Microbial Ecology
https://doi.org/10.1007/s00248-019-01336-4

PLANT MICROBE INTERACTIONS

Plant Identity Influences Foliar Fungal Symbionts More Than Elevation in the Colorado Rocky Mountains

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

Received: 4 June 2018 / Accepted: 22 January 2019

Abstract

Despite colonizing nearly every plant on Earth, foliar fungal symbionts have received little attention in studies on the biogeography of host-associated microbes. Evidence from regional scale studies suggests that foliar fungal symbiont distributions are influenced both by plant hosts and environmental variation in climate and soil resources. However, previous surveys have focused on either one plant host across an environmental gradient or one gradient and multiple plant hosts, making it difficult to disentangle the influence of host identity from the influence of the environment on foliar endophyte communities. We used a culture-based approach to survey fungal symbiont composition in the leaves of nine C_3 grass species along replicated elevation gradients in grasslands of the Colorado Rocky Mountains. In these ecosystems, the taxonomic richness and composition of foliar fungal symbionts were mostly structured by the taxonomic identity of the plant host rather than by variation in climate. Plant traits related to size (height and leaf length) were the best predictors of foliar fungal symbiont composition and diversity, and composition did not vary predictably with plant evolutionary history. The largest plants had the most diverse and distinctive fungal communities. These results suggest that across the ~ 300 m elevation range that we sampled, foliar fungal symbionts may indirectly experience climate change by tracking the shifting distributions of plant hosts rather than tracking climate directly.

Keywords Climate · C_3 grass · *Epichloë* · Foliar endophytes · Horizontally transmitted endophytic fungi · Microbiome · Mountain ecosystems · Plant host

Introduction

Fungal symbionts inhabit the intracellular spaces of leaves on every plant species surveyed to date [1], where they can function along a symbiotic continuum from pathogenic to mutualistic (e.g., [2]). When beneficial to their host plant, foliar fungal symbionts can confer tolerance to drought by decreasing plant water loss [2], regulating osmotic potential [3], or lowering stomatal conductance [4]. Foliar fungal symbionts can also confer heat tolerance to plants [5] and protect them from herbivory [6] and pathogens [7] by producing secondary metabolites [8]. Because foliar fungal symbionts can promote plant tolerance to environmental conditions, they may improve plant persistence in a changing climate. For example, in a previous meta-analysis of > 400 studies that manipulated global change factors, we highlighted the critical role of foliar fungal symbionts in plant drought tolerance [9]. Yet the ecology of these fungi in natural communities is difficult to predict because relatively little is known about the interactive effects of plant host species identity and climate on foliar fungal symbiont distributions, diversity, or composition.

Factors known to affect the biogeography of foliar fungal symbionts often relate to spatial and temporal context. In the largest geographic survey of foliar fungal symbionts to date, diversity was negatively correlated with latitude across a gradient of three plant communities (boreal forest, temperate forest, and tropical forest; [10]), possibly owing to shifts in plant species composition, climate, or both. Similarly, in a meta-analysis focused on mountain ecosystems, foliar fungal symbiont richness decreased and composition shifted with

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00248-019-01336-4) contains supplementary material, which is available to authorized users.

✉ Stephanie N. Kivlin
skivlin@utk.edu

[1] Department of Biology, University of New Mexico, Albuquerque, NM 87114, USA

[2] Rocky Mountain Biological Laboratory, Crested Butte, CO 81224, USA

[3] Department of Ecology and Evolutionary Biology, University of Tennessee, Knoxville, TN 37996, USA

Published online: 04 February 2019

increasing elevation [11]; however, the influence of host association versus environmental conditions could not be determined because in most prior studies, elevational gradients are confounded with differences in the identity of the plant species that were sampled. In studies focused on single plant species, historical and contemporary climate were the largest drivers of foliar fungal symbiont composition at the regional scale [2, 12, 13]. Furthermore, foliar fungal symbiont composition in a single host plant can vary on intra- and inter-annual time scales that track environmental variation [14]. However, within-site spatial heterogeneity in foliar fungal symbiont diversity and composition can also be large [1, 15], and composition may vary with aspect (e.g., north versus south facing slopes) or microclimate [16].

Current knowledge of host plant influences on fungal symbiont composition is limited because leaf fungal symbiont surveys at regional scales often do not include multiple plant species that are each sampled across multiple environments. Because it is intractable to survey the entire vascular plant phylogeny of > 300,000 taxa [17], identifying key plant traits associated with fungal symbiont composition could enable generalizations across plant taxa. Indeed, functional trait approaches have expanded our understanding of trade-offs in habitat tolerance and longevity for a range of plant [18] and free-living microbial taxa [19]. Moreover, trait-based approaches may define ecological strategies for unculturable microbial taxa [20]. Some trait-based efforts are beginning to be applied to describe the habitat preferences of foliar fungal symbionts. For example, existing evidence suggests that foliar fungal symbiont composition can vary among plant species due to differences in leaf chemistry [21], plant size [22], leaf surface texture (e.g., glabrous versus hirsute [23]), or the plant's environmental origin [24] or habitat breadth (e.g., broad versus narrow altitudinal distributions). For example, plant species restricted to high elevation habitats may have less diverse and more specialized fungal symbiont taxa than generalist plant species that occupy broad elevation ranges. Variation in plant traits may explain why the composition of foliar fungal symbionts often differs across broad plant clades [9, 25, 26] and within plant families [27]. However, some surveys suggest that foliar fungal symbiont composition varies little among plant species that co-occur at a single location [1, 28].

Here we sampled the leaf fungal symbiont community from nine plant species at six locations representing replicated pairs of high/low elevation sites. In order to distinguish the role of the environment versus plant host in determining fungal symbiont distributions, diversity, and composition, we specifically asked:

(1) What is the influence of plant identity versus elevation in shaping foliar fungal symbiont diversity and composition?

(2) How do plant traits, climate variables, geographic distance and elevation correlate with foliar fungal symbiont composition and diversity?

Methods

Study Sites

We sampled foliar fungal symbiont diversity and composition in the Colorado Rockies at the Rocky Mountain Biological Laboratory, Gunnison Co., Colorado, USA (38° 57′ N, 106° 59′ W). This region has predictable altitudinal declines in air temperature (*c.* 0.8 °C per 100 m) and soil nutrients but increases in precipitation [29–31]. The region is warming at rates of 0.5–1.0 °C per decade [32].

To capture environmental and spatial variation, we sampled foliar fungal symbionts from each of two elevations (low ~ 2900 m, high ~ 3200 m) in three separate watersheds in the Upper Gunnison Basin (East River, Washington Gulch, and Slate River) located within 10 km of each other. Vegetation at all sites was dominated by grasses, but three low elevation-restricted grass species were not present at the high elevation sites. We created an interpolated regional climate model to predict the average number of growing degree days (GDD, base 0 °C), mean annual temperature (MAT), and mean annual precipitation (MAP) for each of the six sites [33].

Host Plant Species

We focused on grasses because they dominate subalpine meadows in the Rocky Mountains. In addition, some individual grass species can span the entire elevational range in our study system [34], whereas tree species and most forbs do not. At each location, we sampled nine adult individuals from up to nine grass species, representing five genera (Poaceae, subfamily Pooideae). Within each grass genus, we sampled one species with a broad distribution that occurred at both high and low elevation sites and one species with a narrow distribution that occurred only at the low elevation sites (Table S1). Thus, our sampling design allowed us to disentangle the effects of species' habitat range (broad distribution generalist versus low elevation specialist) on foliar fungal symbiont communities while accounting for the effects of some aspects of shared plant evolutionary history (e.g., at the genus level). Adult plant size varied among plant species surveyed. To understand if variation among species in plant size could influence foliar fungal symbionts, we collected data on the species minimum, mean, and maximum height and leaf length from the USDA PLANTS database [35]. Note that these traits were

not directly measured on plant samples, so the traits are an approximation of differences among plant species. Because intraspecific differences in plant traits are typically smaller than interspecific differences, mean species trait values often provide quantitatively similar results to empirically measured traits at a given study location [36]. Furthermore, others have analyzed fungal endophyte composition as a function of mean species leaf traits in tropical forests [37].

Sampling Methods

In September 2013, individual plants were collected from each of the six sites and stored at 4 °C for up to 1 week. As soon as possible after collection, leaves were surface sterilized in a biosafety cabinet for 15 s in 95% ethanol, 2 min in 1% sodium hypochlorite, 2 min in 70% ethanol, and then rinsed in sterile water. Leaves from each plant were then cut into six ~ 5-mm sections using sterile technique and plated on potato dextrose agar with penicillin/streptomycin to prevent bacterial contamination. Individual fungal isolates were counted; morphotyped by shape, color, and texture; and subcultured as they grew out of leaf segments. Leaf samples yielding contaminant-free fungal symbiont cultures are presented in Table S2.

Molecular Methods

We sequenced representative cultures from fungal morphotypes containing multiple isolates to ensure that fungal species designations based on morphotyping accurately captured fungal species. Fungal DNA from at least one representative of each non-singleton fungal morphotype was extracted from a 5 × 5-mm agar plug using the Promega Wizard genomic DNA purification kit following the manufacturer's protocol (Madison, WI, USA). DNA was amplified using ITS1FL and TW13 primers [38] with illustra PuReTaq Ready-To-Go PCR beads (GE Healthcare Life Sciences, Pittsburgh, PA), 5 μL of DNA template (~ 10 ng/μL), 0.25 μL of each primer (50 μM), and 19.5 μL of nanopure water. We used the following PCR conditions: initial denaturation of 96 °C for 2 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 40 s, and extension of 72 °C for 2 min, with a final extension at 72 °C for 10 min. PCR products were then sequenced via Sanger sequencing at Beckman Coulter Genomics (Danvers, MA, USA).

Bioinformatics

Sequences were trimmed to exclude primers, and forward and reverse reads were assembled (~ 550 bases). Sequences were then grouped into OTUs ($N = 75$) at the 97% threshold using the UCLUST algorithm with default settings in QIIME [39]. When morphotypes contained more than one OTU at the 97% level, we re-sequenced all isolates assigned to those morphotypes to confirm accurate species delineation. Our OTU approach performs similarly to newly-applied exact sequence variants (ESVs [40]) and is a more accurate approach for delineating species richness for fungi, which can vary in ribosomal operon copy number [41] and number of nuclei [42]. Furthermore, fungi display substantial within-individual ITS sequence variation across repeats of the nuclear ribosomal tandem array, meaning that the use of ESVs would overinflate diversity estimates [43, 44]. A representative sequence from each OTU was assigned to a putative fungal identity using the BLAST algorithm [45] against the GenBank database with an e value of $< 1e^{-09}$ and a coverage of at least 80% (Table S1). Non-fungal reads were discarded. Sequences were then deposited in GenBank with accession numbers (MK415848-MK415922). In addition, fungal guild was estimated for each OTU using the FUNGuild algorithm [46], which provided a first pass on possible functional roles.

Statistical Analysis

Because sampling effort was standardized among all samples (i.e., we sampled the same number of leaf fragments per individual plant), we did not rarefy our OTU matrix. We estimated how much of the foliar fungal symbiont community we had sampled using the specpool function in vegan [47] in R [48]. Plant species varied in leaf size; however, correcting for the percentage of leaf tissue sampled for each plant species did not influence foliar fungal symbiont diversity or composition ($P > 0.05$).

What is the influence of plant identity versus elevation in shaping foliar fungal symbiont diversity and composition?

Fungal alpha diversity was assessed with Shannon's Diversity index [49] and was highly correlated with total richness and Simpson's diversity index ($r > 0.8$, $P < 0.001$). For analysis of diversity responses, we examined the fixed effects of elevation, watershed, plant genus, and genus × elevation, which allowed us to test the elevation × host identity interaction, which we could not do if we used plant species rather than genus. Models included the random effect of site (nested within watershed and elevation) to account for the non-independence of high and low elevation sites on the same watershed and of multiple collections from different species at the same site. General linear mixed models were constructed in lme4, using maximum likelihood estimation [48, 50]. To compare fungal diversity among grass species, we ran a similar model that excluded interactions between host identity and elevation and replaced the effect of plant genus with plant

species. We also analyzed the host identity × elevation interaction for broadly distributed species that spanned both high and low elevations. We decomposed differences among plant genera and species with *post-hoc* pairwise contrasts that were corrected for multiple comparisons using the false discovery rate ($\alpha = 0.05$).

To understand how fungal composition varied across samples, we tested for the relative influence of plant genus, elevation, and genus × elevation and included the random effects of watershed and site (nested within watershed and elevation) using nested permutational multivariate analysis of variance (PERMANOVA) on the matrix of abundances of each fungal morphotype (here called OTU) for each individual plant we collected (Primer v. 6 [51]). We also analyzed the host species identity × elevation interaction separately for the set of broadly distributed species that spanned both high and low elevations. We did not additionally analyze proportional abundances of OTUs because the culturing effort was identical across all samples. In all analyses of composition, we used a Bray–Curtis distance metric and 9999 permutations of residuals under a reduced model (Primer v. 6, [51]). Pseudo-*F* statistics were calculated with a type III sums of squares. While PERMANOVA is the most robust approach when sample numbers vary across treatments [52], these results should be interpreted with caution as unequal sampling effort among elevations and/or plant species could artificially inflate our power to detect differences among these groups. However, analysis of multivariate dispersion (PERMDISP, 9999 permutations, Primer v. 6) showed no significant heterogeneity in dispersion by elevation ($P = 0.46$) or by plant genus, with the exception of genus *Trisetum*, which trended toward lower dispersion than all other genera (pairwise, $P < 0.067$) because only one species in this genus occurred in our study region. We visualized community composition using non-metric multidimensional scaling (NMS) analysis with 500 restarts and a Bray–Curtis distance metric (three-dimensional stress = 0.01).

Because the influence of elevation on fungal composition depended on plant genus (PERMANOVA, genus × elevation, pseudo-$F_{4,258} = 1.3$, $P = 0.04$), we then conducted analyses and visualizations separately for each plant genus to determine which plant clades showed elevational patterns in foliar endophyte composition. PERMANOVA models for each genus included the fixed effects of elevation and species (excepting the *Trisetum* model, which lacked a species factor) and the random effect of gradient. We also analyzed the host species × elevation interaction for broadly distributed species that spanned both high and low elevations.

To understand which fungal taxa varied among plant species, elevation, and watersheds, we performed indicator species analysis using the indval function (labdsv v. 1.8-0; [53]). Indicator species values were considered significant when $P < 0.05$ after correcting for false discovery rate ($\alpha = 0.05$).

How do plant traits, climate variables, geographic distance and elevation correlate with foliar fungal symbiont composition and diversity?

To assess potential mechanisms of spatial (latitude, longitude, elevation), climatic (GDD, MAT, MAP), and plant trait relationships (minimum, average, and maximum plant height and leaf length) using continuous variables as predictors of foliar fungal symbiont composition, we used distance-based redundancy analysis (dbRDA) combined with step-wise model selection procedures based on the *AICc* (Vegan v. 2.4–5; [47]). Final climatic and plant trait predictor variables in each model were chosen from the wider subset of metrics above when they explained the most variance in fungal symbiont composition and did not covary by more than 75%. Furthermore, variables included in the final models had a variable inflation factor (*VIF*) less than 10 ([54]; Table S3). Environmental vectors were then overlaid onto canonical correspondence analysis (CCA) ordinations with centroids either representing each plant species or each fungal guild as determined by the FUNGuild algorithm [46]. In addition, we independently assessed if plant traits were phylogenetically conserved using Blomberg's *K* on a plant phylogeny constructed in Ranelli et al. [55].

Results

We obtained a total of 690 foliar fungal symbiont subcultures that were grouped into 152 morphotypes, 75 of which were non-singletons and sequenced. These sequenced OTUs spanned 23 families, 34 genera, and 6 putative fungal guilds. Depending on the metric, we sampled up to 79% (bootstrap) of the total foliar fungal symbiont community for focal grasses in the study region (Fig. S1). Most of the fungal symbionts were Ascomycota (96%) in the Trichocomaceae (28%; Table S4) and *Aspergillus*, *Cladosporium*, and *Chaetomium* genera. Only 49% of taxa could be assigned to guilds using FUNGuild, but of these, 49% were assigned to pathogens, 22% were designated decomposers, and 14% were endophytes. The remaining taxa (15% of those to which guilds were assigned) had multiple guild associations (Table S4). These functional group assignments should be interpreted cautiously given the sparse data available on the function of leaf fungal symbionts.

What is the influence of plant identity versus elevation in shaping foliar fungal symbiont diversity and composition?

Overall, foliar fungal symbiont diversity varied among plant genera (Table 1A, $P = 0.025$). *Elymus* plants had the highest diversity and *Festuca* and *Poa* the lowest, with a difference in

Table 1 Statistics from general linear mixed effects models on (A) foliar endophyte diversity or (B) foliar endophyte composition for models including all genera where plant identity (ID) = genus, elevation, watershed, plant identity × elevation, and site (nested within watershed and elevation) and separately for each individual plant genus (plant ID = species) by elevation and watershed. Bolded values indicate significance as $P < 0.05$. In (A), results are from analysis of deviance using log-likelihood X^2 values from models fit with maximum likelihood estimation. In (B), F values are pseudo-F from permutational MANOVA (Primer v. 6)

(A)	Plant ID		Elevation		Watershed		Plant ID × elevation		Site (watershed × elevation)	
	X^2	P	X^2	P	X^2	P	X^2	P	X^2	P
All genera	**17.761**	**< 0.001**	0.107	0.744	3.379	0.185	2.059	0.725	0.001	0.999
Achnatherum	0.786	0.375	0.686	0.408	**6.220**	**0.045**				
Elymus	0.495	0.482	0.835	0.361	2.316	0.314				
Festuca	**15.978**	**0.001**	0.008	0.929	0.047	0.977				
Poa	0.040	0.841	0.026	0.872	2.249	0.325				
Trisetum			0.162	0.687	0.559	0.756				
(B)	Plant ID		Elevation		Watershed		Plant ID × elevation		Site (watershed × elevation)	
	F	P	F	P	F	P	F	P	F	P
All genera	**1.525**	**0.003**	1.071	0.443	1.318	0.211	**1.305**	**0.039**	1.322	0.087
Achnatherum	**2.528**	**0.002**	**2.640**	**0.001**	**1.731**	**0.006**				
Elymus	**2.961**	**0.001**	1.524	0.089	**2.145**	**0.001**				
Festuca	**2.064**	**0.008**	1.477	0.346	1.909	0.216				
Poa	**1.707**	**0.013**	1.236	0.175	**1.343**	**0.049**				
Trisetum			1.240	0.243	**1.720**	**0.027**				

diversity among these grass genera of > 75% (Fig. 1a). Fungal symbiont diversity also varied among plant species (X^2 = 34.186, $P < 0.001$) in the analysis that did not include an interaction with elevation. *F. saximontana* plants consistently had fewer foliar fungal symbiont taxa than *E. elymoides*, *E. trachycaulus*, or *T. spicatum*. *F. saximontana* plants also

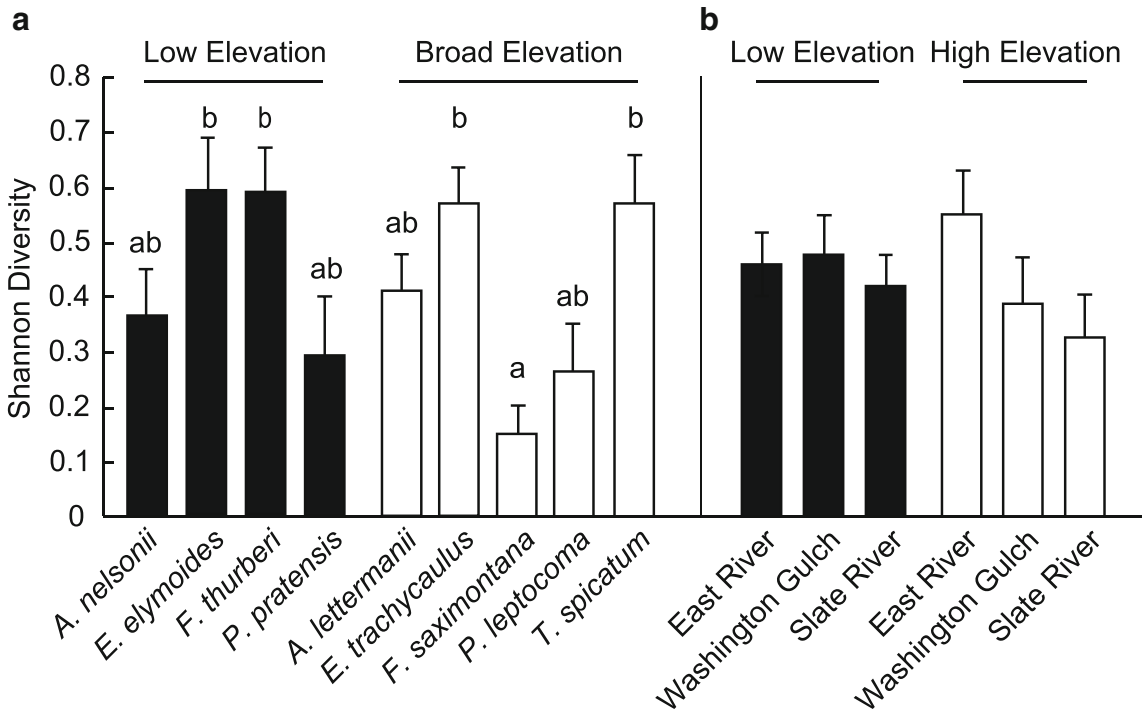

Fig. 1 Shannon diversity of the fungal symbiont community in leaves **a** for each plant species, separated by species restricted to low elevations and those with broad elevation distributions, and **b** by elevation and watershed (arranged East to West). Bars show means ± s.e. Different letters represent significant differences among species with Bonferroni corrections of paired contrasts of 95% confidence intervals

had significantly lower diversity ($H = 0.15 \pm 0.10$) than its congener *F. thurberi* ($H = 0.59 \pm 0.12$) (Table 1A). For the subset of plant species that spanned both high and low elevations (*A. lettermanii*, *E. trachycaulus*, *F. saximontana*, *P. leptocoma*, and *T. spicatum*), there was no significant interaction between plant species identity and elevation for fungal diversity ($X^2 = 1.676$, $P = 0.795$), and the only significant factor affecting symbiont diversity was plant species identity ($X^2 = 11.578$, $P = 0.021$).

Fungal symbiont abundance also varied among plant hosts ($X^2 = 30.600$, $P < 0.001$). When the overall abundance of fungal isolates cultured from each plant species was considered, *F. thurberi* plants consistently yielded more culturable isolates than the *Poa* species (*P. leptocoma*, $P = 0.048$; *P. pratensis*, $P = 0.058$). *E. elymoides* ($P = 0.009$) and *T. spicatum* ($P = 0.014$) plants yielded more culturable taxa than *F. saximontana*. Abundance of culturable fungal symbionts also varied by watershed ($X^2 = 8.688$, $P = 0.013$) with the East River having higher abundance (2.538 ± 0.152 fungal isolates/sample) than Slate River (1.979 ± 0.153 fungal isolates/sample), whereas Washington Gulch was intermediate (2.044 ± 0.174 fungal isolates/sample). There was no significant effect of elevation on fungal symbiont isolate abundance ($X^2 = 0.497$, $P = 0.481$) nor a significant interaction of elevation × watershed ($X^2 = 2.494$, $P = 0.646$).

Host plant identity at the genus or species level explained more of the variability in foliar fungal symbiont composition than did either elevation or gradient (Table 1). In genus-specific models, the identity of the host clade had the strongest influence on community composition and was significant for all genera (Figs. 2 and 3; Table 1; all $P < 0.013$). The next largest influence was spatial watershed, which also had a significant influence on fungal composition for all genera except for *Festuca* (Table 1). Elevation influenced foliar fungal symbiont composition in only two genera, significantly in *Achnatherum* (Table 1; $P = 0.001$) and marginally in *Elymus* (Table 1; $P = 0.089$). For the subset of plant species that spanned both high and low elevations, composition varied strongly among plant species (PERMANOVA, pseudo-$F = 1.627$, $P = 0.002$). There was no significant overall effect of elevation (pseudo-$F = 1.241$, $P = 0.346$), but there was a significant interaction between plant species identity and elevation (pseudo-$F = 1.689$, $P = 0.001$). Only one genus, *Achnatherum*, had a significant influence of elevation (Table 1B) on foliar endophyte composition. Specifically, in the broadly distributed species, *Achnatherum lettermanii,* foliar endophyte composition significantly differed between low and high elevation sites (pseudo-$F = 2.677$, $P = 0.002$). Endophyte composition in *Achnatherum lettermanii* was also significantly more dispersed (larger variability in composition among individual samples) at high elevation sites (dispersion = 67.1) than at low elevation sites (dispersion = 60.4) (PERMDISP, pseudo-$F = 6.060$, $P = 0.018$).

Indicator species analysis revealed that the fungal taxa that differed the most among plant species were mostly putative pathogens (Table S5). Overall, *F. thurberi* had the most fungal indicator taxa ($N = 3$), including one potentially beneficial endophyte, *Clonostachys rosea*, and two functionally ambiguous taxa, *Aspergillus niger* and *Phaeosphaeria caricis*. *Poa pratensis* and *T. spicatum* each had two indicator species (*P. pratensis*—*Paecilomyces variotii*, *Phaeosphaeria caricis*; *T. spicatum*—*Drechslera poae*, *Alternaria infectoria*) and *E. elymoides* had one (*Alternaria mali*), the majority of which are putative pathogens. Only one taxon, *Alternaria malorum*, a putative human pathogen, was more abundant at low elevations than high elevations. Spatial variation among watersheds was high for some fungal taxa, but these patterns did not conform to predefined fungal guilds: *Aspergillus niger* and *Phaeosphaeria caricis* abundance was highest in the East River, and

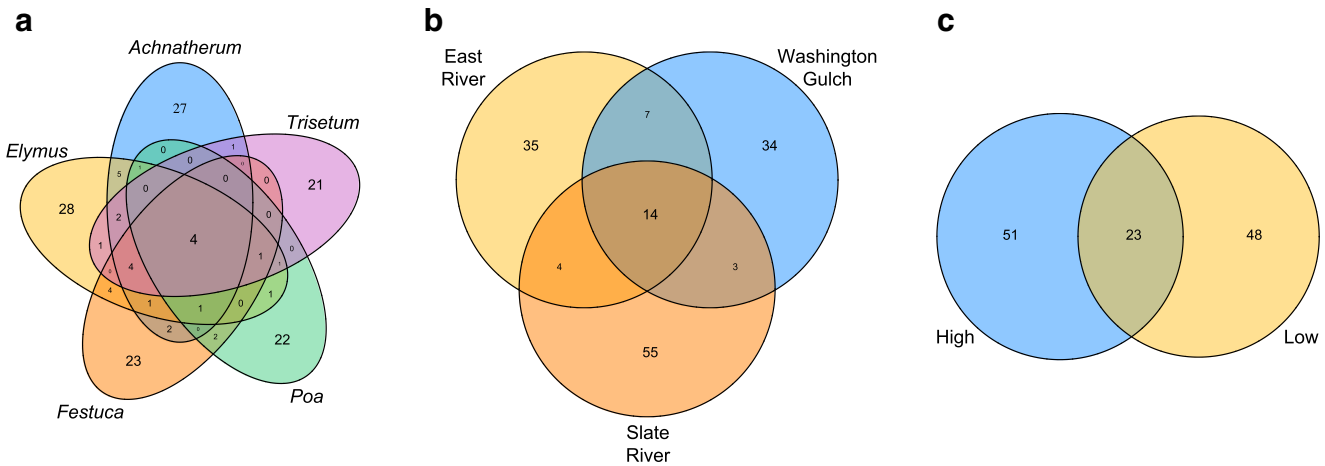

Fig. 2 Venn diagrams for fungal taxa overlap among **a** plant genera, **b** watersheds, and **c** elevations. Fungal taxa most strongly varied among plant genera (Table 1)

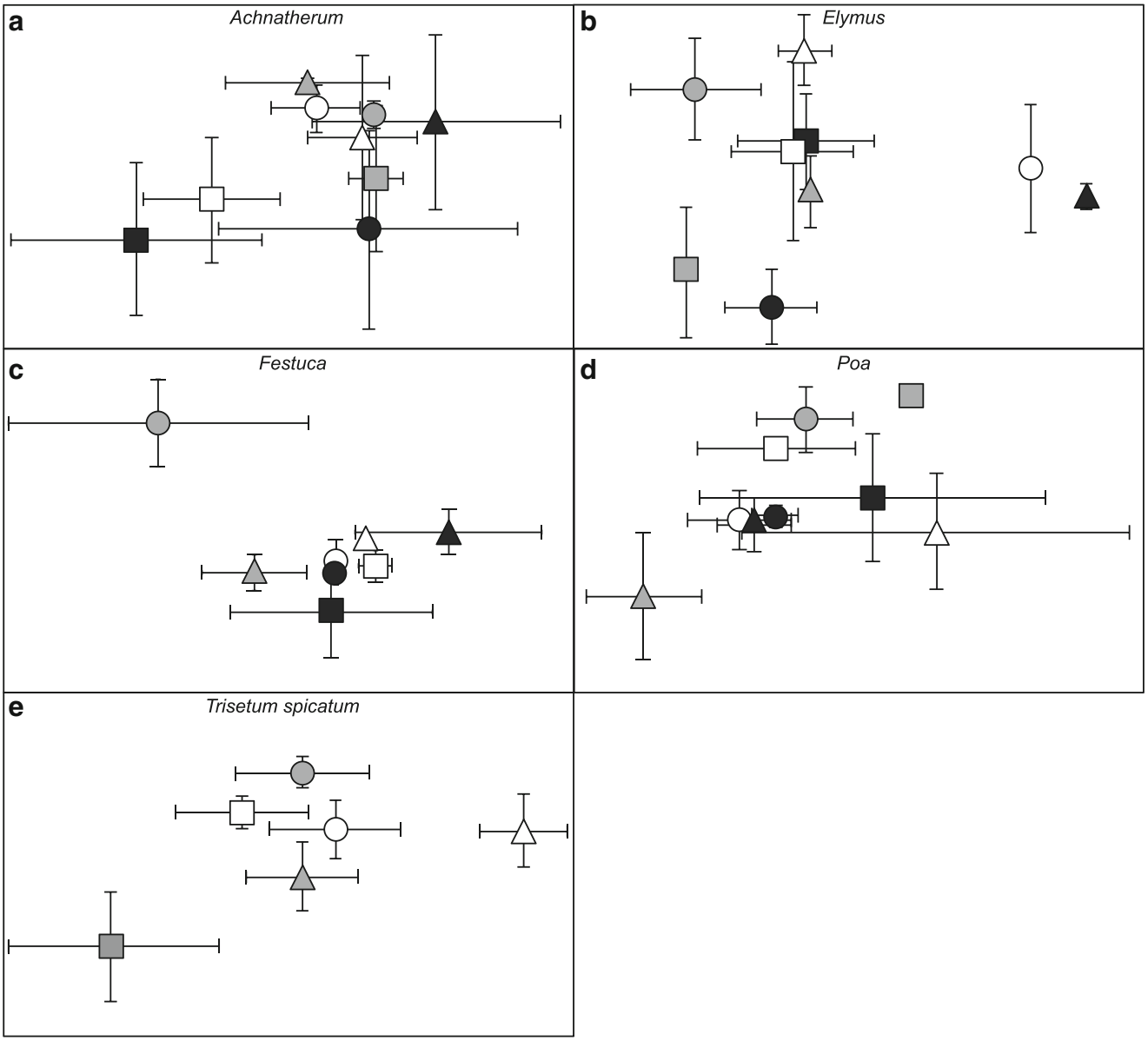

Fig. 3 NMS ordination of foliar fungal symbiont composition in each plant species separated by genus, showing the centroid ± s.e. Within each panel, broadly distributed plant species are designated white when they occur in high elevation sites and gray in low elevation sites. Species restricted to low elevations are in black. Symbol shapes indicate replicate watersheds (circle = East River, triangle = Washington Gulch, and square = Slate River)

Alternaria mali and *Penicillium ochrochloron* abundance was highest in Washington Gulch. There were no indicator fungal taxa as a function of interactions among plant species, elevation, or watershed.

What variables correlate with the influence of plant identity (traits), climate, or geographic distance and elevation on foliar fungal symbiont composition and diversity?

Foliar fungal symbiont composition varied the most with minimum plant height (Fig. 4a; Table 2). In addition, minimum plant leaf length was a predictor of foliar fungal symbiont composition. These traits differed among plant species (Fig. 4a) but were not phylogenetically conserved: Closely related plant hosts did not have more similar plant traits than expected by chance ($P > 0.05$).

Spatial and elevational factors also co-varied with foliar fungal symbiont composition (Table 2). Unexpectedly, latitude and longitude outweighed the association of composition with elevation and climate, despite the strong change in MAP and MAT with elevation. When fungal taxa were grouped into guilds, there were no effects of plant hosts or spatial drivers on composition ($P > 0.05$; Fig. 4b).

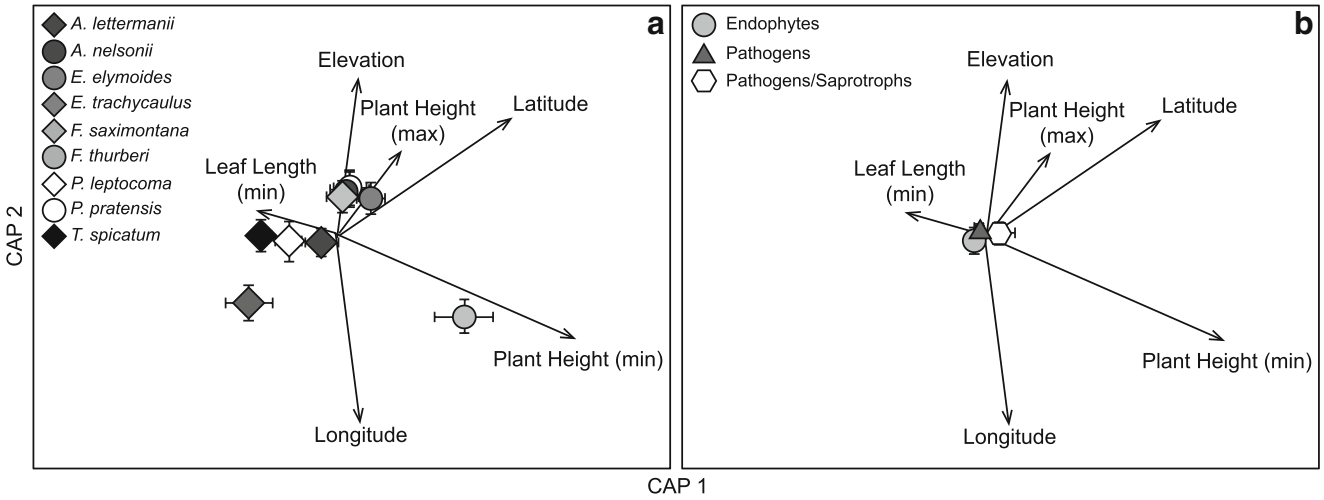

Fig. 4 Distance-based redundancy analysis of continuous factors affecting foliar fungal symbiont composition by **a** plant species and **b** fungal guild. Only statistically significant geographic variables and plant traits are shown with vectors. Vector length indicates the relative influence of the factor on foliar fungal symbiont composition

Discussion

Foliar fungal symbiont diversity and composition mainly tracked differences among host plant species that may be explained by plant traits, such as plant height. In addition, fungal symbiont composition varied over space with latitude, longitude, and with elevation among plant genera. In contrast, we found no evidence that variation in climatic parameters predicted shifts in foliar fungal symbiont diversity or composition. In a previous survey of foliar fungal symbiont abundance in the same system, Ranelli et al. [55] documented plant species-specific patterns of fungal colonization of leaves that mostly declined with increasing elevation.

Altogether, this evidence suggests that projected warming in this region may affect foliar fungal symbiont communities indirectly via shifts in host plant distributions (reviewed by [56]). A resurvey of plant communities at sites in the Gunnison basin originally surveyed from 1948 to 1952 [57] revealed an average upward elevational migration of grass hosts by 41 m [58]. Our evidence suggests that shifts in plant communities will be followed by subsequent dispersal of horizontally transmitted foliar fungal symbionts. The degree to which foliar fungal symbionts may be able to shift ranges in concert with their plant hosts is still unknown. However, underlying spatial variation in fungal symbiont composition with latitude and longitude—even within 10 km at our sites—may indicate that dispersal limitation can hinder foliar fungal symbiont host tracking.

Most of the indicator foliar fungal symbiont taxa that significantly varied among plant hosts were identified by FUNGuild as putative pathogens. However, fungal symbionts often vary from detrimental to beneficial [2] depending on environmental conditions and plant hosts [59], and pathogens are better studied and documented than commensals or mutualists. Furthermore, FUNGuild assigned function to < 50% of the isolates from our survey, so this result should be interpreted cautiously. The one putatively beneficial endophyte identified, *Clonostachys rosea*, has been documented to increase plant biomass and tissue phosphorus concentrations in agricultural grasses [60], suggesting that this fungus may play a role in nutrient acquisition in long-lived *F. thurberi* plants. Greenhouse trials that manipulate these foliar fungal symbionts alone and in combination with root fungal symbionts in a variety of plant hosts and environmental conditions are necessary before function can ultimately be assigned to these fungal isolates.

Many mechanisms may explain why foliar fungal symbiont communities varied with plant species identity and plant size in our system. Individual C_3 grasses are long-lived in this region. However, it remains unknown whether most foliar fungal symbionts can persist in plant meristems during winter or must recolonize every year following snowmelt. The exception is the genus *Epichloë,* which overwinters and remains as a systemic infection. Plant species may differ in chemical compounds either produced directly or by vertically

Table 2 Redundancy analysis of all significant factors affecting foliar endophyte communities

Factor	*df*	*F*	*P*
Latitude	1	2.166	0.004
Longitude	1	2.464	0.002
Elevation	1	2.183	0.006
Minimum height	1	2.893	0.002
Maximum height	1	1.726	0.025
Minimum leaf length	1	1.795	0.015
Residual	266		

transmitted endophytes in *Festuca thurberi* and *Trisetum spicatum* [61]. In previous studies, vertically transmitted leaf endophytes negatively affected the abundance of other microbial symbionts in plants (e.g., [62]). However, we did not observe systematically lower diversity of culturable foliar fungal symbionts in species known to host *Epichloë* spp. Instead the smallest plant species, *Festuca saximontana,* had the lowest diversity. This may indicate that competition for space or plant carbon, which can vary with the size and age of photosynthetic tissue [63]), dictates how many foliar fungal symbiont taxa plants can host at a given time and that interspecific interactions among fungal symbionts are only ancillary components of community assembly.

We did not observe differences in fungal symbiont diversity or composition among plant species restricted to low versus broad elevational distributions. This may suggest that, in general, foliar fungal symbionts have high environmental tolerance to variation in temperature and rainfall, which vary systematically with elevation in our study region. Our variance partitioning results support this finding as MAP and MAT were not strong predictors of foliar fungal symbiont composition. Alternatively, foliar fungal symbiont response to differences in plant traits may override any environmental-based filtering in plant hosts with broad elevational distributions.

Most previous studies indicating environmental based filtering on foliar fungal symbiont composition have occurred over larger spatial scales than our survey [2, 12, 14]. Thus, dispersal limitation may be part of the reason that foliar fungal symbionts are environmentally structured at these larger spatial scales (see [64]). We also observed some evidence consistent with dispersal limitation among watersheds, which are separated longitudinally, albeit with much less impact on foliar fungal symbiont communities than host plant identity. Small-scale dispersal limitation may be common for fungal symbiont communities in grassland/forest ecotones [1, 16] that predominate our study sites, where air current eddies are more turbulent and less linear than in than homogeneously structured plant communities [65].

While our overall sampling effort resulted in more than 600 initial cultures, we likely missed some cultivable and many unculturable fungal symbiont taxa. Nevertheless, the dominant trend of plant host influence on foliar fungal symbionts that we found here was also captured in surveys conducted with next-generation Illumina sequencing along some of the same elevation gradients [66]. Moreover, our results may be specific to long-lived grasses and host structuring of foliar fungal symbionts may not occur as frequently in plants with annual or biennial life cycles where foliar fungal symbiont communities must re-assemble more frequently (e.g., [2, 14]). Additionally, 2013 was a relatively wet year in our study region [33] and thus environmental filtering of foliar fungal symbionts may be stronger under more stressful environmental conditions. Finally, we did not characterize all of the environmental variables in our study region (e.g., heavy metals). Therefore, unmeasured environmental variables may also affect foliar fungal symbiont diversity and composition along elevational gradients.

Conclusions

Here we showed that foliar fungal symbiont diversity and composition varied most strongly with plant species identity along replicated elevational gradients. This result may be driven by differences in plant traits, in particular plant height. While we also observed spatial variation in foliar fungal symbiont composition, climate was never a strong predictor of this variation. Thus, our results suggest that rather than responding to direct influences of climate, foliar fungal symbionts may experience strong indirect effects of climate change via changes in plant host distributions, which are already occurring in many mountain ecosystems (e.g., [57]). Because foliar fungal symbionts may mitigate negative responses of plants to climate change [9], understanding how plant and foliar fungal symbiont dispersal trajectories and environmental tolerances are linked will be required to project future plant and fungal composition in mountain ecosystems.

Acknowledgments We thank K. Anderson and B. McCormick for help maintaining the foliar endophyte culture collection and A. Chung and J. Bell for assistance with DNA extraction and sequencing.

Funding Information This work was supported by National Science Foundation grant number DEB1354972 to Rudgers, Taylor and Kivlin and RMBL fellowships to Kivlin, Rudgers, and Lynn.

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