM: $\chi^2 = 0.232$, $P = 0.63$, CFI=1.0, $RMSEA < 0.0001$; N:P ratio SEM: $\chi^2 = 0.41$, $P = 0.52$, $CFI = 1.0$, $RMSEA < 0.0001$), while the nitrogen model had poor model fit ($\chi^2 = 26.3$, $P < 0.0001$, $CFI = 0.96$, $RMSEA = 0.284$) and was dropped from further consideration. Both the phosphorus and N:P ratio models had similar magnitudes and directions of the path coefficients. However, the plant N:P ratios suggested nitrogen limitation or nitrogen and phosphorus co-limitation (Appendix A: Figure S2.C), so we chose the N:P model (Figure 6) for further interpretation (see Appendix A: Figure S3 for phosphorus model output).

We also examined the consistency of the N:P SEM model among each species and each site separately. Models with data from individual species showed similar trends to the model using all species, but had poor model fit, presumably due to the lower sample size ($N < 100$), and we do not consider them further. Similarly, models that included data from each site separately had poor model fit, except for the model that included data from only the southern site ($\chi^2 = 0.82$, $P = 0.36$, $CFI = 1.0$, $RMSEA < 0.0001$). We were particularly interested in SEM of the southern site because it has much

higher nutrient availability (Appendix A: S1) and the heating treatments were beginning to experience extreme drought conditions at the time of plant collection (Figure 2).

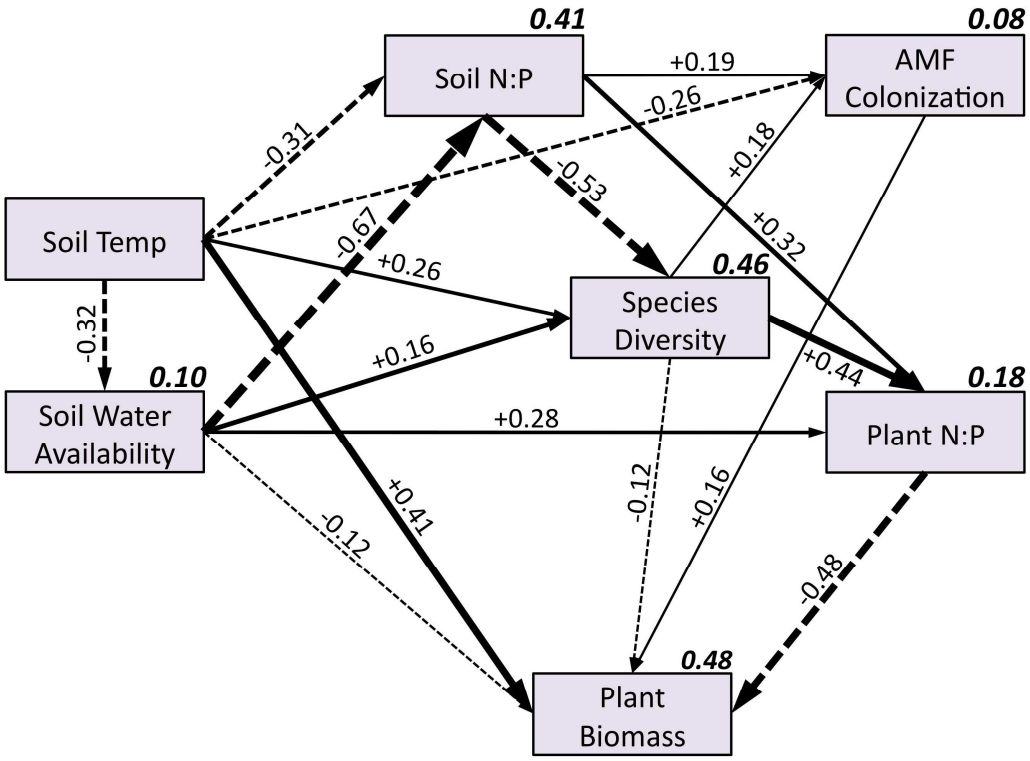

Figure 6. Overall SEM including all sites and species for N:P ratios. Each box represents a variable in the model, while the number above each arrow represents the value of the standardized path coefficients. The width of each arrow corresponds with the magnitude of the path coefficient, solid lines indicate positive effects, and dashed lines indicate negative effects. Path coefficients not significant at $P < 0.10$ are not shown. The italicized, bold number above each box represents the total explained variance (R^2) of each variable.

The overall SEM was fairly successful in explaining the variance in soil N:P (R^2 = 0.41), species diversity (R^2 = 0.46), and plant biomass (R^2 = 0.48), but was less successful in explaining the plant N:P ratio (R^2 = 0.18) and AMF abundance (R^2 = 0.08). Though all but three predicted path coefficients from our *a priori* N:P model (Figure 3) were significant (Figure 6), we focus only on the direct and indirect effects on AMF colonization and plant biomass to simplify our presentation.

As we hypothesized (Figure 3), there were many indirect effects of temperature on AMF colonization which were mediated by soil water availability, soil N:P, and plot diversity (Figure 6). Soil N:P and plot diversity had moderate direct positive effects on AMF colonization. Soil water availability did not have a significant direct effect on AMF colonization, though it did have considerable indirect effects which were mediated by both soil N:P and plot diversity. Interestingly, because some indirect pathways were positive and some were negative, the total indirect effect of temperature mediated by other variables was negligible (Table 2). Thus, the overall effect of temperature on AMF colonization was predominately a direct effect.

Similarly, there were many indirect effects of temperature on the host plant biomass, which were mediated by soil water availability, soil N:P, plant diversity, AMF colonization, and plant N:P ratio. However, similar to AMF colonization, the various negative and positive indirect effects canceled each other out, and the total effect of temperature on plant biomass was largely a direct effect (Table 2). AMF colonization had a slight positive effect on plant biomass, which was also driven by direct, rather than indirect effects (Table 2, Figure 7). Contrary to what we expected, AMF colonization did

not affect plant N:P ratios, though plant N:P ratios had a strong negative effect on plant biomass.

Table 2. Standardized direct, indirect, and total effects of the overall N:P ratio SEM.

Effect of Variable 1	on	Variable 2	Direct Effect	Indirect Effect	Total Effect
Soil Temp	→	Soil Water Avail.	-0.32	N/A	-0.32
Soil Temp	→	Soil N:P	-0.31	0.21	-0.10
Soil Temp	→	Diversity	0.26	0.00	0.26
Soil Temp	→	AMF colonization	-0.26	0.04	-0.23
Soil Temp	→	Plant N:P	N/A	0.01	0.01
Soil Temp	→	Plant Biomass	0.41	-0.03	0.38
Soil Water Avail.	→	Soil N:P	-0.67	N.A	-0.67
Soil Water Avail.	→	Diversity	0.16	0.35	0.51
Soil Water Avail.	→	AMF colonization	-0.03	-0.03	-0.06
Soil Water Avail.	→	Plant N:P	0.28	0.01	0.29
Soil Water Avail.	→	Plant Biomass	-0.12	-0.15	-0.27
Soil N:P	→	Diversity	-0.53	N/A	-0.53
Soil N:P	→	AMF colonization	0.19	-0.10	0.09
Soil N:P	→	Plant N:P	0.32	-0.24	0.09
Soil N:P	→	Plant Biomass	-0.09	0.04	-0.05
Diversity	→	AMF colonization	0.18	N/A	0.18
Diversity	→	Plant N:P	0.44	-0.01	0.43
Diversity	→	Plant Biomass	-0.12	-0.18	-0.30
AMF colonization	→	Plant N:P	-0.05	N/A	-0.05
AMF colonization	→	Plant Biomass	0.15	0.03	0.18
Plant N:P	→	Plant Biomass	-0.48	N/A	-0.48

Even though the southern site SEM had fewer significant pathways and path coefficients that were different in magnitude (and occasionally direction), the general outcomes were very similar to the overall SEM (Appendix A: Figure S4). The effect of temperature on AMF colonization was largely a direct negative effect, as indirect effects

canceled out. The effect temperature on plant biomass was also predominately a positive direct effect (Appendix A: Table S6).

The southern site SEM was different in that there was a stronger negative effect of temperature on AMF colonization, and AMF colonization had a much stronger positive effect on plant biomass. The total explained variance of AMF colonization was higher than the overall SEM (19% compared to 8% Figure 6 and Figure S4). The total effect of temperature on plant biomass was, however, nearly identical to the overall SEM (0.39 compared to 0.38, Figure 7), as was the total explained variance in plant biomass (50% compared to 48%, Figure 6 and Figure S4).

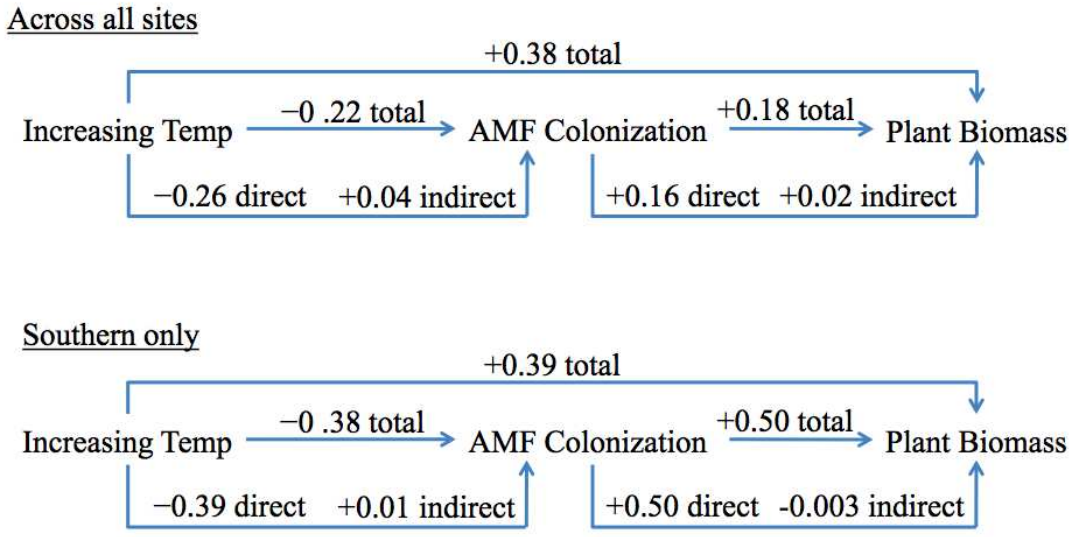

Figure 7. Simplified scheme of direct, indirect and total effects of temperature on AMF colonization and plant biomass for the overall SEM and southern only SEM. Total effect is the sum of direct and indirect effects. Numbers used are extracted from Table 2 and Appendix A: Table S6, respectively.

Discussion

Our results show that there was a decrease in AMF colonization in response to temperature across sites and species. We confirmed our original predictions that the effect of temperature on AMF and the plant hosts was mediated by indirect interactions with soil water availability, soil nutrients, and plant species diversity. However, because of both negative and positive interactions, these indirect effects canceled out, and the total effect of temperature was driven by the direct effects (Figure 7). We also demonstrate that this result was regionally consistent across the Mediterranean climate gradient, despite local site effects of soil type demonstrated in the greenhouse experiment. We are confident this result was not driven primarily by innate site differences because the southern site SEM had similar effects of temperature, despite some differences in causal pathways (Appendix A: Figure S4). Moreover, the ANOVA results from the field experiment support the finding of a negative heating effect across sites and species (Figure 5).

To our knowledge, this is the first manipulative climate change study to examine the regional response of AMF interactions. Interestingly, our results challenge the positive response to temperature that many other studies have reported. Many of these studies, however, were either performed in a greenhouse or at a single site, potentially limiting the transferability of their results. Our research highlights how studies at the ecosystem and regional level are needed to make reliable generalizations about the response of AMF-plant interactions to climate change.

Greenhouse and Field Experiments

We used a greenhouse experiment to isolate the effects of soil type on AMF colonization and host plant response. The results indicated that soil type is a strong control over AMF colonization in this region. The pattern follows what was expected, with higher colonization in the soils with low nutrient availability (Mosse and Phillips, 1971; Smith and Read, 2008). Though colonization was higher in the central and northern sites, plants were consistently smaller, suggesting that increased colonization did not fully compensate for the large differences in nutrient availability between the southern and other two sites.

In the field experiment, we saw a different trend from the greenhouse, with AMF colonization either highest in the southern site or showing few differences across sites. Although there was a general trend for plants at the northern site to be smaller, differences in plant size between the southern and central site were not consistent among species. This result suggests that the effects of climate may overwhelm the effect of soil type and nutrient availability on AMF colonization and plant biomass, although we cannot exclude the possibility that other site-level factors were important.

The most intriguing result from the field experiment, however, is the consistent decrease in colonization in the heating treatment, which is opposite from the majority of similar warming studies (Compant et al., 2010). There also was a general trend of increased aboveground biomass in the heating treatments, which is consistent with aboveground NPP collected at the plot level (data not shown). Because of this unexpected result, we further investigated various factors that could explain the decrease in AMF abundance.

Direct and Indirect Effects of Temperature

We used SEM to determine the direct and indirect effects of temperature on AMF colonization and plant biomass. In the overall SEM, which included data from all sites and species, increasing temperatures had a negative total effect on AMF colonization and a positive total effect on plant biomass, in agreement with the ANOVAs. Contrary to our initial hypothesis, however, the effect of temperature on both variables was almost entirely a direct effect. Though many indirect interactions mediated the effect of temperature on AMF colonization and plant biomass, these effects essentially cancelled each other out (Figure 7). However, our analysis was limited to a single growing season after less than two years of heating. Over time, the effect of increasing temperature could make these indirect effects stronger or alter the balance among them. Additionally, 2011 was a La Niña year with greater spring precipitation than in other weather patterns. Moreover, the PNW and Mediterranean regions globally are predicted to experience increasingly severe summer drought and heavier winter rains over the 21st century (Mote and Salathe, 2010; Ruffault et al., 2012). Thus, indirect effects mediated by soil water moisture could become more prominent in the future. Given these considerations, we examine the direct and indirect pathways in some detail below.

Indirect Effects

The total effect of temperature on soil N:P was close to neutral because the direct effect was negative (-0.31) and the indirect effect was positive (+0.21) (Table 2 and Figure 6). However, a scatter plot (data not shown) suggests that the negative direct effect was driven by site differences, since temperatures during this time period actually

increased from south to north (Appendix A: Table S4), and soil N:P was much higher in the southern site than the northern sites due to innate differences in soil type.

The positive indirect effect of soil temperature on soil N:P was driven by the negative effect of soil temperature on soil water availability, which in turn had a strong negative effect on soil N:P (resulting in a net positive effect). This positive effect agrees with our nutrient data (Appendix A: Figure S1), where we saw an increase in soil N:P in the heating treatments in two of the three sites. Additionally, in the southern only SEM there was only a positive effect of soil temperature on soil N:P (Appendix A: Figure S4).

Assuming the negative direct effect was mainly driven by innate differences in soil type among the sites, our results suggest that increasing soil temperatures caused a shift toward phosphorus limitation due to a decrease in soil water availability. This may reflect the much greater mobility of nitrate (the predominant form of inorganic nitrogen in our sites) than phosphorus in soils. Increasing soil N:P had a moderate direct positive effect on AMF colonization, and it has been shown that plants in phosphorus-limited soils tend to have increased colonization and produce more exudates known to attract AMF (Ostertag, 2001; Yoneyama et al., 2012). The positive effect of warming on AMF colonization that most other studies have found could have been due to increased phosphorus limitation mediated by soil water availability (Rillig et al., 2002; Staddon et al., 2003). The relative limitation of phosphorus and nitrogen has been previously suggested as an important driver of AMF responses (Johnson, 2009). Testing all three of the *a priori* SEMs revealed that the N:P ratio was a better predictor of AMF colonization than the availability of soil nitrogen or phosphorus alone.

Though it makes logical sense that increasing soil phosphorus limitation would increase AMF colonization, the overall effect was cut in half by the negative indirect effect mediated via species diversity. Consistent with previous studies of the effect of plant diversity on AMF (Vandenkoornhuyse et al., 2003; Johnson et al., 2004), species diversity had a positive effect on AMF colonization. Because increasing soil N:P had a strong negative effect on species diversity, this indirect effect of soil N:P on AMF colonization was negative.

We found that species diversity was a better predictor of AMF colonization than various measures of net primary productivity (see *Plot Measures*). While it has been suggested that increased productively should directly effect AMF by increasing belowground carbon allocation (Pendall et al., 2004), it has also been shown that nutrient and carbon allocation are not shared equally among the plant and fungal symbionts within a community (Klironomos, 2003; Van Der Heijden et al., 2003; Leake et al., 2004). Higher plant diversity may provide an improved root network that accommodates both higher colonization and AMF diversity (Van Der Heijden et al., 2003; Leake et al., 2004).

Direct Effects

The direct negative effect of temperature could have been a direct physiological response of the AMF (Koltai and Kapulnik, 2010). However, the total explained variance in AMF colonization for both the overall and southern only SEM was small (9% and 19%, respectively), and the direct effect may have been mediated by something we did not measure. Increased temperatures have been shown to decrease extraradical hyphae,

presumably due to higher decomposition and turnover rates (Rillig et al., 2002; Rillig, 2004; Wilson et al., 2009). Because extraradical and internal root colonization are often positively correlated (Wilson et al., 2009; Barto et al., 2010; van Diepen et al., 2010), we likely would have observed a decrease in extraradical hyphae had it been measured. A decrease in extraradical hyphae drastically decreases glomulin production, a glycoprotein that has been shown to increase soil stability (Rillig, 2004). Decreased AMF colonization could have serious consequences to overall ecosystem functions by destabilizing soil aggregates (Wilson et al., 2009).

We saw a positive total effect of temperature on plant biomass in both the overall and southern SEM, which was also primarily driven by the direct effect. This is also consistent with the positive effect of heating on NPP at the plot level (data not shown). We found a modest positive effect of AMF colonization on plant biomass in the overall SEM, also driven primarily from the direct effect (Figure 7). The same was true for the southern site SEM, but the effect of AMF colonization on plant biomass was much stronger (Appendix A: Figure S4). Though the total effect of heating on biomass was positive, over time the indirect negative effect on plant biomass (via the negative temperature effect on AMF colonization) could dampen the total positive effect of temperature on plant biomass, in addition to other ecosystem consequences.

AMF Community Data

AMF colonization did not have any effect on plant N:P ratios (Figures 6 and S4) or plant phosphorus content (Appendix A: Figure S3). Though AMF are well known for enhancing phosphorus uptake, it has been shown that enhanced uptake via the AMF

symbiont is not necessarily correlated with the degree of AMF colonization or the phosphorus content in the plant (Smith et al., 2004). However, plant species diversity had a relatively strong effect on plant N:P (negative effect in the southern only SEM and positive effect in the overall SEM), which could have been mediated by the community of AMF, rather than the overall colonization (Van der Heijden et al., 1998; Klironomos et al., 2000; Van Der Heijden et al., 2003).

Though our community data was limited and was not further investigated due to logistical constraints, we do have evidence that there was a diverse community of AMF across and within the sites given that our small data set (from one host plant species, *Eriophyllum*) spans most major families of the Glomeromycota (Appendix A:Figure S5, Table S1). Different species of AMF have been shown to mediate host plant nutrient uptake (Van Der Heijden et al., 2003), so it would be interesting to further investigate the links between species diversity, AMF community, and plant nutrient uptake under climate change.

Conclusion

We found that the direct effect of increasing temperatures caused a decrease in AMF colonization, and this appeared to be regionally consistent across the Mediterranean climate gradient. A suite of complicated indirect effects mediated this response, though these effects canceled out due to both positive and negative effects. However, because of the fine balance of indirect effects, this region could potentially be quite sensitive to climate change. Over time, a shift in the relative strengths of different indirect effects could either exacerbate or mitigate the negative direct effect of temperature on AMF

colonization. Furthermore, we cannot rule out the possibility that the direct effect may have been mediated by other variables we did not measure, such as glomulin secretion and related effects on soil stability. AMF colonization appears to be most important for plant biomass production in the southern site, the most extreme site in terms Mediterranean seasonality. Thus, as ecosystems in Mediterranean climates experience even more intense droughts and heavier rains, a decrease in AMF colonization could have substantial consequences for plant communities and ecosystem function.

CHAPTER III

CONCLUSIONS

We found that the direct effect of increasing temperatures caused a decrease in AMF colonization. This could have serious consequences for both natural and human-managed ecosystems. In the final chapter, I summarize the results and discuss possible consequences of our findings in terms of Mediterranean climates and imperiled prairie ecosystems. I also address practical applications of my research in terms of agriculture and discuss further research that is needed to understand how community structure, in addition to the amount of colonization, of AMF may respond to climate change.

Our results show remarkable consistency of the effect of temperature across the region and among the plant species. From this we can conclude that future climatic warming in this region will likely result in a decrease in AMF colonization and an increase in plant productivity. The negative effect of heating was strongest in the southern site. The widely dispersed sites differed in many ways, as one would expect, but one of the most important differences was that the southern site had the most severe Mediterranean climate. This suggests that the direct negative effect of temperature on AMF colonization may become stronger as this region experiences more extreme seasonality, with largely unknown consequences for plant growth and ecosystem function.

Though indirect effects of temperature mediated through other variables canceled each other out, over time there may be a shift to either exacerbate or mitigate the negative direct effect of temperature. As Mediterranean climates experience even more intense

droughts and heavier rains, soil moisture availability may become more important in mediating the effect of temperature on AMF colonization. Our SEMs show that AMF colonization may be most important to plants in the southern site, which was beginning to experience drought at the time of collection. With an earlier onset of drought, a decrease in colonization from increased temperatures may have serious consequences to overall plant performance, especially if the decrease is experienced during the usual growing season.

Mediterranean ecosystems harbor a large percentage of the terrestrial plant diversity (Cowling et al., 1996), and prairies of the Pacific Northwest are particularly endanger of losing much of their native biodiversity (Noss et al., 1995; Stanley et al., 2011). We saw a strong negative effect of soil N:P on plant diversity in the southern Oregon model, though it is difficult to determine if this was a direct causation without specifically measuring how the plant community responds to changes in nutrient limitation. Changes in nutrient availability have been shown to affect both plant and AMF community composition (Egerton-Warburton and Allen, 2000). Higher N:P ratios can be due to either decreasing relative phosphorus availability or increasing relative nitrogen availability. An increase in nitrogen availability has been shown to favor invasive plant species (Lejeune and Seastedt, 2002; Lowe et al., 2003).

Prairies of the PNW have become endangered ecosystems due to habitat fragmentation and conversion, fire suppression, and species invasions (Noss et al., 1995). Much effort is being put forth to improve methods for restoring these habitats (Stanley et al., 2011). If this region experiences a general a decrease in AMF colonization in future climates, restorations of these prairies may benefit from amendments of AMF inoculum.

A study by Smith et al. (1998) showed that in a recently disturbed tall-grass prairie, plots seeded with an addition of an AMF inoculum reproduced from a native prairie had a larger percent cover of seeded native species than control plots without inoculum. It has also been shown native species perform better with local AMF symbionts than non-local symbionts (Johnson et al., 2010). Thus, developing appropriate AMF inoculums for site-specific restorations could be challenging.

Agricultural practices may also need to consider mycorrhizal amendments in future climates. Conventional agriculture practices such as tilling, the application of biocides, genetically engineers crops, fertilization, crop rotation with non-mycorrhizal hosts, have been shown to decrease AMF abundance and diversity (Oehl et al., 2003; Rillig, 2004; Cheeke et al., 2012; Douds and Seidel, 2012). A decrease in hyphae colonization, specifically extraradical hyphae, has been shown to dramatically decrease glomulin secretion (Rillig et al., 2002; Wilson et al., 2009).

Glomulin is a glycoprotein that incases soil particles and enhances soil aggregate formation and stability (Rillig, 2004). Enhanced soil aggregation and stability increases soil organic matter, water holding capacity, nutrient availability, and erosion resistance (Brady and Weil, 2004). A decrease in AMF colonization in response to climate change, especially if it results in a decrease in glomulin production, could further exacerbate the already damaging effects of conventional practices on soil quality. However, adoption of no-till practices, crop rotation, and overwintering, have not only been shown to increase soil quality but may also increase both AMF abundance and diversity (Douds and Seidel, 2012).

Though we found that AMF colonization had a moderately positive effect on plant biomass, there was no effect of colonization on the plant N:P ratio or plant phosphorus content, despite the known importance of AMF for phosphorus uptake. However, it has been shown that the community structure of AMF can determine the relative uptake of nutrients among co-occurring plants (Van Der Heijden et al., 2003) and is a major determinant of plant diversity and productivity (Van der Heijden et al., 1998; Hartnett and Wilson, 1999; Klironomos et al., 2000). It is likely that traditional experiments that use a single isolate, which is easily cultured and propagated, may have been substantially biased by the specific AMF-plant association (Klironomos, 2003). Further large-scale, ecosystem based studies of mycorrhizal community responses to climate change may provide more accurate information from which to make generalizations and predictions (Russell et al., 2012).

AMF have been notoriously difficult to study because their extremely fine and delicate hyphae are not easy to quantify and they are largely unculturable (Rillig, 2004). However, recent advances in molecular techniques may enhance our understanding of AMF community structure and functional diversity of AMF (Husband et al., 2002; Anderson and Cairney, 2004; Montesinos-Navarro et al., 2012). In the past year, the phylogeny of AMF has been substantially modified (Krüger et al., 2012), and the global diversity has been found to be much larger than previously estimated (Kivlin et al., 2011).

It is clear these organisms are highly important in mediating plant responses to climate change, but more studies are needed to understand how they may mitigate or enhance these effects. Our study provides evidence that a decrease in AMF colonization

in response to warming temperatures may have serious consequences in Mediterranean climates, and the plant-AMF symbiosis may become even more important under the greater predicted seasonal intensity of moisture and temperature stress over the coming century. However, further study of how AMF community structure, extraradical hyphae colonization, and glomulin production will respond to increasing temperatures is needed to better understand the consequences of climate change effects on AMF.

APPENDIX A

SUPPLEMENTAL FIGURES AND TABLES

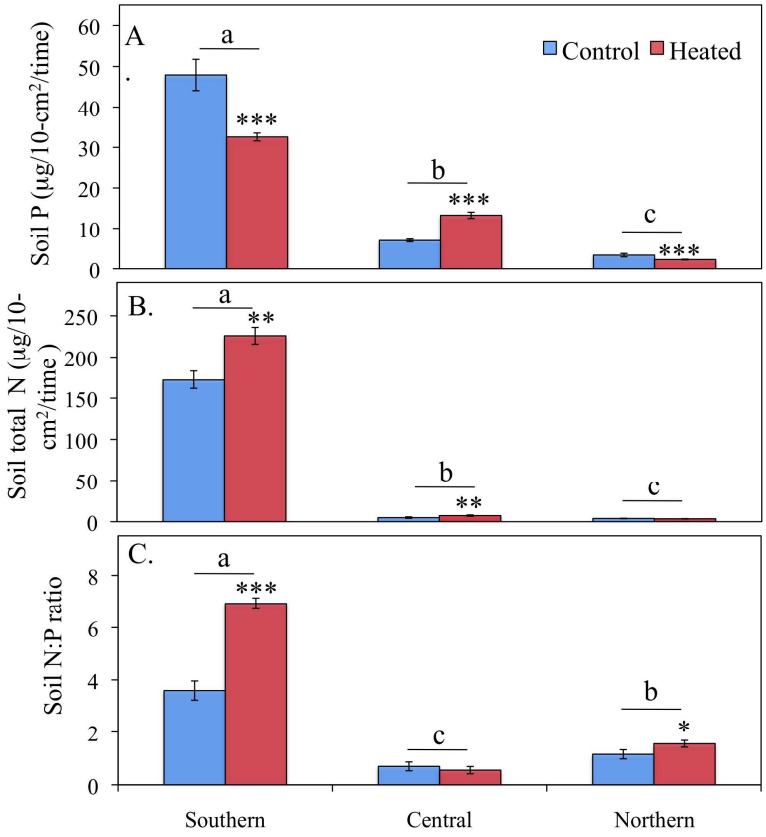

Figure S1. Soil phosphorus availability (A), total inorganic nitrogen availability (B), and ratio of the two nutrients (C) from anion and cation exchange resins in the ground from April to July, 2011. Colored bars represent control and heated treatments across the three sites. Different letters indicate significant differences among sites. Asterisks represent significant differences between control and heated treatments (*** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.1$). Error bars are represented as +/- one SE.

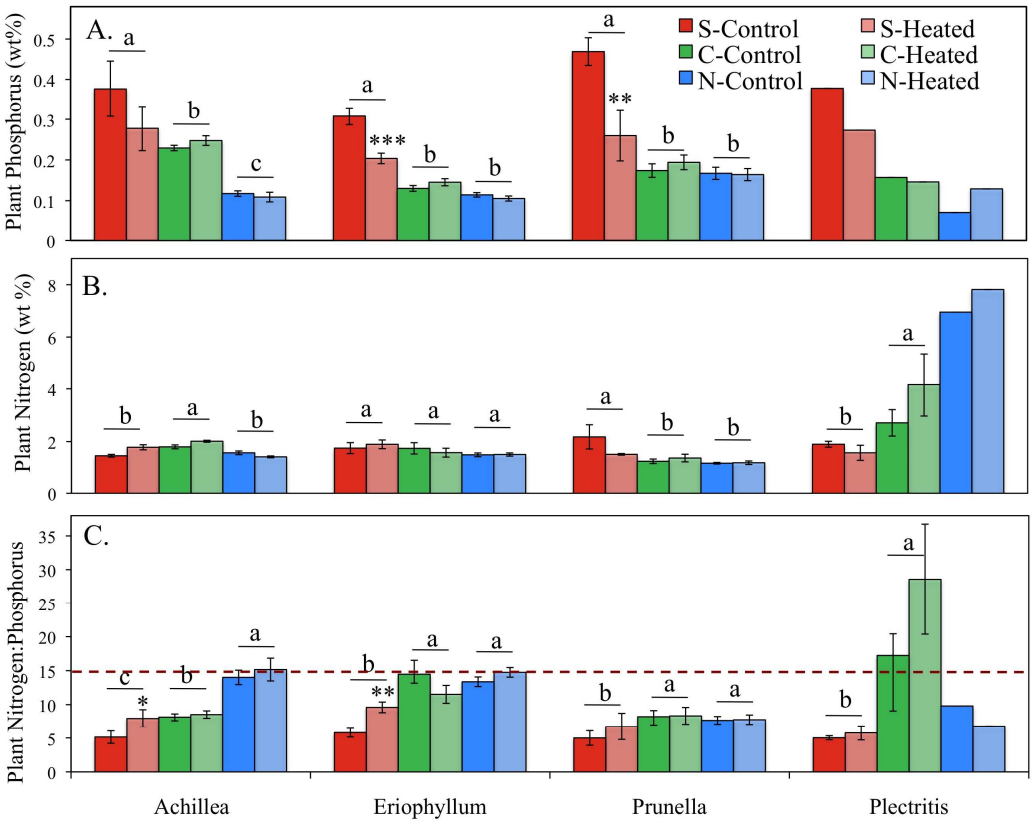

Figure S2. Percentage of phosphorus (A), total nitrogen (B), and the nitrogen to phosphorus ratio (C) of plant material across sites and species. Darker colored bars represent control treatments while lighter colored bars represent heated treatments. Different letters indicate significant differences among sites within a species. Asterisks represent significant differences between control and heated treatments (*** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.1$). Error bars are represented as +/- one SE. *Plectritis* lacks errors bars for treatments with replicates of N=1. Dashed line represents the approximate point at which phosphorus and nitrogen are co-limited.

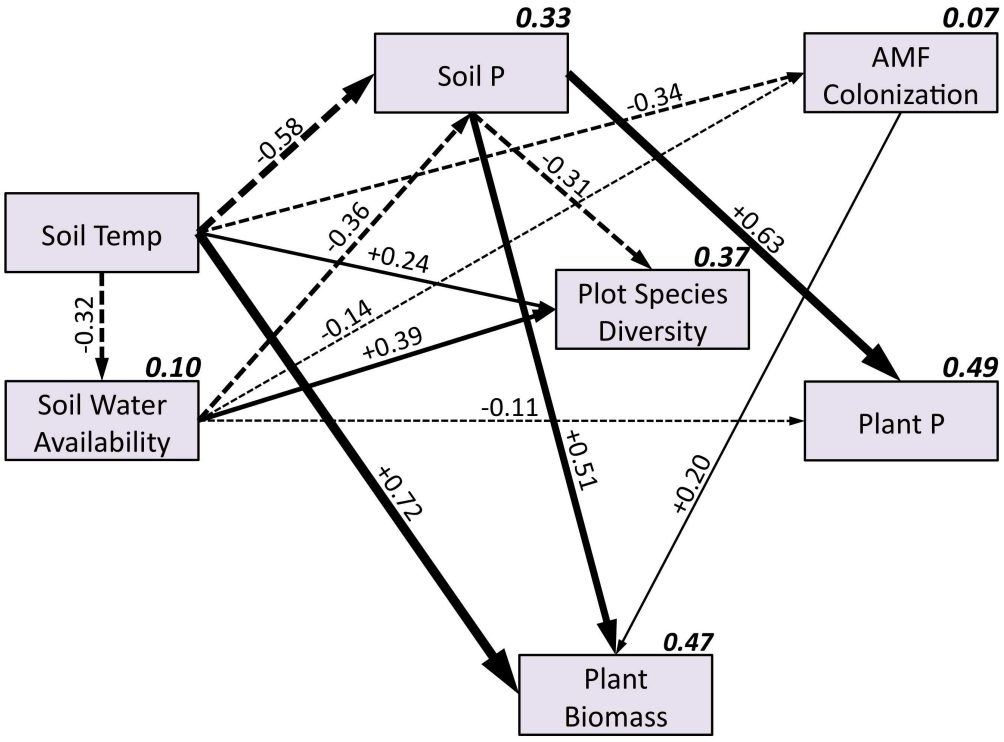

Figure S3. Phosphorus SEM including data from all sites and species. Each box represents a variable in the model, and while the number above each arrow represents the value of the standardized path coefficients. The width of each arrow corresponds with the magnitude of the path coefficient, solid lines indicate positive effects, and dashed lines indicate negative effects. Path coefficients that were not significant to $P < 0.1$, are not shown. The italicized number above each box represents the total explained variance (R^2) of each variable.

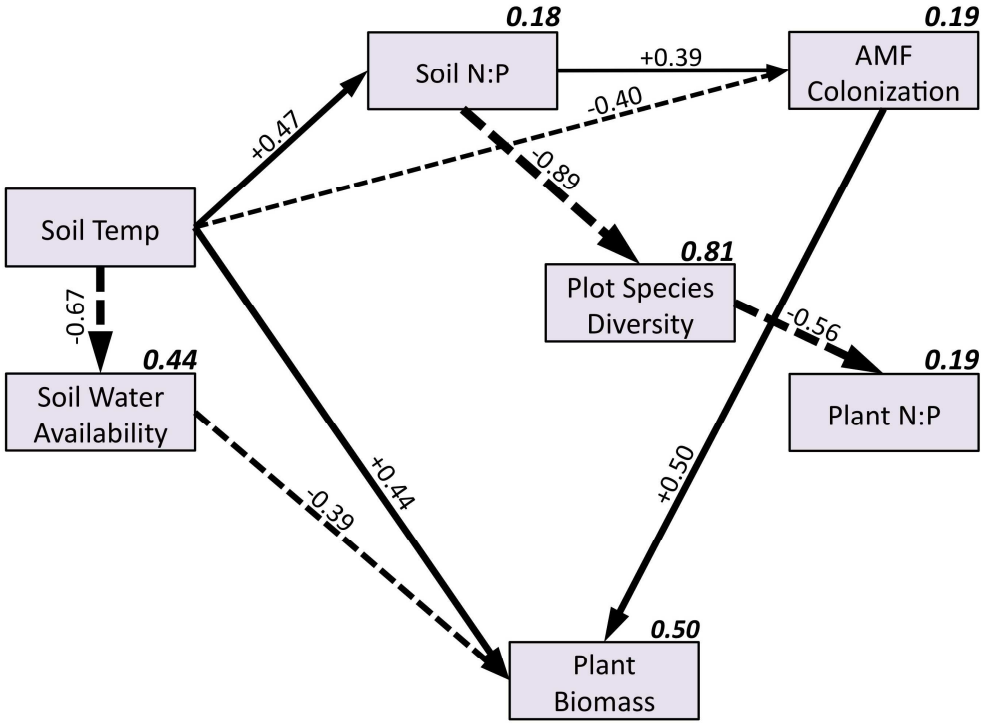

Figure S4. Southern N:P SEM including only data from the southern site, but all species. Each box represents a variable in the model, and while the number above each arrow represents the value of the standardized path coefficients. The width of each arrow corresponds with the magnitude of the path coefficient, solid lines indicate positive effects, and dashed lines indicate negative effects. Path coefficients that were not significant to $P < 0.1$, are not shown. The italicized number above each box represents the total explained variance (R^2) of each variable.

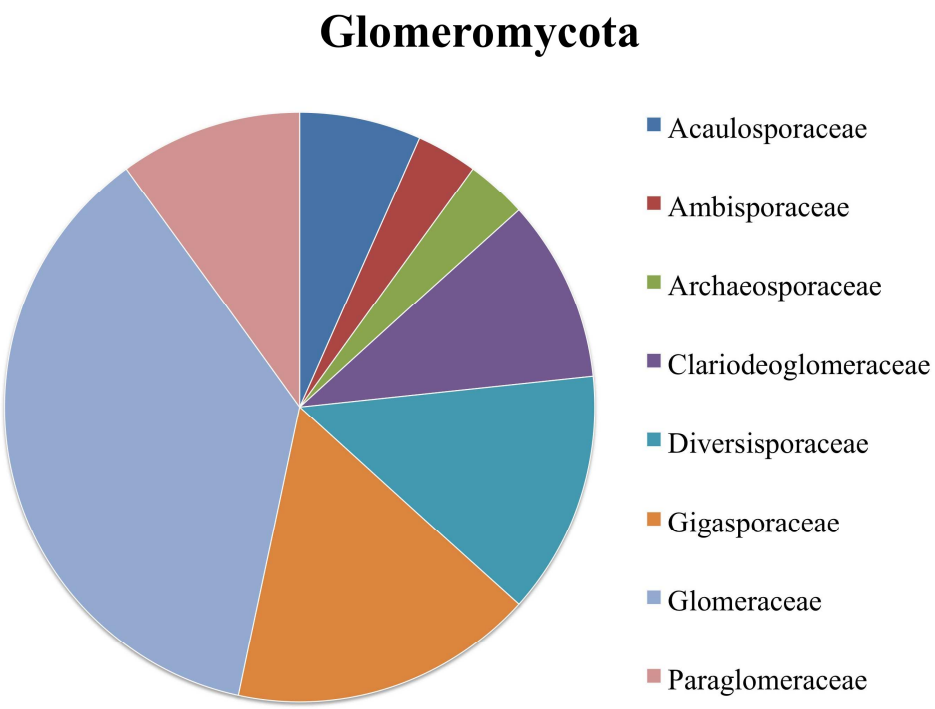

Figure S5. AMF species from the host plant *Eriophyllum lanatum* identified by the trail *Illumina* sequencing run grouped by family. Only 1% of original sequence data were matched to AMF, though these species range across most families of AMF.

Table S1. Species of AMF identified from host species *Eriophyllum lanatum* in the trial *Illumina* sequencing run.

Species Identified
Acaulospora lacunosa
Acaulospora sp.
Ambispora sp.
Archaeospora trappei
Claroideoglomus claroideum
Claroideoglomus lamellosum
Claroideoglomus luteum
Diversispora aurantia
Diversispora epigaea
Diversispora epigaea
Diversispora sp.
Gigaspora rosea
Scutellospora biornata
Scutellospora calospora
Scutellospora gregaria
Scutellospora sp.
Funneliformis caledonius
Funneliformis coronatus
Funneliformis fragilistratus
Funneliformis geosporus
Funneliformis verruculosus
Glomus albidum
Glomus indicum
Rhizophagus clarus
Rhizophagus fasciculatum
Rhizophagus intraradices
Rhizophagus manihotis
Paraglomus brasilianum
Paraglomus majewskii
Paraglomus occultum
Acaulospora lacunosa

Table S2. Three-way ANOVA table of the field experiment for AMF colonization.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	7.6	23	0.3	5	<0.0001
Site	0.5	2	0.3	3.8	0.023
Treatment	1.2	1	1.2	17.8	<0.0001
Species	3.8	3	1.3	18.9	<0.0001
Site * Treatment	0.1	2	0.1	0.8	0.438
Site * Species	1.8	6	0.3	4.4	<0.0001
Treatment * Species	0.4	3	0.1	2	0.121
Site*Treatment*Species	0.4	6	0.1	1.1	0.392
Error	18.6	281	0.1		
Total	26.2	304			

Table S2. Three-way ANOVA table of the field experiment for plant biomass.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	335.2	23	14.6	103.3	<0.0001
Site	15.7	2	7.9	55.8	<0.0001
Treatment	0.3	1	0.3	2.1	0.149
Species	193.5	3	64.5	457.1	<0.0001
Site * Treatment	1.3	2	0.7	4.6	0.01
Site * Species	16.6	6	2.8	19.6	<0.0001
Treatment * Species	1.5	3	0.5	3.5	0.016
Site*Treatment*Species	2.1	6	0.3	2.5	0.024
Error	40.9	290	0.1		
Total	376.1	313			

Table S4. Means and standard deviations of SEM variables used to test the three *a priori* models.

		Soil Temp.	Water Avail.	Plant P	Plant N	Plant N:P	Soil P	Soil N	Soil N:P	Diversity	AM Col.	Biomass
Site		**(°C)**	**Matric ψ**	**wt%**	**wt%**	**wt%**	*****	*****	*****	**(1/D)**	**%**	**(g)**
Southern												
Control	Mean	14.6	-460.8	0.39	1.83	4.61	53.4	187.7	3.59	4.2	78.0	2.96
	SD	1.4	-0.3	0.16	1.12	1.69	26	74.5	2.1	1.6	9.0	2.83
Heated	Mean	16.7	-1009.7	0.24	1.77	7.49	32.9	233.4	6.93	2.22	59.0	3.87
	SD	2	-0.4	0.09	0.51	1.62	5.3	55.5	1.21	0.76	10.0	0.75
Central												
Control	Mean	15.5	-30.1	0.17	1.95	10.33	7.5	7.2	0.69	6.85	68.0	2.35
	SD	1.8	-0.9	0.05	1.38	1.79	2.8	7.6	2.35	1.76	7.0	3.45
Heated	Mean	18.4	-36.9	0.19	2.12	9.31	14.6	9	0.55	6.43	57.0	3.47
	SD	1.4	-0.6	0.06	1.9	2.18	5.7	5.8	2.97	1.09	9.0	5.35
Northern												
Control	Mean	17	-64.9	0.12	2.8	18.92	4.2	4.4	1.15	7.62	70.0	0.74
	SD	1.6	-0.4	0.05	2.43	2.76	3.3	2.2	2.38	1.79	7.0	0.95
Heated	Mean	20.2	-455.9	0.13	3.13	18.27	2.4	3.8	1.55	7.69	63.0	1.31
	SD	1.7	-0.3	0.04	2.89	2.29	0.7	1.6	1.67	0.61	7.0	1.11
Total												
Control	Mean	15.8	-83.1	0.21	2.22	10.28	18.8	55.4	1.3	6.4	0.72	1.95
	SD	1.9	-0.3	0.14	1.82	2.54	25.3	90	2.88	2.21	0.08	2.78
Heated	Mean	18.8	-210.8	0.17	2.47	11.75	13.3	53.2	1.44	6.09	0.6	2.64
	SD	2.2	-0.2	0.08	2.27	2.31	12.2	95.7	3.28	2.23	0.08	4.24

***** units of soil N and soil P: μg/10cm^2/April-July.

Table S5. Pearson's correlations of SEM variables. Boldface represents P < 0.01, italicized represents P < 0.05.

Correlations	AM Col %	Biomass	Soil N	Soil P	Soil N:P	Plant N:P	Plant N	Plant P	Soil Temp	Water Avail.
AM Col.	1.00									
Biomass	0.05	1.00								
Soil N	0.09	**0.25**	1.00							
Soil P	0.04	**0.28**	**0.77**	1.00						
Soil N:P	0.10	0.10	**0.74**	*0.13*	1.00					
Plant N:P	-0.03	**-0.55**	**-0.42**	**-0.50**	*-0.13*	1.00				
Plant N	0.03	**-0.57**	*-0.13*	*-0.14*	-0.04	**0.83**	1.00			
Plant P	0.09	**0.27**	**0.61**	**0.68**	**0.23**	**-0.66**	**-0.17**	1.00		
Soil Temp	**-0.23**	**0.39**	**-0.38**	**-0.47**	-0.10	-0.02	**-0.27**	**-0.30**	1.00	
Water Avail.	0.03	**-0.36**	**-0.49**	**-0.18**	**-0.57**	**0.27**	**0.24**	**-0.24**	**-0.32**	1.00
Diversity	-0.02	**-0.17**	**-0.80**	**-0.50**	**-0.64**	**-0.34**	*0.13*	**-0.42**	**0.26**	**0.32**

Table S6. Standardized direct, indirect, and total effects of the southern site only SEM.

Effect of Variable 1	on	Variable 2	Direct Effect	Indirect Effect	Total Effect
Soil Temp	→	Soil Water Avail.	-0.67	N/A	-0.67
Soil Temp	→	Soil N:P	0.47	-0.05	0.42
Soil Temp	→	Diversity	-0.07	-0.32	-0.39
Soil Temp	→	AMF colonization	-0.38	0.01	-0.37
Soil Temp	→	Plant N:P	N/A	0.24	0.24
Soil Temp	→	Plant Biomass	0.44	-0.05	0.39
Soil Water Avail.	→	Soil N:P	0.08	N/A	0.08
Soil Water Avail.	→	Diversity	-0.08	-0.07	-0.14
Soil Water Avail.	→	AMF colonization	0.10	-0.01	0.09
Soil Water Avail.	→	Plant N:P	-0.13	0.06	-0.07
Soil Water Avail.	→	Plant Biomass	-0.39	0.00	-0.39
Soil N:P	→	Diversity	-0.89	N/A	-0.89
Soil N:P	→	AMF colonization	0.40	-0.22	0.18
Soil N:P	→	Plant N:P	0.20	0.49	0.69
Soil N:P	→	Plant Biomass	-0.22	0.00	-0.23
Diversity	→	AMF colonization	0.24	N/A	0.24
Diversity	→	Plant N:P	-0.56	-0.01	-0.57
Diversity	→	Plant Biomass	0.13	0.07	0.20
AMF colonization	→	Plant N:P	-0.03	N/A	-0.03
AMF colonization	→	Plant Biomass	0.50	0.00	0.50
Plant N:P	→	Plant Biomass	0.09	N/A	0.09

APPENDIX B

SOIL AND PLANT NUTRIENT RESULTS

Soil Nutrient Data

Soil phosphorus availability, nitrogen availability and their ratio differed among sites, with the southern site having nearly 10-fold higher nutrient availability (Appendix A: Figure S1). The effect of the heating treatment depended on site (Tables S7-S9). Heating decreased phosphorus availability in the southern and northern sites but increased it in the central site ($P < 0.0001$). Heating increased nitrogen availability in the southern and central sites ($P \leq 0.004$) with no effect in the northern site. Heating also increased the N:P ratio in the southern and northern sites ($P \leq 0.023$), suggesting a shift toward phosphorus limitation in response to warming.

Table S7. Two-way ANOVA table of soil phosphorus availability.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	68.6	5	13.7	374.0	<0.0001
Site	61.6	2	30.8	839.6	<0.0001
Treatment	0.0	1	0.0	0.8	0.4
Site * Treatment	3.7	2	1.8	50.0	<0.0001
Error	11.3	308	0.0		
Total	79.9	313			

Table S8. Two-way ANOVA table of soil nitrogen availability

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	154.7	5	30.9	478.1	<0.0001
Site	150.0	2	75.0	1159.3	<0.0001
Treatment	0.5	1	0.5	8.1	0.005
Site * Treatment	0.8	2	0.4	6.1	0.002
Error	19.9	308	0.1		
Total	174.6	313			

Table S9. Two-way ANOVA table of soil N:P ratio.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	37.3	5	7.5	62.9	<0.0001
Site	36.7	2	18.4	154.6	<0.0001
Treatment	0.8	1	0.8	6.8	0.009
Site * Treatment	1.8	2	0.9	7.5	0.001
Error	36.6	308	0.1		
Total	73.9	313			

Plant Nutrient Data

Plant phosphorus content differed among sites and species, and these were interdependent ($P < 0.0001$, Table S10). Plant phosphorus content was highest in the southern site for all three perennial species, with a similar trend in the annual species, *Plectritis*. The effect of the heating treatment depended on site ($P < 0.0001$), and there was a marginal three-way interaction among treatment, site, and species ($P = 0.09$). The heating treatment decreased phosphorus content only in the southern site for *Eriophyllum* and *Prunella* ($P \leq 0.013$, Appendix A: Figure S2.A).

Plant nitrogen content differed by site and species, and they were interdependent ($P < 0.0001$, Table S11). There were no direct or interactive effects of heating (Appendix A: Figure S2.B). All species had relatively constant levels across sites and species, with the exception of *Plectritis*, which showed a dramatic increase in nitrogen from south to north.

The plant N:P ratio differed by site and species, and they were interdependent ($P < 0.0001$, Table S12). The plant N:P ratio differed among species but generally plants tended to have lower N:P ratios in the southern site ($P < 0.0001$, Appendix A: Figure S2.C). Plants with a ratio < 10 and > 20 are considered to be N limited and P limited, respectively (Güsewell, 2004). By these criteria, plants appear to be generally nitrogen

limited or co-limited by the two nutrients. The effect of the heating treatment depended on site ($P = 0.01$), where heating increased the N:P ratio in the southern site ($P < 0.1$).

Table S10. Three-way ANOVA table of plant phosphorus content.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	0.34	23	0.015	29.0	<0.0001
Site	0.177	2	0.088	172.8	<0.0001
Treatment	0.008	1	0.008	16.2	<0.0001
Species	0.025	3	0.008	16.5	<0.0001
Site * Treatment	0.023	2	0.012	22.6	<0.0001
Site * Species	0.018	6	0.003	5.9	<0.0001
Treatment * Species	0.002	3	0.001	1.0	0.37
Site*Treatment*Species	0.006	6	0.001	1.9	0.09
Error	0.148	290	0.001		
Total	0.488	313			

Table S11. Three-way ANOVA table of plant nitrogen content.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	7.985	23	0.347	27.8	<0.0001
Site	0.398	2	0.199	15.9	<0.0001
Treatment	0.002	1	0.002	0.1	0.72
Species	2.183	3	0.728	58.2	<0.0001
Site * Treatment	0.01	2	0.005	0.4	0.66
Site * Species	2.745	6	0.457	36.6	<0.0001
Treatment * Species	0.011	3	0.004	0.3	0.83
Site*Treatment*Species	0.072	6	0.012	1.0	0.46
Error	3.589	287	0.013		
Total	11.573	310			

Table S12. Three-way ANOVA table of plant N:P ratio.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	33.303	23	1.448	31.5	<0.0001
Site	10.539	2	5.269	114.7	<0.0001
Treatment	0.079	1	0.079	1.7	0.19
Species	5.39	3	1.797	39.1	<0.0001
Site * Treatment	0.401	2	0.201	4.4	0.01
Site * Species	7.098	6	1.183	25.8	<0.0001
Treatment * Species	0.177	3	0.059	1.3	0.28
Site*Treatment*Species	0.306	6	0.051	1.1	0.36
Error	13.181	287	0.046		
Corrected Total	46.484	310			

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