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Shrub range expansion alters diversity and distribution of soil fungal communities across an alpine elevation gradient

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Abstract

Global climate and land use change are altering plant and soil microbial communities worldwide, particularly in arctic and alpine biomes where warming is accelerated. The widespread expansion of woody shrubs into historically herbaceous alpine plant zones is likely to interact with climate to affect soil microbial community structure and function; however, our understanding of alpine soil ecology remains limited. This study aimed to (i) determine whether the diversity and community composition of soil fungi vary across elevation gradients and to (ii) assess the impact of woody shrub expansion on these patterns. In the White Mountains of California, sagebrush (*Artemisia rothrockii*) shrubs have been expanding upwards into alpine areas since 1960. In this study, we combined observational field data with a manipulative shrub removal experiment along an elevation transect of alpine shrub expansion. We utilized next-generation sequencing of the ITS1 region for fungi and joint distribution modelling to tease apart effects of the environment and intracommunity interactions on soil fungi. We found that soil fungal diversity declines and community composition changes with increasing elevation. Both abiotic factors (primarily soil moisture and soil organic C) and woody sagebrush range expansion had significant effects on these patterns. However, fungal diversity and relative abundance had high spatial variation, overwhelming the predictive power of vegetation type, elevation and abiotic soil conditions at the landscape scale. Finally, we observed positive and negative associations among fungal taxa which may be important in structuring community responses to global change.

KEYWORDS
alpine, fungi, global change, joint distribution model, shrub expansion, soil

1 | INTRODUCTION

Changes in global climate and land use are having significant impacts on above- and belowground organisms worldwide including plants and soil microbes (Wolters, Silver, Bignell, Coleman, & Lavelle, 2000). This is particularly true in cold arctic and alpine biomes where warming is occurring at an accelerated pace (Pepin et al., 2015; Rammig, Jonas, Zimmermann, & Rixen, 2010). Alpine environments have been relatively poorly studied when considering the impacts of global change on belowground soil organisms (Lazzaro, Hilfiker, & Zeyer, 2015). This is especially true for soil fungi, as a large majority of soil microbial studies in alpine environments focus on bacteria and archaea (Siles & Margesin, 2016). Therefore, it is necessary to improve our baseline knowledge of how soil fungal communities change across elevation and climate gradients to better understand and predict how these patterns are being altered by global change.

Soil fungal communities generally change across elevation gradients; however, these patterns are variable and mechanisms are still not well understood (Sundqvist, Sanders, & Wardle, 2013). In particular, the relative importance of different mechanisms including soil abiotic conditions, plant associations and interactions among taxa is difficult to disentangle. Soil fungal diversity often declines with increasing elevation in alpine environments (Körner, 2003; Schinner & Gstraunthaler, 1981). This is primarily due to a decline in plant species richness in high-elevation ecosystems, as belowground fungal communities are known to be closely tied to plant species diversity and identity (Bahram, Põlme, Kõljalg, Zarre, & Tedersoo, 2012; C Körner, 2003; Peay, Baraloto, & Fine, 2013). In addition, fungal community composition in soils also changes along elevation gradients. Schinner and Gstraunthaler (1981) were among the first to describe this pattern in the central European Alps whereby soil fungal communities declined in diversity as elevation increased and fungal community composition and species dominance paralleled shifts in plant communities along this same gradient. More recent studies have confirmed these patterns, as soil fungal diversity declined strongly with elevation and paralleled declines in plant and bacterial diversity in tropical montane forests (Nottingham et al., 2016). Other research however has suggested that fungal diversity and richness have no clear relationship with altitude (Coince et al., 2014; Siles & Margesin, 2016) or that fungal community composition, but not alpha diversity and richness, varies across elevation gradients (Lanzén et al., 2016; Shen et al., 2014). In general however, it is known that elevation plays an important role in structuring fungal communities worldwide (Kivlin, Hawkes, & Treseder, 2011; Tedersoo et al., 2014). More studies of soil fungi across elevation gradients are required to understand how these patterns differ globally and the mechanisms that drive elevation–diversity relationships.

In addition to changing plant diversity and composition, the abiotic environment also changes significantly with elevation and may have important effects on fungal communities (He, Hou, Liu, & Wen, 2016; Körner, 2003; Körner, 2007). For example, mean annual temperature (MAT), soil moisture, soil organic carbon (SOC) and nitrogen (SON) and soil pH all influence the diversity and community structure of soil fungi along elevation gradients (Sundqvist et al., 2013), although not always in consistent ways. Fungal diversity may decline with mean annual temperature (MAT) at high-elevation sites (Nottingham et al., 2016), or may increase due to greater soil moisture at high elevations irrespective of temperature (Pellissier et al., 2014). Soil pH was the most important predictor of fungal community structure in alpine soils in Northeast China (Shen et al., 2014), and equally important as MAT for root-associated fungi in the French Alps and Pyrenees (Coince et al., 2014). Changes in abiotic soil parameters may also interact with vegetation in their effects on soil fungi (Sundqvist et al., 2013). For example, fungal diversity was inversely related to SOC and total soil N in alpine steppe of the Tibetan Plateau; however, this trend was reversed in nearby alpine meadows, displaying a strong interaction between the dominant vegetation type and soil nutrients on fungal diversity (Zhang et al., 2017).

Fungal groups may differ in their responses to elevation gradients due to their environmental tolerances and plant associations. For example, mycorrhizal fungi tend to decline in diversity at higher elevations because of declines in plant species hosts at high-elevation sites (Bahram et al., 2012; Shen et al., 2014; Tedersoo et al., 2014; Wu, Hogetsu, Isobe, & Ishii, 2007). However, dark septate endophytes (DSE) maintain high abundances in alpine environments where in general mycorrhizal abundance is low (Körner, 2003; Newsham, 2011; Schmidt, Naff, Lynch, & Newsham, 2012). In addition, ectomycorrhizal (ECM) and arbuscular mycorrhizal fungi (AMF) may have contrasting elevational patterns with AMF richness declining and ECM richness increasing with altitude (Kivlin, Lynn, Kazenel, Beals, & Rudgers, 2017). Free-living fungal taxa are also likely to vary across elevation gradients due to changes in abiotic conditions and plant resource quality and quantity, but these relationships are more poorly studied than for mycorrhizal groups. Two notable examples include evidence that Archaeorhizomycetes have higher abundance at high elevation in tropical montane forests (Nottingham et al., 2016) and that Agaricomycete fungi increase in abundance with elevation at a global scale (Tedersoo et al., 2014).

Given the importance of plant communities for shaping fungal distributions, the expected shifts in alpine plant communities due to climate change could have large effects on fungal biogeography. One prevalent shift in alpine plant communities is that of woody plants, mainly shrubs and trees, expanding into historically herbaceous-dominated alpine grasslands and fellfields (Cannone, Sergio, & Guglielmin, 2007; Myers-Smith et al., 2011). Woody plant encroachment can occur through a variety of global change drivers including warming temperatures, altered precipitation and changes in grazing regimes. Because fungi are the primary decomposers of woody and other recalcitrant plant material, shifts in plant communities from herbaceous to woody species are likely to have strong impacts on fungal diversity and community structure (Bardgett, Hopkins, & Usher, 2005; De Boer, Folman, Summerbell, & Boddy, 2005; Harmon et al., 1986; Nielsen, Wall, & Six, 2015). Shifts from herbaceous to woody plant cover may directly impact fungal communities by altering the quantity and quality of litter substrates, and indirectly by affecting the abiotic soil environment including carbon and N pools, pH and water availability (Archer, Boutton, & Hibbard, 2001; Hollister, Schadt, Palumbo, James Ansley, & Boutton, 2010). In arctic tundra, Ascomycota and Chytridiomycota were more abundant in grass tussock soils than in shrub soils, while Zygomycete and Basidiomycete fungi were more abundant in shrub soils (Wallenstein, McMahon, & Schimel, 2007). This is likely due to higher levels of woody and lignin-rich litter in shrub soils, which promotes saprotrophic wood decomposer fungi common to the Basidiomycota (Boddy & Watkinson, 1995). Because of the similarities in the "shrubification" of arctic and alpine ecosystems with global climate and land use change (Myers-Smith et al., 2011), we may expect similar patterns in fungal communities under alpine shrub expansion scenarios.

Finally, interactions among members within microbial communities have become increasingly recognized as an important

determinant of microbial community structure that is often missing from traditional analyses (Cordero & Datta, 2016; Little, Robinson, Peterson, Raffa, & Handelsman, 2008; Wardle, 2006). Both negative interactions such as resource competition and chemical antagonism and positive interactions including complementarity in enzyme production can be important drivers of community assembly and spatial aggregation of soil fungi (Bell, Callender, Whyte, & Greer, 2013; Gessner et al., 2010). Further, the stress-gradient hypothesis (SGH) is beginning to be applied to microbial interactions in soil communities and proposes that interactions between microbial taxa shift from competitive (negative) to facilitative (positive) as the abiotic stress of the soil environment increases (Callaway & Walker, 1997; Li et al., 2013; Maestre, Callaway, Valladares, & Lortie, 2009). Indeed, in biological soil crusts, interactions among microbial species were more neutral to positive in nutrient-poor soils but shifted to strongly competitive as nutrient availability increased (Li et al., 2013). In alpine environments, facilitation among plant species in response to severe abiotic conditions is a well-established driver of plant community structure (Anthelme, Cavieres, & Dangles, 2014; Cavieres, Hernández-Fuentes, Sierra-Almeida, & Kikvidze, 2016). Soil microbial communities in alpine soils may similarly tend towards positive interactions; however, interactions among microbial taxa are still poorly understood, particularly within natural communities (Bell et al., 2013). How these interactions may change over abiotic stress gradients and with global change is an important next step in microbial ecology.

Overall, this study aimed to (i) determine whether the diversity and community composition of soil fungi vary across elevation gradients and to (ii) assess the impact of woody shrub expansion on these patterns. Alpine environments contain steep elevation gradients that offer a unique opportunity to understand how soil organisms respond to variability in both climate and vegetation (Sundqvist et al., 2013). We tested three primary hypotheses: (i) fungal diversity decreases and community composition changes with increased elevation in alpine soils; (ii) vegetation more strongly influences fungal diversity and community structure than abiotic soil parameters as soil fungi are closely related to plant identity; and (iii) interactions among fungal taxa will further shape community structure and positive interactions will be more prevalent than negative interactions due to high abiotic stress in alpine soils.

To test these hypotheses, we combined observational field data with a manipulative shrub removal experiment along an elevation transect of alpine shrub expansion. We utilized next-generation sequencing and joint distribution modelling to tease apart effects of the environment and intracommunity interactions on soil fungi.

2 | MATERIALS AND METHODS

2.1 | Soil sampling

Soils were sampled in August 2015 at the peak of the growing season in the White Mountains of California, near Crooked Creek (3,094 m; 37°29′56″N, 118°10′19″W) and Barcroft (3,800 m; 37°34′59″N, 118°14′14″W) research stations. This mountain range runs up the far eastern side of California into Nevada and flanks the western edge of the Great Basin. It has a cold and dry climate receiving 150–450 mm of precipitation annually. Mean annual temperature and precipitation at the two ends of our sampling transect are 0.9°C and 327 mm at Crooked Creek Station and −1.7°C and 456 mm at Barcroft Station (Hall 1991). Sampling took place within a transition zone from subalpine sagebrush steppe into alpine fellfields dominated by prostrate cushion plants and perennial bunchgrasses. As described by Taylor (1976) and Travers (1993), plant communities here include *Artemisia* shrubland at low elevations and a mixture of *Trifolium andersonii* and *Carex* sp.–*Eriogonum ovalifolium* communities at high elevations. Artemisia shrubland (below 3,657 m elevation) contains seventeen plant species with the three most common being *Trifolium andersonii*, *Leptosiphon nuttallii* and *Koeleria macrantha*. *Trifolium andersonii* communities have a very similar species composition to *Artemisia* shrubland but with only 12 plant species present and no shrubs. *Trifolium andersonii and Carex incurviformis* are the two most common species. Finally, *Carex* sp.–*Eriogonum ovalifolium* communities have very low species diversity and are dominated by *Carex incurviformis* interspersed with *Eriogonum ovalifolium*.

We sampled under and outside sagebrush canopies, and in 1-m^2 sagebrush removal plots where shrubs were cut at the base of the stem and trimmed back yearly since 2011 at three elevation sites: 3,200, 3,500 and 3,800 m (however, sagebrush removal plots were only at 3,200 and 3,800 m elevations). This elevation gradient spans the observed sagebrush range expansion from subalpine (<3,500 m) to alpine (>3,500 m) areas over the last 50 years (Kopp & Cleland, 2014). In 1961, *A. rothrockii* was not present at the 3,800-m site and was found in moderate-to-low densities at the 3,500-m site, while the subalpine (3,200 m) site had historically high sagebrush cover (Mooney, St. Andre & Wright 1962; Kopp & Cleland, 2014). Therefore, this elevation gradient can be considered a chronosequence, spanning a gradient from historically continuous cover of sagebrush at low elevations to recently established patches at high elevations. All sampling locations have granitic soils and east/southeast-facing slopes to control for edaphic and aspect variation. Two replicate soil cores (1.3 cm diameter × 10 cm deep) were collected from directly under and outside five sagebrush individuals at each elevation site. In addition, two replicate soil cores were taken from five sagebrush removal plots at the low (3,200 m)- and high (3,800 m)-elevation sites. Soil was placed in sterile specimen cups and stored at −80°C prior to analysis.

For soils characterizing nonshrub communities, cores were taken between 1 and 5 m from the edge of each sagebrush canopy, based on the sagebrush density at each site and distance to the next closest shrub canopy. We aimed to sample at distances outside the direct influences of the sagebrush species. For shrub removal plots, only aboveground sagebrush biomass was removed to prevent significant disturbance to soil structure. Removal plots examined whether sagebrush has long-lasting effects on soil fungi or whether fungal communities are able to recover quickly to a preshrub composition.

2.2 | Soil abiotic properties

Volumetric water content (VWC) and pH were measured at the same time and location of each soil core with a Campbell Scientific HS2 Hydrosense II probe (Campbell Scientific, Logan, UT, USA) and an Extech PH100 ExStik pH meter (Extech instruments, Nashua, NH, USA) at 10 cm depth. Total organic carbon and nitrogen (TOC, TON) for each soil sample were calculated using 5 g of field moist soil and 0.5 M K_2SO_4 extraction through a 1.2-μm glass fibre filter (Thomas C5500; Thomas Scientific, Swedesboro, NJ). Extracts were shipped overnight and analysed on a Shimadzu TOC-L autoanalyzer (Shimadzu Scientific Instruments, Inc., Carlsbad, CA) at the EcoCore Analytical Facility at Colorado State University, Fort Collins, CO. We also measured microbial biomass C and N from the same samples using chloroform fumigation–extraction (Brookes, Landman, Pruden, & Jenkinson, 1985). We subtracted unfumigated TOC/TON from paired fumigated samples and divided it by the kEC and kEN coefficients of 0.45 and 0.69, respectively (Joergensen & Mueller, 1996; Wu, Joergensen, Pommerening, Chaussod, & Brooks, 1990).

2.3 | Molecular analyses

We extracted microbial DNA from 0.25 g of soil (±0.025 g) using a MO BIO PowerLyzer PowerSoil DNA Isolation Kit (MO BIO Laboratories Inc., Carlsbad, CA, USA) and quantified the extracted DNA using a NanoDrop 2000 (Thermo Fisher Scientific Inc., Wilmington, DE, USA). We used modified versions of the universal fungal primers ITS1F and ITS2 described in Smith and Peay (2014) improved as part of the Earth Microbiome Project (Walters et al., 2015). While currently considered the most accurate for species identification of fungi, these primers do have certain limitations, particularly low resolution for arbuscular mycorrhizal fungi (*Glomeromycota*) (Öpik, Davison, Moora, & Zobel, 2014; Schoch et al., 2012) and potentially poor phylogenetic resolution (Lindahl et al., 2013; Yarza, Yilmaz, Panzer, Glöckner, & Reich, 2017).

2.4 | PCR

We performed PCR amplification in 25 μl reactions including 1 μl of 10 μM for each primer (forward and reverse), 1 μl DNA, 12.5 μl of Taq 2X Master Mix (New England Biolabs) and 9.5 μl diH2O. Thermocycler settings were 94°C for 3 min, followed by 35 cycles of 94°C for 45 s, 50°C for 60 s and 72°C for 90 s, followed by 72°C for 10 min. Forward primers contained unique 12-base Golay barcodes as described in Walters et al. (2015) (see also Hamady, Walker, Harris, Gold, & Knight, 2008). We then did PCR clean-up using a NucleoSpin Gel-Extraction kit (Macherey-Nagel GmbH & Co. KG). Purified samples were pooled in equimolar concentrations and sequenced in a multiplexed 2- × 150-bp paired-end sequencing run on the Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA) at the University of California Riverside (UCR) Genomics Core Facility.

2.5 | Bioinformatics

Sequences were demultiplexed and processed with the split_libraries_fastq.py from QIIME 1.9.1 (Caporaso et al. 2010). OTUs were generated from the forward ITS1 reads using open reference OTU assignment implemented in pick_open_reference_otus.py (QIIME 1.9.1) using UCLUST (Edgar, 2010) and comparing to the v7 (ver7_dynamic_s_22.08.2016) database of UNITE (Kõljalg et al., 2005). OTUs were assigned taxonomy using QIIME BLAST at 97% similarity as defined by the UNITE v7 database. BIOM table files were generated from OTU tables and diversity analyses performed with QIIME core_diversity_analyses.py script where samples were rarefied to 10,000 sequences per sample for alpha and beta diversities. For analysis of differential abundance of taxa (HMSC; see below), samples were normalized using cumulative sum scaling (CSS) in the metagenomeSeq (BIOCONDUCTOR) package in R.

2.6 | Statistical analyses

Alpha and beta diversities were calculated using outputs from the core_diversity_analyses.py function in QIIME v1.9.1. For alpha diversity, we used both the number of observed OTUs (richness) and the Chao1 diversity metric for each sample. We used linear mixed-effects models to test the relationship between alpha diversity (Chao1 diversity and OTU richness) and elevation, vegetation type and abiotic soil parameters. For abiotic models, only TOC was used for soil nutrients, as TOC and TON were correlated across samples ($r = .56$, $p < .001$). All the above predictors were included as fixed effects, while core replicate pair was included as a random effect of sampling location within sites. Models were fit using the "lmer" function in the LME4 package in R (R Core Team 2015). Models were assessed individually for significance of parameters and pairwise comparisons were run on significant predictors using a Tukey's post hoc test in the "glht" function of the MULTCOMP package in R. In addition, because many abiotic variables covary with both elevation and vegetation type (Figure S1), we used a model selection approach for these parameters, assessing delta AIC of partial and full models via the "AICtab" function in the BBMLE package in R. Models are described in Table 1.

Beta diversity (community composition) was assessed using non-metric multidimensional scaling (NMDS) of the Bray–Curtis dissimilarity metric and permutational multivariate analysis of variance (PERMANOVA) in the vegan function "adonis" in R (999 permutations; Oksanen, Blanchet, Kindt, Legendre, & O'Hara, 2016). Vegetation type, elevation and their interaction were included as predictor variables and checked for within-group heterogeneity using the vegan functions "betadisper" and "permutest." Additionally, abiotic parameters (TOC, pH, VWC) were tested in separate models and checked for heteroscedasticity of predictors using a Breusch–Pagan test. Elevation was used as a blocking variable (strata) to restrict permutations to within sites, and the relative influence of abiotic parameters vs. vegetation type was assessed using an interaction term (Table 1).

TABLE 1 Model structure for alpha- and beta-diversity analyses. Linear mixed-effects models were used for alpha diversity (Chao1) and richness (observed_otus) via the function "lmer." Elevation, vegetation type, their interaction and abiotic soil variables were considered fixed effects and core replicate pair (core) was included as a random effect of sampling location within sites. Alpha diversity models are listed in order of best fit using delta AIC. Beta diversity (community composition) was assessed using nonmetric multidimensional scaling (NMDS) of the Bray–Curtis dissimilarity metric (bray) and permutational multivariate analysis of variance (PERMANOVA) via the function "adonis." Elevation, vegetation type and their interaction were used as predictor variables. For abiotic models, elevation was used as a blocking variable (strata) to restrict permutations to within sites, and the relative influence of abiotic parameters vs. vegetation type was assessed using an interaction term. Beta diversity models are listed in order of best fit using R^2 values

Alpha diversity		**ΔAIC**	**R^2**	***df***
Full model	chao1~ soil.pH + VWC + TOC + vegetation*elevation + core	0	.20	13
	observed_otus ~ soil.pH + VWC + TOC + vegetation*elevation + core	0	.273	13
Interaction	chao1 ~ vegetation*elevation + core	22.1	.167	10
	observed_otus ~ vegetation*elevation + core	19.1	.254	10
Abiotic variables	chao1~ soil.pH + VWC + TOC + core	61.1	.152	6
	observed_otus ~ soil.pH + VWC + TOC + core	57.9	.202	6
Elevation	chao1 ~ elevation + core	67.2	.088	5
	observed_otus ~ elevation + core	62.5	.154	5
Vegetation type	chao1 ~ vegetation + core	70.4	.026	5
	observed_otus ~ vegetation + core	68.5	.038	5
Beta diversity			**R^2**	***df***
Elevation	bray ~ elevation		.126	2
Vegetation type	bray ~ vegetation		.087	2
Interaction	bray ~ elevation*vegetation		.081	3
Abiotic variables	bray ~ VWC*vegetation, strata = elevation		.07	1
			(.054–int)	2
	bray ~ TOC*vegetation, strata = elevation		.047	1
			(.046–int)	2
	bray ~ soil.pH*vegetation, strata = elevation		.022	1
			(.038–int)	2

2.7 | Joint distribution models

CSS-normalized read abundance of fungal OTUs at different taxonomic levels was analysed using multivariate, joint distribution models (HMSC package in R; Ovaskainen et al., 2017). This approach uses a hierarchical Bayesian framework to fit a joint distribution model to occurrence and/or abundance data from multispecies communities. This approach is increasingly favoured for analysing plant and animal community data but is just beginning to be used for microbial community (sequencing) data (Aivelo & Norberg, 2017). The primary motivation for these models is to simultaneously quantify the importance of environmental filtering (abiotic factors), biotic filtering (species interactions) and neutral processes (random effects) for shaping species distributions and structuring communities (Ovaskainen et al., 2017). Specifically, these models estimate fixed effects of environmental covariates, positive and negative species associations via a covariance matrix, and random effects based on study design.

We ran these community models using CSS-normalized read abundance data aggregated at the fungal class, order and family levels. We included elevation, vegetation type, soil pH, VWC, TOC, TON and microbial biomass C and N as fixed effects, and specified soil core replicate and sampling location ("block") as random effects. We used the default (flat) priors and Gibbs sampler as described in the supporting information of Ovaskainen et al. (2017) and ran models with a Gaussian distribution. MCMC chains were run for 10,000 iterations with the first 1,000 discarded and the remainder thinned for a total of 900 posterior samples. We checked for model convergence using visual assessment of trace plots and used the posterior distributions of each environmental covariate to calculate the probability that it was different from zero. We considered parameters to be "significant" when their posterior probabilities had a >90% probability of being different from zero ($p < .1$). We calculated the relative proportion of the total model variance that could be attributed to each of our fixed and random effects using the "variPart" function in the HMSC package. Finally, we estimated residual taxon associations using the "corRandomEff" function, which calculates pairwise correlation (r) matrices for all taxa. These associations represent the positive or negative associations among taxa after having accounted for environmental effects and may be influenced by both direct interactions among taxa and common responses to unmeasured environmental variables.

3 | RESULTS

3.1 | Molecular sequencing

Sequencing of soil fungal communities via the ITS1F and ITS2 yielded 1,590,851 total sequences and an average sequencing depth

of 28,924 reads. Overall, these sequences made up 12 phyla, with Ascomycota making up the largest percentage (59.7%), followed by Basidiomycota (20.8%), unidentified fungi (8.5%), Zygomycota (2.9%), Chytridiomycota (0.3%), Glomeromycota (0.3%), Protists (Cercozoa) (0.5%) and Microsporidia-like organisms (Rozellomycota) (0.5%). Seven percent of the total sequences had no blast hit, so taxonomy could not be assigned.

3.2 | Alpha diversity

Fungal diversity and OTU richness decreased significantly with elevation; however, elevation effects on richness were stronger than diversity (diversity: $df = 30.39$, t-value = −2.063, $p = .047$; richness: $df = 32.76$, t-value = −2.555, $p = .015$). The low-elevation (3,200 m) site had significantly higher richness than both the middle (3,500 m)- and high (3,800 m)-elevation sites, while the latter two sites were not significantly different from each other (3,500 vs. 3,200: z-value = −2.531, $p = .031$; 3,800 vs. 3,200: z-value = −2.555, $p = .028$; Figure 1) and Chao1 diversity was slightly lower at the high- vs. low-elevation site (3,800 vs. 3,200: z-value = −2.063, $p = .097$).

Vegetation type (shrub, shrub interspace, shrub removal) influenced fungal richness and diversity most at the high-elevation site, where shrub soils had overall lower richness ($df = 22.97$, t-value = −3.310, $p = .003$; Figure 1) and diversity ($df = 21.610$, t-value = −2.688, $p = .013$) and lower richness than shrub removal soils (z-value = −2.345, $p = .049$; Figure 1). Within vegetation types, shrub soils at the high-elevation site had lower richness than shrub soils at the low-elevation site (z-value = −2.455, $p = .037$; Figure 1) and shrub interspace soils at the middle-elevation site had lower richness than shrub interspace soils at the low-elevation site (z-value = −2.380, $p = .045$; Figure 1).

For abiotic predictors, the full model incorporating VWC, TOC and soil pH with elevation and vegetation type was the strongest model for both diversity and richness, and it was significantly better than the elevation x vegetation interaction model (ΔAIC: 22.1 diversity, 19.1 richness; Table 1). Additionally, the model including all abiotic parameters was significantly better than both elevation-only and vegetation type-only models for diversity and richness (ΔAIC>>2; Table 1). No single abiotic parameter was a significant predictor of alpha diversity alone.

3.3 | Beta diversity–community composition

Fungal community composition varied significantly across the elevation gradient. Beta diversity (Bray–Curtis dissimilarity) varied by vegetation type, elevation and their interaction (vegetation: $df = 2$, $F = 2.92$, $p = .001$, $R^2 = .087$; elevation: $df = 2$, $F = 4.19$, $p = .001$, $R^2 = .125$; interaction: $df = 3$, $F = 1.79$, $p = .001$, $R^2 = .081$, respectively; Figure 2). Across vegetation types, shrub soil community composition was different from both interspace soils ($p = .001$) and shrub removal soils ($p = .001$); however, shrub removal soils were not different than shrub interspace soils. Across elevations, low-elevation soils differed from both middle ($p = .001$)-and high ($p = .001$)-elevation soils, and middle-elevation soils also differed from high-elevation soils ($p = .001$).

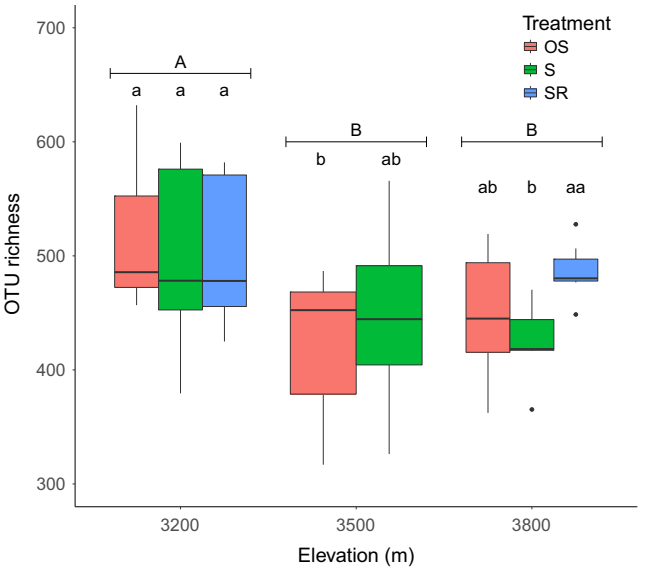

FIGURE 1 Alpha diversity (OTU richness) results by vegetation type (OS = shrub interspace; S = shrub; SR = shrub removal) at three elevation sites (3,200, 3,500 and 3,800 m asl). Capital letters denote significant differences between elevation sites, while lowercase letters denote significant differences between vegetation types within a site and for the same vegetation type across sites

For abiotic drivers, VWC and TOC were significant predictors of fungal community composition (VWC: $df = 1$, $F = 4.379$, $p = .001$, $R^2 = .070$; TOC: $df = 1$, $F = 2.755$, $p = .001$ $R^2 = .047$), while soil pH was not. In addition, both VWC and TOC had significant interactions with vegetation type on beta diversity, but the effect of vegetation type was stronger than either abiotic variable (VWC int: $df = 2$, $F = 1.686$, $p = .004$, $R^2 = .054$; TOC int: $df = 1$, $F = 2.755$, $p = .001$, $R^2 = .047$).

3.4 | Joint distribution modelling

Joint distribution models fit using the HMSC package provided information on the relative abundance of different fungal taxa in soils across our elevation and shrub expansion gradient. At the class level, relative abundance of different fungal taxa did not differ across elevation or vegetation types. At finer scales, the order Phyllachorales, a group commonly known to be foliar parasites (Silva-Hanlin & Hanlin, 1998), was more abundant in shrub interspace soils ($p = .098$). The order Rhizophlyctidales, a soil-inhabiting, cellulose-degrading chytrid (Letcher et al., 2008), was more abundant in shrub soils ($p = .083$). The corresponding families Phyllachoraceae and Rhizophlyctidaceae were also more abundant in interspace and shrub soils, respectively ($p = .11$, .102), although these probability estimates were slightly higher than our proposed cut-off (Tables 2 and S1).

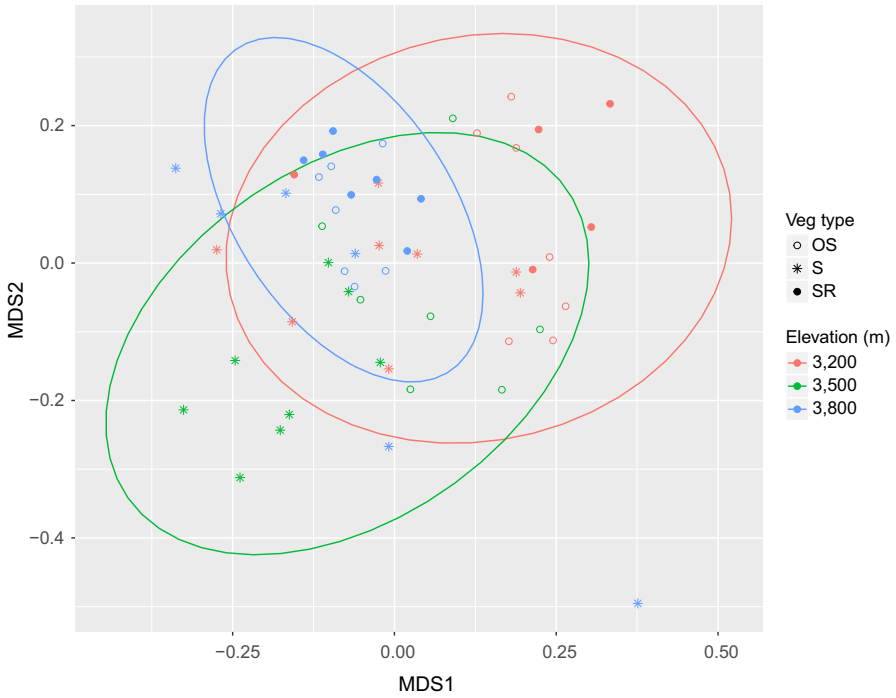

FIGURE 2 Nonmetric multidimensional scaling (NMDS) of community dissimilarity (Bray–Curtis) of soil fungi (stress value = 0.145). Each point corresponds to a soil sample collected from one of three vegetation types (shape) or elevation sites (colour). Points which are close together signify samples with similar fungal community composition. Coloured ovals represent 95% confidence intervals of sample ordination grouped by elevation

Family-level models also revealed that the Pucciniaceae, a Basidiomycete rust fungal pathogen, were more abundant in shrub interspace soils (p = .07), while the Pluteaceae, a family in the Agaricomycota closely related to Amanita, were more abundant in shrub soils (p = .095). In shrub removal soils, both Lachnocladiaceae and Auriscalpiaceae, two families in the Russulales order of Agaricomycota, had higher relative abundance (p = .1, .08), as well as the Thelotremataceae, a lichenized Pezizomycotina (p = .07) (Tables 2 and S1).

Across elevation, there was an increase in relative abundance for the family Botryobasidiaceae, an Agaricomycete of the Cantharellales order (p = .054). In addition, this family and closely related Cantharellales (family incertae sedis) increased in relative abundance with higher pH (p = .05, .077) (Tables 2 and S1).

3.5 | Variance partitioning

Variance partitioning of the relative abundance of different fungal classes revealed that random effects including sampling location (block) and core replicate (core) explained the majority of variation in the data. Block explained between 34% and 99% of the variation dependent on fungal class with an average of 74%, while core explained between 0.4% and 37% of the variance with an average of 10% (Figure 3, Table S2). Overall, fixed effects including elevation, vegetation type and biotic and abiotic soil parameters explained between 1% and 50% of total variation (Figure 3, Table S2). Vegetation type explained ~3.6% of the variation (shrub and shrub interspace only), while elevation explained ~2%. Other biotic and abiotic soil parameters explained on average 2% of the variation in the data, with microbial biomass N explaining the

TABLE 2 Description of significant fungal families from the HMSC analysis and relevant citations

Fungal family	Larger taxonomic group	Increased relative abundance in	Known function	Citation
Pucciniaceae	Basidiomycota (Pucciniomycetes)	Shrub interspace	Rust pathogen	James et al. (2006)
Phyllachoraceae	Ascomycota (Sordariomycetes)	Shrub interspace	Foliar parasite	Silva-Hanlin and Hanlin (1998)
Rhizophlyctidaceae	Chytridiomycota (Chytridiomycetes)	Shrub	Cellulose degradation	Letcher et al. (2008)
Pluteaceae	Basidiomycota (Agaricomycetes)	Shrub	Saprotroph, litter decomposition	Justo et al. (2011)
Lachnocladiaceae	Basidiomycota (Agaricomycetes)	Shrub removal	Wood decomposition	Cannon and Kirk (2007)
Auriscalpiaceae	Basidiomycota (Agaricomycetes)	Shrub removal	Saprotrophic, wood decomposition	Larsson and Larsson (2003)
Thelotremataceae	Ascomycota (Lecanoromycetes)	Shrub removal	Lichenized	Mangold, Martín, Lücking, and Lumbsch (2008)
Botryobasidiaceae	Basidiomycota (Agaricomycetes)	High elevation	Wood, litter decomposition	Larsson (2007)

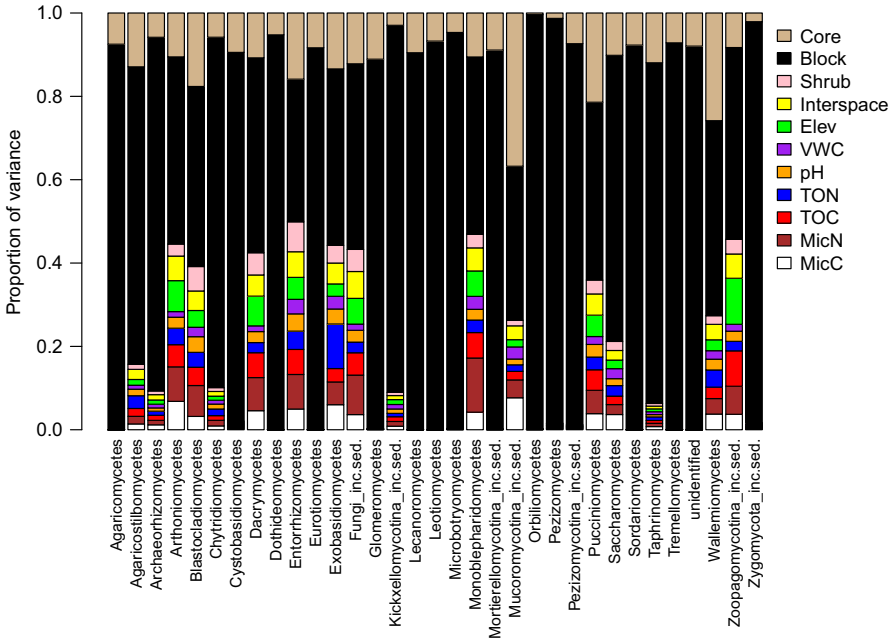

FIGURE 3 Results of variance partitioning for the variation in fungal relative abundance (at the class level) in response to vegetation type (shrub and interspace), elevation, soil pH, VWC, TOC, TON and microbial biomass C and N. Core replicate and sampling location ("block") were included as random effects

most (~2.7%) and volumetric water content (VWC) explaining the least (~1%).

3.6 | Taxon associations

After accounting for fixed effects, there were significant positive and negative associations among individual fungal taxa. At a correlation (*r*) level of ± .3 or greater, 25 fungal orders showed varying positive and negative relationships (Figure 4). Out of these 25, 10 fungal orders had correlations (*r*) of ± .4 or greater. Specifically, the Wallemiales order was negatively correlated with four other taxa including Pezizomycotina (inc. sedis), Mytilindiales, Hymenochetales and Arthoniales, as well as positively correlated with Myriangiales and Amylocorticales. The Pezizomycotina (inc. sedis) were positively correlated with four other taxa including Mytilinidiales, Diversisporales, Coniochaetales and Agaricostilbales (Figure 4, Table S3).

4 | DISCUSSION

In this study, we assessed how elevational patterns in soil fungal diversity and community composition are altered by global change-driven shrub expansion in an alpine environment. We found at least partial support for our three hypotheses. First, we observed that fungal diversity declined and community composition shifted with elevation as has been demonstrated in other alpine research. Next, both vegetation type and abiotic soil parameters were important predictors of fungal alpha diversity and community composition. Vegetation type however was a better predictor of beta diversity, explaining more variation than any abiotic parameter. Finally, we found both positive and negative associations among fungal taxa after controlling for environmental covariates. Positive associations were more common, implying that facilitation, and to a lesser degree competition, may mediate how fungal communities are structured and adapt to abiotic stress in alpine soils. Understanding how soil fungal communities respond to global change, both directly through abiotic controls and indirectly through plant species range shifts, will be critical as alpine ecosystems continue to undergo rapid warming and land use changes.

4.1 | Alpha and beta diversities

Fungal diversity, including the Chao1 index and OTU richness, declined with increasing elevation, exhibiting the strongest decline from the subalpine (3,200 m) to alpine sites (3,500, 3,800 m). Beta diversity (community composition) was also distinct among elevations, and high-elevation soils had less variation in community composition compared to low-elevation sites (reflected in the width of respective circles in the NMDS plots; Figure 2). These results are in accordance with previous studies highlighting that fungal diversity declines with increasing elevation (C Körner, 2003; Sundqvist et al., 2013). Elevation, in and of itself, is not a mechanistic driver (Christian Körner, 2007), but is nonetheless useful in determining large-scale patterns in fungal communities in response to biotic and abiotic factors. Instead, a decline in plant species diversity and biomass at high elevations is a proposed mechanism for changing fungal diversity across elevation gradients (Tedersoo et al., 2014). Indeed, the plant community at our study sites changes from more speciose subalpine *Artemisia* shrubland at low elevations to lower diversity alpine grasslands at high elevations, including the *Carex* sp.–*Eriogonum ovalifolium* and *Trifolium andersonii* vegetation communities (Taylor 1976, Travers 1993).

Vegetation type and abiotic soil properties also influenced fungal diversity and community composition, and their relative importance

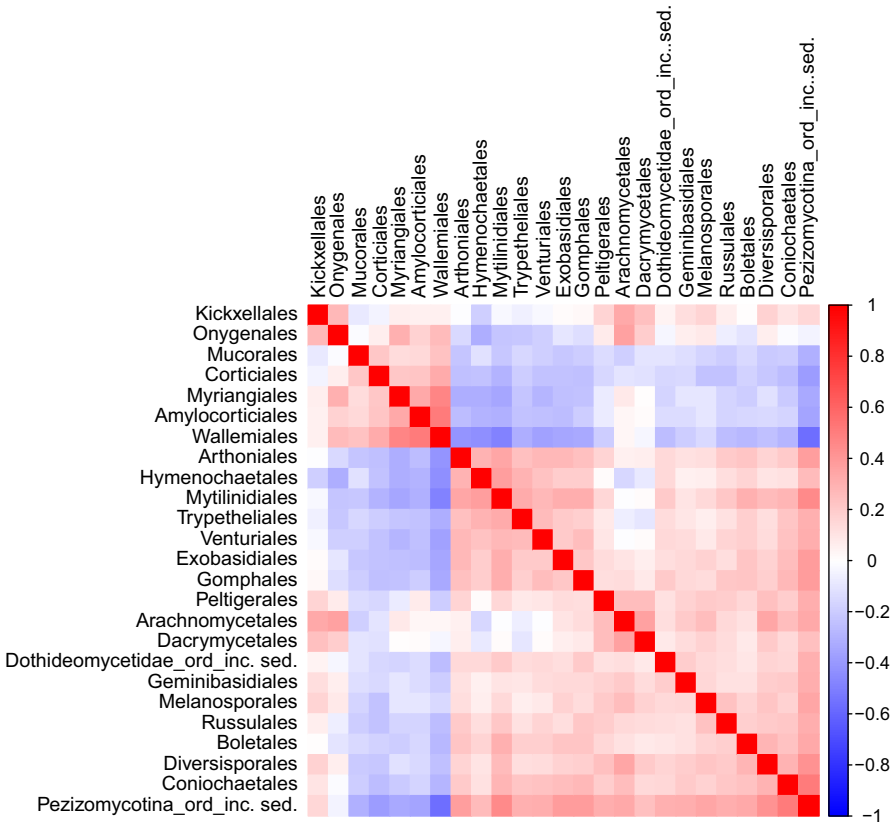

FIGURE 4 Estimates of associations among fungal taxa (at the order level) based on residual correlations after accounting for fixed predictors. Taxa have a positive (red), negative (blue) or neutral (white) association with other taxa, and the strength of this association (Pearson correlation coefficient, *r*) is depicted by the shade of the colour. Shown here are the subset of fungal orders (25 total) that had at least one association that was greater than or less than ±0.3. Eighty-five fungal orders did not have any associations meeting this criterion

differed for alpha and beta diversities. Shrub soils at high-elevation sites had lower richness than shrub soils at the low-elevation site, and at the high-elevation site, shrub soils had the lowest OTU richness of all three vegetation types (although not significantly lower than shrub interspace soils). This supports the idea that fungal diversity at high-elevation sites declines even further as shrubs move into alpine areas. Additionally, shrub removal soils had higher alpha diversity than shrub soils, and equally high diversity as shrub interspace soils at the high-elevation site, suggesting that alpine fungal communities can change rapidly in response to plant community shifts.

Among abiotic variables, VWC, TOC and soil pH all influenced alpha diversity in combination, although no abiotic parameters were significant predictors alone. This was confirmed by our model selection approach in which the best model incorporated abiotic parameters, elevation and vegetation type. This model was a significant improvement to the elevation-by-vegetation interaction model, implying that the combined abiotic conditions of the soil environment directly influence fungal community diversity in addition to the influence of elevation and plant community.

Fungal beta diversity was also regulated by VWC in addition to TOC, but VWC was a better predictor, explaining a higher proportion of model variation (7% vs. 4.7%). VWC significantly increases with elevation along our transect, primarily due to increased precipitation as snow at high elevations; therefore, soil moisture likely plays a key role in the observed diversity–elevation trend in soil fungi. Hawkes et al. (2011) found that fungal diversity decreased with increased precipitation in a rainfall manipulation experiment in California grasslands. We see similar results for both alpha diversity and beta diversity in that low-elevation sites were more taxonomically diverse within and across sampling locations. Because this is a dry alpine ecosystem, drought is common, particularly in subalpine sites with low annual precipitation. This abiotic stress may ameliorate competition and promote coexistence among different fungal groups, thereby increasing overall taxonomic diversity and diversity across the landscape (Hawkes et al., 2011). Another potential mechanism is that dry soil increases heterogeneity of the soil matrix via decreased connectivity among soil pores. This may increase resource hot spots and diversity of niches, promoting more distinct fungal communities across sites (Classen et al., 2015; Frey, 2007).

There is reasonable evidence that fungi are more closely associated with plant species identity than other microbial groups, particularly bacteria, which are predominantly regulated by abiotic soil properties (Cassman et al., 2016; Nielsen, Osler, Campbell, Burslem, & van der Wal, 2010). This is likely due to the major role that soil fungi play in plant litter decomposition, especially because fungi produce lignin-degrading enzymes absent in most bacteria (De Boer et al., 2005; Hammel, 1997; Thorn & Lynch, 2007). Our data partially supported this hypothesis, with vegetation type being a better predictor (higher R^2) of fungal community composition than any abiotic factor alone. Abiotic soil parameters including soil moisture and organic nutrients (but not pH) also interacted with vegetation type in their influence on fungal community structure, suggesting indirect effects of plants on soil fungi via shifts in the soil environment. In a previous study, we found that both soil moisture and TOC/TON are

enhanced in soils below sagebrush canopies and that this indirectly affects soil bacterial diversity and richness (Collins, Carey, Aronson, Kopp, & Diez, 2016). Similarly, our data suggest that shrub expansion may affect fungal community structure through shifts in soil organic nutrient pools, likely resulting from the accumulation of low-quality woody litter. In addition, enhanced soil moisture below shrub canopies may further promote decomposition of soil organic matter and impact fungal community composition in this arid environment. Alpine cushion plants can similarly influence soil fungal communities via enhanced soil moisture and nutrients as well as buffer fluctuations in soil pH (Roy et al., 2013). We found that water and nutrients had a larger influence than pH on fungal community composition, however each parameter may reflect the significant role of plants on the abiotic soil conditions for fungi.

4.2 | Joint modelling of fungal communities

The amount of taxon-specific distribution data generated from sequencing, combined with the joint distribution modelling approach, offers remarkable new potential to understand what controls the distributions of soil organisms. In particular, we can begin assembling a unique understanding of the relative importance of environmental variation, species interactions and random spatial assembly processes for determining belowground communities.

This approach showed that the relative abundance of particular fungal taxa differed among vegetation types and elevations and that there are significant residual associations (positive and negative) among many taxa. The trends varied by classification level (i.e., class vs. order vs. family). At the class level, no significant trends were detected; however, taxa at the order level, and especially at the family level, had increased relative abundance in soils across elevation and vegetation types. Our ability to detect stronger trends at finer taxonomic scales posits that these broader groups (class, order) contain taxa with distinct environmental responses (Lu et al., 2016) and therefore are not ecologically equivalent. This is likely most relevant for very large classes with many fungal families, such as the Agaricomycetes or Sordariomycetes, which was corroborated in our variance partitioning analysis (below).

Relative abundance of fungal taxa across elevation and vegetation types presented several trends. First, Agaricomycetes and close relatives had higher relative abundance in shrub, shrub removal and high-elevation soils. Agaricomycetes are commonly saprotrophic, wood- or litter-decaying fungi (Lyncht & Thorn, 2006; Zak, Pregitzer, Burton, Edwards, & Kellner, 2011) and also include mycorrhizal species. They are important decomposers in cold, dry environments, as has been shown in arctic studies (Ludley & Robinson, 2008), and are dominant in forest floor communities (Edwards & Zak, 2010). The increased relative abundance of these fungal groups may result from increased woody litter accumulation from shrubs, both above- and belowground, and may provide important substrate for decomposer fungi, particularly at high-elevation sites. This is especially relevant in shrub removal soils as root systems decompose gradually after aboveground sagebrush removal. Shrub removal therefore is likely to promote an initial proliferation in wood decay fungi which will decline over time. By sampling 4 years after shrub removal, we were able to characterize how fungal communities may recover after disturbance.

Next, shrub interspace soils had increased relative abundance of two pathogenic fungal families: the Pucciniaceae, a known plant pathogen of rust fungi, and the Phyllachoraceae, an Ascomycete family of mostly foliar parasites. Higher relative abundance of pathotrophs in shrub interspace soils was consistent across elevations. Members of the family Pucciniaceae are particularly strong plant pathogens which are commonly used as biocontrol for agricultural weeds (Stubbs & Kennedy, 2012). Because shrub interspace plant communities have been historically present in alpine environments, species-specific soil pathogens may have developed over time in the rhizosphere of these plants but have not yet accumulated underneath the newly arrived shrubs (Colautti, Ricciardi, Grigorovich, & MacIsaac, 2004; Diez et al., 2010).

In addition, we observed increased relative abundance of a cellulose-degrading chytrid (Rhizophlyctidaceae) in shrub soils and lichenized Pezizomycotina (Thelotremataceae) in shrub removal soils. The high relative abundance of Rhizophlyctidaceae suggests that shrub soils provide substrates that promote saprotrophic decomposer taxa such as these cellulose-degrading, soil-inhabiting chytrids (Letcher et al., 2008). Additionally, the Thelotremataceae is a large family in the Lecanoromycetes, known to form soil crusts on bare soil surfaces. Sagebrush removal led to high levels of newly exposed soil, which is ideal for lichen establishment. Zumsteg et al. (2012) found this group to be an important colonizer of barren substrate after glacial retreat, revealing its opportunistic life strategy and tolerance of cold, dry environments.

Interpretation of joint distribution model results needs to be made cautiously however, as read abundances of fungal OTUs are normalized relative to the sequence count within a given sample. While CSS-normalized read abundances account for several common issues including amplification biases and undersampling (Paulson, Stine, Bravo, & Pop, 2013), any attempt to estimate true biological abundance from sequence read abundance is imperfect (Weiss et al., 2017). Nonetheless, careful use and interpretation of these differential abundance data can provide useful insights into how environmental variation affects microbial composition (Ghanbari, Shahraki, Kneifel, & Domig, 2017; Timonen et al., 2017).

4.3 | Variance partitioning

Despite the significant effects of measured predictors on fungal communities, variance partitioning revealed that sampling location "block" was a substantially better predictor of the relative abundance of fungal groups than any measured environmental covariate. In addition, replicate core pair was the second best predictor of relative group abundance, suggesting that the particular spatial location within the landscape is more influential than abiotic soil properties, plant community or elevation. These results suggest that there is remarkable heterogeneity in the relative abundance of fungal taxa at

the landscape scale, which may be related to both small microsite variation in environmental variables and processes such as dispersal limitation and priority effects. Feinstein and Blackwood (2012) also found high spatial variation in forest floor fungal communities and little explanatory power of plant traits or plant species identity. Rather, neutral models (zero-sum) had the highest predictive power for species abundance and distribution, indicating the critical role of neutral processes in community assembly of saprotrophic fungi. This parallels observed patterns at a global scale, where community composition of soil fungi is highly variable, with often very few shared OTUs across geographic regions (Meiser, Bálint, & Schmitt, 2014). Nonetheless, fixed effects explained up to half of the total variation in relative abundance for some taxa, so it appears that the relative importance of environmental and stochastic effects may vary among taxa. We found stronger environmental effects for the more narrow or smaller taxonomic groups, such as the Entorrhizomycetes, a fungal class with a single order and family (Figure 3), in which individual members are likely to have more similar environmental responses (Lu et al., 2016).

4.4 | Taxon associations

After controlling for direct responses to environmental variation, many significant positive and negative associations remained among taxa, reflecting either important interactions among fungal groups or common responses to unmeasured environmental variables (Clark, Gelfand, Woodall, & Zhu, 2014; Ovaskainen et al., 2017). As hypothesized, interactions tended to be positive rather than negative (Figure 4), suggesting the importance of facilitative interactions among taxa in this stressful alpine environment. Facilitative interactions among fungi are common during decomposition, including the process by which some taxa break down complex plant tissues (lignin, cellulose) into simpler forms which in turn are decomposed by other taxa (Gessner et al., 2010). For example, in our study the Pezizomycotina (*inc. sedis*) tended to have positive associations with other fungal orders. Pezizomycotina are among the most abundant and diverse group of ascomycete fungi in forest floor communities (Edwards & Zak, 2011) and proliferate during and directly after peak plant biomass in alpine soils (Zinger, Shahnavaz, Baptist, Geremia, & Choler, 2009), offering a potentially important facilitative role for this group of saprotrophic soil fungi (Damon et al., 2010). Although less common, we observed negative interactions among several fungal orders; in particular, the Wallemiales had primarily negative associations with other orders. This small group is comprised of highly xerophilic Basidiomycete fungi (Zalar, Sybren Hoog, Schroers, Frank, & Gunde-Cimerman, 2005). Surviving in very dry environments is a rare trait for Basidiomycota, and establishment of these hyphal forming fungi may prevent colonization by other more common xerophilic taxa, in particular Ascomycetes. Colonization of a substrate (e.g., leaf, piece of wood) by saprophytic fungal taxa may prevent other fungi from utilizing that same substrate, highlighting a negative (competitive) interaction between taxa within a trophic guild (Wardle, 2006). Certainly priority effects of colonizing fungal taxa are influential in structuring subsequent community composition in saprotrophic wood rot communities via either direct spatial exclusion or alteration of resource pools (Hiscox et al., 2015; Maynard et al., 2017). Release of microbial antibiotics or allelochemicals into the surrounding soil matrix is another example of such interaction, common for lichens in particular (Stark, Kytöviita, & Neumann, 2007). Thus, these results suggest that both positive and negative interactions among taxa may help regulate community structure, and positive interactions may help to buffer abiotic stress for soil fungi in this ecosystem. The underlying causes of these associations, including potentially unmeasured environmental factors, will remain uncertain using observational data, but regardless can prove useful in developing further hypotheses about interactions among specific taxa that may then be experimentally tested.

4.5 | Comparison across microbial groups

Because soil bacterial and fungal communities are intricately linked and play synergistic roles in decomposition (De Boer et al., 2005) as well as interactions with plants in the rhizosphere (Artursson, Finlay, & Jansson, 2006), it is important to know how our results compare to other microbial groups, particularly bacteria. Our previous work in this alpine ecosystem has shown that bacterial diversity and community composition are weakly influenced by elevation and that shrub expansion increases bacterial alpha diversity. Shrub expansion also altered bacterial community composition indirectly by causing shifts in abiotic soil parameters including soil moisture and organic nutrients. In addition, pH was a strong driver of bacterial community structure, as has been shown in other research (Lauber, Hamady, Knight, & Fierer, 2009; Siles & Margesin, 2016), although pH was not correlated with elevation or vegetation type (Collins et al., 2016). This contrasts with the patterns observed in fungal communities, in that elevation was a much better predictor of alpha diversity and that shrub expansion created a *decrease* in fungal diversity, particularly at high-elevation sites. Additionally, unlike for bacteria, pH was a relatively unimportant abiotic driver for soil fungi; however, interactions between vegetation type and soil water and nutrients did similarly influence fungal community structure. Finally, both bacterial and fungal communities in this ecosystem showed remarkable community resilience and were able to revert back to similar levels of diversity and community composition after 4 years of shrub removal (Collins et al., 2016).

In other ecosystems, comparisons of soil bacterial and fungal communities across elevation gradients are likewise complex. Siles and Margesin (2016) observed that bacterial diversity decreased from submontane to alpine sites while fungal diversity did not change but the relative abundance of soil fungi increased. Across two subalpine mountain transects in China, bacterial diversity peaked at mid-elevations rather than at either end of the climatic gradient and differences in relative abundance of taxa across the transect were much stronger for bacteria than fungi (Meng et al., 2013; Ren et al., 2018). Due to inconsistencies across studies, it has been argued that bacteria simply do not exhibit the elevation–diversity

patterns present in other eukaryotic organisms (Fierer et al., 2011); however, high spatial heterogeneity within soil sampling locations as well as low sampling intensity within transects may obscure the ability to detect trends across larger elevation gradients (Nottingham et al., 2016; Rowe & Lidgard, 2009). We also observed that sampling location and spatial heterogeneity across the landscape were dominant drivers of soil fungal community structure and abundance, and it is likely that increased sampling intensity could help explain a larger proportion of the variation in these communities.

Although not examined in this study, seasonal fluctuations are another important driver of soil microbial community structure and relative abundance of taxa in alpine environments (Lazzaro et al., 2015). While elevation and vegetation type significantly affect abiotic soil properties, seasonal fluctuations in resources can be equally important predictors of microbial community composition (Lazzaro et al., 2015; Shahnavaz, Zinger, Lavergne, Choler, & Geremia, 2012). Further, bacteria and fungi respond very differently to seasonal events including snowpack, snowmelt and peak growing season (Lazzaro et al., 2015; Zinger et al., 2009). In general, annual cycles of biomass, diversity and turnover of particular taxa are more pronounced for bacteria than for fungi, as fungi tend to be more cold-tolerant and can utilize more recalcitrant plant compounds to maintain their biomass under winter snowpack (Lazzaro et al., 2015; Zinger et al., 2009). If interannual climate variability increases with climate change as projected (Nicholls & Alexander, 2007), these annual cycles may become much less predictable, increasing our need to understand the mechanisms driving diversity and biogeographic patterns of alpine soil microbial communities.

5 | CONCLUSION

Overall, we found support for our hypothesis that soil fungal diversity declines and community composition changes with increasing elevation. In addition, both abiotic factors (particularly soil moisture and soil organic C and N) and woody sagebrush range expansion had significant effects on these patterns. In the context of global change, it is particularly striking that the negative effect of shrubs on alpha diversity was strongest in high-elevation sites where shrubs have only recently colonized. However, fungal communities displayed a relatively rapid ability to recover this diversity after just 4 years of shrub removal. Moreover, the increased relative abundance of saprotrophic Agaricomycete fungi at high elevations portends ongoing changes to soil community function as shrubs continue moving uphill. Nevertheless, while fungal diversity and distribution were significantly affected by vegetation type, elevation and abiotic conditions, the residual spatial variation overwhelmed these fixed effects, highlighting the extreme heterogeneity in fungal communities at the landscape scale. Finally, positive and negative associations between fungal taxa may be important in structuring community responses to environmental change, particularly facilitative interactions in alpine environments. These within-community interactions are difficult to quantify and typically absent in studies of microbial biogeography (Kivlin et al., 2011; Martiny et al., 2006). As more studies integrate sequencing data, manipulative experiments and joint distribution models, we may test more general hypotheses about the nature and importance of these associations and how they are affected by global change.

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AUTHOR CONTRIBUTION

C.G.C. and J.M.D. designed the experiment. C.G.C. and N.P. conducted laboratory analyses of soils prior to sequencing, and J.E.S. and N.P. performed bioinformatics on returned sequences. C.G.C., J.M.D. and S.E.W. performed statistical analyses. C.G.C. carried out field sampling, reviewed relevant literature, created tables and figures and wrote the manuscript. All authors contributed to further manuscript writing and editing.

DATA ACCESSIBILITY

Raw data for all nonmolecular analyses including sampling location, soil nutrient, moisture, pH and microbial biomass may be found in the Dryad repository https://doi.org/10.5061/dryad.rh61t5g. Raw sequences may be found in the NCBI Short Read Archive (SRA) Accession no. SRP133697.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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