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Culturable root endophyte communities are shaped by both warming and plant host identity in the Rocky Mountains, USA

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ABSTRACT

Understanding the biogeographic patterns of root-associated fungi and their sensitivity to temperature may improve predictions of future changes in terrestrial biodiversity and associated ecosystem processes, but data are currently limited. Anticipating change will require combining observational data, which predict how climatic factors limit current species distributions, with direct manipulations of climate, which can isolate responses to specific climate variables. Root endophytes are common symbionts of plants, particularly in arctic and alpine environments, yet their responses to climate warming are not resolved. Here, we directly cultured endophytic fungi from roots collected along altitudinal gradients in replicated mountain watersheds and from a 27 y field warming experiment in the Rocky Mountains, USA, to improve understanding of climate impacts on fungal root endophytes. Fungal taxa that were common at high elevations declined most under climate warming, whereas low elevation dominants responded neutrally or increased with experimental warming. Altitudinal gradients in fungal communities were strongly specific to the plant host species. Specifically, *Poa* species had 25–60% greater fungal isolate abundance and 25–38% greater fungal diversity at high elevations than at low elevation sites. In contrast, *Festuca thurberi* had 64% lower fungal diversity on roots at high elevation than at low elevation. Our results help to improve understanding of the potential for climate change to alter plant-fungal interactions in mountain ecosystems.

1. Introduction

Under the business-as-usual scenarios, climate models predict a 2–4 °C increase in global mean annual temperature over the next century (IPCC, 2014; Masson-Delmotte et al., 2018) with intensified warming at high elevations (Rangwala and Miller, 2012; Wang et al., 2016). Many species experience shifts in their geographic distributions as a consequence of contemporary climate change (Parmesan, 2006; Chen et al., 2011). In particular, many plant species are moving up mountainsides into cooler, higher elevation climates (Lenoir et al., 2008; Engler et al., 2011). The ability to anticipate future range shifts will require a combination of observational data, to predict how climatic factors limit current species distributions, and direct manipulations of climate, to isolate response mechanisms to specific climate variables (Knapp et al., 2018; Urban, 2019).

For plants and animals, observational and experimental data on the effects of climate change on species distributions and community structure are becoming widely available (e.g., Diamond et al., 2012; Liu et al., 2018), but such data remain limited for fungi and other microorganisms (Miyamoto et al., 2018; Kazenel et al., 2019). Understanding the biogeographic patterns of root-associated fungi and their sensitivity to temperature may improve predictions of future changes in terrestrial biodiversity and associated ecosystem function under climate warming. Root endophytes are common symbionts of plants, particularly in arctic and alpine environments (Haselwandter and Read, 1980; Porras-Alfaro and Bayman, 2011). These fungi can have important ecological functions by improving plant nutrient uptake (Newsham, 2011) and buffering plants under warming or drought (Kivlin et al., 2013). The abundance, diversity, and species composition of plant-associated fungi often follow

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altitudinal patterns (reviewed by Kivlin et al., 2017), suggesting that fungi are sensitive to temperature in mountain ecosystems. For example, both root colonization by arbuscular mycorrhizal fungi (AMF) and the diversity of AMF declined with elevation (Gardes and Dahlberg, 1996; Wu et al., 2007; Gai et al., 2012), although exceptions to these patterns also exist (Ruotsalainen et al., 2004; Zubek et al., 2009). Despite their putative importance in plants, altitudinal patterns in the composition and colonization of non-mycorrhizal root-associated fungi are poorly resolved (Read and Haselwandter, 1981; Schmidt et al., 2008), representing just 31 of 374 records in our meta-analysis of altitudinal patterns in fungal symbiosis in plants (Kivlin et al., 2017). This lack of information limits our ability to predict how these fungi, and their host plants, may respond to climate change.

Traditionally, abiotic determinants of species distributions, such as climate, are assumed to operate over broad spatial scales, while biotic interactions, such as those among plants and fungi, are expected to have the strongest influence at local scales (Bowman et al., 2017). However, accumulating evidence suggests that biotic interactions can be important broad-scale determinants of species distributions (Thuiller et al., 2008; Van der Putten et al., 2010; Lewis et al., 2017; Lynn et al., 2019). Indeed, several examples for root-associated fungi suggest an overriding influence of host plant identity on fungal composition, abundance, or diversity (Davison et al., 2016; Põlme et al., 2018; Ramirez et al., 2019). Host plants may affect the distributions of root endophytes if variation in plant species traits such as root architecture (Maherali, 2014) or chemical quality and stoichiometry (Averill et al., 2019) are correlated with fungal communities.

In this study, we assessed the abundance, diversity, and composition of culturable root endophytes in replicated pairs of high and low elevation sites and in a 27 y warming experiment in the Rocky Mountains, USA. Plant species in this region have moved, on average, ~40 m higher in elevation during the past 65 y (Zorio et al., 2016). Likewise, plant species composition has responded to experimental warming. For instance, an increase of 2 °C during 25 y of year-round infrared heating increased sedges, decreased grass abundance, and increased AMF and root endophyte colonization for some grass species (Rudgers et al., 2014). Here, we focused on fungi that are culturable (i.e., non-obligate symbionts) because they can be isolated, stored, and manipulated. These taxa have potential to play roles in land management and restoration, improving practices such as assisted migration or myco-remediation. We used a combination of culturing and Sanger sequencing to assess whether: (1) altitude acts as a determinant of abundance, diversity, and composition of culturable root endophytic fungi; and (2) culturable root fungi respond to experimental warming. By combining observational and experimental datasets, we then evaluated whether (3) fungal responses to direct warming are predictable from their altitudinal distributions within a mountain ecosystem.

2. Materials and methods

2.1. Focal host plant species

We selected four pairs of congeneric grass species, plus an additional species *Trisetum spicatum,* that lacked a congener but is abundant over a broad altitudinal range (Table 1). Within congeneric pairs, one species had a broad altitudinal distribution and the other was restricted to low elevations (Lynn et al., 2019).

2.2. altitudinal survey

We collected plants within three replicate watersheds (Slate River, Washington Gulch, and East River) in the Gunnison National Forest near the Rocky Mountain Biological Laboratory (RMBL), Gunnison County, Colorado, USA. Each watershed had a high and a low elevation subalpine meadow site (Table 2, Supporting Material Fig. S1). Sites were chosen based on the presence of the focal grass species at low (~2800–3000 m) or high (~3200–3400 m) elevation.

Table 1
List of focal plant species for the Altitudinal Gradients Survey at high elevation (H) or low elevation (L) sites and in the Warming Meadow Experiment (W). Plant species are grouped by Tribe/Subtribe within the Poaceae, then alphabetically by genus. Distribution indicates the typical altitudinal range of each species in the Upper Gunnison Basin based on plant surveys (Lynn et al., 2019).

Tribe-Subtribe	Distribution	Plant Species	Collection
Stipeae	broad	*Achnatherum lettermanii*	H/L/W
Stipeae	low	*Achnatherum nelsonii*	L
Triticeae	broad	*Elymus trachycaulus*	H/L
Triticeae	low	*Elymus elymoides*	L
Poeae-Loliinae	broad	*Festuca thurberi*	H/L/W
Poeae-Lollinae	low	*Festuca saximontana*	L
Poeae-Poinae	broad	*Poa leptocoma*	H/L
Poeae-Poinae	low-mid	*Poa pratensis*	H/L/W
Aveneae	broad	*Trisetum spicatum*	H/L

2.3. warming meadow experiment

The warming experiment was established across a small glacial moraine spanning 10 m along an east-west oriented gradient (38.95136240 N, −106.98635923 W, elevation 2885 m, Supporting Material Fig. S1) (Harte and Shaw, 1995; Price and Waser, 2000), with ~1–2 m change in elevation along the moraine. Approximately 60 herbaceous perennials and a few annuals have been identified in the plots (Price and Waser, 2000; De Valpine and Harte, 2001). The higher and drier, western portion of the moraine was dominated by sagebrush (*Artemisia tridentata*), rabbitbrush (*Ericameria parryi*), and bunchgrasses (*Festuca thurberi* and *Achnatherum* spp.) while the wetter, eastern portion was dominated by willow (*Salix* spp.) and monkshood (*Aconitum columbianum*). We collected root samples from three grass species that were present in both the Altitudinal Survey and the Warming Meadow. These were: *A. lettermanii, P. pratensis,* and *F. thurberi* (Table 1).

The warming experiment was designed to simulate conditions under a doubling of atmospheric CO_2 (Harte and Shaw, 1995). Along the moraine, ten plots (10 × 3 m) alternated between a warmed or control treatment. Each plot was oriented with the long axis of the plot running west to east from higher to lower elevation and drier to wetter soil conditions, respectively. Beginning fall 1990, two electric heaters (15 W m^{-2} infrared radiation) were suspended on aircraft cable ~1.5–2.5 m above the center of the long axis of the warmed plots. Heating began January 6, 1991, and has run continuously until July 2019. A third heater was added between two existing heaters in May 1993, increasing the total flux to 22 W m^{-2}. Heaters shaded only 2% of the soil surface, and there was no experimental control for the presence of heating infrastructure. The treatment warmed the top 15 cm of soil ~2 °C on average, decreased soil water content by 10–20%, and extended the duration of snow-free soil conditions at each end of the growing season by ~12 d (Harte and Shaw, 1995; Saleska et al., 2002; Harte et al., 2015).

Heating had the strongest effects on abiotic conditions, plant communities, and fungal colonization of roots at the upper, drier end of the moraine within each plot. Of particular importance to this study, graminoid (grass and sedge) species composition diverged between warmed and control plots over time (Rudgers et al., 2014). In 2011, sedges occupied a larger percentage of sampled quadrats under warming in the upper and mid-elevation (drier) portions of the plots, while grasses generally declined in these drier areas. At the upper, drier end of the moraine, AMF root colonization increased in one grass

Table 2
Geographic coordinates (Latitude = Lat., Longitude = Long.) of the root collection locations for the Altitudinal Gradients Survey. High and Low elevation sites are shown for each watershed, the East River, Washington Gulch, and Slate River. Elevation is given in m.

	East River			Slate River			Washington Gulch		
	Lat.	Long.	Elev.	Lat.	Long.	Elev.	Lat.	Long.	Elev.
High	38.97529	−106.978	3358	38.97073	−107.059	3210	38.97018	−107.03	3381
Low	38.96642	−106.99	3001	38.93282	−107.05	2812	38.9346	−107.011	2945

species and in sagebrush, but did not change in sedges. Intraradical non-AMF fungi, characterized by septate hyphae, did not differ between control and warming plots within individual graminoid species. However, due to increases in the abundance of some graminoid species with large colonization by septate fungi, the abundance of septate fungi increased at the plot scale (Rudgers et al., 2014).

2.4. Sampling methods

Altitudinal Survey. We sampled grasses between 11 and 17 July 2017 from naturally occurring populations. All species were sampled from low-elevation habitats, while only species with broad distribution were sampled from high-elevation habitats (Table 1). Within a site, six individuals of each species were uprooted using a bleach-sterilized soil knife. Care was taken to obtain roots from the base of the plant to the edge of rooting depth. We removed as much soil and litter as possible from the roots and then combined all six individuals per species into a single, sterile Whirl-pak® (Nasco, Fort Atkinson, WI, USA). Whirl-paks were stored on ice and returned to the lab.

Warming Meadow Experiment. We sampled grasses during 12−18 July 2017 from the warming experiment (Table 1). Due to the sensitive nature of the long-term warming experiment, roots from just three individuals per species per plot were collected and with as little disturbance as possible by wedging a bleach-sterilized soil knife under one edge of the plant root system. Since entire plants could not be removed from the plot, we were cautious to collect only live roots that were attached to the base of the plant. We replaced disturbed soil and caused no plant mortality. Roots of each replicate plant were placed into separate sterile Whirl-paks, placed on ice, and returned to the lab.

2.5. Fungal cultures

Culturing occurred within 24 h for all samples. Root samples were cultured under a biological safety cabinet using standard sterile technique. Samples were surface-sterilized by submerging in 95% ethanol for 1 min, then in 1% sodium hypochlorite solution for 2 min. Roots were then rinsed three times with sterile, DI water. Root fragments were cut into smaller segments (~3 mm) using a flame-sterilized scalpel, then placed onto 10 cm diameter Petri plates containing malt extract agar with penicillin and streptomycin (50 mg/L). Plates were sealed with parafilm then incubated at room temperature. We checked plates daily and transferred unique morphotypes to 50 mm Petri dishes of the same media to create pure cultures. The last round of sub-culturing for unique morphotypes took place until August 4, 2017 (18 days after the first round of sub-culturing). The original plates were monitored until the end of August, at which point the plates were saturated and no new fungi appeared. We maintained and counted all replicate morphotypes per species per treatment. All isolates were archived in sterile water in replicate reference collections at University of New Mexico and Trinity University.

2.6. Fungal identification

DNA extraction and PCR. Multiple subcultures of each unique morphotype were randomly selected for sequencing. We extracted DNA with the Qiagen Dneasy Plant Kit and amplified via PCR with the ITS1-FL and TW13 primers (White et al., 1990; Taylor et al., 2008). Cultures that contained the 50−500 bp intron between the ITS1-FL and the ITS1 region were additionally amplified using the ITS1 (White et al., 1990) and TW13 primers. Each PCR used the Phusion High-Fidelity DNA polymerase kit (ThermoFisher Scientific, Waltham, MA). We used 5 μL of 5x High fidelity buffer, 0.5 μL of dNTP, 0.25 μL of each primer at 25 μM, 18.75 of nanopore water and 0.25 μL of Phusion, and 1 μL of template (~10 ng/μL). The thermocycler conditions for the ITS1-FL and TW13 pair consisted of denaturing at 96 °C for 2 min, 27 cycles of denaturing 94 °C for 30 s, annealing at 60.6 °C for 40 s, extending at 72 °C for 2 min, and a final extension at 72 °C for 10 min. Thermocycler conditions for the ITS1 and TW13 pair were identical except the annealing temperature was increased to 64 °C and the number of cycles was increased to 29 cycles. Each amplicon was Sanger sequenced by Genewiz (South Plainfield, NJ).

Bioinformatics. We trimmed the raw sequences to exclude low-quality bases (quality score < 20) and assembled the sequences using 4 peaks (Griekspoor and Groothuis, 2018) and Geneious (2018). The assembled sequences were aligned using MAFFT v7.407 (Katoh and Standley, 2013) and trimmed to include only the ITS region by removing nucleotides beyond the ITS1 and ITS4 primer regions. The sequences were clustered into operational taxon units (OTUs) at 97% similarity with UCLUST implemented in USEARCH (v10.0.240, 32 bit) (Edgar, 2010). If subcultures assigned to the same morphotype yielded different OTUs, we split the subcultures into separate morphotypes and the new morphotypes were subsequently sequenced to confirm inclusion in an OTU. Representative sequences for each OTU was generated in USEARCH and the taxonomy for each predicted using the "sintax" command (Edgar, 2016) with the Utax (v7.2) sequence dataset (UNITE Community, 2017). In addition, we used the BLAST algorithm (Altschul et al., 1990) against the GenBank database with an e value of < 1e-09 and a coverage of at least 80% to obtain more detailed taxonomy for each isolate that was sequenced. Sequences are hosted in GenBank under accession numbers MT820041:MT820113.

2.7. Statistical analysis

2.7.1. Are there altitudinal gradients in the composition or diversity of culturable root fungi?

To evaluate sensitivity of fungal diversity, the number of isolates cultured (abundance), and fungal OTU composition to altitude, we used elevation (high/low) and focal species identity or plant genus (Table 1, nine species, five genera) as fixed effects, with the random effect of replicate watershed (Table 2, three watersheds) in general linear mixed effects models. To assess the number of culturable isolates, we counted and tracked all isolates on each plate. Fungal isolates were characterized into morphotypes by visual assessment, followed by Sanger sequencing of similar morphotypes. For fungal community composition, we used a Bray-Curtis distance matrix to conduct permutational MANOVA in Primer version 6 (Clarke and Gorley, 2009). Pseudo-*F* statistics were calculated with Type *III* sums of squares. We visualized community composition using non-metric multidimensional scaling (NMDS) analysis with 500 re-starts (3-dimensional stress = 0.12 following the exclusion of one outlier sample that had low fungal abundance (*P. leptocoma* at East River

low). Analysis of multivariate dispersion (permDISP, 9999 permutations, Primer v. 6, Clarke and Gorley, 2009) showed no significant heterogeneity in dispersion by elevation or by plant species (Table 3a).

To assess fungal diversity responses, we calculated the Shannon diversity index, OTU richness, and the inverse Simpson Evenness index in the <vegan> package (Oksanen et al., 2018) in R (R Core Team, 2018) from an OTU matrix in which samples were rows, OTUs were columns, and each cell was the number of times we isolated each OTU from the root sample. For diversity metrics, we built mixed effect models using <lmer> in the lme4 (v1.1-19) package (Bates et al., 2015) with the fixed effects of elevation x genus, as well as plant species nested within genus and watershed as a random effect. Models were evaluated using analysis of deviance with function <Anova> in the car package (Fox and Weisberg, 2019). For significant main effects, *post-hoc* comparisons and 95% confidence intervals were obtained using the emmeans package (Lenth, 2018) with a false discovery rate correction for multiple comparisons.

2.7.2. Do culturable root fungi respond to experimental warming?

We applied a similar analysis framework to the warming experiment. Models for fungal diversity metrics were built with <lmer> with the fixed effects of the warming treatment (warmed/control) and plant species identity (Table 1, three species), warming x plant species, and the random effect of block (spatially paired warm/control plots). Fungal OTU composition was analyzed with the same model structure implemented in perMANOVA (Clarke and Gorley, 2009). Analysis of multivariate dispersion (permDISP, 9999 permutations, Primer v. 6) showed no significant heterogeneity in dispersion by treatment or plant species (Table 3b), indicating similar degrees of turnover in OTUs among individual samples within a treatment or plant species group.

2.7.3. Are fungal responses to direct warming predictable based on their altitudinal distributions?

A significantly positive correlation between a species decline with low elevation and its decline under experimental warming would indicate that fungal responses to warming were predictable from their altitudinal distributions. A subset of 11 fungal taxa co-occurred in a sufficient number of sites and plots (at least 3 of each per study) to compare their sensitivities to both elevation and experimental warming (see Results: Fungal composition, Fig. 1). We determined a measure of effect size for each OTU using the Relative Interaction Intensity metric (*RII*) (Armas et al., 2004) because it scales symmetrically from −1 to 1. We calculated *RII* for the altitudinal survey as: (mean abundance at low elevation − mean abundance at high elevation)/(mean abundance at low elevation +mean abundance at high elevation). Thus, OTUs that were most abundant at low elevation had a positive *RII*, and taxa that were most abundant at high elevation had negative *RII*. For the warming meadow experiment, *RII* = (mean abundance in warmed plots − mean abundance in control plots)/(mean abundance in warmed plots +mean abundance in control plots). Thus, fungal taxa that increased with warming had a positive *RII* and those that declined with warming had a negative *RII*. We then used Pearson product-moment correlation to evaluate whether responsiveness to warming was predictable from the change in abundance with elevation, using each OTU as an observation. Both variables were normally distributed.

3. Results

3.1. Fungal composition

We cultured 1517 fungal isolates from the altitudinal survey and the warming experiment combined. We sequenced 606 of these isolates, which were clustered to 77 OTUs (Supporting Material, Table S1). Ascomycetes were the most common phylum of fungi, comprising 84% of the OTUs (Supporting Material, Table S1). Basidiomycota comprised 12% of OTUs and Mortierellomycota were 4%. Common orders were Pleosporales (30% of OTUs), Helotiales (23%), and Hypocreales (13%) (Table S1).

The five most abundant OTUs were consistent across both the altitudinal survey and the warming experiment. OTU1 *Fusarium tricinctum* (Ascomycota: Hypocreales) comprised 18% of isolates for altitudinal samples and 27% in the warming experiment and is considered to be a weak plant pathogen with a broad host range and geographical distribution (Norberg et al., 2014). Unidentified taxa were common: OTU23 (an unidentified Helotiales) comprised 15% of isolates for altitudinal samples and 12% for the warming experiment, OTU12 (an unidentified Pleosporales) comprised 11% of isolates for altitudinal samples and 8% in the warming experiment, and OTU45 (another Helotiales) was 6% of isolates in the altitude study and 9% in the warming experiment. A *Mycena* sp. (OTU34, Basidiomycota: Agaricales: Mycenaceae) was the fifth most abundant taxon in the survey and fourth in the warming experiment, comprising 4% and 10% of isolates, respectively. *Mycena* form small saprotrophic mushrooms that are typically gray or brown (Smith, 1947), and have been previously characterized from grass roots and litter (Geesteranus, 1991; Osono, 2010; Tejesvi et al., 2013). An *Ophiosphaerella* sp. (Ascomycota: Pleosporales: Phaeosphaeriaceae) − a genus containing common turfgrass pathogens − was 4% of altitudinal survey isolates, and a *Plectania* sp. (Ascomycota: Pezizales: Sarcosomataceae), commonly associated with melting snow, was 4% of isolates in the altitudinal survey and 3% of isolates in the warming experiment.

Spatial variation in fungal composition among watersheds was larger than variation associated with altitude. Of the 54 taxa collected from the large spatial scale of the altitudinal survey (~10 km), ~30% (17/54) were shared between the high and low elevation sites (Fig. 1A) and just 15% (8/54) were present in all three watersheds (Fig. 1B). At the small spatial scale of the warming experiment (40 m × 100 m), ~40% (23/54) of the taxa cultured were present in both warmed and control plots (Fig. 1C). In addition, within each study, plant species shared some taxa but also had many unique fungal OTUs (Fig. 1D&E). In the altitudinal study, just seven percent (4/54) of taxa were shared among all six grasses surveyed whereas 17% (9/54) of taxa were shared among the three grass species sampled in the small footprint of the warming experiment.

Both altitude (Fig. 2) and experimental warming (Fig. 3) had marginally non-significant effects on the community composition of culturable root endophytes (Table 3, $0.05 < P < 0.1$). Across the altitudinal survey, plant species identity did not strongly structure fungal composition ($P > 0.4$), but spatial patterns were evident from the significant effect of watershed identity (Table 3a), indicating sufficient statistical power for this dataset. At the small spatial scale of the warming experiment, warming had a weaker effect on fungal composition than the identity of plant species (Fig. 3); the three grass species significantly diverged in fungal composition (Table 3b, $P < 0.03$). Neither warming nor elevation altered the dispersion of fungal communities among samples (Table 3).

3.2. Fungal diversity

Overall, OTU fungal abundance and diversity did not significantly vary with elevation (Table 4a, "Altitudinal Gradient") and did not decline under experimental warming (Table 4b, "Treatment"). However, altitudinal gradients in diversity indices were detected for some grass species, and these patterns diverged based on plant identity (Fig. 4, Table 4a, "Altitudinal Gradient X Genus"). Specifically, *Poa* species had 25−60% greater fungal isolate abundance (Fig. 4A, Elevation, $X^2 = 16.7$, $P < 0.0001$) and 25−38% greater fungal

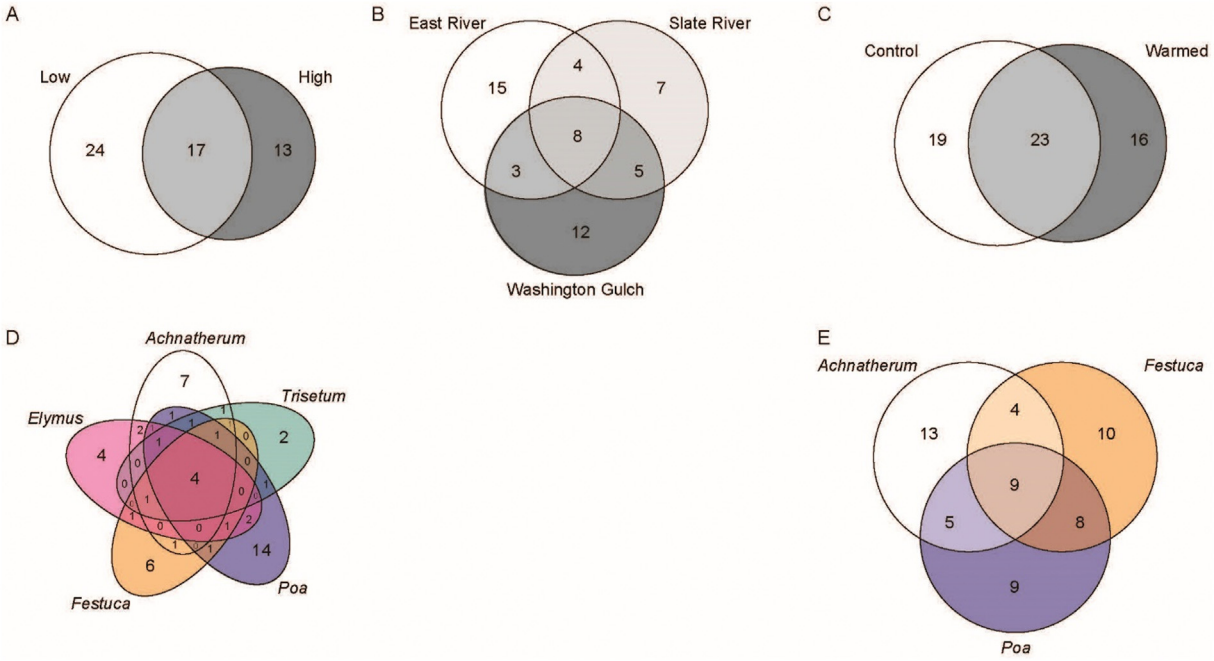

Fig. 1. Venn diagrams indicating the amount of overlap in fungal OTU composition for root samples in the Altitudinal Gradients Survey and Warming Meadow Experiment. Abiotic Effects: (A) Overlap in fungal taxa between Low and High elevations, (B) among watersheds, and (C) in control and warmed plots. Biotic Effect: D and E show overlap in fungal taxa among the six species of the Altitudinal Gradients Survey and (E) among the three species in control and warmed plots in the Warming Meadow Experiment. All numbers are numbers of OTUs.

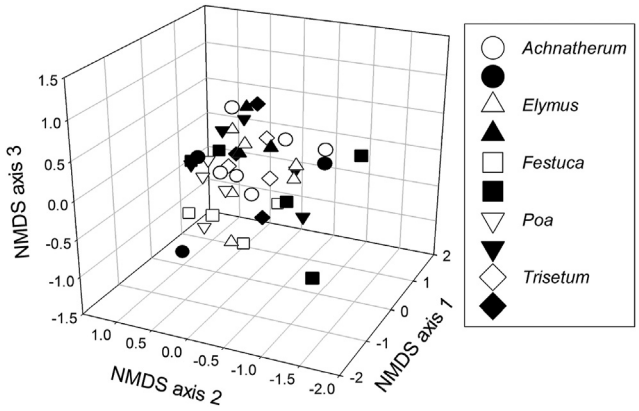

Fig. 2. Nonmetric multidimensional scaling plots depicting variation in fungal community composition for Altitudinal Gradients Survey. Each point is a unique root sample as described in the methods. Open symbols are low elevation sites. Filled symbols are high elevation sites. 3D stress = 0.12. Elevation: $P = 0.0867$ (Table 3a). Genus and species were *n. s.*

diversity (Fig. 4B, $X^2 = 27.5$, $P < 0.0001$) at high elevation than low elevation sites. The diversity increase for *Poa* was driven by a combination of ~1.67 more OTUs per sample for both *Poa* species (richness $X^2 = 27.5$, $P < 0.0001$) and 15–48% greater evenness (Elevation, $X^2 = 19.2$, $P < 0.0001$; Fig. 4). In contrast, *F. thurberi* had 64% *lower* fungal diversity on roots at high elevation (Fig. 4B, Elevation, $X^2 = 14.7$, $P = 0.0001$), driven by 2.3 fewer OTUs ($X^2 = 9.6$, $P = 0.0020$) and 70% lower evenness ($X^2 = 19.4$, $P < 0.0001$). No other plant taxon had significant altitudinal patterns in fungal diversity or abundance (Fig. 4).

Plant species also trended toward divergence in fungal diversity and evenness on the small spatial scale of the warming meadow experiment (<10% probability that data support the null hypothesis of difference: Table 4b, Plant Species, $0.05 < P < 0.1$). *Poa pratensis* had the highest fungal diversity and evenness in roots (Shannon $H' = 0.98 \pm 0.10$, Inverse Simpson $1/D = 2.64 \pm 0.23$) relative to either *A. lettermanii* ($H' = 0.77 \pm 0.10$, $1/D = 2.20 \pm 0.22$) or *F. thurberi* ($H' = 0.79 \pm 0.10$, $1/D = 2.19 \pm 0.22$) (Table 4b).

3.3. Individual fungal taxa

The response of individual fungal taxa to experimental warming was positively correlated with their altitudinal distributions (Fig. 5, Pearson $r = 0.61$, $P = 0.046$). Specifically, fungal taxa that were most abundant in warmed plots were also most abundant at lower elevations. The two taxa that were more abundant at high elevations also declined under warming. *Clonostachys rosea* (Bionectriaceae, Hypocreales, OTU26) was absent from warmed plots and present only in *F. thurberi*. OTU23 (unidentified Helotiales) declined with elevation and under warming (*RII* = −0.31) and was a generalist present in all three warming meadow grass species (Supporting Information, Table S1). Generally, those fungi that were more abundant at low elevation had positive or neutral responses to experimental warming. The strongest increases under warming were two taxa present in all three plant species: OTU3 – *Cadophora* sp. (Helotiales, *RII* warming = 0.76) and OTU6 – *Lachnum* sp. (Hyaloscyphaceae, Helotiales, *RII* warming = 0.48). In addition, one fungus that was absent from *F. thurberi* (OTU5 – *Fusarium redolens* (Nectriaceae, Hypocreales)) had strong positive response to warming (*RII* = + 0.27) across the other two grass species (Table S1).

4. Discussion

4.1. Are there altitudinal gradients in the composition or diversity of culturable root fungi?

The majority (68%) of root endophytes varied with elevation but caused relatively small shifts in overall fungal composition between high and low elevation sites. This pattern was not driven by changes in

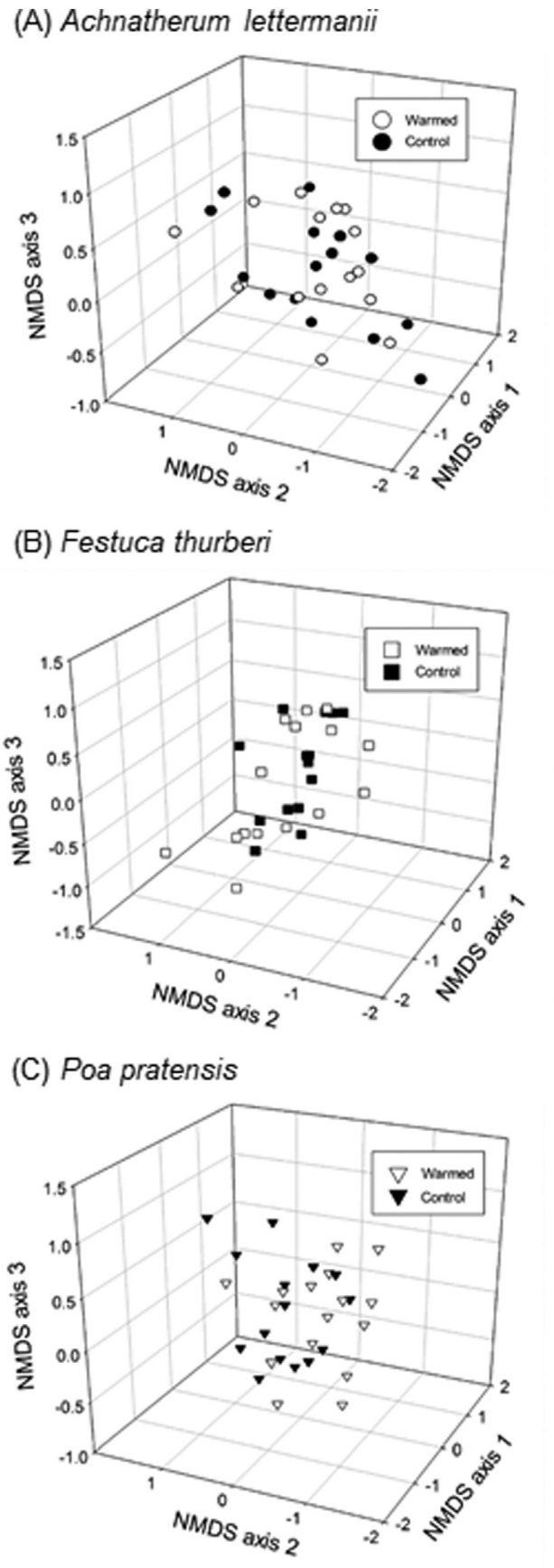

Fig. 3. Nonmetric multidimensional scaling plots depicting variation in fungal community composition in the Warming Meadow Experiment for (A) *Achnatherum lettermanii*, (B) *Festuca thurberi*, or (C) *Poa pratensis*. Each point is a unique root sample from an individual plant. Open symbols are warmed plots. Filled symbols are control plots. 3D stress for the ordination (all species together) = 0.13. Plant species identity ($P = 0.0062$) had a stronger influence than warming ($P = 0.0955$, Table 3b).

root endophyte diversity (gains or losses of species), but instead by individual root endophyte taxa that appear to be sorted by the steep altitudinal gradients as well as by plant host identity. Importantly, plant species identity shaped the relationships between fungal endophyte communities and altitude, even though there was not a strong overall signature of plant species identity on root endophyte composition. Specifically, root endophyte abundance and diversity peaked at high elevation sites in *Poa* spp. but peaked at low elevations in *F. thurberi*. In previous culture-based and next-generation sequencing surveys of the same plant species, their *leaf* fungal endophytes similarly showed host-specific altitudinal trends in diversity and abundance (Kazenel et al., 2019, Kivlin et al., 2019). Additionally, the abundance of AMF colonizing roots displayed species-specific trends with elevation in an earlier study (Ranelli et al., 2015).

Several interacting mechanisms may explain the variation in fungal diversity and composition among plant species over environmental gradients. For example, these trends may reflect differential plant host filtering for fungal symbionts throughout portions of their altitudinal range that vary in conspecific host densities (Cobian et al., 2019). Plants may select fungi based on their context-dependent costs and benefits under different abiotic environments (Afkhami et al., 2014). Alternatively, environmental selection for suitable abiotic and biotic conditions may structure plant-fungal symbiont communities along multivariate resource gradients (Kivlin et al., 2017). Additional manipulative studies that study more than one plant host, such as ours with temperature, could help disentangle these potential interactive effects.

4.2. Do culturable root fungi respond to experimental warming?

Individual root fungal endophytes responded to 27 y of experimental warming. For example, OTU26 — *Clonostachys rosea* and OTU23 only occurred in control plots whereas OTU3, OTU5, and OTU6 were mostly found in warmed plots. These effects were fungal-taxon specific and did not translate into broader patterns of shifts in fungal diversity or composition with warming. Previous results from next-generation sequencing of these plots confirm this trend: The overall abundance, diversity, and composition of root fungal endophyte did not vary with warming (Kazenel et al., 2019). In other warming experiments, species-specific response of root fungal endophytes have been common (Fujimura et al. 2008), and sensitivities may vary among fungal functional guilds, with increases in dark septate endophytes and decreases in other fungal endophytes in warmed versus cool environments (Olsrud et al., 2010). Aboveground, foliar fungal symbionts also had idiosyncratic responses to warming in our host species. Leaf fungal endophyte abundance was only sensitive to warming in one plant host (*Festuca thurberi*), and neither culture-based nor next-generation sequencing revealed significant effects of the warming treatment on *foliar* fungal endophyte diversity or composition (Kazenel et al., 2019; Kivlin and Rudgers, 2019). Instead, individual fungal endophytes may shift their metabolism to better-acclimate to warmer conditions (Kivlin and Rudgers, 2019); unfortunately, such plasticity in fungal responses to climate is not yet predictable given the limited data available.

Table 3
Statistical results for fungal root endophyte community composition for (a) Altitudinal Gradients Survey and (b) Warming Meadow Experiment. *n.t.* indicates that block effects on community dispersion were not tested. *P*-values < 0.05 shown in bold and *P* < 0.10 in italics.

	PERMANOVA			PERMDISP		
(a)	pseudo-*F*	*df*	*P*	*F*	*df*	*P*
Altidudinal Gradient	1.57	1	*0.0867*	0.64	1,42	0.5642
Watershed	1.59	2	**0.0284**	1.23	2,41	0.3526
Plant species	1.01	8	0.4498	1.23	2,35	0.6413
(b)						
Treatment	1.60	1	*0.0955*	0.06	1,89	0.819
Plant species	1.98	2	**0.0062**	1.06	2,88	0.4169
Plant species X Treatment	0.83	2	0.6834	1.03	5,85	0.3773
Block	2.05	4	**0.0004**	*n.t.*	*n.t.*	*n.t.*

Table 4
Statistical results for fungal root endophyte abundance and diversity indices for (a) Altitudinal Gradients Survey and (b) Warming Meadow Experiment. Evenness was the inverse Simpson index. *P*-values < 0.05 shown in bold and *P* < 0.10 in italics.

	Isolate abundance		Shannon *H'* (log)		Species richness		Evenness (log)	
(a)	X^2	*P*	X^2	*P*	X^2	*P*	X^2	*P*
Altitudinal Gradient	0.65	0.4195	0.8	0.3702	0.39	0.53	1.86	0.1731
Genus	1.75	0.7822	8.81	0.0659	5.49	0.2492	5.37	0.2511
Altidudinal Gradient X Genus	8.12	*0.0874*	12.65	**0.0131**	11.11	**0.0254**	13.21	**0.0103**
Plant species (Genus)	5.5	0.2398	1.75	0.7809	3.24	0.5192	5.05	0.282
(b)								
Treatment	0.24	0.6221	0.12	0.7318	0.02	0.8903	0.04	0.8503
Plant species	0.21	0.9002	5.08	*0.0787*	2.67	0.2632	5.41	*0.0669*
Plant species X Treatment	0.71	0.7015	1.5	0.4723	1.03	0.5966	2.11	0.3479

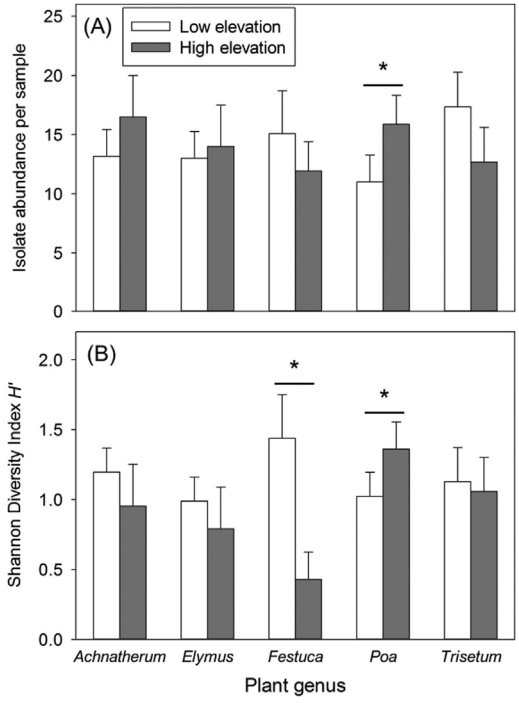

Fig. 4. Altitudinal Gradients Survey means ± s. e. from linear mixed effects models evaluating the influence of elevation (low or high) on (A) culturable root fungal abundance or (B) fungal diversity (Shannon index, *H'*). Asterisks indicate pairwise comparisons within a genus with *P* < 0.05.

4.3. Are fungal responses to direct warming predictable from their altitudinal distributions?

For common root fungal endophytes, altitudinal distributions could provide inference into their sensitivity to warming: Fungal taxa that declined with warming were also less abundant at lower than higher elevation sites across the altitudinal survey. This is one of the first indications that space-for-time substitution can partially inform fungal root endophyte responses to warming. However, this effect was not predictable based on limited data available for fungal evolutionary histories, life-history strategies (e.g., decomposer versus pathogen), or growth form. Specifically, the four main responders to warmer climates were *Clonostachys rosea* (Hypocreales), *Cadophora* sp. (Helotiales), *Lachnum* sp. (Helotiales) and *Fusarium redolens* (Hypocreales). While all of these species are within phylum Ascomycota, the taxa come from two distinct subphyla (Pezizomycetes and Sordariomycetes). Furthermore, whereas some of these taxa are usually beneficial to plant hosts, protecting plants from pathogens (e.g., *Clonostachys rosea*), others can be pathogenic (e.g., *Cadophora*, *Fusarium redolens*), or have wide distributions on a variety of substrates (e.g., *Lachnum*). The specific ecological roles of these species in association with host plants in our study remain unknown. Thus, a predictive framework for fungal symbiont winners and losers with climate change is still elusive.

Determining the ecological outcomes of specific plant-fungal symbioses will be critical if plant success in new environments depends on interactions with fungal symbionts (Kivlin et al., 2013; Ramirez et al., 2019). For example, plant altitudinal shifts with climate change may be hindered via establishment lags if certain

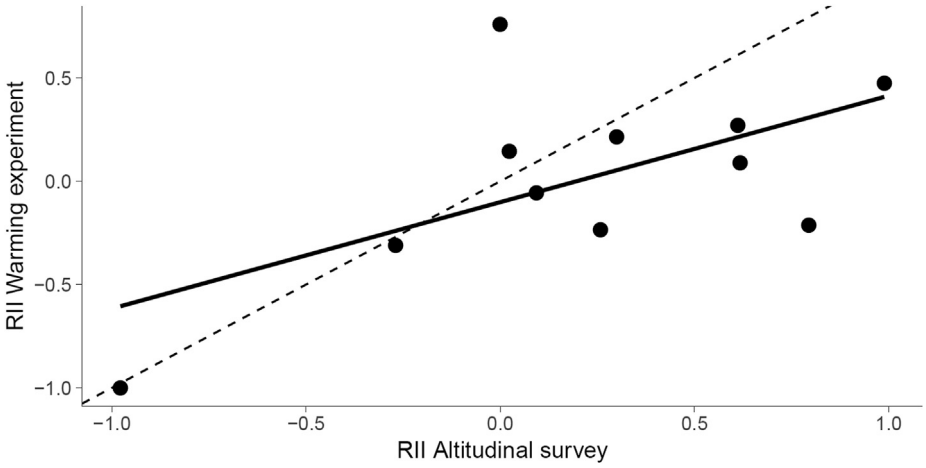

Fig. 5. Relationship between fungal sensitivity to elevation (*RII* Altitudinal survey) versus sensitivity to experimental warming (*RII* Warming experiment). Pearson's $r = 0.61$, $P = 0.046$, slope estimate $\beta = 0.52 \pm 0.22$. The dashed line shows the 1:1.

biotic interactions are absent from the novel species range (Alexander et al., 2018). Similarly, reciprocal transplants of plants and their fungal symbionts along altitudinal gradients have shown a home field advantage, where plants grow largest with symbionts that originate from their local elevation. However, the outcomes of specific plant-fungal symbioses are also commonly dependent on the degree of environmental stress (Wagg et al., 2011; Yang et al., 2018). In range-shifting plant species, the successional development of specific plant-fungal interactions common to the home range may require several generations of plant-soil feedback to rebuild (Chung et al., 2018; Collins et al., 2018; Van Nuland et al., 2019). Mismatches between plants and their microbial communities could slow rates of upward plant range expansion in mountain ecosystems and increase the likelihood of plant local extirpation or wholesale extinction under climate change.

5. Conclusion

Root-associated fungi, like their host plants (Smith et al., 2009), likely follow a hierarchy of responses to altitudinal gradients, natural warming, and global environmental change (Bardgett et al., 2013). Results of this study must be analyzed with caution as samples were collected over a 2 week period at the beginning of the growing season and fungal isolates were grown on a single culture medium for all plant species across all treatments. Nonetheless, several trends emerge. First, individual organisms can respond through changes in physiology, morphology, growth or reproduction. Our culture-based study revealed both winner and loser fungal taxa under long-term experimental warming, providing evidence of this first-level response. Second, global change can cause community reordering, altering whole community composition and the rank abundances of species. We found only weak support for re-ordering, with marginally non-significant changes in the fungal composition of plant roots subjected to long-term warming. The influence of abiotic conditions was generally weaker than that of host plant species identity, suggesting that non-mycorrhizal root endophytes may be relatively resilient to climate warming. This may be good news where host-symbiont associations benefit the fitness of native grass species. Third, species loss and immigration can cause the largest ecological responses to global change. Experimental manipulations may lack power to detect such responses because taxa can disperse across the small spatial scales of experimental plots. Our study detected just one species (*Clonostachys rosea*) that was present in control plots but absent under warming. However, general concordance between the altitudinal distributions of fungal OTUs and the magnitude of their responses to warming provides some optimism that geographic patterns in fungal abundances can help to predict their future sensitivities to warming (but see Kazenel et al., 2019). Importantly, our work supports other recent studies (Becklin et al., 2012; Li et al., 2014; Davison et al., 2016; Põlme et al., 2018; Cobian et al., 2019; Rudgers et al., n.d.) documenting strong plant-species specificity in the biogeography of plant-associated fungi, indicating that studies of single plant taxa will not reveal general patterns for Ascomycota and other non-mycorrhizal endophytes in plant roots.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.funeco.2020.101002.

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