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Altitudinal gradients fail to predict fungal symbiont responses to warming

Melanie R. Kazenel [1,2,4] Stephanie N. Kivlin,[1,2,3] D. Lee Taylor,[1] Joshua S. Lynn,[1,2] and Jennifer A. Rudgers[1,2]

[1]*Department of Biology, The University of New Mexico, Albuquerque, New Mexico 87131 USA*
[2]*Rocky Mountain Biological Laboratory, Crested Butte, Colorado 81224 USA*
[3]*Department of Ecology and Evolutionary Biology, The University of Tennessee, Knoxville, Tennessee 37996 USA*

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Abstract. Climate change is shifting altitudinal species ranges, with potential to disrupt species interactions. Altitudinal gradient studies and warming experiments can both increase understanding of climate effects on species interactions, but few studies have used both together to improve predictions. We examined whether plant–fungal symbioses responded similarly to altitude and 23 yr of experimental warming. Root- and leaf-associated fungi, which can mediate plants' climate sensitivity, responded divergently to elevation vs. warming. Fungal colonization, diversity, and composition varied with altitude, but climate variables were generally weak predictors; other factors such as host plant identity, plant community composition, or edaphic variables likely drive fungal altitudinal distributions. Manipulated warming altered fungal colonization, but not composition or diversity. Leaf symbionts were more sensitive to climate and experimental warming than root symbionts. Altitudinal patterns and responses to warming differed among host plant species and fungal groups, indicating that predicting climate effects on symbioses will require tracking both host and symbiont identities. Combining experimental and observational methods can yield valuable insight into how climate change may alter plant–symbiont interactions, but our results indicate that altitude does not always serve as an adequate proxy for warming effects on fungal symbionts of plants.

Key words: climate change; community composition; diversity; elevation gradient; endophytes; mycorrhizal fungi; next-generation sequencing; plant–fungal interactions; symbiosis; warming experiment.

Introduction

Climate change is shifting the altitudinal ranges of species worldwide (Chen et al. 2011, Wolf et al. 2016). Although the implications of these range shifts for community composition are well recognized, range shifts may also disrupt species interactions, creating communities and species interactions that lack contemporary analogs (van der Putten 2012, Classen et al. 2015, Tylianakis and Morris 2017). Disruption of plant–fungal symbioses may be particularly important because they can alter plant community structure, ecosystem-scale productivity, carbon storage, and nutrient dynamics (Hodge et al. 2001, Rudgers et al. 2007, Hodge and Fitter 2013, Bender et al. 2015, Bell-Dereske et al. 2017). Fungal symbionts can also alter plant sensitivities to climate change (Compant et al. 2010), for instance, by buffering their hosts against warming or drought (reviewed by Kivlin et al. 2013). Yet, disruption of these interactions has often been overlooked because fungal communities are thought to contain high functional redundancy (Allen et al. 1995, Talbot et al. 2014).

Both altitudinal gradients and warming experiments are widely used tools for understanding ecological responses to climate change (Sundqvist et al. 2013). A limited number of studies have used both approaches together to test whether altitudinal patterns predict responses of a single species or taxonomic group to experimental warming (e.g., Dunne et al. 2003, Elmendorf et al. 2015). However, little work has done so for species interactions, and to our knowledge, no prior study of plant–fungal symbioses has compared altitudinal patterns against experimental heating responses (reviewed by Kivlin et al. 2017). Past studies have reported fungal responses to either altitude (e.g., Gai et al. 2012, Kivlin et al. 2017) or warming (e.g., Gray et al. 2011, Rudgers et al. 2014), with trends often differing between the two, suggesting the value of conducting comparisons within a single system. Agreement of altitudinal and warming patterns would support the utility of altitudinal gradients for predicting plant-microbe responses to future temperatures, and trends may align if organisms

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[4] E-mail: mkazenel@unm.edu

exhibit rapid ecological or evolutionary responses to warming over short time frames. However, short-term warming experiments may fail to recapitulate altitudinal patterns when environmental factors other than temperature alone drive altitudinal species distributions, or when altitudinal patterns reflect long-term ecological or evolutionary dynamics and low potential for rapid physiological responses to climate.

Furthermore, individual metrics, such as the extent of a particular fungal symbiont's colonization within its host, might respond to global change on different timescales than community metrics, such as fungal diversity and composition, with individual physiological responses occurring first, followed by reordering of community composition, and then ultimately by local extinctions and immigration (Smith et al. 2009). Gradient studies often assume that physiological responses, dispersal, and community reordering will be rapid for microbes, and we lack data on the relative paces of these processes for most systems. Comparing the degree to which fungal symbiont colonization vs. diversity or composition differ in their responses to elevation and experimental warming could help to illuminate the potential for acclimatization vs. wholesale shifts in community composition over short vs. long timescales, respectively. This information is vital to making informed predictions about species interactions under future climates.

In addition, relatively few studies have considered responses to climate for multiple symbionts co-occurring within a single host (Kivlin et al. 2017). Belowground, likely all plant species are colonized by arbuscular mycorrhizal fungi, endophytes, or both (Partida-Martínez and Heil 2011), which often improve plant nutrient uptake or stress tolerance (Smith and Read 2008, Porras-Alfaro and Bayman 2011). Aboveground, plants can host both systemic and localized foliar endophytes (Wani et al. 2015), which can increase plant resistance to herbivores and pathogens (Bacon and White 2016). Examining climate responses of multiple symbionts may therefore be important to predicting the community-level consequences of global change.

This study combined surveys along eight replicate altitudinal gradients with a 23-yr warming experiment to examine whether gradient studies can predict how plant–fungal symbioses respond to direct temperature manipulation. Specifically, we addressed: (1) Do altitudinal patterns in fungal symbioses correspond with fungal responses to experimental warming, and do above- and belowground fungal symbionts exhibit similar trends? We hypothesized that fungal symbionts would respond similarly to warming and altitude if temperature was an important driver and that aboveground leaf symbionts would be more sensitive to temperature than belowground root symbionts because of their greater exposure to temperature extremes. (2) How closely do fungal colonization responses to altitude or warming match shifts in fungal community composition? We hypothesized that fungal colonization responses would match those of composition, if fungal taxa respond to altered temperature over relatively short timescales. By evaluating these questions for four fungal functional types and three host plant species, we also gauged whether altitude and warming responses were consistent across host plant or fungal taxa. Using replicated altitudinal gradients allowed us to consider fungal symbiont trends across a much wider temperature range than warming experiments can capture.

Methods

Detailed methods are included in the Supporting Information (see Appendix S1).

Study system

Achnatherum lettermanii, *Festuca thurberi*, and *Poa pratensis* are cool-season, perennial grasses that host arbuscular mycorrhizal fungi (AMF) and root fungal endophytes (RFE) belowground and leaf fungal endophytes (LFE) aboveground. *Festuca thurberi* also hosts an undescribed, systemic foliar endophyte (*Epichloë* sp.) in its leaves and seeds. These grasses are abundant and co-occur in the Upper Gunnison Basin, Colorado, USA. In the Rocky Mountains, air temperature declines by ~0.8°C per 100-m elevation increase, and precipitation increases with altitude (Kittel et al. 2002, Pepin and Losleben 2002, Dunne et al. 2003).

Altitudinal gradient survey

To test how fungal colonization and composition varied with elevation, we collected roots and leaves from six plant individuals/species/site along independently replicated altitudinal gradients in 2012 and 2014 (see Appendix S2: Fig. S1 for an illustration of study design). We sampled eight altitudinal gradients in total, with sites spaced every ~100–200 m from 2,700 to 3,700 m above sea level (Appendix S3: Figs. S1–S3; Tables S1–S3). In a given year, a site was sampled only once.

To examine whether climate variables predicted fungal distributions, we aggregated climate data from regional weather stations (Appendix S3: Table S4), including mean cumulative growing degree days (GDD, a measure of temperature combined with growing season length; Frank and Hofmann 1989), mean annual precipitation (MAP), which included both rain and snow, and mean snow depth (MSD). Using model selection procedures as defined by Burnham and Anderson (2002), we built equations to predict climate variables from elevation, slope, and aspect, and then interpolated climate for each collection site (Lynn et al. 2018).

Warming experiment

To test how fungal colonization and composition in plants varied with warming, we collected samples from

plots established at the Rocky Mountain Biological Laboratory (RMBL) in 1991 (see Harte and Shaw 1995, Saleska et al. 2002). The experiment (elevation 2,920 m) consists of 10 plots (10 × 3 m), which alternate spatially between warmed and control (ambient temperature) treatments (n = 5; Appendix S2: Fig. S1). Warmed plots receive continuous infrared radiation from suspended heating lamps (22 W/m^2). 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 (Harte et al. 2015). On 30 June–2 July and 25–30 September 2014, for each of the three plant species, we collected roots and leaves from six plant individuals/plot, to match replication from the altitudinal survey. We also collected leaf samples on 16–18 June and 12 September 2015 to provide additional estimates of leaf colonization.

Fungal symbiont colonization and community composition

To assess fungal colonization of grass roots and leaves, we stained tissue samples and scored colonization via light microscopy (McGonigle et al. 1990, Bacon and White 1994). To characterize fungal composition in surface-sterilized plant tissue, we used barcoded paired-end Illumina MiSeq v.3 sequencing of the nuclear ribosomal repeat unit, with fungal-selective primers targeting the ITS2 region for LFE and RFE (Taylor et al. 2016), and FLR3-FLR4 primers targeting ~300 base pairs (bp) in the 28S region for AMF (Gollotte et al. 2004). Sequencing was performed by the Genomic Sequencing and Analysis Facility at The University of Texas at Austin (https://sites.cns.utexas.edu/cbrs/genomics).

Bioinformatics

We processed sequence reads in QIIME v.1.9.1 (Caporaso et al. 2010), using standard scripts to join paired end reads, remove any unjoined sequences, remove forward and reverse primers, and filter out sequences if they had a quality score <25. We then used UCLUST (Edgar 2010) to create operational taxonomic units (OTUs) at 93% identity (Taylor et al. 2016), and removed OTUs represented by <10 reads (Cline et al. 2017). For OTU identifications, we used the RDP Classifier (Wang et al. 2007) with the fungal LSU training set 11 for the 28S data (Liu et al. 2012) and the UNITE fungal ITS training set for the ITS2 data (Nilsson et al. 2018). Glomeromycota OTUs were removed from the ITS data set prior to analyses because we resolved AMF using 28S. We performed all subsequent analyses in R (R Core Team 2017) unless otherwise specified. Prior to diversity analyses ('vegan' package, Oksanen et al. 2015), we rarefied the root ITS data to 5,000 sequences/sample, the leaf ITS data to 50 sequences/sample, and the 28S data to 1,000 sequences/sample, averaging across 1,000 independent rarefactions for each data set ('EcolUtils' package, Salazar 2019). A threshold of 50 sequences/sample was chosen for the leaf ITS data because leaf fungal colonization rates were relatively low at some of our sampling sites (Ranelli et al. 2015). For composition, we expressed each OTU as a proportion of the total reads/sample.

Data analysis: fungal colonization and diversity

Altitudinal patterns.—We used linear mixed effects models to examine relationships between fungal responses (colonization, Shannon diversity) and elevation, both across and within individual grass species. Models included year and sampling date (fixed effects) and replicate gradient identity (random effect; 'nlme' package, Pinheiro et al. 2014). If diversity responses were significant, we decomposed diversity into rarefied richness or evenness (Shannon J) to examine whether diversity changes were due to the number of species present vs. their relative dominance. We considered nonlinearities by comparing Akaike's information criterion (AICc) values of models with or without a quadratic term for elevation.

Climate predictors along altitudinal gradients.—To explore the importance of abiotic correlates, for each fungal group (AMF, RFE, or LFE), we used AICc-based model selection (Burnham et al. 2011) to compare individual models that, respectively, included the effects of GDD, MAP, or MSD. This approach identified which climate variable best predicted fungal variation along altitudinal gradients (Ranelli et al. 2015), without the complication of multicollinearity. Models included host species, year, and sampling date (fixed effects) and gradient identity (random effect). We also created separate models for each host species × fungal type combination.

Warming experiment.—We used factorial mixed model analysis of variance (ANOVA) to test the effects of warming, host plant identity, and sampling date (June/July vs. September) on fungal colonization/diversity, including plot as the random effect. For significant host species × warming interactions, we tested a priori contrasts for the warming treatment within each host species ('emmeans' package, Lenth et al. 2018).

Altitudinal gradient vs. warming comparison.—When fungi responded to experimental warming but not altitude, we used linear mixed effects 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 ~2°C difference (lapse rate) to match the temperature increase created by experimental warming. Models examined fungal responses as a function of the average altitude of site pairs and the site location (low/high). We then tested a priori contrasts for the low/high contrast within each average altitude set ('emmeans').

Data analysis: fungal composition

Altitudinal patterns.—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). To evaluate climate variables, we used AICc-based model selection in DISTLM to compare models that included GDD, MAP, or MSD. For each of the 30 proportionally most abundant OTUs, we also regressed relative abundance against elevation, adjusting *P* values with false discovery rate (FDR) correction.

Warming experiment.—We used PERMANOVA with a factorial mixed model (as above) to test warming effects on OTU composition (Primer v. 6, Clarke and Gorley 2009). We also calculated relative interaction intensity (RII, Armas et al. 2004) for each of the 30 most proportionally abundant OTUs to estimate the effect size of warming, adjusting *P* values with FDR correction.

Results

Fungal symbiont colonization, diversity, and composition varied both along altitudinal gradients and under experimental warming (Table 1). Leaf endophytes exhibited greater change with altitude or warming than fungi colonizing roots. However, trends present along gradients were largely absent under warming, and vice versa. The direction and magnitude of responses differed among host plant species, fungal functional groups, and metrics of fungal response.

Colonization: leaf fungi

Leaf colonization varied with altitude in two host species and responded to warming in one. For *A. lettermanii*, leaf colonization declined 44% per 1-km increase in elevation (Fig. 1A). Colonization was greater under longer, hotter growing seasons and lower MAP (Appendix S3: Table S5) but did not increase with warming (Fig. 1B). Similarly, colonization of *F. thurberi* leaves by *Epichloë* sp. decreased 69% per 1-km increase in elevation (Fig. 1C; Appendix S3: Table S6) and was greater under longer, hotter growing seasons (Appendix S3: Table S5). However, in the warming experiment, *Epichloë* sp. colonization of *F. thurberi* was 78% lower in warmed than control plots, although the effect was marginally nonsignificant (Fig. 1D; Appendix S3: Table S7). Paired site analysis suggested a possible mid-elevation peak in *Epichloë* colonization, which was 61% less at lower (warmer) than higher elevations within the lowest-elevation pair of sites (low/high contrast: df = 41, $t = 1.58$, $P = 0.12$), which best matched the warming experiment.

Colonization: root fungi

AMF colonization varied with elevation in only one host species, *P. pratensis*, peaking at midelevation (AICc quadratic = 81.55, AICc linear = 87.81; Fig. 1E). However, climate variables were not strong predictors of this trend (Appendix S3: Table S5), and there was no similar responsiveness to experimental warming (Fig. 1F). In the warming experiment, *A. lettermanii* was singularly responsive in AMF colonization, with 31% higher colonization in warmed than control plots (Fig. 1H). There was no similar trend between the lowest altitudinal pair of sites (2°C lapse rate) that best matched the experiment's treatments (low/high contrast: df = 3, $t = 1.61$, $P = 0.21$), nor was there a pattern along gradients overall (Fig. 1G). In contrast with AMF, colonization by root endophytes (septate hyphae) did not vary with altitude, climate, or warming (Appendix S3: Tables S5–S7).

Diversity: leaf fungi

Leaf fungal diversity was greater at cooler sites across hosts, but declined with elevation for one grass species and did not respond to experimental warming

Table 1. Summary of fungal symbiont responses to altitude and experimental warming for *Achnatherum lettermanii*, *Festuca thurberi*, and *Poa pratensis*.

		Leaf endophytes		AMF		Root endophytes	
Species	Variable	Altitude	Warming	Altitude	Warming	Altitude	Warming
A. lettermanii	Colonization	**0.0094** ↑	NS	NS	**0.0385** ↑	NS	NS
	Diversity	NS	NS	NS	NS	NS	NS
	Composition	NS	NS	**0.0637** Δ	NS	NS	NS
F. thurberi	Colonization	**0.0023** ↑	**0.0673** ↓	NS	NS	NS	NS
	Diversity	NS	NS	**0.0607** ↓	NS	NS	NS
	Composition	**0.0033** Δ	NS	NS	NS	NS	NS
P. pratensis	Colonization	NS	NS	**0.0431** Δ	NS	NS	NS
	Diversity	**0.0322** ↑	NS	NS	NS	NS	NS
	Composition	**0.0503** Δ	NS	NS	NS	NS	NS

Notes: *P* values for significant or marginally nonsignificant effects are listed in bold. Symbols indicate whether the fungal response metric increased (↑), decreased (↓), or changed (Δ) as temperature increased. NS = not significant. AMF = arbuscular mycorrhizal fungi.

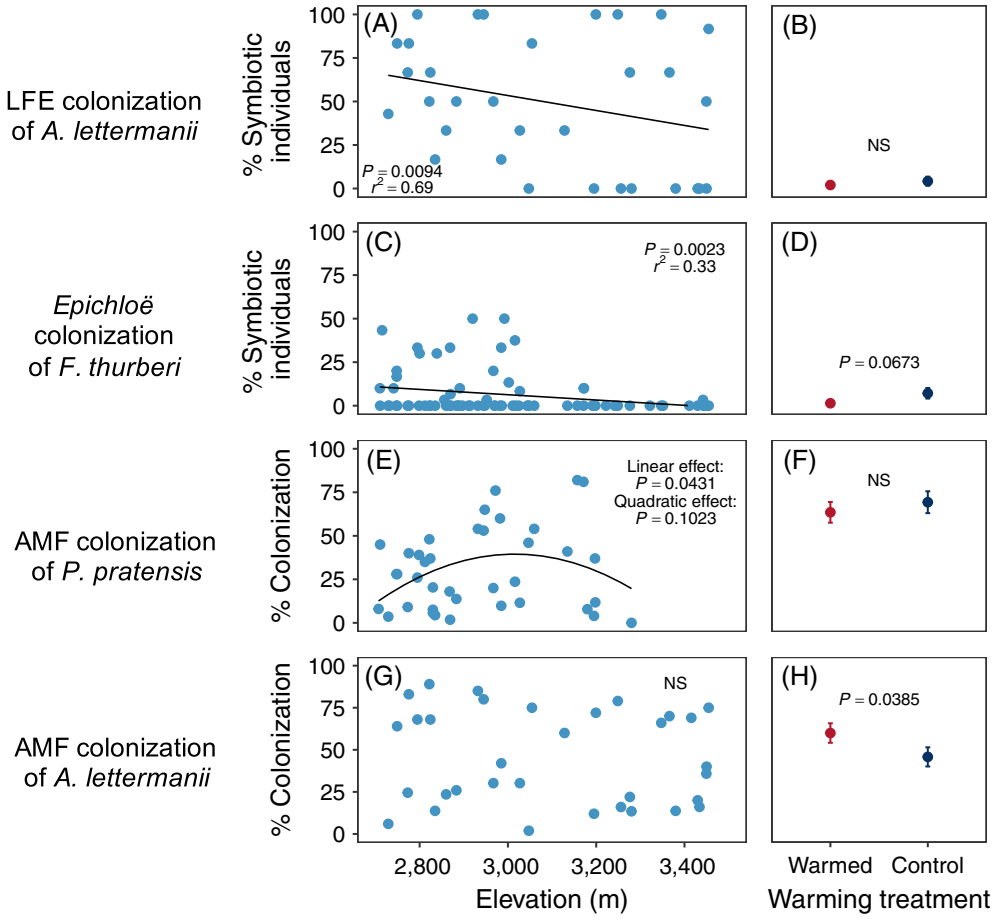


FIG. 1. Variation with elevation and experimental warming in (A), (B) mean percentage of *Achnatherum lettermanii* individuals symbiotic with leaf fungal endophytes (LFE; elevation effect: $F_{1,22} = 8.1$, $P = 0.0094$, marginal $r^2 = 0.69$, conditional $r^2 = 0.69$); (C), (D) mean percentage of *Festuca thurberi* individuals symbiotic with *Epichloë* sp. (elevation effect: $F_{1,65} = 10.1$, $P = 0.0023$, marginal $r^2 = 0.33$, conditional $r^2 = 0.33$; warming effect: $F_{1,12} = 4.0$, $P = 0.0673$); (E), (F) mean percentage colonization of *Poa pratensis* roots by arbuscular mycorrhizal fungi (AMF; elevation linear effect: $F_{1,25} = 4.54$, $P = 0.0431$; elevation quadratic effect: $F_{1,25} = 2.9$, $P = 0.1023$; marginal $r^2 = 0.81$, conditional $r^2 = 0.81$); and (G), (H) mean percentage colonization of *A. lettermanii* roots by AMF (warming contrast: df = 12, $t = -2.32$, $P = 0.0385$). NS = not significant. In (E), the depicted quadratic model better predicted colonization than a linear model (AICc quadratic = 81.55, AICc linear = 87.81). In (A), (C), the listed r^2 values are conditional r^2 values for the full models that we constructed, which included the fixed effects of sampling date and year, and the random effect of gradient identity.

(Appendix S3: Tables S8–S11). We identified 4,156 ITS2 OTUs (excluding Glomeromycota), with 29% overlap in OTUs between roots and leaves (Fig. 2A). For the 1,223 leaf fungal OTUs, we found 24% overlap between altitudinal gradients and experimental warming, the lowest overlap of any fungal symbiont group (Fig. 2B). Across hosts, LFE Shannon diversity decreased under longer, warmer growing seasons and lower MAP and MSD, and there was a marginally nonsignificant elevation × host species interaction ($P = 0.1260$; Appendix S3: Tables S8 and S9). In *P. pratensis*, LFE diversity declined at higher elevations ($F_{1,17} = 5.4$, $P = 0.0322$, $r^2 = 0.35$), driven by decreasing evenness ($F_{1,17} = 12.8$, $P = 0.0023$, $r^2 = 0.72$) but not by climate (Appendix S3: Table S10). LFE diversity did not change with altitude in *A. lettermanii* or *F. thurberi*.

Amongst the 30 most common LFE taxa, 20% responded to experimental warming (Fig. 3A), but none varied with altitude (Appendix S3: Table S12). Four taxa decreased in abundance under warming, and two increased (Fig. 3A). Four were the same taxa that responded to warming in roots (see next). However, while one of these taxa responded in the same direction in roots and leaves (Fig. 3A, C, Dothideomycetes 1), three responded oppositely (Agaricomycetes 1, Pleosporales 2 and 3).

Diversity: root fungi

AMF Shannon diversity increased with altitude in one grass species but did not respond to warming; however, 27% of common AMF taxa declined with warming and

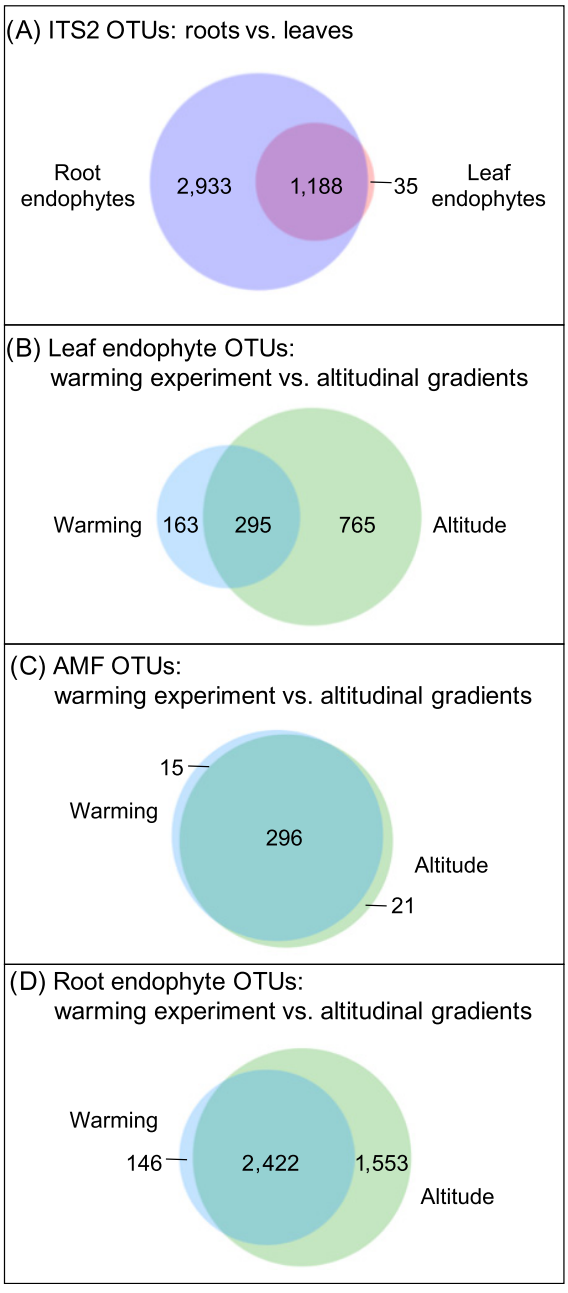


FIG. 2. Venn diagrams indicating (A) overlap in ITS2 operational taxonomic units (OTUs) between roots and leaves, and (B)–(D) overlap in leaf fungal endophyte, arbuscular mycorrhizal fungi (AMF), and root fungal endophyte OTUs between the altitudinal gradient and experimental warming studies.

7% increased. We detected 332 Glomeromycota (AMF) 28S OTUs, with 89% overlap between altitude and warming (Fig. 2C). Thus, AMF diversity was especially high within the 300-m^2 warming experiment, given that maximum global AMF richness has been estimated at ~1,300 OTUs (Kivlin et al. 2011, Öpik et al. 2013). There was a marginally nonsignificant increase in AMF diversity with elevation for *F. thurberi* ($F_{1,23}$ = 3.9, P = 0.0607, r^2 = 0.40), driven by variation in AMF evenness ($F_{1,23}$ = 2.8, P = 0.1060, r^2 = 0.28) rather than richness; however, climate variables were not important predictors (Appendix S3: Table S10). AMF diversity, richness, and evenness did not vary with elevation or climate for the other two grasses, or with warming in any grasses (Appendix S3: Table S11). Among the 30 most abundant AMF OTUs, 8 decreased in relative abundance under warming, and 2 increased (Fig. 3B), but none varied with altitude (Appendix S3: Table S12).

Common root fungal endophyte OTUs changed in abundance under experimental warming, but RFE diversity did not vary with altitude, climate, or warming (Appendix S3: Tables S8, S9, and S11). We identified 4,121 ITS2 root endophyte OTUs, with 59% overlap between altitude and warming (Fig. 2D). Under warming, 6 of the 30 common OTUs (20%) decreased in relative abundance, and 6 other OTUs (20%) increased (Fig. 3C). However, none of the common OTUs varied with elevation (Appendix S3: Table S12).

Composition: leaf and root fungi

Across host plants, both LFE and AMF composition varied with altitude, but fungal composition did not correlate with climate or respond to experimental warming in any fungal group (Appendix S3: Tables S11, S13–S15). Aboveground, LFE composition varied with altitude across grasses (P = 0.0042, r^2 = 0.20; Appendix S3: Table S13) and differed with elevation for *F. thurberi* (pseudo-F = 3.2, P = 0.0033, r^2 = 0.33) and *P. pratensis* (pseudo-F = 1.5, P = 0.0503, r^2 = 0.38) but not for *A. lettermanii* (pseudo-F = 1.0, P = 0.47). Similar to LFE, across hosts, AMF composition varied with altitude (P = 0.0031, r^2 = 0.22; Appendix S3: Table S13); within individual hosts, there was marginally nonsignificant compositional change with elevation for *A. lettermanii* (pseudo-F = 1.9, P = 0.0637, r^2 = 0.22), but not for *F. thurberi* (pseudo-F = 1.4, P = 0.19) or *P. pratensis* (pseudo-F = 0.9, P = 0.44). RFE composition also varied with elevation across hosts (P = 0.0045, r^2 = 0.22; Appendix S3: Table S13), but trends were nonsignificant in each grass species alone.

DISCUSSION

Our study is unique in that we examined variation in fungal symbiont colonization, diversity, and composition along eight replicate altitudinal gradients and under experimental warming, considered both above- and belowground fungal symbionts, and sampled the majority of the altitudinal ranges of two of three focal grass species (*A. lettermanii* and *F. thurberi*). Fungal symbiont patterns along altitudinal gradients generally did not correspond with their responses to experimental warming across this robust data set, and we conclude that factors other than climate largely drive fungal variation along these gradients.

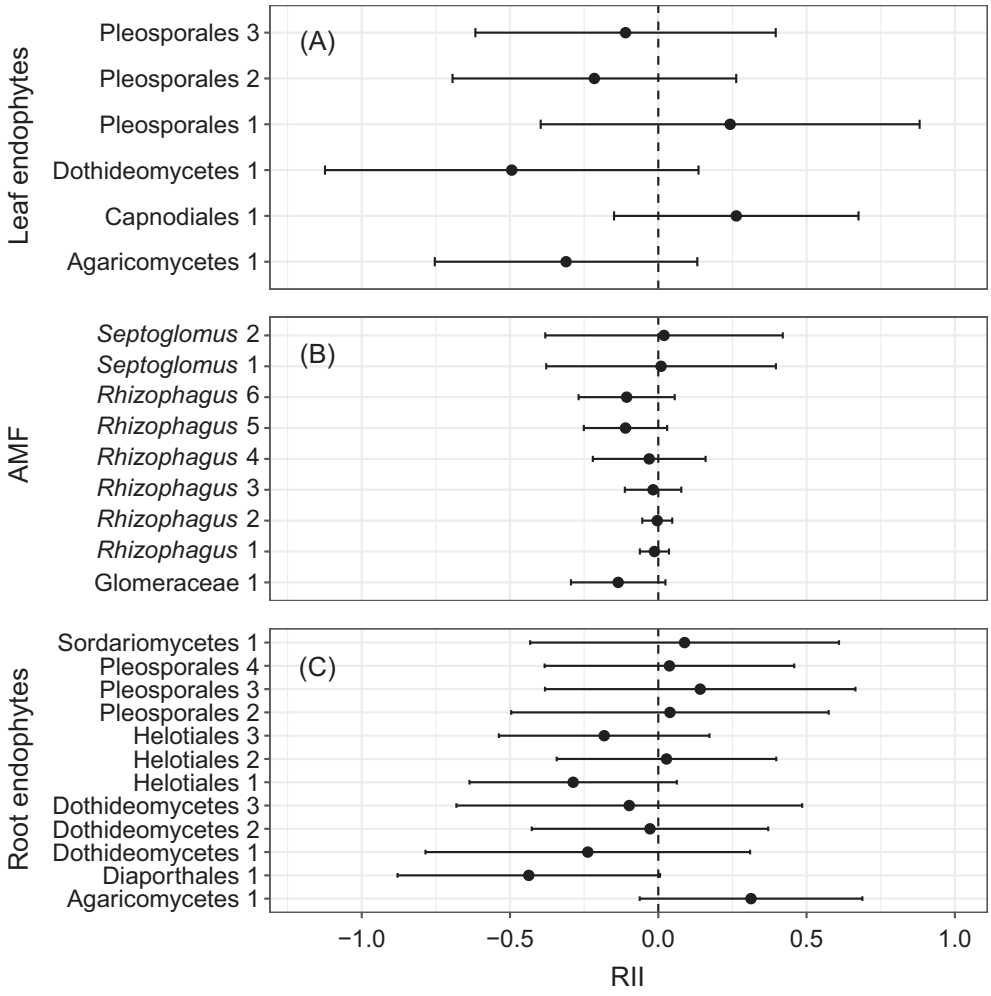


FIG. 3. Magnitude and direction of relative abundance responses to experimental warming for individual fungal operational taxonomic units (OTUs), with positive RII values indicating an increase in abundance under warming, and negative values indicating a decrease. Fungal OTUs are grouped by functional type: (A) leaf fungal endophytes, (B) arbuscular mycorrhizal fungi (AMF), and (C) root fungal endophytes. Each OTU is listed as the finest taxonomic level to which it could be identified, along with a unique identification number. One AMF taxon that responded to warming, *Rhizophagus* 7, is not depicted due to its high variance (RII = −0.71, 95% confidence interval = −2.1, 0.67).

Leaf fungi varied more strongly with altitude or warming than root fungi, suggesting that leaf symbioses may be particularly susceptible to disruption under climate change, perhaps because they are less buffered against air temperatures than belowground fungi. Patterns differed among host plant species and fungal groups, demonstrating that the ecologies of specific plant–fungal symbioses are more important than general behaviors of fungal functional groups to predicting future community change.

Altitudinal gradient and experimental warming trends largely did not correspond

For most fungal responses, shifts with elevation and warming were nonconcordant, which is surprising, given the range of fungal functional groups in our data set and the high degree of overlap in OTU composition between the altitude and warming studies. Why might altitudinal patterns and warming responses differ? First, factors other than temperature are likely more important determinants of symbiont distributions along altitudinal gradients in our ecosystem. Although climate variables drove some fungal responses to altitude, particularly for leaf fungi, they were poor predictors of most trends, despite the large temperature change over the altitudinal gradients (~8°C) relative to experimental warming (~2°C). Abiotic factors such as pH (e.g., Rincón et al. 2015) or soil nutrients (e.g., Johnson et al. 2010) may instead drive fungal symbiont abundance and composition along altitudinal gradients. Variation in plant community composition may also influence fungal

distributions, particularly given that a majority of the fungi in our study are horizontally transmitted among plants (Wani et al. 2015, Vályi et al. 2016).

Second, species can respond divergently to experimental warming in different parts of their geographic ranges (Pelini et al. 2012, Stuble et al. 2013), and biotic interactions can be highly context-dependent (Chamberlain et al. 2014). Fungi might thus respond differently to 2°C of temperature change at a low elevation site within our study system, such as the RMBL warming experiment, than at a high elevation site. Alternatively, fungi in the Rocky Mountains may be adapted to the level of stress imposed by a 2°C temperature increase, but might respond to the larger temperature change captured by altitudinal gradients, especially given the ample evidence of fungal sensitivity to temperature (Alster et al. 2018, Glassman et al. 2018).

Third, divergence between altitudinal pattern and response to warming might reflect differing sensitivities to environmental change over long vs. short timescales, consistent with the hierarchical-response framework developed for plants (Smith et al. 2009). Under this framework, change begins with individual physiological responses, then progresses to a reordering of species abundance ranks, and finally results in extinctions/immigrations that reorganize community composition. Our contrasting colonization and composition results suggest that warming has potentially induced individual physiological responses of fungi, but has not reordered communities or caused extinctions. The fungal communities in our altitudinal survey may represent a later stage of response to the environment, in which community turnover occurs, consistent with the findings of Geml et al. (2014), and may thus better reflect how gradual fungal responses to warming could occur over longer timescales. Longer-term warming experiments (beyond 23 yr) might reconcile the strengths and weaknesses of the two methods compared here to predict responses to climate change more accurately.

Fungal functional groups differed in sensitivity to environmental drivers

Our findings suggest that broadly defined fungal functional groups will respond divergently to climate change. For leaf fungi, our data predict susceptibility to warming, with shifts in colonization and community composition and possible decreases in diversity. Leaf endophytes may be less buffered by the soil environment relative to belowground symbionts and thus influenced more strongly by temperature changes. Our findings also suggest susceptibility of AMF to future warming, indicating that climate change may increase colonization frequency, decrease diversity in some hosts, and alter community composition. In contrast, our RFE results predict that warming may have little effect on colonization of hosts but may instead shift taxonomic composition broadly, across hosts. Until now, we have had limited inference on these patterns because most prior studies have examined fungal symbionts in just one host tissue type (i.e., roots or leaves). Our new results are consistent with the work of Coince et al. (2014), who found that different environmental drivers explained contrasting patterns in root vs. leaf symbiont composition.

Our work also indicates that individual, dominant fungal taxa may shift in abundance under climate change. For instance, the common AMF taxa that responded to experimental warming were largely in the Glomerales, members of which may confer greater protection against pathogens relative to other AMF orders (Powell et al. 2009). Among RFE, relative abundance changed for three common taxa in the Helotiales, a functionally diverse group that also includes ericoid and ectomycorrhizal taxa, plant pathogens, and saprotrophs (Vralstad et al. 2002). With environmental changes, RFE may shift along the mutualism–parasitism continuum (Mandyam and Jumpponen 2015). Overall, there is a need for improved databases and increased phylogenetic resolution for fungi in mountain ecosystems to enable higher-resolution identification of the taxa that are sensitive vs. insensitive to climate shifts.

Host specificity in fungal symbiont responses to temperature

Plant species strongly differed in their sensitivity to climate-induced disruption of symbioses, in line with our prior observational work (Ranelli et al. 2015). We predict that fungal symbionts in *A. lettermanii* will have high sensitivity to warming, given their colonization responses (leaf colonization declined with altitudinal increase and climate variables, and AMF colonization increased with warming) and compositional change (AMF composition varied with elevation). Because we sampled the majority of *A. lettermanii*'s documented altitudinal range (Taylor 2000), our data set likely captured much of the natural variation in its symbiont community. We also expect that symbionts of *F. thurberi* will be sensitive to climate change, but the divergence between experimental and survey results complicates this prediction (i.e., *Epichloë* colonization increased at warmer sites but declined with experimental warming). As with *A. lettermanii*, we sampled the majority of *F. thurberi*'s altitudinal range (Meyer 2009). Because *F. thurberi* is a community dominant that stabilizes soils (Langenheim 1962, Meyer 2009), symbiont shifts could have cascading effects on plant communities and ecohydrology. Symbionts of *P. pratensis* were the least sensitive to climate, and of the three grasses, this species declined most strongly under experimental warming (Rudgers et al. 2014). We sampled only the upper altitudinal edge of *P. pratensis*' broad distribution across North America (U.S. Department of Agriculture, Natural Resources Conservation Service 2016), and we lack information on this species' genotype/ecotype and length of evolutionary history in our study system, both

of which can be highly variable (Barkworth et al. 2007). *Poa pratensis* may have wide ecological amplitude with low reliance on specific beneficial fungi, as indicated by prior work (Hetrick et al. 1988).

Conclusions

Our findings indicate that leaf fungal symbionts may be less resilient to climate change than root symbionts. Understanding climate change effects on plant–fungal symbioses thus requires separate consideration of above- and belowground fungal communities. Our results also suggest that caution should be exercised in predicting organismal responses to global change based on environmental gradient or experimental warming trends alone. Combining experimental and observational methods can yield valuable insight into how plant–fungal interactions will respond to future climates.

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MRK completed field and laboratory work, analyzed the data, and wrote the manuscript. JAR and SNK conceived the study and contributed to data collection and analyses. DLT and SNK contributed to molecular work and bioinformatics. JSL collected field data. All authors helped to revise the manuscript. We thank C. Takacs-Vesbach and the Rudgers-Whitney laboratory at the University of New Mexico for valuable manuscript feedback. We also thank K. Clausen, C. Forrester, W. Hendricks, and L. Ranelli for field and laboratory assistance. Work was funded by National Science Foundation (NSF) grant DEB-1354972, the Rocky Mountain Biological Laboratory (NSF grants DBI-0753774 and OIA-0963529), and the University of New Mexico Department of Biology.

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