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Altitudinal gradients do not predict plant-symbiont responses to experimental warming

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ALTITUDINAL GRADIENTS DO NOT PREDICT PLANT-SYMBIONT RESPONSES TO EXPERIMENTAL WARMING

by

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DEDICATION

To Mom and Nana, for instilling within me your love of learning and the natural world.

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ABSTRACT

Fungal symbionts, ubiquitous inhabitants of above- and belowground plant tissues, can play important roles in increasing plant tolerance to abiotic and biotic stress. Disruption of plant-fungal interactions may therefore have important consequences for plant responses to climate change. Both altitudinal gradients and warming experiments can be useful tools for understanding responses of symbioses to climate shifts, but the degree to which altitudinal patterns will predict species responses to warming has received little attention for plant-symbiont interactions. This study combined surveys along replicated altitudinal gradients with a long-term warming experiment at the Rocky Mountain Biological Laboratory (RMBL) in Colorado, USA to test the potential for disruption of plant-fungal symbioses under future climate conditions. Because multiple symbioses within the same host individual may result in complex plant-fungal responses to climate change, we examined the full mycobiome in leaves and roots. Altitudinal patterns in fungal symbioses largely did not correspond to fungal responses to experimental warming, suggesting limited utility of these frequently used methods for predicting fungal responses to climate warming. Variation in temperature influenced fungal colonization, composition, or diversity for some fungal groups and host species.

However, our work indicates that effects of climate change on plant-fungal symbioses will depend on host plant identity and fungal functional group, with some associations weakened or disrupted, others affected weakly, and yet others enhanced under climate warming. Predicting how climate change will alter ecologically important symbioses should therefore involve attention to the identity and ecology of both hosts and symbionts. Our approach suggests the strength of comparing environmental gradients to warming experiments in order to gain a more nuanced perspective on how climate change may alter communities and ecosystems.

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Introduction

Climate change will cause a 2-4.5°C increase in global mean annual temperature by the year 2100, along with major shifts in precipitation patterns (IPCC 2014). These changes will have important consequences for terrestrial ecosystems, with potential to disrupt species interactions and create communities or species interactions that lack contemporary analogs (van der Putten 2012). As a result, complex and unanticipated ecological responses to climate change may occur (Walther 2010).

Fungal symbionts, ubiquitous inhabitants of plant above- and belowground tissues, can play important roles in increasing plant tolerance to abiotic and biotic stress, and have been shown to alter plant responses to climate change (Compant et al. 2010, Chakraborty et al. 2012, Mohan et al. 2014). In a recent meta-analysis, both above- and belowground fungal symbionts buffered plants against warming and drought (Kivlin et al. 2013), thus influencing plant resistance and resilience to climate variability (see also Bourguignon et al. 2015, Hu et al. 2015). Disruption of plant-fungal interactions may therefore have important consequences for plant responses to global change. First, if fungi and plants differ in their physiological responses to climate change in ways that affect their survival, growth, reproduction, or phenology, their distributions may become decoupled. Second, plants and their symbionts might have similar physiological tolerances to climate, but could realize different distributions due to disparities in their dispersal abilities. Third, climate change could alter the context-dependent outcomes of

symbioses (Chamberlain et al. 2014), leading to shifts in the fitness consequences of these interactions. For example, symbionts in plants can shift from having neutral or positive effects on hosts to pathogenesis (Johnson et al. 1997). An alternative hypothesis is that climate-induced disruptions will be less likely to occur in symbioses than in non-symbiotic species interactions because symbioses are more tightly coupled. For example, vertically transmitted symbionts can co-disperse with seed to new environments, reducing potential for mismatched distributions, and symbionts can potentially buffer hosts against climate change (Kannadan and Rudgers 2008).

In addition, multiple symbioses within the same host individual may result in complex plant-microbe responses to climate change (Afkhami et al. 2014). For example, belowground, arbuscular mycorrhizal fungi (AMF) and dark septate endophytes (DSE) colonize the roots of up to 80% of plant species, often improving nutrient uptake and stress tolerance (Smith and Read 2008, Porras-Alfaro and Bayman 2011). Aboveground, plants host systemic foliar endophytes in the Clavicipitaceae (Ascomycota) as well as localized foliar endophytes (LFE) (mainly Ascomycota) (Rodriguez et al. 2009). Multiple symbionts may interact synergistically to promote host resilience to abiotic stress (Kivlin et al. 2013, Afkhami et al. 2014). Alternatively, antagonisms among symbionts could reduce net benefits to hosts (e.g., Müller 2003), adding complexity to predicting plant-symbiont responses to global change and necessitating studies that examine the full mycobiome. In addition, systemic foliar endophytes in the genus *Epichloë* are obligate symbionts of living plants (Schardl et al. 2008), as are AMF (Smith and Read 2008), whereas LFE and DSE are facultative plant associates that can grow in other environments (Menkis et al. 2004, Osono 2006). These differences have potential to

further influence how different fungal groups respond to abiotic conditions, co-disperse with their hosts, and affect host fitness.

Both altitudinal gradients and warming experiments can be useful tools for understanding responses of symbioses to climate shifts (Sundqvist et al. 2013). However, the degree to which altitudinal patterns will predict species responses to warming (space for time substitution) has received little attention for plant-symbiont interactions. Past studies have examined either fungal symbiont distributions along altitudinal gradients or fungal symbiont responses to warming. For instance, in roots, AMF colonization and diversity often decline with increasing elevation (Wu et al. 2007, Gai et al. 2012, Mohan et al. 2014). DSE colonization and richness typically increase with elevation (Read and Haselwandter 1981, Schmidt et al. 2008, Geml et al. 2014). Limited data are available for LFE (Helander et al. 2013, Coince et al. 2014), with studies indicating differing trends with altitude for different fungal taxa (Hashizume et al. 2008, Cordier et al. 2012). Data are also limited for *Epichloë*, although some studies suggest altitudinal declines (Granath et al. 2007, Gonzalo-Turpin et al. 2010, Ranelli et al. 2015).

Prior studies also report physiological or abundance responses of individual fungal taxa to experimental warming (Kytoviita and Ruotsalainen 2007, Hawkes et al. 2008, Gray et al. 2011, Zavalloni et al. 2012, Deslippe et al. 2012, Li et al. 2013, Jumpponen and Jones 2014, McCulley et al. 2014, Geml et al. 2015), with most work focused on AMF (reviewed by Compant et al. 2010). For AMF, results suggest declines in colonization with warming for some host plants (Gray et al. 2011), but no change (Barrett et al. 2014) or increases in colonization with warming for others (Zavalloni et al. 2012, Rudgers et al. 2014, Vega-Frutis et al. 2014). Few studies have examined responses

of DSE to warming, although increases with warming for root endophytic fungi have been found recently (Geml et al. 2015). Responses of LFE and *Epichloë* have also been little considered; one study showed a negative effect of warming on LFE diversity (Bálint et al. 2015) and another found no response of *Epichloë* frequency (McCulley et al. 2014). Warming can also alter fungal community structure, with different functional groups responding in different directions (Deslippe et al. 2012, Geml et al. 2015). For instance, warming has been shown to increase AMF richness and diversity (Kim et al. 2015) but to decrease richness of other functional groups, including ectomycorrhizal fungi (Geml et al. 2015).

While parallels between elevation patterns and warming responses have been observed for plants (Dunne et al. 2003), to our knowledge, no study of fungal symbionts of plants to date has compared altitudinal patterns with responses to experimental warming to test whether space-for-time substitution is a reliable predictor of warming trends. Agreement of altitudinal patterns and warming responses would support the use of altitudinal gradients as tools for predicting how plant-fungal symbioses will respond to future temperatures. But differences in altitude and warming trends would suggest that factors other than temperature, such as other climate variables or edaphic factors, drive altitudinal distributions of symbionts, implying that space-for-time substitutions may not be appropriate for inferring the effects of temperature change. Therefore, testing whether altitudinal patterns align with responses to warming is important to making informed predictions about responses of plant-fungal symbioses to future climates.

This study combined surveys along replicated altitudinal gradients with a long-term warming experiment at the Rocky Mountain Biological Laboratory (RMBL) in

Colorado, USA to test the potential for disruption of plant-fungal symbioses under future climates, and to examine whether space-for-time substitution can be used to make predictions. Specifically, we examined how fungal symbiont colonization rates and community composition changed with elevation and experimental warming by addressing the following questions: (1) Do different plant-associated fungal symbiont groups show divergent altitudinal gradients in colonization, composition, and diversity? (2) Do altitudinal patterns in fungal symbioses correspond with fungal responses to experimental warming (space-for-time substitution)? We hypothesized that (*i*) Fungal groups (*Epichloë*, LFE, AMF, and non-AMF root fungi, including DSE) show divergent altitudinal distributions that are coupled with altitudinal variation in temperature and precipitation; (*ii*) Altitudinal gradients in fungal symbiont abundance and composition depend upon host plant identity; and (*iii*) Fungal responses to experimental warming will be similar to altitudinal patterns if temperature is an important driver of fungal colonization and composition.

Methods

Study System

Achnatherum lettermanii, *Festuca thurberi,* and *Poa pratensis* are cool-season, perennial grasses that are abundant in the Rocky Mountains of Colorado, USA. *Achnatherum lettermanii* and *F. thurberi* both have relatively broad altitudinal distributions and were found at elevations up to 3700 m in our surveys. *Poa pratensis* is more abundant at low-to-mid elevations, and was not found above 3600 m in our surveys. These grasses form symbioses with both leaf- and root-associated fungal symbionts, and all three species host AMF and non-AMF root fungi belowground and LFE aboveground. In addition, *F. thurberi* hosts an undescribed *Epichloë* species in its leaves and seeds.

The study was conducted in the Upper Gunnison Basin near the Rocky Mountain Biological Laboratory (RMBL) in Gothic, Colorado, where *A. lettermanii, F. thurberi*, and *P. pratensis* occur. In the Rocky Mountains, air temperature, atmospheric pressure, and soil nutrients decline with altitude, while precipitation increases (Kittel et al. 2002, Dunne et al. 2003). Declines in air temperature with altitude are of ~0.8°C per 100 m increase in elevation (Kittel et al. 2002). Climate data for the southern Rockies show significant warming trends (0.5-1°C per decade) including higher air temperatures and steeper surface temperature lapse rates (Pepin and Losleben 2002, McGuire et al. 2012, Rangwala and Miller 2012).

Altitudinal Gradient Survey

To test how colonization and composition of fungal symbioses change with elevation, we collected grass samples along independent altitudinal gradients. We sampled in both 2012 and 2014, which allowed us to compare a relatively normal year

(2014) to a warm, dry year (2012) (Ryan and Doesken 2013) representative of the increased drought severity predicted to occur under climate change in the Rocky Mountains (Kittel et al. 2002). In both 2012 and 2014, we collected roots and leaves of *A. lettermanii*, *F. thurberi* and *P. pratensis*. For only *F. thurberi*, we also collected leaf samples in 2008, 2009, and 2011 to assess colonization by *Epichloë* over a broader geographical range. In each year, between July and September, we collected samples from 3-10 sites per gradient at sites spaced at ~200 m intervals from the mountain summit of each gradient to the gradient's base. Across years, we sampled a total of eight altitudinal gradients, with sites ranging in elevation from 2700 m to 3700 m (see Tables S1-S3 for site locality information). To eliminate possible confounding effects of sampling date and altitudinal phenology, each full gradient was sampled on a single day or within a few days, with replicate gradients sampled over the course of the growing season.

We collected six individual plants per species per site, choosing plants that were spaced a minimum of 5 m apart along a 50 m transect oriented horizontally with respect to the altitudinal gradient. From each individual plant we collected a minimum of two asymptomatic tillers and ~5 g of roots (fresh weight). To ensure that roots came from the correct plant individual, we only collected roots that were directly connected to the target plant. Plant material collected in 2012 was refrigerated the same day it was collected and processed within ~1 week. Plant material collected in 2014 was frozen at -20°C the day it was collected because we sampled at more sites than we could process within 1 week of collection.

At each site, we also measured phosphorus (P) content of the soil because of the well documented role of arbuscular mycorrhizal fungi in P acquisition (Smith and Read 2008). In 2012, we collected ~50 mL soil (at 10–15 cm depth) at each 5 m interval along the 50 m transect at each site. Soil samples were pooled by volume at the site level and analyzed at the Michigan State Soil Testing Laboratory, East Lansing, Michigan, USA for available phosphorus [Bray P1 by ascorbic acid, spectrophotometer (U.S. Environmental Protection Agency 1993)]. In 2014, we deployed Plant Root Simulator Probes (Western Ag, Saskatoon, SK, Canada) at each site to measure plant-available phosphate. Four probes were placed at each site and were spaced 20 m apart from one another. Probes were deployed on 13-23 July 2014, collected between 23 September and 8 October 2014, and analyzed by Western Ag (Saskatoon, SK, Canada) using inductively-coupled plasma spectrometry.

To examine whether climate variables predict fungal symbiont distributions, we aggregated climate data from 29 weather stations in Gunnison and Pitkin Counties, Colorado (Table S4). We calculated mean cumulative growing degree days (GDD) from 1981-2015 using a base temperature of 0°C for grasses (Frank and Hofmann 1989), mean annual precipitation (MAP), and mean snow depth (MSD) for each weather station over the full period of record. Mean cumulative growing degree days was included as a measure of temperature combined with the length of the growing season. We used model selection based on *AICc* following Anderson (2008) to obtain equations to predict each climate variable from elevation, longitude, and latitude. We then used these equations to interpolate climate for each sample collection site (nlme package, Pinheiro et al. 2014, R

Core Team 2014). Each model explained substantial variation in the climate variable under consideration (GDD: $r^2 = 0.98$; MSD: $r^2 = 0.94$; MAP: $r^2 = 0.93$).

Warming Experiment

To test how fungal symbiont colonization and composition change with warming, we collected *F. thurberi*, *A. lettermanii*, and *P. pratensis* samples from long-term experimental warming plots established at RMBL in 1991 (see Harte and Shaw 1995, Saleska et al. 2002). The RMBL warming experiment (38.95806°N, 106.9894°W, elev. 2920 m) consists of ten plots (10 m x 3 m), which alternate spatially between warmed and control (ambient temperature) treatments ($n = 5$). Warmed plots receive continuous infrared radiation from suspended heating lamps (22 W/m^2). Plots are located along a moraine that spans a natural gradient in soil moisture and vegetation composition. Heating has warmed the top 15 cm of the soil by ~2°C, dried it by 10-20% during the growing season, and extended the growing season by ~2 weeks on each end on average (Harte et al. 2015). Following 23 y of warming, grass cover had declined by 25% (Rudgers et al. 2014). *Poa pratensis* declined by 66% and *A. lettermanii* declined by 45%; there was a smaller and non-significant decline for *F. thurberi*. Warming also reduced local grass species richness by 40% (Rudgers et al. 2014).

On 30 June and 2 July 2014, and again on 25-30 September 2014, roots and leaves from six randomly chosen individuals of each grass species were collected from each plot, to match the replication from the altitudinal surveys. We collected a minimum of two asymptomatic tillers and ~5 g of roots from each plant, refrigerated the material the day it was collected, and processed it within ~1 week. We collected additional leaf

samples on 16-18 June 2015 and 12 September 2015, using the same collection and processing methods.

Fungal Symbiont Colonization

Root Colonization. To assess fungal colonization of grass roots, equal amounts of root tissue from the six plants collected for a given species × site or warming plot combination were pooled by volume. Samples were placed into plastic tissue cassettes and soaked in 10% w:v KOH for 4-10 d, depending on the species. Samples were removed from KOH and rinsed in water (5 min, three times) then soaked in 0.1N HCl for ~10 h. Samples were then soaked in preheated 5% Parker blue ink and vinegar stain for 20 min, and finally rinsed in water (5 min, three times) (McGonigle et al. 1990, Vierheilig et al. 1998). Root colonization by AMF (aseptate hyphae with vesicles and/or arbuscules) and non-AMF fungi, including DSE (dark, melanized, septate hyphae) were scored via light microscopy with the magnified intersections method at 200x magnification (McGonigle et al. 1990) to obtain percentage colonization out of 100 views per sample.

Leaf Colonization. We used staining and microscopy to assess fungal symbiont colonization of grass leaves. Thin sections from the inner leaf sheaths of culms collected from each plant were mounted on a microscope slide and stained with aniline blue lactic acid (Bacon and White 1994). Slides were scored via light microscopy at 200–400x magnification. For the altitudinal gradient samples, we stained and scored approximately two thin sections from the inner leaf sheaths of each of two separate culms from each collected plant (Ranelli et al. 2015). We scored *Epichloë* and LFE presence/absence for each individual plant, and calculated the percentage of symbiotic individuals for each

grass species × site combination to obtain one estimate per population. For the warming experiment samples, we stained and scored 1-3 thin sections from the inner leaf sheath of each of 1-6 separate culms from each collected plant, depending on tissue availability. We scored *Epichloë* and LFE presence/absence for each individual plant, and calculated the percentage of symbiotic individuals for each grass species × plot combination. We also positioned an ocular grid with 100 squares over the leaf peel from each culm, and recorded the number of grid squares that contained *Epichloë* and LFE for 18 grid views (1800 squares) per population (Sylvia 1992). For each grass species × plot combination, we calculated average mm hyphae/mm^2 leaf tissue for *Epichloë* and LFE.

Fungal Symbiont Community Composition

We used Illumina MiSeq amplicon sequencing to examine the composition of fungal symbiont communities associated with grass roots and leaves. In preparation, collected root and leaf samples were surface sterilized (1 minute in 95% ethanol, 2 minutes in 1% sodium hypochlorite solution, and 2 minutes in 70% ethanol). Warming experiment samples from 2014 and altitudinal gradient samples from 2012 were frozen at -20°C following surface sterilization. For the altitudinal gradient samples from 2014, surface sterilization was performed over ice to keep frozen samples from thawing; following surface sterilization, these samples were rinsed in purified water (Milli-Q Integral Water Purification System, EMD Millipore Corporation, Billerica, MA), stored in RNAlater, and refrigerated. All samples were then frozen in liquid nitrogen and ground using a mortar and pestle. Total DNA was extracted from ~50 mg of ground sample using QIAGEN DNeasy plant extraction kits (QIAGEN Inc., Valencia, CA).

We characterized fungal composition using barcoded paired-end Illumina sequencing of fungal nuclear ribosomal ITS amplicons, the approved barcode for Fungi (Schoch et al. 2012). For *Epichloë*, LFE, and non-AMF root fungi, we used fungal-selective primers targeting the ITS2 region (Taylor 2014). For AMF, we used selective FLR3-FLR4 primers that amplify ~300bp in the 28S region (Gollotte et al. 2004). Each PCR reaction contained 5 μL of ~1-10 ng/μL DNA template, 21.5 μL of Platinum PCR SuperMix (Thermo Fisher Scientific Inc., Waltham, MA), 1.25 μL of each primer (10 μM), 1.25 μL of 20 mg/mL BSA, and 0.44 μL of 25mM $MgCl_2$. For the ITS2 primers, each reaction began with an initial denaturing step at 96°C for 2 min, followed by 24 cycles of 94°C for 30 sec, 51°C for 40 sec, and 72°C for 2 min, with a final extension at 72° for 10 min. For the 28S primers, each reaction began with an initial denaturing step at 93°C for 5 min, followed by 33 cycles of 93°C for 1 min, 55°C for 1 min, and 72°C for 1 min, with a final extension at 72° for 10 min.

For each sample, three PCR replicates were pooled and were then cleaned and concentrated using a ZR-96 DNA Clean & Concentrator-5 (Zymo Research Corporation, Irvine, CA). PCR was then carried out on all samples to add dual indexes and Illumina sequencing adaptors; each reaction began with an initial denaturing step at 98°C for 30 sec, followed by 7 cycles of 98°C for 30 sec, 62°C for 30 sec, and 72°C for 30 sec, with a final extension at 72° for 5 min. Sequencing was performed by the Genomic Sequencing and Analysis Facility at The University of Texas at Austin using Illumina MiSeq v3 chemistry (Illumina, Inc., San Diego, CA). We aimed to obtain a minimum of 30,000 reads/sample for the ITS2 region and 20,000 reads/sample for the 28S region. [Sample sizes – warming experiment: 3 grass species x 10 plots x 2 tissues (roots, leaves) x 2

sampling dates (September and June 2014) = 120 samples; altitudinal gradient experiment: 18 *A. lettermanii* samples + 16 *F. thurberi* samples + 14 *P. pratensis* samples x 2 tissues (roots, leaves) = 96 samples; total number of samples across experiments = 216 x 2 primer sets for roots (ITS2 and 28S) and one for leaves (ITS2) = 324].

Bioinformatics

Quality Filtering and OTU Clustering. For the 28S samples, we obtained a total of 4,148,559 sequences, and for the ITS2 samples we obtained a total of 15,041,420 sequences. Sequence reads were processed in QIIME (Caporaso et al. 2010) using standard scripts. For both the ITS2 and 28S sequences, we joined paired end reads and removed any unjoined sequences. We then filtered out sequences if they had a quality score < 25, contained < 200 bases or > 1000 bases, had > 1 mismatch in the either the forward or reverse primer region, or contained homopolymers of > 6 bases. These quality filtering steps yielded 2,661,732 28S sequences and 4,151,333 ITS2 sequences. We then used Sumaclust (Mercier et al. 2013) to create operational taxonomic unites (OTUs) at 97% identity (Blaalid et al. 2013). Data were normalized using DESeq2 (Love et al. 2014).

Taxonomy: 28S Sequences. For the 28S data, sequences that were singletons in the dataset were removed, and remaining representative sequences of each OTU were aligned in PASTA (Mirarab et al. 2014) using the MAFFT algorithm. This alignment was used to create OTUs at the 97% level using the mothur algorithm (Schloss et al. 2009), to ensure that alignment errors did not inflate the diversity of our dataset. We used the RDP Classifier with the fungal LSU training set 11 (Wang et al. 2007) to derive OTU identifications, and removed ~300 non-fungal sequences. We then created a phylogeny in

PASTA with the RAXML algorithm using known AMF reference sequences (Krüger et al. 2012), and retained only taxa that were monophyletic with known AMF clades. We then ran USEARCH (Edgar 2010) to identify putative chimeric sequences, and assigned taxonomy to the OTUs based on the phylogeny and RDP output. These steps resulted in 1833 28S OTUs (1,975,738 sequences).

Taxonomy: ITS2 Sequences. For the ITS2 data, singletons were removed, and representative sequences were chosen in QIIME using default settings. We used the RDP Classifier with the UNITE fungal ITS trainset (Wang et al. 2007) to derive OTU identifications, and removed all sequences with less than 60% identity to a fungal phylum. For sequences with 60-70% identity to a fungal phylum in the UNITE database and sequences that UNITE failed to classify to a fungal phylum, we used NCBI BLAST to search GenBank (Benson 2004) to confirm sequence identity, and removed non-fungal OTUs. Two additional OTUs were discarded due to low sequence abundance (355 reads removed). We then ran USEARCH (Edgar 2010) to identify putative chimeric sequences, and assigned taxonomy to the OTUs based on the RDP output. These steps resulted in resulted in 972 ITS2 OTUs. Because we used the 28S data to consider the AMF, and the ITS region alone poorly resolves this clade (Lindahl et al. 2013), we removed Glomeromycota OTUs from the ITS dataset prior to analysis. Doing so resulted in 921 ITS2 OTUs (1,309,861 sequences), 911 of which were present in roots and 354 of which were present in leaves. Only 10 OTUs were found only in leaves, whereas 567 OTUs were found only in roots.

We repeated all 28S and ITS2 analyses using a 93% similarity threshold for OTU clustering, which resulted in 200 28S and 431 ITS2 OTUs. We recognize that using a

97% similarity threshold on the 28S (AMF) data resulted in an unusually high number of OTUs (1833). However, because results of subsequent statistical analyses were qualitatively similar for the 93% and 97% OTUs, the 97% OTU results are presented here.

Data Analysis

Colonization and Diversity: Altitudinal Patterns. For each fungal functional group, we calculated Shannon OTU diversity in R ('vegan' package, R Core Team 2014, Oksanen et al. 2015). For the altitudinal gradient survey, we used general linear mixed models to examine relationships between fungal colonization/Shannon diversity and elevation, both across the three grasses (with species as a fixed factor that interacted with elevation) and within individual host species, always including sampling date (Julian day) as a continuous variable and gradient identity as a random factor. For the colonization data, we also included sampling year (2012 versus 2014) as a fixed factor, along with its interactions with elevation and host species. For the diversity data, when responses were statistically significant, we decomposed diversity into richness and evenness (Shannon *J*) to understand the mechanisms underlying shifts in diversity. Fungal colonization data were logit transformed to improve normality of residuals and homogeneity of variances (Warton and Hui 2011).

Colonization and Diversity: Environmental Predictors along Altitudinal Gradients. To explore the relative importance of abiotic correlates, for each fungal group, we used model selection based on *AICc* (Burnham et al. 2011) to compare individual models that respectively included the fixed, continuous effects of mean cumulative growing degree days, mean annual precipitation, mean snow depth, or mean soil phosphorus content. We used a separate model for each predictor rather than multiple regression analysis due to

multicollinearity issues. To facilitate comparison of sensitivity to different predictors, climate data were *z*-scored prior to analyses (mean = 0, standard deviation = 1), and phosphorus data from each year (2012 and 2014) were *z*-scored separately to standardize for differences between analytical methods used. For *Epichloë* colonization of *F. thurberi*, we did not include phosphorus in our models, as we lacked phosphorus data for the 2008, 2009, and 2011 collections. For colonization, we created models for each host species × fungal group combination to explore potential drivers of host-specific fungal responses. The null model for colonization included year and sampling date (fixed effects) and gradient (random effect), and the null model for diversity (data for 2012 only) included sampling date and host species identity (fixed effects) and gradient (random effect). Analyses were performed in R using the 'nlme' package (Pinheiro et al. 2014, R Core Team 2014).

Colonization and Diversity: Warming Experiment. We used factorial mixed model ANOVA to test the effects of warming on fungal colonization/diversity, including the fixed effects of warming treatment, host species identity, and sampling date, with all possible interactions ('nlme' package, Pinheiro et al. 2014, R Core Team 2014). Because the plots span a natural gradient in soil moisture and vegetation composition, we blocked the plots spatially into pairs, each consisting of a control plot and an adjacent warmed plot, and included the random effect of block in our models. LFE colonization data were logit transformed and *Epichloë* colonization data were square root transformed to improve normality of residuals and homogeneity of variances. If the host species × warming treatment interaction was significant, we tested *a priori* contrasts for treatment effects within each host species in R ('lsmeans' package, R Core Team 2014, Lenth

2016). For the diversity data, when responses were statistically significant, we decomposed diversity into richness and evenness (Shannon *J*) to understand the mechanisms underlying shifts in diversity.

Colonization and Diversity: Altitudinal Gradient and Warming Experiment Comparison. For a particular host species, when we observed fungal responses to experimental warming but not to altitude, we used general linear mixed models to test whether fungal colonization/diversity differed between paired high and low sites along altitudinal gradients. Sites in each pair were spaced ~250 m apart to represent a ~2°C difference in temperature, the change induced by the RMBL warming experiment. Models included the fixed effects of pair identity (median altitude of the pair of sites), site location within the pair (high or low), sampling date, and year (where applicable), as well as the random effect of gradient identity. We also included the interaction between pair identity and site location when possible.

Composition: Altitudinal Patterns. For the altitudinal gradient data, we used distance-based linear models (DISTLM) to test for shifts in symbiont composition with elevation, host species, sampling date, and gradient identity (Primer v. 6, Clarke and Gorley 2009). When elevation affected OTU composition, we calculated the Spearman correlation between each OTU's abundance and elevation in R (‘agricolae’ package, de Mendiburu 2014, R Core Team 2014). We also used model selection in DISTLM to examine the importance of mean cumulative growing degree days, mean annual precipitation, mean snow depth, or mean soil phosphorus content in predicting OTU composition, with a separate model for each predictor due to multicollinearity. The null model contained the effects of host species, gradient, and sampling date.

Composition: Warming Experiment. We used non-metric multidimensional scaling analysis (Primer v. 6, Clarke and Gorley 2009) to visualize fungal OTU composition. We used perMANOVA to test effects of warming on fungal composition, using block as a random factor, as above (Primer v. 6, Clarke and Gorley 2009). Where there were significant effects of host species, we conducted analyses for each species individually. We also used indicator species analysis (SIMPER) to identify OTUs that contributed strongly to differences in OTU composition among host species, warming treatments, and sampling dates (Primer v. 6, Clarke and Gorley 2009).

Results

Altitudinal Gradient Survey

Arbuscular Mycorrhizal Fungi. *Colonization*. Patterns in AMF colonization with elevation varied among host grasses and also by year (Table S5). For *P. pratensis,* colonization changed non-linearly with altitude, peaking at mid-elevation, as indicated by the improvement in quadratic model fit (*AICc* quadratic = 81.55, *AICc* linear = 87.81) (elevation linear effect: $F_{1,25} = 4.54$, $P = 0.0431$; elevation quadratic term: $F_{1,25} = 2.88$, $P = 0.1023$; Fig. 1A). Trends in AMF colonization with elevation were not present for the other two host species in either year. Across the three host species, colonization by AMF hyphae was on average ~75% lower in the hot, dry year of 2012 relative to the more average climate year of 2014 ($df = 102$, $t = -12.54$, $P < 0.0001$). However, neither climate variables nor soil phosphorus content were strong predictors of AMF colonization for the three host species (Fig. 2A-C; Table S6), leaving open the question of what factors drive variation in AMF colonization.

Diversity. Of the 1,833 28S (AMF) OTUs that were identified, 850 were present in the altitudinal gradient survey samples, representing a total of nine AMF genera in the families Ambisporaceae, Claroideoglomeraceae, Glomeraceae, Diversisporaceae, Paraglomeraceae, and Scutellosporaceae. Only three of these genera were present in 10 or more samples; for all three genera, sequence abundance decreased with elevation, although none of these relationships were statistically significant (Table S7; Fig. S1). Across AMF taxa there was no change in Shannon OTU diversity with elevation ($P = 0.1418$; Table S8) or among host grass species ($P = 0.9752$; Table S8), and climate

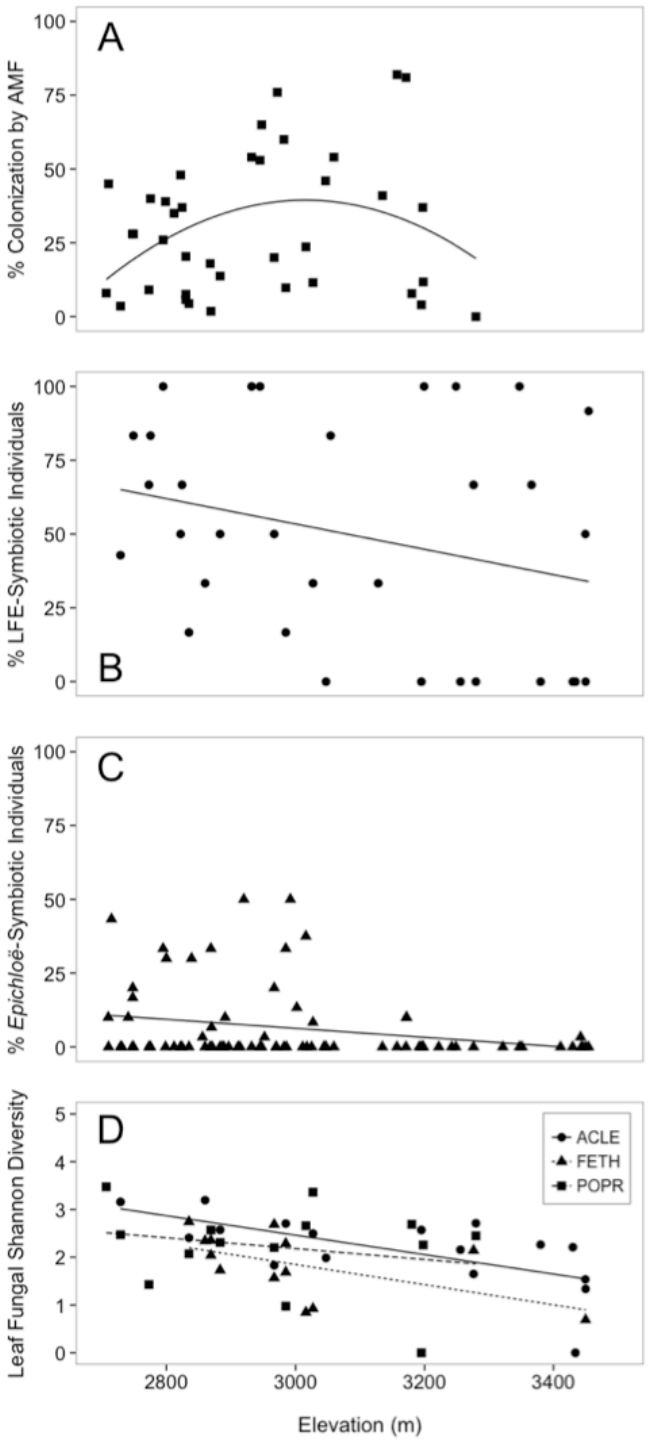

Figure 1. Change with elevation in A) mean percentage colonization of *Poa pratensis* roots by arbuscular mycorrhizal fungi (AMF), B) mean percentage of *Achnatherum lettermanii* individuals symbiotic with localized foliar endophytes (LFE), C) mean percentage of *F. thurberi* individuals symbiotic with *Epichloë,* and D) Shannon OTU diversity of leaf fungi (LFE and *Epichloë*) for each host plant species. Legend: ACLE = *A. lettermanii*, FETH = *F. thurberi*, POPR = *P. pratensis*.

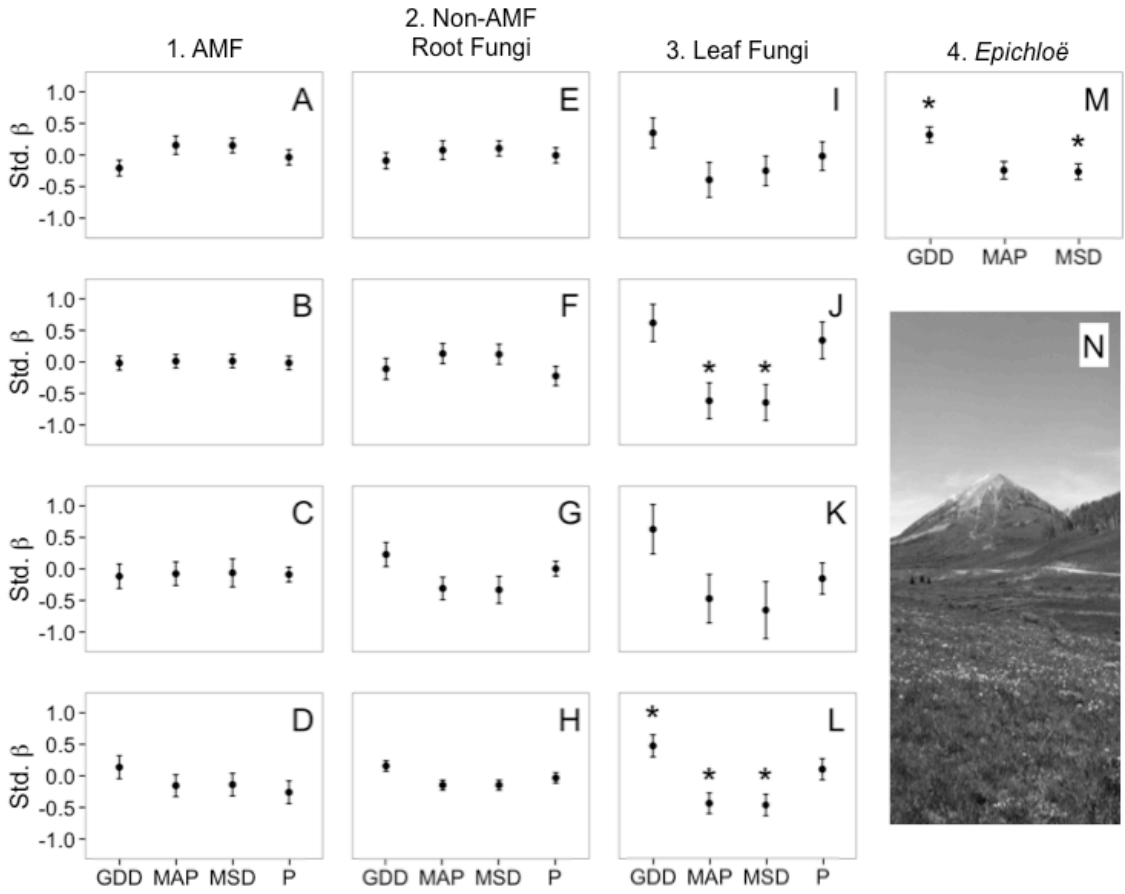

Figure 2. Standardized β values (± s.e.) from general linear mixed models predicting fungal responses along altitudinal gradients based on mean cumulative growing degree days (GDD), mean annual precipitation (MAP), mean snow depth (MSD), or mean soil phosphorus content (P). (1) Arbuscular mycorrhizal fungi (AMF) colonization of A) *F. thurberi* (FETH), B) *A. lettermanii* (ACLE), and C) *P. pratensis* (POPR), and D) AMF Shannon diversity (all plant species); (2) non-AMF root fungi colonization of E) FETH, F) ACLE, and G) POPR, and H) non-AMF root fungi Shannon diversity (all plant species); (3) localized foliar endophyte colonization of I) FETH, J) ACLE, and K) POPR, and L) leaf fungi Shannon diversity (all plant species); and (4) M) *Epichloë* colonization of FETH. N) Low elevation field site in the altitudinal gradient survey. Asterisks denote β estimates that were significantly different from zero, indicating important predictors of the fungal response.

variables and soil phosphorus were not strong predictors of AMF Shannon diversity (Fig. 2D; Table S9).

Composition. There was marginal change in AMF OTU composition with elevation (perMANOVA, $P = 0.0746$; Table S10), driven by decreases with elevation in the abundances of the genera *Rhizophagus*, *Claroideoglomus*, and *Glomus* (Table S7). This effect was present across host species identity, which did not influence AMF OTU composition ($P = 0.1488$; Table S10). Model selection suggested that climate variables and soil phosphorus were not important predictors of variation in AMF OTU composition (Table S11).

Non-AMF Root Fungi. *Colonization.* There was no altitudinal trend in colonization of roots by non-AMF fungi ($P = 0.4338$; Table S5). Similar to AMF, non-AMF root fungal colonization was on average ~62% lower in 2012 than 2014 ($df = 102$, $t = -8.42$, $P < 0.0001$), indicating there was appropriate statistical power to detect biologically important shifts in colonization. Neither climate nor soil phosphorus strongly predicted non-AMF root fungal colonization (Fig. 2E-G; Table S6).

Diversity. Of the 911 ITS2 non-AMF root fungal OTUs, 479 were present in the altitudinal gradient survey samples, representing a total of 47 identifiable fungal orders. Eighteen fungal orders were present in 10 or more samples (Table S7). The orders with the strongest relationships between abundance and elevation were Cantharellales (negative, $P = 0.1836$) and Coniochaetales (positive, $P = 0.0655$), though neither relationship was statistically significant (Table S7). Shannon OTU diversity of non-AMF root fungi did not change with elevation ($P = 0.1346$; Table S8). Neither climate

variables nor soil phosphorus explained significant variation in non-AMF root fungal OTU diversity (Fig. 2H; Table S9).

Composition. Non-AMF root fungal OTU composition changed with elevation for *A. lettermanii* ($P = 0.0456$) but not for *F. thurberi* ($P = 0.1111$) or *P. pratensis* ($P = 0.3677$) (Table S10). All three host grasses differed in non-AMF composition from one another ($P = 0.0001$; Table S10). Climate variables over the period of record were not important predictors of non-AMF root fungal OTU composition (Table S11).

Leaf Fungi. *Colonization.* Across both years, colonization of *A. lettermanii* leaves by LFE declined by ~44% per 1 km increase in elevation ($F_{1,22} = 8.11$, $P = 0.0094$, $r^2 = 0.69$; Fig. 1B). Elevation did not influence colonization by LFE for the other two grass species. For *A. lettermanii*, mean snow depth and mean annual precipitation were both significant predictors of LFE colonization: colonization was higher with lower snow depth and precipitation (Fig. 2J, Table S6). Climate variables and soil phosphorus did not predict LFE colonization of *F. thurberi* (Fig. 2I; Table S6) or *P. pratensis* (Fig. 2K; Table S6).

Across all years, colonization of *F. thurberi* leaves by *Epichloë* decreased ~69% per 1 km increase in elevation ($P = 0.0023$, $r^2 = 0.33$; Fig. 1C; Table S5). Model selection suggested that colonization was higher under longer, hotter growing seasons and lower mean snow depth (Fig. 2M; Table S6).

Diversity. Of the 354 ITS2 leaf fungal OTUs, 164 were present along altitudinal gradients. Shannon diversity of leaf-associated fungi decreased as elevation increased across all three host species ($P = 0.0050$, $r^2 = 0.26$; Fig. 1D; Table S8). Decomposing this effect, richness decreased with altitude ($P = 0.0053$, $r^2 = 0.31$), while evenness did not

change (P = 0.1433). Climate variables were important predictors of leaf fungal Shannon diversity (Table S9). The Shannon diversity increased with greater growing degree days and decreased with higher mean annual precipitation and snow depth (Fig. 2L; Table S9), suggesting higher diversity under hotter, drier climates.

Composition. Leaf fungal OTU composition did not change with elevation (P = 0.7091) or host species identity (P = 0.1453) (Table S10). Similarly, climate variables and soil phosphorus were not important predictors of OTU composition (Table S11).

Warming Experiment

Arbuscular Mycorrhizal Fungi. *Colonization*. AMF colonization increased with warming, but only for one of three host plant species. For *A. lettermanii,* colonization by AMF hyphae was ~31% higher in warmed relative to control plots on average over both sampling dates (contrast: df = 12, t = -2.32, P = 0.0385; Fig. 3A). There was no similar trend between pairs of sites 2°C apart in elevation in the altitudinal gradient study, however (Fig. 3B), suggesting controls other than temperature at play along altitudinal gradients. AMF hyphal colonization did not significantly differ between control and warmed plots for *F. thurberi* or *P. pratensis* (Fig. 3A). Responses of other AMF structures (vesicles and arbuscules) varied by host species and sampling date, but did not respond to warming (Table S12).

Colonization phenology differed among host plant species. Between June and September, there were decreases in AMF colonization of ~35% for *A. lettermanii* (contrast: df = 44, t = 3.58, P = 0.0104) and ~61% for *F. thurberi* (contrast: df = 44, t = 7.31, P < 0.0001) (Fig. 4A). In contrast, AMF colonization increased during the growing season ~46% for *P. pratensis* (contrast: df = 44, t = -4.03, P = 0.0028; Fig. 4A),

indicating potential variation among host species in the seasonal dynamics of colonization.

Diversity. Of the 1,833 28S (AMF) OTUs, 1,811 occurred in the warming experiment, indicating high AMF diversity within the 1000 m^2 area of the warming meadow. Overlap in OTUs between the warming experiment and altitudinal gradient survey was high: 828 OTUs were found in both. AMF Shannon diversity differed among host species ($P < 0.0001$) and between sampling dates ($P = 0.0080$), but there was no significant effect of warming (Table S13). AMF Shannon diversity was ~9% higher in *F. thurberi* relative to *A. lettermanii* ($df = 44$, $t = -4.18$, $P = 0.0004$) and ~12% higher in *F. thurberi* relative to *P. pratensis* ($df = 44$, $t = 5.29$, $P < 0.0001$). While AMF diversity did not change over the course of the growing season for *F. thurberi,* there were marginally non-significant decreases in diversity between June and September for the other two grass species (Fig. 4B).

Composition. AMF OTU composition did not respond to experimental warming, but differed among host species ($P = 0.0001$) and between sampling dates ($P = 0.0003$); thus, the analysis was not lacking in statistical power (Table S13). *Festuca thurberi*'s AMF community differed in composition from those of *A. lettermanii* ($t = 2.93$, $P = 0.0001$; Fig. S2A) and *P. pratensis* ($t = 3.04$, $P = 0.0001$; Fig. S2A). Indicator species analysis suggested that higher abundances of several OTUs in genus *Rhizophagus* were of greatest importance in contributing to the unique assemblage in *F. thurberi* (Table S14).

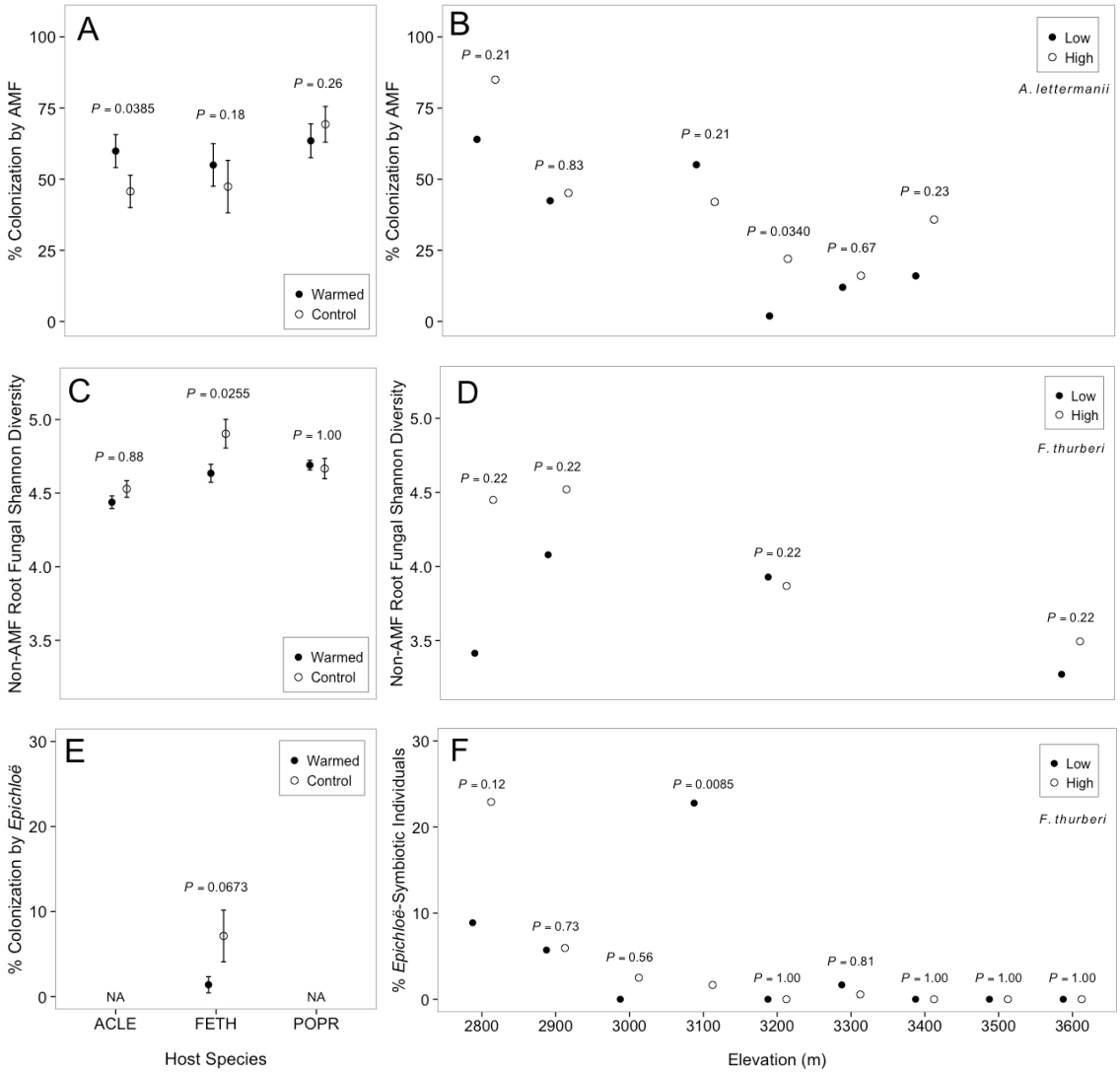

Figure 3. Mean fungal colonization/diversity (± s.e.) for the three plant species in warmed versus control plots in the RMBL warming experiment (A,C,E) and, for the host species that responded to warming, mean fungal colonization/diversity in pairs of sites 2°C apart in elevation in the altitudinal gradient survey (B,D,F). Host species: ACLE = *Achnatherum lettermanii*, FETH = *Festuca thurberi*, POPR = *Poa pratensis.* Fungal responses in each graph: A) and B) colonization by arbuscular mycorrhizal fungi (AMF); C) and D) non-AMF root fungal Shannon diversity; E) and F) colonization by *Epichloë*. Host species in altitudinal gradient graphs: B) ACLE; D) FETH; F) FETH.

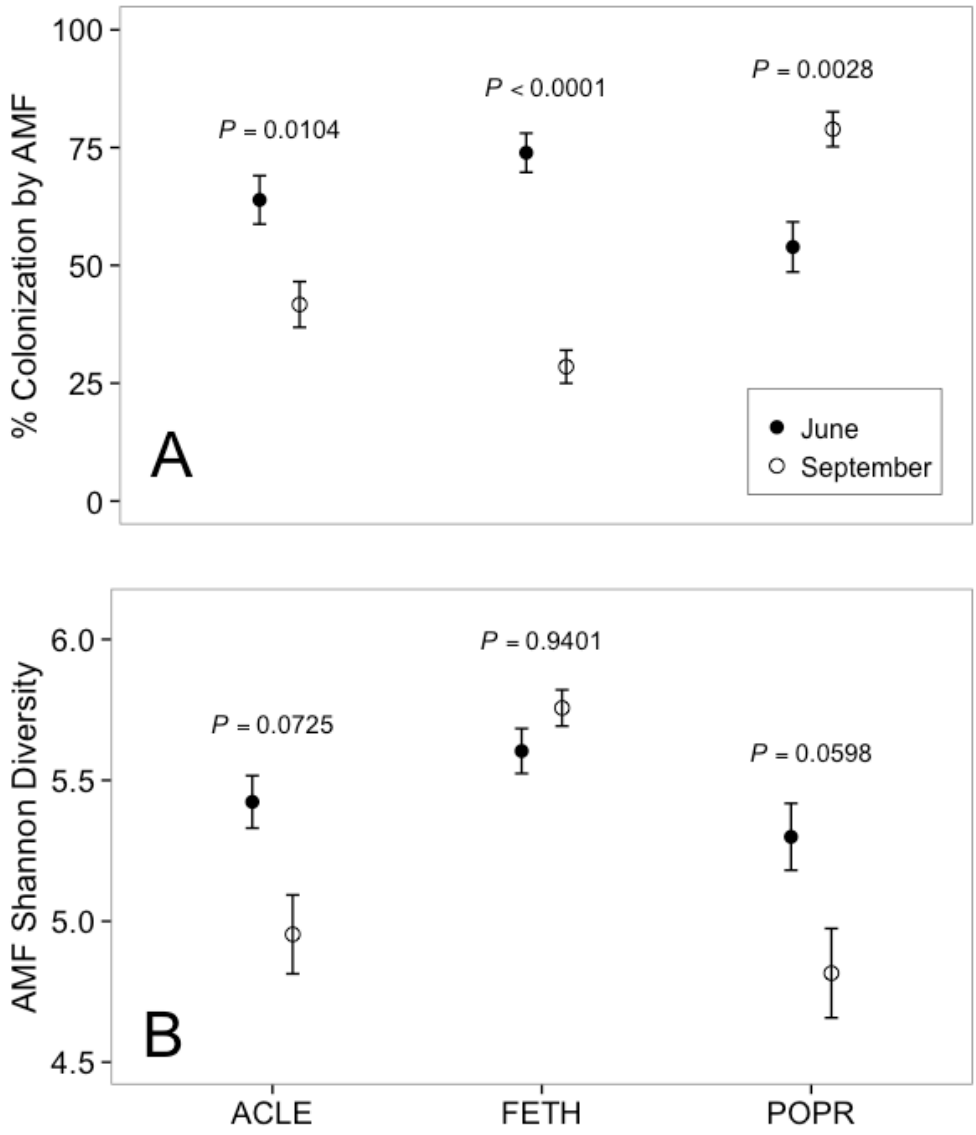

Figure 4. A) Mean percentage colonization (± s.e.) and B) mean Shannon diversity (± s.e.) for arbuscular mycorrhizal fungi (AMF) in the three host grass species in June versus September in the RMBL warming experiment. ACLE = *Achnatherum lettermanii*, FETH = *Festuca thurberi*, POPR = *Poa pratensis.*

Non-AMF Root Fungi. *Colonization.* Colonization of roots by non-AMF fungi varied among host species ($P = 0.0001$) but did not respond to warming or change between sampling dates (Table S12).

Diversity. Of the 911 non-AMF root fungal OTUs, 903 were present in the warming experiment, and 471 of these were present in both studies. Across the three host species, the Shannon diversity of non-AMF root fungi was lower under experimental warming relative to ambient conditions ($P = 0.0240$; Table S13). This trend was driven by root fungi in *F. thurberi*, for which Shannon diversity was ~6% lower in warmed relative to control plots ($df = 44$, $t = 3.25$, $P = 0.0255$; Fig. 3C), and OTU richness was ~26% lower ($F_{1,12} = 6.43$, $P < 0.0001$), but evenness did not respond to warming ($F_{1,12} = 0.60$, $P = 0.4420$). There was a similar but non-significant pattern among paired sites in the altitudinal gradient survey; in the lowest altitudinal pair of sites (the pair most comparable to the temperature change represented by the warming experiment), non-AMF root fungal Shannon diversity in *F. thurberi* was ~23% lower at lower (warmer) altitudes relative to higher ones (contrast: $df = 2$, $t = 1.76$, $P = 0.2197$; Fig. 3D).

Despite the trend in *F. thurberi,* warming did not significantly alter Shannon diversity of non-AMF root fungi for *A. lettermanii* or *P. pratensis* (Fig. 3C). Non-AMF Shannon diversity was lower in *A. lettermanii* relative to *F. thurberi* ($df = 44$, $t = -4.88$, $P < 0.0001$) and *P. pratensis* ($df = 44$, $t = -3.34$, $P = 0.0049$), which did not differ in diversity from one another ($df = 44$, $t = 1.54$, $P = 0.2798$).

Composition. Non-AMF root fungal OTU composition significantly differed between warmed and control plots for both *A. lettermanii* and *F. thurberi* (Fig. 5). Indicator species analysis showed that the fungi of greatest importance in contributing to

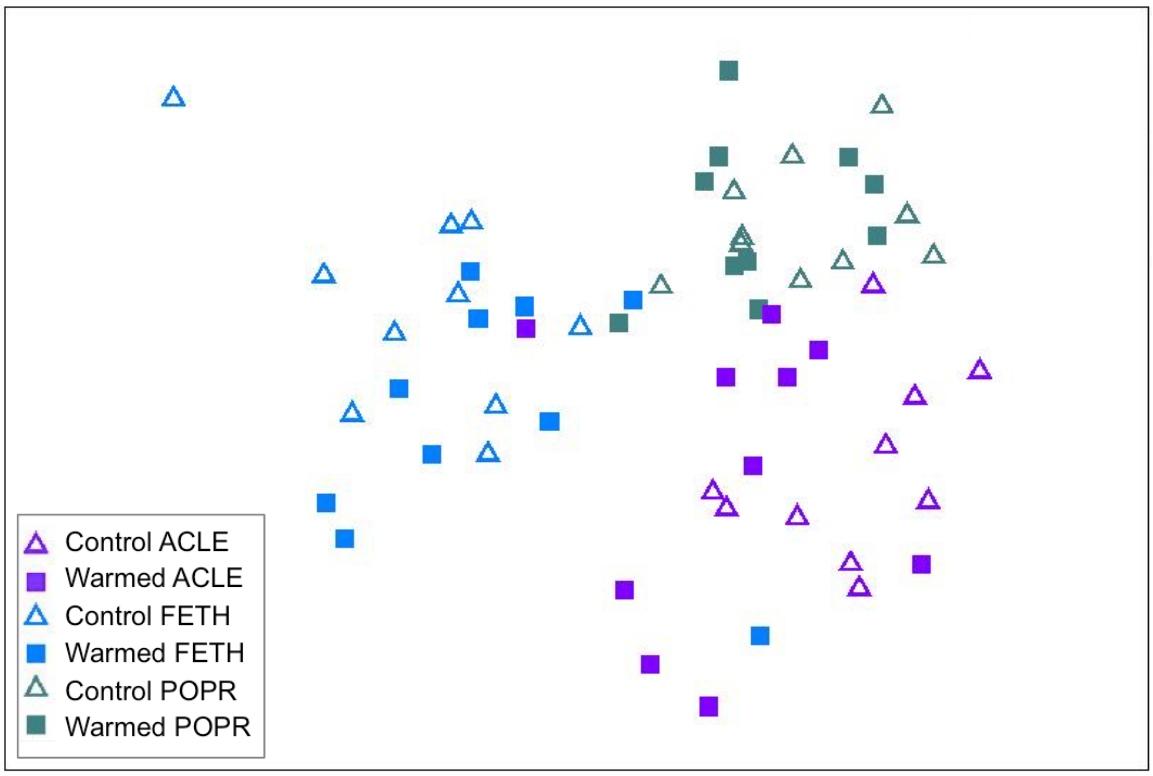

Figure 5. NMDS plot indicating differences in non-arbuscular mycorrhizal root fungal OTU composition among warming treatments (warmed versus control) and host species in the RMBL warming experiment. ACLE = *Achnatherum lettermanii*, FETH = *Festuca thurberi*, POPR = *Poa pratensis.* OTU composition differed between warming treatments for *A. lettermanii* ($t = 1.30$, $P = 0.0162$) and *F. thurberi* ($t = 1.40$, $P = 0.0100$) but not for *P. pratensis* ($t = 1.14$, $P = 0.1458$). 2D stress = 0.18, number of iterations = 325, number of restarts = 500.

treatment differences for *A. lettermanii* were in the Helotiales (Table S15). For *F. thurberi*, differences were driven by change in both the Capnodiales and Helotiales (Table S15). Warming did not significantly alter non-AMF root fungal OTU composition for *P. pratensis* (Fig. 5). OTU composition also differed among all pairs of host species ($P = 0.0001$; Fig. 5; Table S13) and by sampling date ($P = 0.0001$; Table S13).

Leaf Fungi. *Colonization.* Across host species, neither warming nor other measured variables influenced colonization of leaves by LFE (Table S12). However, in *F. thurberi*, leaf colonization by *Epichloë* was ~78% lower in warmed relative to control plots, although the effect was marginally non-significant ($P = 0.0673$; Fig. 3E; Table S12). Mirroring this result, *Epichloë* colonization was ~61% lower at lower (warmer) elevations relative to higher ones within the lowest elevation pair of sites, although this trend was not significant (Fig. 3F).

Diversity. Of the 354 ITS2 leaf fungal OTUs, 322 were present in the warming experiment, and 132 of these were present in both studies. Shannon diversity of leaf fungi did not differ between warming treatments, among host species, or between sampling dates (Table S13).

Composition. For leaf fungi, OTU composition did not differ between warming treatments ($P = 0.6973$), but host species differed ($P = 0.0001$) as did sampling dates ($P = 0.0001$) (Table S13). Composition differed between all pairs of host species, and changed between June and September for all three grasses (Fig. S2B).

Discussion

Along altitudinal gradients and under experimental warming, trends with temperature change differed among host species as well as among symbiont groups. Thus, predicting responses to future climate warming will require attention to both host identity and symbiont functional role. Importantly, altitudinal patterns in fungal symbioses largely did not correspond to fungal responses to experimental warming, suggesting the potential limitations of using gradients to predict symbiont responses to climate change.

Altitudinal gradient and experimental warming trends largely did not mirror one another.

For most symbiont responses, shifts with elevation versus warming were non-concordant. There were even opposing trends between altitudinal gradients and experimental warming for some host-symbiont associations. For instance, *Epichloë* colonization of *F. thurberi* decreased with increased temperature in the warming experiment but increased with temperature along altitudinal gradients. Furthermore, patterns with altitude were often absent under experimental warming and vice-versa. For example, along altitudinal gradients, AMF colonization of *P. pratensis* peaked at mid-elevations, AMF OTU composition changed, LFE colonization of *A. lettermanii* decreased with elevation, and leaf fungal diversity decreased with elevation, but these responses were all absent in the warming experiment. Conversely, under experimental warming, AMF colonization of *A. lettermanii* increased and non-AMF fungal diversity decreased for *F. thurberi*, but trends were absent for these symbioses along altitudinal gradients. In addition, we found that fungal community differences among host species were largely present in the warming experiment but absent in the altitudinal gradient study. Change in non-AMF OTU composition for *A. lettermanii* was the one exception

where responses were concordant, which is surprising given the broad range of fungal taxa represented in our dataset. Furthermore, our data suggest that divergence in diversity and community composition trends was not due to lack of overlap in OTUs between the two studies.

Why do altitudinal patterns and warming responses diverge? First, factors other than temperature might be driving altitudinal patterns, and as such gradient data cannot determine causality. For example, Rincón et al. (2015) found that overall soil fungal richness varied with pH but not with climate variables along an altitudinal gradient. Alternatively, our gradient study may be capturing broader-scale temperature trends not present with experimental warming alone because temperature change over the full altitudinal gradient (~8°C) exceeded temperature change induced by experimental warming (~2°C). Third, warming chamber experiments at different latitudes have shown varying responses of species to warming in different parts of their geographic ranges (Pelini et al. 2012, Stuble et al. 2013); our warming results could similarly be influenced by the geographic placement of the experiment. In our altitudinal survey, for *F. thurberi,* differences in non-AMF root fungal diversity and *Epichloë* colonization between pairs of sites 2°C apart at low elevations mirrored warming responses, though overall altitudinal patterns did not. Fungal responses to 2°C of temperature change at a lower elevation site, such as the location of the RMBL warming experiment, might therefore differ from responses to 2°C of change at a higher elevation. This observation possibly explains the divergent trends we observed in *Epichloë* colonization between the gradient survey and warming experiment.

Fourth, imposing experimental warming causes an immediate step change in temperature and could therefore influence plant-fungal symbioses in ways that gradual temperature gradients or real-world warming will not. For instance, in the RMBL warming experiment, soil organic carbon decreased sharply in heated plots during the first four years of the experiment, but then increased slowly afterward (Harte et al. 2015), whereas in control plots there has been a slow decline in soil organic carbon over time, indicative of a gradual response associated with increased temperatures. Plant-fungal symbioses could exhibit similarly divergent responses between experimental warming and natural climate gradients.

Studies of other taxa have also found a lack of correspondence between responses to experimental warming and natural climate trends. For instance, a study of ant communities in the Colorado Rocky Mountains found shifts along a natural climate gradient that were absent under experimental warming (Menke et al. 2014). Similarly, in Germany, Backhaus et al. (2014) found that plants experienced differing growth responses to temperature change in a warming experiment versus in a transplant experiment along a latitudinal gradient. And in Australia, shifts in arthropod community composition with experimental warming were absent along a natural altitudinal gradient (Nash et al. 2013). While a number of mechanisms might contribute to the lack of congruence in our study between altitudinal gradient and warming trends, our results indicate that space-for-time substitution does not apply in our system, suggesting the potential limitations of frequently used methods for predicting species responses to climate change.

Host specificity in response to temperature

Our data support the prediction that plant species will differ in their sensitivity to climate-induced disruption of symbioses, as prior work in our system indicated (Ranelli et al. 2015). Some host species experienced stronger responses of their fungal communities to temperature than others. Depending on the importance of symbioses to plant fitness, such effects could alter plant community dynamics.

Of the host species considered, we predict that *A. lettermanii* will show the highest sensitivity of fungal symbionts to climate change. Symbionts of *A. lettermanii* responded particularly strongly to temperature change. For this host, AMF colonization increased by ~31% under experimental warming, LFE colonization declined by ~44% per 1 km increase in elevation, and non-AMF root fungal composition changed both along gradients and under warming. *Achnatherum lettermanii* can occupy a range of mountain habitats, including mid-elevation to subalpine grasslands, the understories of *Populus tremuloides* and conifer stands, and sagebrush-dominated communities (Cronquist et al. 1977). We focused our sampling efforts on grasslands and meadows, including communities with sagebrush present. Considering additional habitat types could therefore yield different trends. However, we collected samples from the majority of *A. lettermanii*'s documented altitudinal range (Taylor 2000), suggesting the strength of our dataset in capturing the scope of variation in *A. lettermanii*'s symbiont community.

For *F. thurberi*, *Epichloë* colonization decreased ~69% per km increase in elevation, but was ~78% lower in warmed relative to control plots in the warming experiment. Under experimental warming, non-AMF richness also decreased by ~26%, and non-AMF community composition changed. These results suggest that *F. thurberi*

might also be substantially influenced by changes in its fungal symbionts under climate change. As with *A. lettermanii*, we collected samples from the majority of *F. thurberi*'s altitudinal range (Meyer 2009), improving our likelihood of capturing broad patterns in plant-fungal symbioses. *Festuca thurberi* is a dominant species in the western Colorado Rocky Mountains, and with its rapid root growth and dense litter production, it can help to stabilize soils and prevent erosion (Langenheim 1962, Meyer 2009). Changes in *F. thurberi*'s distribution or abundance with symbiont shifts under climate warming could thus substantially alter plant communities and hydrogeological dynamics.

For *P. pratensis*, AMF colonization peaked at mid-elevation in the altitudinal gradient study, but other fungal groups and community metrics were less responsive to temperature change. Therefore, with climate change, *P. pratensis* might be less influenced by shifts in its associations with fungal symbionts relative to the other two host species. However, *P. pratensis* is distributed widely across North America, occurring throughout the continental U.S. and in Alaska and Canada (USDA, NRCS 2016). We sampled only the upper altitudinal edge of *P. pratensis*' broad distribution. Thus, we did not sample comprehensively enough to detect trends in symbiont change along environmental gradients relevant to the entire species range. The broad geographic distribution of *P. pratensis* may indicate a wide ecological amplitude with high tolerance of environmental conditions and low reliance on specific beneficial fungi, as is suggested by the low mycorrhizal dependence of *P. pratensis* that has been identified in prior work (Wilson and Hartnett 1997).

Fungal groups showed differential sensitivity to environmental drivers.

We found responses to temperature change within all of the fungal functional groups that we considered. However, fungal groups differed from one another in the magnitude and direction of their responses.

Arbuscular Mycorrhizal Fungi. Our results suggest that climate warming will alter AMF colonization frequency and community composition, but that AMF diversity will remain unchanged. Colonization by AMF could show varying patterns with climate shifts depending on host species identity, as indicated by the differing trends for *P. pratensis* along altitudinal gradients versus *A. lettermanii* in the warming experiment. Changes in AMF community composition along altitudinal gradients were largely due to decreases in abundance of the three most abundant AMF genera (*Rhizophagus*, *Claroideoglomus*, and *Glomus*) as elevation increased. Warmer temperatures may therefore lead to increased dominance of these taxa in AMF communities, along with changes in colonization frequency. All three of these genera are in the Glomerales; this order is known to extensively colonize host roots, which can have positive effects on plant performance (Powell et al. 2009). The Glomerales may also confer higher protection against pathogens to their hosts relative to other AMF orders (Powell et al. 2009). Given predictions of increased plant pathogen abundance and severity under climate change (Harvell et al. 2002, Tylianakis et al. 2008), increased association with members of the Glomerales could benefit host plants in future climates. However, plant species are known to differ in their growth responses to different AMF taxa, meaning that change in AMF community composition could affect plant community structure in ways that are difficult to predict

(van der Heijden et al. 1998). Our results therefore suggest the potential for complex community-level consequences of shifts in symbioses with AMF under climate change.

Non-AMF Root Fungi. In contrast to AMF, our results for non-AMF root fungi predict that warmer temperatures will have little effect on colonization of hosts, but instead will shift taxonomic composition and decrease diversity.

In the warming experiment, OTUs in the Helotiales and Capnodiales were among the taxa that contributed most strongly to non-AMF root fungi compositional differences between warmed and control plots for both *A. lettermanii* and *F. thurberi*. Both fungal orders increased in abundance under experimental warming and showed similar relationships with temperature change along altitudinal gradients. Other studies have similarly documented changes in the Helotiales and Capnodiales under experimental warming. In studies of Arctic soil fungi, Helotiales proportional abundance increased under warming (Deslippe et al. 2012), members of the Helotiales were indicator species in both warmed and control plots (Geml et al. 2015), and Capnodiales OTU richness increased under warming (Semenova et al. 2015). The Helotiales is a functionally diverse group that includes DSE, ericoid and ecotomycorrhizal taxa, plant pathogens, and saprotrophs (Vralstad et al. 2002). Within the Capnodiales, *Davidiella tassiana* contributed most to differences in our study between warmed and control plots; this species can also have varied functional roles, including DSE, saprotroph, and plant pathogen (Schubert et al. 2007). With changes in environmental conditions, DSE may shift along the mutualism-parasitism continuum, becoming more or less beneficial to hosts (Mandyam and Jumpponen 2015). How climate change affects both the abundances

and the functional roles of important non-AMF root fungal taxa could therefore influence plant responses to global change in important ways.

Our results also suggest that compositional changes may be accompanied by decreased richness of the non-AMF root fungal community, as we found for *F. thurberi* in the warming experiment. In the moist tussock tundra of the Arctic, ectomycorrhizal richness was similarly lower under experimental warming, with some species increasing in abundance while others disappeared (Morgado et al. 2015). Together, our composition and diversity findings highlight the important roles that dominant non-AMF root fungal taxa may play in determining plant responses to climate warming.

Leaf Fungi. For leaf fungal symbionts, similar to AMF, our results suggest susceptibility to changes in colonization with climate change; LFE and *Epichloë* colonization both varied with climate variables. The declines we observed with elevation for both LFE and *Epichloë* align with the results of prior studies documenting decreases in the frequency of both *Epichloë* (Granath et al. 2007, Bazely et al. 2007, Kirkby et al. 2011, Ranelli et al. 2015) and other leaf endophytes (Hashizume et al. 2008, Helander et al. 2013) with altitude, though different leaf symbiont taxa may exhibit opposing trends (Hashizume et al. 2008).

In contrast to the community composition responses that we observed for both types of root fungi, leaf symbiont composition did not change with elevation, with the measured climate variables, or under experimental warming. However, with temperature increase along altitudinal gradients, leaf symbiont OTU richness increased, changing in the opposite direction from non-AMF fungal diversity in the warming experiment. Most prior studies have been restricted to examining fungal symbionts in one type of host

tissue (i.e., roots or leaves); however, our results are consistent with the work of Coince et al. (2014), who found differing patterns in root and leaf symbiont composition that were explained by different environmental drivers. Differential responses of root and leaf symbiont communities could lead to complex host plant and community responses to climate change, particularly if multiple symbionts in host plants interact with each other (Afkhami et al. 2014).

Climate variables predicted some variation in fungal colonization but little variation in fungal composition or diversity.

Along altitudinal gradients, fungal group responses to elevation were largely driven by climate variables, especially for leaf fungi; this was the case for LFE colonization of *A. lettermanii*, *Epichloë* colonization of *F. thurberi,* and leaf fungal diversity across host species. However, climate variables were poor predictors of fungal colonization for the other plant-symbiont associations that we examined, and were overall weak predictors of symbiont composition and diversity. For some associations, climate might therefore be determining the extent of symbiosis within a host plant with elevation change, but not necessarily playing a role in determining symbiont presence/absence. Gai et al. (2006) similarly found changes in AMF colonization but no variation in species richness or diversity in a study of sedges along an environmental gradient in the Tibetan Plateau. Our findings highlight the importance of considering additional factors that might structure symbiont distributions along altitudinal gradients, such as other climate variables, availability of nutrients other than P, geographic or geological factors, and plant community composition.

Extent and composition of symbioses changed through time.

We found evidence for both interannual variability and differences in phenology among symbioses. In the altitudinal gradient survey, root symbiont colonization was lower for all three host grasses in 2012 relative to 2014, suggestive of potential effects of a warmer, drier climate on fungal colonization. A number of studies have documented increased root colonization by AMF under dry conditions, in contrast with our observations, although other work showed decreased AMF colonization with drought (reviewed by Augé 2001), suggesting that responses to altered soil moisture are system-specific. In addition, drought can change fungal symbiont community composition (Shi et al. 2002, Zhang et al. 2016) and decrease symbiont richness (Gehring et al. 2014). Our work therefore supports predictions of altered symbiont communities under more arid future conditions in the Rocky Mountains, and demonstrates the value of sampling in multiple years to gain insight into variation in host-symbiont patterns.

Furthermore, root fungal colonization and diversity changed between June and September in the warming experiment, with patterns differing among host species. This finding suggests potential differences in the phenology of fungi associated with different hosts, though we lack replication of sampling date, as we only sampled during one growing season. Other studies documented similar patterns, however. For instance, Ruotsalainen et al. (2002) found host species-specific patterns in colonization by both AMF and DSE over the course of one growing season, suggestive of differing fungal phenology among host species. Studies also found that fruiting is occurring later in the growing season in response to temperature increase for both mycorrhizal and saprotrophic fungi, but with variation in trends among fungal species (e.g., Kauserud et al.

2012, Diez et al. 2013). Understanding differences in phenology among plant-fungal associations will therefore be important in predicting species responses to future global change.

Conclusions

Our work suggests that climate change will have effects on plant-fungal symbioses that will depend on host plant identity and fungal functional group, with some associations weakened or disrupted, others affected weakly, and yet others enhanced by climate warming. Predicting how climate change will alter ecologically important symbioses should therefore involve attention to the identity and ecology of both hosts and symbionts. Our results also indicate that caution should be used in predicting species responses to global change based on altitudinal gradient and experimental warming trends. Our approach suggests the strength of comparing environmental gradient studies to warming experiments in order to gain a more nuanced perspective on how climate change may alter communities and ecosystems.

APPENDICES

APPENDIX A. SUPPLEMENTARY TABLES

Table S1. Locality information of sites in the altitudinal gradient study at which *Festuca thurberi* (FETH) samples were collected in 2008, 2009, and 2011, along with date of sampling.

	Gradient	Elevation (m)	Latitude	Longitude	Species Collected	Collection Date
2008						
	East River	2970	38.9609	106.9816	FETH	8/9/2008
	East River	3172	38.9681	106.9844	FETH	8/15/2008
	East River	3411	38.9766	106.9772	FETH	8/9/2008
2009						
	Cinnamon	2891	38.9153	107.0016	FETH	7/8/2009
	Cinnamon	2952	38.9336	107.0195	FETH	7/8/2009
	Cinnamon	3044	38.9454	107.0279	FETH	7/8/2009
	Cinnamon	3242	38.9272	106.9858	FETH	7/21/2009
	Cottonwood Pass	2741	38.8273	106.8489	FETH	6/19/2009
	Cottonwood Pass	2856	38.7810	106.7617	FETH	6/28/2009
	Cottonwood Pass	2871	38.8074	106.7406	FETH	6/28/2009
	Cottonwood Pass	2914	38.8390	106.8426	FETH	6/19/2009
	Cottonwood Pass	2970	38.8377	106.7160	FETH	6/28/2009
	Cottonwood Pass	3002	38.8412	106.8420	FETH	6/19/2009
	East River	2731	38.8735	106.9537	FETH	7/5/2009
	East River	2870	38.9349	106.9698	FETH	7/13/2009
	East River	2911	38.8585	106.9239	FETH	7/12/2009
	East River	2920	38.9609	106.9906	FETH	7/11/2009
	East River	3172	38.9681	106.9844	FETH	8/15/2009
	East River	3195	38.9717	106.9839	FETH	6/26/2009
	East River	3442	38.9747	106.9761	FETH	6/26/2009
	Ruby	2715	38.8570	107.1437	FETH	7/17/2009
	Ruby	2986	38.8552	107.0904	FETH	7/17/2009
	Teocalli	2889	38.9005	106.8849	FETH	7/22/2009
	Treasury	2748	38.9247	107.0413	FETH	6/20/2009
	Treasury	2887	38.9385	107.0533	FETH	6/20/2009
2011						
	Cinnamon	2800	38.8970	106.9789	FETH	8/4/2011
	Cinnamon	3011	38.9413	107.0238	FETH	8/4/2011
	East River	2710	38.8652	106.9124	FETH	8/4/2011
	East River	2839	38.9444	106.9806	FETH	8/4/2011
	East River	2992	38.9626	106.9852	FETH	8/4/2011
	East River	3222	38.9715	106.9841	FETH	8/18/2011
	Treasury	2748	38.9199	107.0368	FETH	8/4/2011

Table S2. Locality information of sites in the altitudinal gradient study at which *Achnatherum lettermanii* (ACLE), *Festuca thurberi* (FETH), and *Poa pratensis* (POPR) samples were collected in 2012, along with date of sampling.

	Gradient	Elevation (m)	Latitude	Longitude	Species Collected	Collection Date
2012						
	Aspen	2985	39.0245	106.8090	ACLE, FETH, POPR	7/24/2012
	Aspen	3198	39.0055	106.8037	FETH, POPR	7/22/2012
	Cinnamon	2707	38.8818	106.9801	POPR	7/31/2012
	Cinnamon	2835	38.8972	106.9786	ACLE, FETH, POPR	7/31/2012
	Cinnamon	3027	38.9411	107.0235	ACLE, FETH, POPR	7/31/2012
	Cinnamon	3180	38.9609	107.0313	POPR	7/31/2012
	Cinnamon	3430	38.9909	107.0646	ACLE	7/30/2012
	Cottonwood Pass	2830	38.8260	106.5696	POPR	7/1/2012
	Cottonwood Pass	3017	38.8460	106.5058	FETH	7/1/2012
	Cottonwood Pass	3445	38.9961	106.4244	FETH	6/29/2012
	East River	2729	38.8652	106.9123	ACLE, FETH, POPR	7/11/2012
	East River	2860	38.9440	106.9799	ACLE, FETH, POPR	8/1/2012
	East River	3016	38.9626	106.9852	ACLE, FETH, POPR	8/1/2012
	East River	3380	39.0315	107.0789	ACLE	8/1/2012
	Ruby	2869	38.8642	107.1206	FETH, POPR	7/16/2012
	Ruby	3256	38.8895	107.1171	ACLE	7/17/2012
	Ruby	3450	38.9022	107.1164	ACLE	7/17/2012
	Teocalli	2883	38.9062	106.8846	ACLE, FETH, POPR	7/10/2012
	Teocalli	3047	38.9441	106.8874	ACLE, FETH	7/10/2012
	Teocalli	3276	38.9478	106.8809	ACLE, FETH	7/10/2012
	Teocalli	3450	38.9504	106.8760	ACLE, FETH	6/27/2012
	Teocalli	3679	38.9562	106.8779	FETH	7/10/2012
	Treasury	2773	38.9206	107.0372	ACLE, FETH, POPR	7/31/2012
	Treasury	2967	38.9739	107.0660	ACLE, FETH, POPR	7/31/2012
	Treasury	3195	38.9712	107.0596	ACLE, POPR	7/31/2012
	Treasury	3280	38.9755	107.0582	ACLE, POPR	7/30/2012
	Treasury	3434	38.9880	107.0655	ACLE	7/30/2012

Table S3. Locality information of sites in the altitudinal gradient study at which *Achnatherum lettermanii* (ACLE), *Festuca thurberi* (FETH), and *Poa pratensis* (POPR) samples were collected in 2014, along with date of sampling.

	Gradient	Elevation (m)	Latitude	Longitude	Species Collected	Collection Date
2014						
	Cinnamon	2749	38.8816	106.9617	ACLE, FETH, POPR	7/31/2014
	Cinnamon	2799	38.8973	106.9790	FETH, POPR	7/30/2014
	Cinnamon	2932	38.9346	107.0108	ACLE, FETH, POPR	7/31/2014
	Cinnamon	3025	38.9454	107.0282	FETH	7/31/2014
	Cinnamon	3366	38.9702	107.0295	ACLE	8/27/2014
	Cinnamon	3416	38.9911	107.0650	ACLE	8/30/2014
	East River	2711	38.8655	106.9124	FETH, POPR	8/4/2014
	East River	2812	38.8652	106.9124	FETH, POPR	8/4/2014
	East River	2896	38.9514	106.9864	FETH	8/4/2014
	East River	2982	38.9622	106.9855	FETH, POPR	8/5/2014
	East River	3135	38.9659	106.9828	FETH, POPR	8/24/2014
	East River	3192	38.9714	106.9843	FETH	9/14/2014
	East River	3347	38.9753	106.9783	ACLE, FETH	9/14/2014
	East River	3455	38.9274	106.9782	ACLE, FETH	9/13/2014
	Hunter's Hill	2824	38.8476	106.8196	ACLE, FETH, POPR	8/1/2014
	Hunter's Hill	3060	38.9037	106.7838	FETH, POPR	8/1/2014
	Hunter's Hill	3171	38.9254	106.7778	FETH, POPR	9/10/2014
	Hunter's Hill	3249	38.9260	106.7923	ACLE, FETH	9/10/2014
	Hunter's Hill	3322	38.9268	106.7911	FETH	9/10/2014
	Hunter's Hill	3430	38.9296	106.7890	FETH	9/12/2014
	Hunter's Hill	3531	38.9330	106.7870	FETH, POPR	9/12/2014
	Hunter's Hill	3629	38.9376	106.7874	FETH	9/11/2014
	Ruby	2822	38.8643	107.0317	ACLE, FETH, POPR	7/30/2014
	Ruby	2945	38.8562	107.0695	ACLE, FETH, POPR	7/30/2014
	Ruby	3055	38.8645	107.1058	ACLE	7/29/2014
	Ruby	3128	38.8742	107.1060	ACLE	7/28/2014
	Ruby	3199	38.8838	107.1133	ACLE	7/28/2014
	Teocalli	2776	38.8957	106.8912	ACLE, FETH, POPR	8/8/2014
	Teocalli	2868	38.9061	106.8838	FETH, POPR	8/7/2014
	Teocalli	2948	38.9288	106.8784	FETH, POPR	8/7/2014
	Teocalli	3047	38.9440	106.8872	FETH, POPR	8/8/2014
	Teocalli	3157	38.9481	106.8902	FETH, POPR	9/19/2014
	Teocalli	3275	38.9476	106.8807	FETH	9/19/2014
	Teocalli	3351	38.9472	106.8776	FETH	9/19/2014
	Teocalli	3443	38.9503	106.8762	FETH	9/19/2014
	Teocalli	3553	38.9534	106.8763	FETH	9/18/2014
	Teocalli	3667	38.9559	106.8779	FETH	9/18/2014
	Treasury	2747	38.9186	107.0363	FETH, POPR	8/5/2014
	Treasury	2795	38.9328	107.0498	ACLE, FETH, POPR	8/5/2014
	Treasury	2972	38.9726	107.0619	POPR	8/6/2014
	Treasury	3197	38.9707	107.0587	FETH, POPR	9/8/2014

Table S4. Locality information of weather stations from which climate data were aggregated, along with data collection period and data source (RMBL = Rocky Mountain Biological Laboratory, NRCS = USDA Natural Resources Conservation Service, SCENIC = Southwest Climate and Environmental Information Collaborative).

Station Name	Elevation (m)	Latitude	Longitude	Data Collection Period	Data Source
Billy Barr	2917	38.9631	106.9933	2011-2015	RMBL
Bison Lake	3316	39.7649	107.3568	1987-2015	NRCS
Brumley	3231	39.0877	106.5417	1981-2015	NRCS
Butte	3097	38.8943	106.9530	1982-2015	NRCS
Chapman Tunnel	3082	39.2622	106.6293	2008-2015	NRCS
Cochetopa Pass	3054	38.1628	106.5988	2005-2015	NRCS
Crested Butte	2702	38.8739	106.9769	1981-2015	SCENIC
Crested Butte 6.2N	2928	38.9603	106.9908	2006-2015	SCENIC
Gunnison 6.6N	2420	38.6391	106.9408	2010-2015	SCENIC
Independence Pass	3231	39.0754	106.6117	1982-2015	NRCS
Ivanhoe	3170	39.2920	106.5492	1993-2015	NRCS
Judd Falls	3004	38.9636	106.9836	2010-2015	RMBL
Kettle Ponds	2860	38.9417	106.9731	2010-2015	RMBL
Kiln	2926	39.3172	106.6145	1981-2015	NRCS
Marble 0.5NNW	2565	39.0791	107.1906	2011-2015	SCENIC
McClure Pass	2896	39.1290	107.2881	1981-2015	NRCS
Mexican Cut	3412	39.0283	107.0636	2010-2015	RMBL
Nast Lake	2652	39.2972	106.6069	1987-2015	NRCS
North Lost Trail	2804	39.0781	107.1439	1986-2015	NRCS
Overland Reservoir	2999	39.0906	107.6347	1990-2015	NRCS
Park Cone	2926	38.8200	106.5897	1981-2015	NRCS
Park Reservoir	3036	39.0464	107.8741	1981-2015	NRCS
Porphyry Creek	3280	38.4888	106.3397	1981-2015	NRCS
Saint Elmo	3213	38.6998	106.3680	2008-2015	NRCS
Sargents Mesa	3514	38.2856	106.3707	2010-2015	NRCS
Schofield Pass	3261	39.0152	107.0488	1986-2015	NRCS
Snodgrass	3396	38.9331	106.9861	2010-2015	RMBL
Taylor Park Colorado	3173	38.9078	106.6017	1989-2015	SCENIC
Upper Taylor	3243	38.9908	106.7542	2010-2015	NRCS

Table S5. Results of statistical models testing the effects of elevation, host species identity (where applicable), sampling year, and their interactions, along with sampling date, on colonization of grasses by hyphae of arbuscular mycorrhizal fungi (AMF), non-AMF root fungi, localized foliar endophytes (LFE), and *Epichloë*. *P*-values < 0.05 are shown in bold.

		AMF			**Non-AMF Root Fungi**			**LFE**			***Epichloë***			
	Num. *df*	Denom. *df*	*F*	*P*	Denom. *df*	*F*	*P*	Denom. *df*	*F*	*P*	Num. *df*	Denom. *df*	*F*	*P*
Elevation	1	102	14.7	**0.0002**	102	0.6	0.4338	103	4.0	**0.0469**	1	65	10.1	**0.0023**
Host ID	2	102	47.6	**< 0.0001**	102	34.1	**< 0.0001**	103	7.1	**0.0013**				
Year	1	102	324.8	**< 0.0001**	102	108.3	**< 0.0001**	103	145.8	**< 0.0001**	4	65	4.8	**0.0018**
Sampling date	1	102	0.1	0.7664	102	2.6	0.1132	103	3.4	0.0665	1	65	2.9	0.0920
Elevation*host ID	2	102	0.8	0.4353	102	0.4	0.6684	103	0.4	0.6661				
Elevation*year	1	102	0.6	0.4432	102	2.4	0.1266	103	0.8	0.3687	4	65	0.7	0.5624
Host ID*year	2	102	3.2	**0.0460**	102	2.6	0.0797	103	3.7	**0.0293**				
Elevation*host ID*year	2	102	2.3	0.1082	102	0.7	0.4813	103	1.0	0.3818				

Table S6. Model selection results for predicting fungal colonization for each host species × fungal group combination along altitudinal gradients. Individual models containing climate variables are compared to the null model. Fungal groups: arbuscular mycorrhizal fungi (AMF), non-AMF root fungi, localized foliar endophytes (LFE) and *Epichloë*. Predictor variables: mean cumulative growing degree days (GDD), mean annual precipitation (MAP), mean snow depth (MSD), and mean soil phosphorus content (P).

	AMF				**Non-AMF Root Fungi**					**LFE**					***Epichloë***				
Model	*K*	R^2	*ΔAICc*	w_i	Model	*K*	R^2	*ΔAICc*	w_i	Model	*K*	R^2	*ΔAICc*	w_i	Model	*K*	R^2	*ΔAICc*	w_i
A. lettermanii																			
Null	5	0.81	0	0.53	Null	5	0.48	0	0.38	MSD	6	0.66	0	0.34					
GDD	6	0.81	2.98	0.12	P	6	0.52	0.68	0.27	MAP	6	0.65	0.46	0.27					
P	6	0.81	2.99	0.12	MAP	6	0.49	2.23	0.12	GDD	6	0.65	0.76	0.23					
MSD	6	0.81	2.99	0.12	MSD	6	0.49	2.36	0.12	Null	5	0.60	2.39	0.10					
MAP	6	0.81	2.99	0.12	GDD	6	0.49	2.51	0.11	P	6	0.62	3.86	0.05					
F. thurberi																			
GDD	6	0.75	0	0.33	Null	5	0.56	0	0.42	Null	5	0.40	0	0.29	GDD	6	0.30	0	0.64
Null	5	0.73	0.35	0.27	MSD	6	0.57	1.77	0.17	GDD	6	0.43	0.29	0.25	MSD	6	0.28	2.33	0.20
MSD	6	0.74	1.18	0.18	GDD	6	0.57	2.07	0.15	MAP	6	0.42	0.44	0.23	MAP	6	0.27	3.92	0.09
MAP	6	0.74	1.74	0.14	MAP	6	0.57	2.31	0.13	MSD	6	0.41	1.35	0.15	Null	5	0.25	4.56	0.07
P	6	0.74	2.85	0.08	P	6	0.56	2.59	0.12	P	6	0.40	2.58	0.08					
P. pratensis																			
Null	5	0.73	0	0.46	MAP	6	0.50	0	0.31	Null	5	0.68	0	0.28					
P	6	0.73	2.18	0.16	Null	5	0.46	0.41	0.26	GDD	6	0.71	0.02	0.27					
GDD	6	0.73	2.40	0.14	MSD	6	0.49	0.63	0.23	MSD	6	0.70	0.52	0.21					
MAP	6	0.73	2.62	0.12	GDD	6	0.48	1.62	0.14	MAP	6	0.70	1.15	0.15					
MSD	6	0.73	2.72	0.12	P	6	0.46	3.22	0.06	P	6	0.69	2.36	0.08					

Table S7. Spearman correlation coefficients for the relationship between sequence abundance and elevation for each genus of arbuscular mycorrhizal fungi (AMF) and each order of non-AMF root fungi found in ≥10 samples in the altitudinal gradient study. *n* is the number of site × species combinations observed for each genus or order.

AMF				Non-AMF Root Fungi			
Genus	Spearman *r*	*P*	*n*	Order	Spearman *r*	*P*	*n*
Glomus	-0.05	0.7557	22	Coniochaetales	0.27	0.0655	25
Claroideoglomus	-0.11	0.4567	27	Agaricales	0.12	0.4076	45
Rhizophagus	-0.18	0.2223	48	Pleosporales	0.08	0.5919	48
				Sebacinales	0.03	0.8312	10
				Chaetothyriales	-0.01	0.9512	47
				Eurotiales	-0.03	0.8216	24
				Helotiales	-0.03	0.8230	47
				Mortierellales	-0.05	0.7423	17
				Filobasidiales	-0.05	0.7115	19
				Trechisporales	-0.05	0.7584	26
				Capnodiales	-0.05	0.7118	41
				Xylariales	-0.06	0.6784	41
				Dothideales	-0.11	0.4461	12
				Tremellales	-0.12	0.4351	14
				Sordariales	-0.13	0.3681	45
				Diaporthales	-0.19	0.2077	27
				Chaetosphaeriales	-0.19	0.1950	33
				Cantharellales	-0.20	0.1836	12

Table S8. Results of statistical models testing the effects of elevation, host species identity, and their interaction, as well as sampling date, on Shannon OTU diversity for arbuscular mycorrhizal fungi (AMF), non-AMF root fungi, and leaf fungi. *P*-values < 0.05 are shown in bold.

		AMF			**Non-AMF Root Fungi**			**Leaf Fungi**		
	Num. *df*	Denom. *df*	*F*	*P*	Denom. *df*	*F*	*P*	Denom. *df*	*F*	*P*
Elevation	1	35	2.3	0.1418	34	2.3	0.1346	33	9.1	**0.0050**
Host ID	2	35	0.03	0.9752	34	3.5	**0.0409**	33	1.7	0.2035
Sampling date	1	35	0.1	0.7985	34	21.2	**0.0001**	33	0.1	0.7626
Elevation*host ID	2	35	1.0	0.3714	34	0.3	0.7739	33	0.4	0.6532

Table S9. Model selection results for predicting Shannon OTU diversity of A) arbuscular mycorrhizal fungi (AMF), B) non-AMF root fungi, and C) leaf fungi along altitudinal gradients. Individual models containing climate variables are compared to the null model. GDD = mean cumulative growing degree days, MAP = mean annual precipitation, MSD = mean snow depth, and P = mean soil phosphorus concentration.

Model	K	R^2	$\Delta AICc$	w_i
A) AMF				
Null	5	0.01	0	0.69
MAP	6	0.11	3.81	0.10
MSD	6	0.10	4.11	0.09
GDD	6	0.10	4.21	0.08
P	6	0.07	5.96	0.03
B) Non-AMF root fungi				
Null	5	0.69	0	0.53
MSD	6	0.79	2.17	0.18
GDD	6	0.80	2.53	0.15
MAP	6	0.80	3.02	0.12
P	6	0.76	6.93	0.02
C) Leaf fungi				
GDD	6	0.26	0	0.31
MAP	6	0.26	0.26	0.27
MSD	6	0.26	0.47	0.24
Null	5	0.09	1.59	0.14
P	6	0.19	4.25	0.04

Table S10. DISTLM results indicating the effects of elevation, host species, and sampling date on OTU composition for arbuscular mycorrhizal fungi (AMF), non-AMF root fungi, and leaf fungi. *P*-values < 0.05 are shown in bold.

	AMF				Non-AMF Root Fungi				Leaf Fungi			
	Pseudo-F	*P*	Regression *df*	Residual *df*	*Pseudo-F*	*P*	Regression *df*	Residual *df*	*Pseudo-F*	*P*	Regression *df*	Residual *df*
Elevation	1.4	0.0746	2	46	1.9	**0.0073**	2	46	0.8	0.7091	2	44
Host species	1.2	0.1488	3	45	2.6	**0.0001**	3	45	1.2	0.1453	3	43
Sampling date	1.0	0.4825	2	46	1.7	**0.0152**	2	46	1.1	0.2907	2	44

Table S11. Model selection results for predicting OTU composition of A) arbuscular mycorrhizal fungi (AMF), B) non-AMF root fungi, and C) leaf fungi along altitudinal gradients. Individual models containing climate variables are compared to the null model. GDD = mean cumulative growing degree days, MAP = mean annual precipitation, MSD = mean snow depth, and P = mean soil phosphorus concentration.

Model	K	R^2	$\Delta AICc$	w_i
A) AMF				
Null	5	0.18	0	0.33
MSD	6	0.21	1.17	0.18
MAP	6	0.21	1.29	0.17
GDD	6	0.21	1.31	0.17
P	6	0.21	1.52	0.15
B) Non-AMF root fungi				
Null	5	0.28	0	0.31
MSD	6	0.32	0.85	0.20
GDD	6	0.32	0.91	0.20
MAP	6	0.32	0.91	0.20
P	6	0.30	2.37	0.09
C) Leaf fungi				
Null	5	0.17	0	0.43
MSD	6	0.19	2.16	0.14
GDD	6	0.19	2.16	0.14
MAP	6	0.19	2.17	0.14
P	6	0.19	2.19	0.14

Table S12. Results of statistical models testing the effects of warming treatment, sampling date, host species identity (where applicable), and their interactions on colonization frequency of arbuscular mycorrhizal fungi (AMF) hyphae, AMF vesicles, AMF arbuscules, non-AMF root hyphae, localized foliar endophyte (LFE) hyphae, and *Epichloë* hyphae. *P*-values < 0.05 are shown in bold.

			AMF hyphae		**AMF vesicles**		**AMF arbuscules**		**Non-AMF root hyphae**		**LFE hyphae**		***Epichloë* hyphae**			
	Num. *df*	Denom. *df*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	Num. *df*	Denom. *df*	*F*	*P*
Warming	1	44	2.2	0.1438	0.07	0.7887	0.2	0.6435	0.002	0.9647	0.9	0.3493	1	12	4.0	0.0673
Date	1	44	15.7	**0.0003**	5.7	**0.0218**	0.9	0.3464	0.3	0.5893	0.5	0.4868	1	12	0.8	0.3990
Host ID	2	44	7.2	**0.0019**	3.4	**0.0434**	5.7	**0.0063**	12.3	**0.0001**	0.3	0.7274				
Warming*date	1	44	0.3	0.5923	0.2	0.6788	0.01	0.9340	0.1	0.7431	0.5	0.4873	1	12	1.0	0.3313
Warming*host ID	2	44	2.7	0.0786	0.1	0.9400	0.9	0.4157	0.3	0.7282	2.2	0.1185				
Host ID*date	2	44	33.4	**< 0.0001**	11.3	**0.0001**	9.2	**0.0004**	7.3	**0.0018**	2.4	0.1041				
Warming*host ID*date	2	44	0.4	0.6817	0.1	0.9101	0.2	0.8165	0.3	0.7472	0.8	0.4585				

Table S13. Results of statistical models testing the effects of warming treatment, sampling date, host species identity, and their interactions on Shannon diversity and OTU composition for arbuscular mycorrhizal fungi (AMF), non-AMF root fungi, and leaf fungi. *P*-values < 0.05 are shown in bold.

			AMF OTU diversity		**AMF OTU composition**		**Non-AMF root fungi OTU diversity**		**Non-AMF root fungi OTU composition**		**Leaf fungi OTU diversity**		**Leaf fungi OTU composition**	
	Num. *df*	Denom. *df*	*F*	*P*	*Pseudo-F*	*P*	*F*	*P*	*Pseudo-F*	*P*	*F*	*P*	*Pseudo-F*	*P*
Warming	1	44	0.02	0.8795	1.0	0.4470	5.5	**0.0240**	2.1	**0.0001**	0.8	0.3900	0.9	0.6973
Date	1	44	7.7	**0.0080**	2.8	**0.0003**	0.3	0.6183	2.4	**0.0001**	0.04	0.8411	3.6	**0.0001**
Host ID	2	44	15.5	**< 0.0001**	6.3	**0.0001**	12.4	**0.0001**	8.1	**0.0001**	2.1	0.1301	5.1	**0.0001**
Warming*date	1	44	0.9	0.3450	1.1	0.2799	3.8	0.0563	1.4	**0.0216**	0.4	0.5272	0.9	0.5720
Warming*host ID	2	44	0.2	0.8092	0.8	0.8800	3.2	0.0517	1.4	**0.0102**	0.8	0.4704	0.7	0.9416
Host ID*date	2	44	4.8	**0.0134**	1.4	**0.0479**	5.2	**0.0094**	1.4	**0.0034**	4.0	**0.0249**	1.7	**0.0013**
Warming*host ID*date	2	44	0.6	0.5455	0.7	0.9759	0.5	0.6013	0.8	0.9120	1.4	0.2547	0.9	0.6184

Table S14. Results of indicator species analysis (SIMPER), indicating the 15 OTUs that contributed most to the average dissimilarity in arbuscular mycorrhizal fungi OTU composition between *F. thurberi* and *A. lettermanii,* and between *F. thurberi* and *P. pratensis.* Included are genus assigned to the OTU, average OTU abundance in each host species, percent difference in abundance of the OTU in *F. thurberi* relative to *A. lettermanii,* average dissimilarity between the host species in OTU abundance, and percent contribution of the OTU to the overall difference in OTU composition between the two species.

		Average OTU Abundance in Host				
OTU	**Genus**	***F. thurberi***	***A. lettermanii***	**% Difference**	**Avg. Dissimilarity**	**% Contribution**
OTU15	*Rhizophagus*	8.04	2.71	197	0.30	0.49
OTU5	*Rhizophagus*	5.41	9.67	-44	0.30	0.49
OTU13	*Rhizophagus*	7.14	2.20	225	0.28	0.46
OTU16	*Rhizophagus*	6.05	1.64	269	0.26	0.42
OTU11	*Rhizophagus*	4.94	8.42	-41	0.24	0.39
OTU123	*Rhizophagus*	5.13	0.87	490	0.22	0.37
OTU6	*Rhizophagus*	1.46	4.46	-67	0.22	0.36
OTU12	*Rhizophagus*	6.69	4.70	42	0.22	0.36
OTU19	*Claroideoglomus*	2.24	4.84	-54	0.22	0.36
OTU129	*Rhizophagus*	4.92	0.75	556	0.21	0.35
OTU8	*Rhizophagus*	4.20	0.44	855	0.21	0.35
OTU148	*Rhizophagus*	4.66	0.48	871	0.21	0.35
OTU7	*Rhizophagus*	1.22	4.17	-71	0.20	0.34
OTU10	*Rhizophagus*	1.85	3.71	-50	0.20	0.33
OTU143	*Rhizophagus*	1.51	4.58	-67	0.20	0.32
		F. thurberi	***P. pratensis***			
OTU13	*Rhizophagus*	7.14	1.01	607	0.34	0.53
OTU15	*Rhizophagus*	8.04	2.52	219	0.31	0.48
OTU16	*Rhizophagus*	6.05	2.15	181	0.29	0.45
OTU11	*Rhizophagus*	4.94	9.86	-50	0.29	0.45
OTU12	*Rhizophagus*	6.69	2.91	130	0.28	0.43
OTU4	*Rhizophagus*	9.36	5.88	59	0.28	0.43
OTU123	*Rhizophagus*	5.13	0.38	1250	0.26	0.40
OTU5	*Rhizophagus*	5.41	8.28	-35	0.25	0.39
OTU19	*Rhizophagus*	2.24	6.71	-67	0.24	0.38
OTU129	*Rhizophagus*	4.92	0.59	734	0.23	0.36
OTU148	*Rhizophagus*	4.66	0.38	1126	0.23	0.36
OTU113	*Rhizophagus*	5.57	2.28	144	0.23	0.36
OTU8	*Rhizophagus*	4.20	1.00	320	0.23	0.36
OTU104	*Rhizophagus*	6.14	2.68	129	0.23	0.35
OTU157	*Rhizophagus*	4.35	0.27	1511	0.22	0.35

Table S15. Results of indicator species analysis (SIMPER), indicating the 15 OTUs that contributed most to the average dissimilarity in non-arbuscular mycorrhizal root fungi OTU composition between warmed and control plots for A) *A. lettermanii* and B) *F. thurberi.* Included are taxon assigned to the OTU, average OTU abundance in the host in warmed and control plots in the RMBL warming experiment, percent difference in abundance of the OTU in warmed relative to control plots, average dissimilarity between the plot types in OTU abundance, and percent contribution of the OTU to the overall difference in OTU composition between the plot types.

		A) Average OTU Abundance in *A. lettermanii*				
OTU	**Taxon**	**Warmed**	**Control**	**% Difference**	**Avg. Dissimilarity**	**% Contribution**
OTU11	Helotiales	5.79	3.05	90	0.73	1.24
OTU4	Helotiales	7.16	6.15	16	0.64	1.08
OTU67	Pyrenulales	2.13	5.94	-64	0.63	1.07
OTU8	Agaricomycetes	6.78	10.62	-36	0.62	1.06
OTU3	Capnodiales	4.69	1.10	326	0.62	1.06
OTU9	Helotiales	5.44	6.49	-16	0.62	1.05
OTU22	Pleosporales	3.34	4.78	-30	0.60	1.01
OTU19	Dothideomycetes	3.46	4.34	-20	0.58	0.99
OTU18	Pleosporales	4.48	6.35	-29	0.57	0.97
OTU14	Agaricales	3.81	1.91	99	0.56	0.95
OTU12	Diaporthales	4.41	4.04	9	0.54	0.92
OTU52	Pleosporales	4.27	2.94	45	0.54	0.92
OTU16	Sordariomycetes	6.29	6.45	-2	0.51	0.87
OTU49	Dothideomycetes	1.40	3.80	-63	0.50	0.85
OTU29	Sordariomycetes	2.80	5.58	-50	0.49	0.84
		B) Average OTU Abundance in *F. thurberi*				
OTU3	Capnodiales	5.75	1.44	299	0.54	0.93
OTU4	Helotiales	8.61	5.90	46	0.51	0.87
OTU64	Agaricales	2.44	5.45	-55	0.49	0.84
OTU11	Helotiales	4.02	7.07	-43	0.48	0.83
OTU8	Agaricomycetes	6.29	7.43	-15	0.45	0.78
OTU7	Pleosporales	3.55	5.51	-36	0.43	0.74
OTU12	Diaporthales	8.57	5.68	51	0.43	0.74
OTU32	Trechisporales	1.03	4.46	-77	0.42	0.72
OTU14	Agaricales	7.57	4.91	54	0.41	0.70
OTU43	Agaricales	4.78	5.91	-19	0.40	0.69
OTU314	Helotiales	0.76	4.37	-83	0.40	0.68
OTU46	Agaricales	3.58	2.99	20	0.37	0.64
OTU51	Thelephorales	3.12	4.54	-31	0.37	0.63
OTU68	Agaricales	4.97	4.97	0	0.36	0.61
OTU394	Helotiales	1.42	2.98	-52	0.36	0.61

APPENDIX B. SUPPLEMENTARY FIGURES

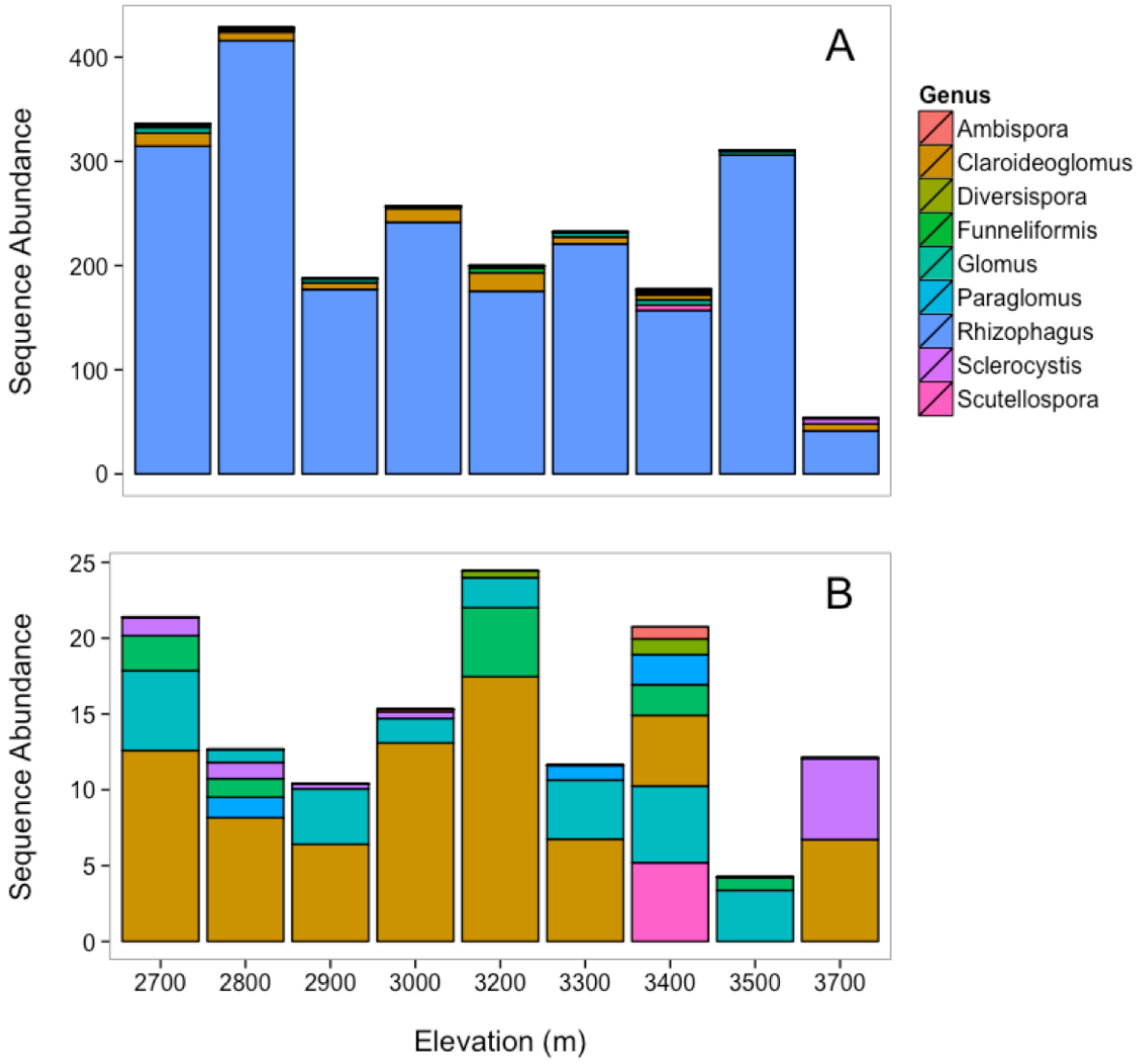

Figure S1. Arbuscular mycorrhizal fungi (AMF) sequence abundance per sample by 100 m elevation interval in the altitudinal gradient survey. A) Sequence abundances for all AMF genera. B) Sequence abundances for AMF genera other than *Rhizophagus*, the most abundant genus.

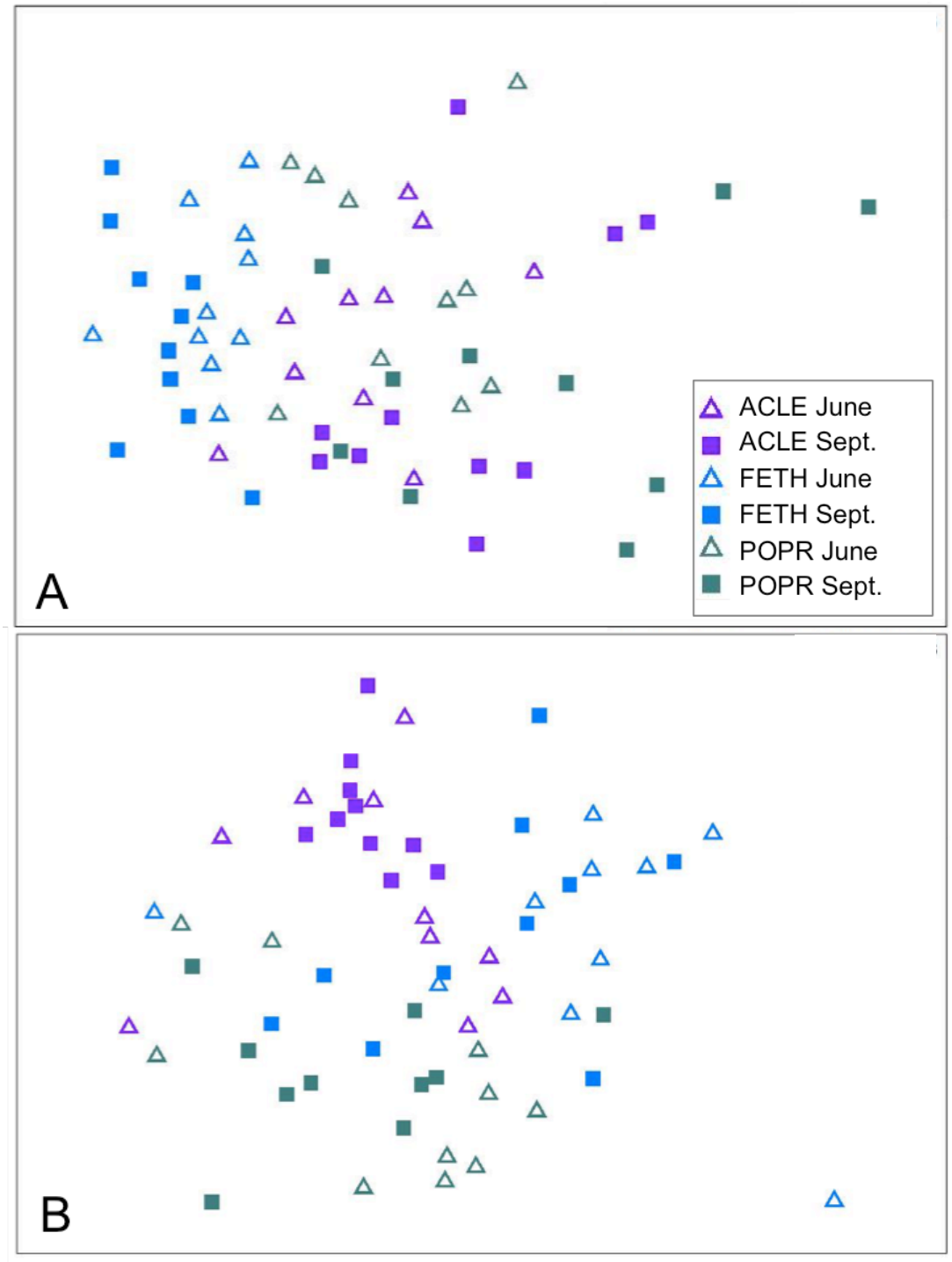

Figure S2. NMDS plots indicating differences in the RMBL warming experiment among host species and sampling dates (June and September) for A) arbuscular mycorrhizal fungi OTU composition (2D stress = 0.16, number of iterations = 239, number of restarts = 500), and B) leaf fungi OTU composition (2D stress = 0.26, number of iterations = 268, number of restarts = 500). ACLE = *Achnatherum lettermanii*, FETH = *Festuca thurberi*, POPR = *Poa pratensis.*

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