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Above- and Below-Ground Consequences of Woody Plant Range Expansion in Alpine Ecosystems

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ABSTRACT OF THE DISSERTATION

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by

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Global change is creating novel communities of organisms around the globe as species shift their geographic ranges to maintain favorable climate conditions. Species range shifts create novel plant species interactions and influence relationships between plants and soil organisms in the new range. One well documented range shift in both arctic and alpine biomes is of woody plants, primarily shrubs, moving upwards in elevation or latitude and establishing in traditionally herbaceous dominated ecosystems. Woody plant range expansion can have significant impacts on belowground ecosystem processes through changes in litter quality and quantity, rooting depth, and interactions with soil microbial communities. In addition, both biotic and abiotic soil conditions can limit the establishment and range expansion of woody plant species. The overarching goal of this dissertation was to understand how plant-soil interactions influence and respond to the range expansion of a sagebrush shrub in the White Mountains of California. I examined multiple belowground processes including soil microbial community structure and function, biogeochemical cycling, and soil feedbacks to other

native plant species. I used field and greenhouse studies, plant demographic monitoring, laboratory assays, functional trait analyses, next generation sequencing and diverse statistical modeling techniques to link ecological processes from the microbial to ecosystem scale. Overall, sagebrush expansion increased bacterial diversity, soil moisture, soil organic carbon and nitrogen pools and microbial biomass, which were all associated with increased microbial activity (substrate induced respiration). Sagebrush did not strongly influence overall fungal diversity but increased the abundance of fungal groups including the saprotrophic Agaricomycete fungi, which are important wood and litter decomposers. Soil microbial communities influenced both the performance and leaf functional traits of sagebrush seedlings through changes in microbial diversity and extracellular enzyme activity, which suggested strong plant-microbial competition for soil nutrients. Finally, sagebrush influenced the demography of two co-occurring herbaceous plant species through negative plant-soil feedbacks which overwhelmed the effects of abiotic facilitation by sagebrush. Taken together the results of this dissertation provide a more complete picture of how multiple aspects of the soil environment both respond to changes in plant community composition, and feedback to influence plant performance and plant species interactions.

Table of Contents

List of Tables

List of Figures

Chapter 4

Introduction

Changes in climate and land use are creating novel communities of organisms around the globe (Tylianakis et al. 2008). This is due largely to species migrations or range shifts, often upwards in latitude or elevation as they attempt to "track" changes in climate (Parmesan et al. 2003, Valéry et al. 2008). Species ranges may become larger (expansion), or smaller (contraction), or simply shift in their distribution along a climate or land use gradient (Sexton et al. 2009, Chen et al. 2011). Range shifts are limited by both environmental and biotic filters as well as species dispersal abilities (HilleRisLambers et al. 2012) and many species who attempt to migrate are unable to do so (Zhu et al. 2012). Species who successfully establish often have significant community and ecosystem consequences in their novel range (Wardle et al. 2011, Alexander et al. 2016, Manrubia et al. 2019).

One prominent example is the global expansion of woody plants into historically herbaceous dominated plant communities (Naito and Cairns 2011). This vegetation conversion is occurring across diverse landscapes including arid grasslands, savannah, artic and alpine ecosystems (Naito and Cairns 2011, Myers-Smith et al. 2011, Saintilan and Rogers 2015). A variety of interacting mechanisms can promote woody plant range expansion including increased temperature and CO_2, prolonged drought and altered fire and grazing regimes (Naito and Cairns 2011, Myers-Smith et al. 2011, Saintilan and Rogers 2015). In addition, range expansions of native plants driven by climate change may benefit from the escape of natural enemies present in their home range, such as

specialized soil pathogens and leaf or root herbivores (Engelkes et al. 2008, Van Grunsven et al. 2010).

Release from species-specific soil pathogens is a touted hypothesis driving the invasion of non-native plant species, however the role of soil enemy release in climate driven range expansions is less understood (Morrien et al. 2010). Engelkes *et al.* (2008) was among the first to show that native plant species moving upwards in latitude were less affected by both insect herbivory and soil pathogens than congeneric natives in the expansion range. A native *Tragopogon* species shifting upwards in latitude also experienced fewer negative soil effects in the expansion range than its home range (Van Grunsven et al. 2010). However, several other studies have found mixed or no support for the enemy release hypothesis in native range expansions (Skou et al. 2011, Mlynarek 2015, Wan and Bonser 2016, Mlynarek et al. 2017). Other factors including lack of appropriate soil mutualists, plant-microbial competition in the rhizosphere, or ability of coevolved soil pathogens to 'catch-up' to the range expanding species may explain this discrepancy (Nuñez et al. 2009, Mlynarek 2015). Therefore, explicitly testing multiple soil microbial mechanisms that may simultaneously affect the successful establishment and spread of range expanding species is crucial.

While several interacting mechanisms can promote or limit woody plant range expansion (aka 'encroachment'), it is also clear that woody plant encroachment results in significant ecosystem impacts, including biotic and abiotic effects on biogeochemical cycles, resident plants and soil microbial communities of newly colonized areas (Jackson et al. 2002, Bühlmann et al. 2014, Yannarell et al. 2014). Increased density and cover of

woody vegetation can affect the cycling of carbon, nutrients, and water via changes in litter quantity and quality, rooting depth, woody biomass production, and other factors (Huxman et al. 2005, Eldridge et al. 2011, Myers-Smith et al. 2011). These changes may create conditions that promote further establishment of woody species and feedback into the global climate system. For example, a global decomposition experiment found that woody plant litter is more recalcitrant than graminoid and forb litter and decomposes more slowly, thereby slowing microbial respiration and soil CO_2 flux into the atmosphere (Cornelissen et al. 2007). Additionally, woody shrubs can trap litter and intercept airborne nutrient rich particulates (Köchy and Wilson 2001) under their canopies while inter-canopy soil is degraded via erosion, creating "islands of fertility" which can promote further woody encroachment (Schlesinger et al. 1990, Ridolfi et al. 2008).

Woody encroachment can also alter soil microbial community structure and function as well as relationships with soil mutualists and pathogens for other (non-shrub) plant species. For example, shrub encroachment into grasslands is associated with increased microbial biomass C, bacterial and fungal community diversity and fungi: bacteria ratios in soils (Liao and Boutton 2008, Hollister et al. 2010, Yannarell et al. 2014). Impacts of woody encroachment on soil microbial communities can differ depending on the microbial group, and most research has compared differences among soil bacteria and fungi (but see Biederman and Boutton, 2009). General patterns suggest that fungi may respond more readily to woody plant encroachment than bacteria, although responses vary among sites and woody plant species (Hollister et al. 2010, Yannarell et al. 2014). Mechanistically, woody plants may alter soil microbial

communities through many pathways such as changes in the quality and quantity of litter substrate entering the soil environment (Cornelissen et al. 2007), altering soil abiotic condition such as temperature, moisture and pH which influence microbial niches (Schimel et al. 2004), or through stimulating microbial activity directly via root exudates or carbon transfer to mycorrhizae (Bengtson et al. 2012). Nonetheless, we still lack a comprehensive understanding of the impacts of woody plant range expansion on soil microbial community structure and function.

Changes in the soil environment caused by woody plant encroachment may feedback on the growth and fitness of neighboring plant species. Plants growing under shrub canopies (grasses, forbs etc.) can have altered mycorrhizal relationships due to shrub-induced changes in soil nutrient availability (Shi et al. 2006) or effects of secondary chemicals from shrub litter (Nilsson et al. 1993, Wardle et al. 1998). Non-native invasive plant species often alter the relative abundance or diversity of soil pathogens and mutualists (Hawkes et al. 2006, Eppinga et al. 2006), or enhance microbial competition for and immobilization of soil nutrients (Craig and Fraterrigo 2017), which can create negative plant-soil feedbacks (PSFs) for other plant species (Klironomos 2002). PSFs play an important role in shaping plant community composition and species coexistence (Revilla et al. 2013), however we compared with non-native invasive plants, we have a poor understanding of how PSFs from native range expanding species may feedback to affect co-occurring plant species in their new range.

Finally, as range expanding species establish in a new area, they compete with resident plant species which can strongly influence species coexistence and productivity

(Körner et al. 2008, Alexander et al. 2015). Woody plant species may compete with herbaceous species in their range expansion zone through increased rooting depth to access soil water, shading plants growing under their canopies or allelopathic mechanisms (Aguiar and Sala 1994, Eldridge et al. 2011). Some of these interactions can also shift towards facilitation depending on the ecosystem, including reducing solar radiation, increasing nutrient and water accumulation below canopies, and insulating from cold temperatures and snow (Schweiger et al. 2015, Macek et al. 2016). Direct plant competition (or facilitation) is likely to interact with PSFs and further influence species coexistence; these concepts have been developed theoretically (Bever 2003) but have not been empirically tested in the context of plant range expansion.

The impacts of range expanding plant species on soil microbial communities is beginning to gain traction as a research topic. Recent reviews have suggested that in general, range expanding status *per se* is not a strong predictor of changes in soil microbial communities (Manrubia et al. 2019, Ramirez et al. 2019), however both of these studies included only herbaceous plant species. Range expanding species of a different plant functional type than the resident plant community is likely to have stronger impacts on soil microbial communities, particularly in the herbaceous to woody (or vice-versa) transition (Wolkovich et al. 2010). Despite the growing interest in the role of soil biota in plant range expansions, many of the relationships between woody plant encroachment and soil microbial community structure and function remain speculative, as direct tests linking these belowground processes are rare (Myers-Smith et al. 2011).

Woody encroachment is occurring in ecosystems across the state of California including shrub expansion into alpine grasslands and fellfields and montane meadows. These type conversions are occurring rapidly, with significant vegetation community shifts being documented in less than 50 years (Kopp and Cleland 2014) and are likely to have significant biotic and abiotic impacts as described above. In particular, Timberline sagebrush (*Artemisia rothrockii)* expansion has been documented in the White Mountains (Kopp and Cleland 2014) moving upwards in elevation from its historic range in subalpine shrublands into high elevation alpine fellfields (Mooney et al. 1962). In areas where sagebrush has expanded, resident grasses, forbs and cushion plants have shown moderate to significant declines in their relative abundances (Kopp and Cleland 2014) however the mechanisms underlying these declines are unknown.

The overarching goal of this dissertation was to understand how plant-soil interactions influence and respond to the range expansion of *Artemisia rothrockii* (sagebrush) in the White Mountains of California. Within this goal, I had four specific research objectives, which correspond to the four chapters of this dissertation: 1) Determine how sagebrush expansion influences soil bacterial community structure and function both directly and indirectly through changes in abiotic soil properties 2) Assess patterns of soil fungal community structure and species interactions across an alpine elevation and sagebrush expansion gradient 3) Test how microbially-mediated plant-soil feedbacks influence sagebrush seedling performance and functional traits in the historic versus expansion range 4) Quantify the influence of sagebrush plant-soil feedbacks and competition on the demography of resident alpine plant species.

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Chapter 1

Direct and indirect effects of native range expansion on soil microbial community structure and function

Abstract

Analogous to the spread of non-native species, shifts in native species' ranges resulting from climate and land use change are also creating new combinations of species in many ecosystems. These native range shifts may be facilitated by similar mechanisms that provide advantages for non-native species and may also have comparable impacts on the ecosystems they invade. Soil biota, in particular bacteria and fungi, are important regulators of plant community composition and below-ground ecosystem function. Compared to non-native plant invasions, there have been relatively few studies examining how soil biota influence—or are influenced by—native species range shifts. Here, we examine how a native range-expanding sagebrush species (*Artemisia rothrockii*) affects below-ground abiotic conditions and microbial community structure and function using next-generation sequencing coupled with other biotic and abiotic soil analyses. We utilized a range-expansion gradient, together with a shrub removal experiment and structural equation models, to determine the direct and indirect drivers of these interconnected processes. Sagebrush colonization increased bacterial and archaeal richness and diversity and altered community composition across the expansion gradient. Soil organic C, N, and soil moisture increased with sagebrush presence; however, results varied across the expansion gradient. We found no relationship between sagebrush and soil pH, however pH strongly influenced microbial richness and diversity. Microbial

(substrate induced) respiration was influenced by soil organic N, as well as microbial diversity and functional group relative abundances, highlighting direct and indirect effects of sagebrush on microbial community structure and function. Microbial community composition of soils after 4 years of sagebrush removal was more similar to communities in shrub interspaces than underneath shrubs, suggesting microbial community resilience. Our results suggest that native range expansions can have important impacts on soil biological communities, soil chemistry, and hydrology which can further impact below-ground ecosystem processes such as nutrient cycling and litter decomposition. The combination of high-throughput sequencing and structural equation modeling used here offers an exciting yet under-utilized approach to understanding how both native and non-native species' range expansions may affect the structure and function of soil ecosystems.

Introduction

Changes in climate and land use, as well as anthropogenic introductions of non-native species, are creating novel communities of organisms around the globe (Tylianakis et al. 2008). This is due largely to changes in species' ranges, which include range expansion, contraction and shifts (Sexton et al. 2009, Chen et al. 2011). In particular, range expansions occur with the introduction of non-native species to new areas, but also with native species expanding their ranges in latitude and/or elevation as they "track" changes in climate (Parmesan et al. 2003, Valéry et al. 2008). Although similar processes govern distributions of native and non-native species, including dispersal ability and

competitive interactions, native range expansions may differ from non-native invasions for several reasons (Morrien et al. 2010, Van der Putten et al. 2010). First, native range expansions tend to be driven by changes in climate or land use, as opposed to intentional or unintentional human introductions, as the human introduction process may select for certain species' traits that affect subsequent spread and impacts (Buckley and Catford 2016). Second, native range-expanders are more likely to share evolutionary history with the communities into which they invade, potentially affecting processes such as enemy release or novel weapons that are important in non-native species' establishment in new areas (Callaway et al. 2008, Hill et al. 2011). Third, natives have closer source populations that may continue to be connected via gene flow to the expanding range edge (Leger & Espeland 2010; Van der Putten et al. 2010; Caplat et al. 2013), which may allow for improved adaptability to environmental conditions in the new range. Together, these differences may affect the mechanisms of range expansion and resulting ecosystem impacts of native and non-native species; however, species origin alone is likely not sufficient to accurately describe these complex ecological interactions (Buckley and Catford 2016).

Both native and non-native range expansions can create significant impacts on below-ground ecosystems by altering nutrient uptake, litter inputs, soil microclimate, and disturbance regimes, which may have important effects on soil microbial communities (Wolfe and Klironomos 2005, Ehrenfeld 2010, Chapin et al. 2011). Altering the quality and quantity of litter inputs can shift the dominance of particular microbial groups within the soil (De Deyn et al. 2008). For example, plant litter with high carbon (C) to nitrogen

(N) ratios often promotes fungal dominance in soils, due to differences in organismal C/N ratios and C-use efficiencies between bacteria and fungi (De Deyn et al. 2008, Bardgett 2011) and may also shift bacterial community dominance to oligotrophic groups with slower growth and turnover and higher nutrient use efficiency (Fierer et al. 2007). Range expanding plants may also alter below-ground competition for water and limiting nutrients with soil biota by shifting rooting depth, below-ground biomass, and phenology during invasion (Schenk 2006, Gioria and Osborne 2014). These novel interactions may have strong influences on microbial community structure (composition and diversity) (Batten et al. 2006, Piper et al. 2015). For example, non-native grasses in California were shown to support higher abundances and diversity of ammonia-oxidizing bacteria than natives (Hawkes et al. 2005), and to reduce mycorrhizal fungal diversity of co-occurring native grass roots (Hawkes et al. 2006). Expansion of native shrubs into grassland ecosystems can also increase soil bacterial and fungal diversity and select for distinct fungal community composition (Hollister et al. 2010, Yannarell et al. 2014). Plant range expansions may also alter microbial activity and ecosystem functioning, such as when non-native invasive plants increase rates of decomposition and N cycling (Liao et al. 2008, Ehrenfeld 2010) or select for microbial species which preferentially degrade their own litter (Austin, Vivanco, Gonzalez-Arzac, & Perez, 2014; R D Bardgett & Wardle, 2010). However, below-ground responses to plant range expansions are highly variable, and can depend on time since establishment, plant trait variation, and the microbial associations of the resident plant community (Liao et al. 2008; Castro-Diez et al. 2014).

Among the native range expansions occurring globally, the encroachment of woody plants into historically herbaceous dominated plant communities is particularly prevalent. Woody plants, in particular shrubs, are observed to be moving upslope in montane and alpine ecosystems and increasing in cover and abundance across diverse landscapes including arid grasslands, savannah, and arctic and alpine ecosystems (Wilson and Nilsson 2009, Naito and Cairns 2011, Myers-Smith et al. 2011, Saintilan and Rogers 2015). Similar to non-native invasions, increased density and cover of native woody vegetation can greatly affect the cycling of C, nutrients, and water via changes in litter quantity and quality, rooting depth, and woody biomass production (Huxman et al. 2005, Eldridge et al. 2011, Myers-Smith et al. 2011) These plant-induced changes can cascade through the soil ecosystem, altering microbial community structure and function. For example, global studies suggest that shrub litter has higher C/N than graminoid and forb litter and decomposes more slowly, thereby slowing microbial respiration and soil CO_2 flux into the atmosphere (Cornelissen et al. 2007, but see Wolkovich et al. 2010). Previous work has shown that shrub encroachment into grasslands can increase microbial biomass C, bacterial and fungal community diversity and fungi: bacteria ratios in soils (Liao and Boutton 2008, Hollister et al. 2010, Yannarell et al. 2014). Mycorrhizal relationships in plants growing under shrub canopies (e.g., grasses and forbs) can also be affected by shrub-induced changes in soil nutrient availability (Shi et al. 2006) or secondary chemicals of shrub litter (Nilsson et al. 1993, Wardle et al. 1998). Shifts in soil microbial community structure and function as described above may persist even after the shrubs are removed or retreat (Kulmatiski and Beard 2011) and have the potential to

create negative plant-soil feedbacks (PSFs) for other species by altering microbial decomposer communities and shifting pathogen to mutualist ratios (Bever 2003, Bardgett and Wardle 2010).

Despite the growing interest in the role of soil biota in plant range expansions (Suding et al. 2013), many of the relationships between abiotic impacts of woody plant encroachment and changes in soil microbial community structure and function remain speculative, as direct tests linking these below-ground processes are rare (Myers-Smith et al. 2011). In addition, general patterns for the effects of native range expansions on soil biota have not been well defined. Recent reviews have examined mechanisms whereby native and non-native range expanding species may be similar or dissimilar in their relationship with soil communities (Morrien et al. 2010, Van der Putten et al. 2010, Van der Putten 2012). While conceptual frameworks such as these and the substantial literature base for PSFs of non-natives can help guide our predictions for (climate and land use driven) native range expansions, empirical tests are critical in order to fully fill this knowledge gap.

In this study we examine how a native subalpine sagebrush species with a documented pattern of range expansion over the last 50 years (Kopp and Cleland 2014) is affecting abiotic soil properties and microbial community structure and function. In particular, we combine biotic and abiotic soil analyses with next-generation sequencing techniques to determine how soil microbial biomass C and N, bacterial and archaeal community structure, function (substrate induced respiration [SIR]), and soil

characteristics including C and N availability, pH, and volumetric water content (VWC) were affected by the presence of sagebrush. The study was conducted across an altitudinal gradient of sagebrush expansion, where higher elevation sites were more recently colonized, allowing a chronosequence analysis of how sagebrush affects soil biotic and abiotic properties. We used structural equation modeling (SEM) to test multiple hypotheses for how the impacts of sagebrush expansion fit into a larger conceptual framework of soil biological communities, soil chemistry, hydrology & below-ground ecosystem processes such as nutrient cycling and decomposition (Fig. 1.1a, b). In addition, to estimate the possible consequences for resident herbaceous species we quantified how arbuscular mycorrhizal fungi (AMF) colonization of other native alpine plant (non-shrub) species was impacted by the presence of sagebrush. Finally, we used a 4-year sagebrush removal study to assess the resilience of soil bacterial and archaeal communities and test for a causal link between sagebrush presence and microbial community shifts.

Methods

Site Description

Research was conducted in the White Mountains of California near Crooked Creek (3094 m; 37° 29’ 56” N, 118° 10’ 19” W) and Barcroft (3800 m; 37° 34’ 59” N, 118° 14’ 14” W) research stations. This mountain range lies on the western edge of the Great Basin Floristic Province and in the rain shadow of the Sierra Nevada range. The climate is cold and dry, receiving between 150 to 450 mm of precipitation annually. Temperature declines with increasing elevation, with a mean annual temperature of 0.9°C

at Crooked Creek Station to -1.7°C at Barcroft Station, while precipitation increases from 327 mm/yr. to 456 mm/yr. respectively (Hall 1991). These mountains contain a steep elevation gradient, ranging from 1220 m at its base in the Owens Valley to 4344 m at the summit of White Mountain Peak.

Due to dramatic changes in elevation, temperature, and precipitation, this range contains five distinct plant communities: cold desert (1220–1980 m), montane (1980–2900 m), sub-alpine (2900–3500 m), alpine (3500–4000 m), and high alpine (4000–4344 m) (Rundel et al. 2008). Our research took place between sub-alpine and alpine communities, within the transition from sagebrush steppe to true alpine plant communities dominated by prostrate cushion plants and perennial bunchgrasses. Recent research has shown that *Artemisia rothrockii* A. Gray (sagebrush) is expanding upwards in elevation at a rate of 30 m/decade over the past 50 years (Kopp and Cleland 2014) and establishing patches up to 10 m wide in alpine zones. This range expansion is likely promoted by changes in climate such as increased temperatures and drought and changes in land use including cessation of grazing in the area (Kopp and Cleland 2014). Experimental warming and contracted snow pack periods have been shown to increase intrinsic growth rates of closely related *Artemisia* species in the intermountain west, thus providing a plausible hypothesis for range expansion in this system (Perfors et al. 2003). In areas of sagebrush encroachment, there has been a decline in abundance and cover of native grasses and cushion plants (Kopp and Cleland 2014).

Experimental Design and Sample Collection

In order to determine impacts of *A. rothrockii* expansion on soil communities, we sampled soils under and outside of sagebrush canopies at three sites located along the altitudinal transect at 3100, 3500, and 3800 m elevation. We also sampled soils in nearby (<500 m distance) plots where sagebrush was manually removed 4 years prior ('sagebrush removal') at 3100 and 3750 m elevations (described below). All sites span the observed gradient of sagebrush expansion from subalpine (<3500 m elevation) to alpine (>3500 m elevation) over the last 50 years (Kopp and Cleland 2014). In 1961, *A. rothrockii* was not present at the 3800 m site, was found in moderate to low densities at the 3500 m site, and high densities at the 3100 m site (Mooney et al. 1962, Kopp and Cleland 2014). Therefore, this gradient is useful in assessing impacts of shrub expansion using the low elevation sites as a historic reference and the high elevation sites as representative of the leading edge of the expansion gradient where *A. rothrockii* transitions from an almost continuous population to isolated patches.

All sampling locations had granitic soils (Colluvium derived from granite) and east/southeast facing slopes to control for edaphic and aspect variation. In addition to consistency in parent material across sites, the middle and high elevation sites are in the same soil series (Pergelic cryobrolls-soakpak family association), while the low elevation site is in the Hartig-Packham family association (Soil Survey Staff 2007). Other site selection criteria included choosing areas that were located outside of designated

wilderness area and Ancient Bristlecone Pine forest so that sagebrush removal and soil coring could be conducted.

We collected soils in August 2015 near the peak of growing season. Five sagebrush individuals (<100 m apart) were sampled from each elevation site, with two replicate soil cores (1.3 cm diameter x 10 cm deep) taken from under (shrub) and outside (shrub interspace) each individual. Shrub interspace cores were taken between 1-5 m from the edge of the canopy of each individual (based on sagebrush density of the site) and less than the distance to any other shrub canopy. In July 2011, 1-m^2 sagebrush removal plots were established at 3100 and 3750 m elevation by cutting sagebrush at the base of the stem and trimming any re-sprouting back yearly. Two replicate soil cores were taken from five sagebrush removal plots (within ~200 m radius) at the low and high elevation sites. The corer was sterilized between each sample with a 10% bleach solution to prevent cross contamination and cores were kept separate to assess within individual variation in soil communities. For molecular analyses, soil was stored in sterile specimen cups and placed on dry ice in the field and then stored in a -80 ◦C freezer prior to analysis. For all other analyses, one soil sample was taken from under and outside the same sagebrush individuals at the same time using a garden trowel marked at 10 cm depth and then stored at 4 ◦C. These soil samples were sieved field-moist (0.5 mm) to remove roots and stones prior to analysis for soil abiotic characteristics, substrate induced respiration (SIR), and microbial biomass C/N.

Additionally, roots of species co-occurring with *A. rockrothii* were sampled for mycorrhizal analyses in September 2014 and August 2015 in order to determine the effect of sagebrush expansion on mycorrhizal communities of alpine plants. Ten individual plants (including roots and intact rhizosphere soil) of a dominant alpine bunchgrass (*Koeleria macrantha)*, and cushion plant (*Eriogonum ovalifolium)* were sampled at each of the three elevation sites, 5 from individuals growing directly below *A. rothrockii* canopies and 5 growing in adjacent shrub interspace. This provided a total of 30 individuals for each species (5 individuals x 3 elevations x 2 locations). Soil samples for total (bulk) soil C and N were taken at the same time and location as individual plants sampled in September 2014 for each elevation site (5 under and 5 outside sagebrush).

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 then ran polymerase chain reaction (PCR) using primers targeting the V3-V4 region of 16S rRNA gene (S-D-Bact-0341-b-S-17 127 and S-D-Bact-0785-a-A-21; Klindworth et al. 2013). Specifically, samples were amplified in duplicate by combining 2.5 µL of DNA template, 5 µL each of 1 µM forward and reverse primers, and 12.5 µL KAPA HiFi HotStart ReadyMix (KAPA Biosystems, Inc., Wilmington, MA, USA). The thermocycler conditions were: 95 °C for 3 minutes, followed by 25 cycles of 95 °C for 30 seconds, 55 °C for 30 seconds, 72°C for

30 seconds, and finally an extension step for 5 minutes at 72 °C. We then conducted post-PCR clean-up using Agencourt AMPure XP Beads (Beckman Coulter Genomics, Danvers, MA, USA), followed by a second round of PCR to attach dual indices to each sample using the Nextera XT Index Kit (Illumina Inc., San Diego, CA, USA). For this second round of PCR, we combined 5 µL DNA, 5 µL each of 1 µM forward and reverse index primers, 25 µL KAPA HiFi HotStart ReadyMix, and 10 µL PCR grade water. The thermocycler conditions were: 95 °C for 3 minutes, followed by 8 cycles of 95°C for 30 seconds, 55 °C for 30 seconds, 72 °C for 30 seconds, and finally an extension step for 5 minutes at 72 °C. We then conducted a second round of post-PCR clean-up (same as described above) on the indexed amplicons and quantified them with the Quant-it PicoGreen® dsDNA assay kit (Life Technologies Inc., Grand Island, NY, USA). As a final step, the samples were pooled in equimolar concentrations and then sequenced on the Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA) at the University of California Riverside (UCR) Genomics Core Facility.

Sequence Analyses

We received the sequences already demultiplexed, and initially processed them using Quantitative Insights into Microbial Ecology (QIIME; Caporaso et al. 2010). We joined the forward and reverse reads (allowing for maximum 20% divergence in the overlap region) and used default QIIME parameters for quality control. Specifically, sequences were discarded if there were less than 75% consecutive high-quality base calls, if there were more than three low-quality base calls in a row, or if there were any

ambiguous calls. Sequences were additionally removed if the Phred score was less than three and if the total length was less than 75 bases (Bokulich et al. 2013). After quality filtering, we used UCLUST (Edgar 2010) to pick operational taxonomic units (OTU) at 97% similarity and the 13_8 version of the Greengenes database to assign taxonomy (McDonald et al. 2012). We used an open reference OTU picking approach, where reads that had no sequence matches in the database were clustered de novo. Two samples were removed due to low read counts, leaving 75 samples and resulting in 7,477,268 total reads. After removing unassigned sequences, we rarefied each sample to an even depth of 41,010.

To provide a robust analysis of alpha diversity for each sample (Haegeman et al. 2013), we calculated both the number of observed OTUs (richness) and Shannon diversity (richness and evenness). In addition, we compared the combined relative abundance of taxa that are generally considered to be copiotrophic (higher N demands, utilize labile C pools) and oligotrophic (lower N demands, utilize more recalcitrant C pools) across treatment and elevation in a similar way. Specifically, Actinobacteria, Beta-proteobacteria, and Firmicutes comprised the copiotrophic group, and Acidobacteria and Verrucomicrobia comprised the oligotrophic group (Fierer *et al*., 2007; Ramirez *et al.*, 2012). We tested the effects of treatment on microbial diversity and the relative abundances of taxa by performing analysis of variance (ANOVA) on data collected from the 3100 m and 3800 m elevation sites, followed by a Tukey post-hoc analysis if the overall model was significant. Here, both treatment (shrubs, shrub removal, interspace) and elevation were included as factors in the model. When there was a significant

treatment by elevation interaction, data was separated by elevation and individual ANOVAs were run. The 3500 m elevation site was analyzed separately as it did not contain a shrub removal treatment. To account for multiple comparisons in the relative abundances of taxa, we performed a sequential Bonferroni correction; given this approach can be quite conservative (Moran 2003) we present both uncorrected and corrected values in the results and focus on the uncorrected values in the discussion. Beta diversity was visualized using non-metric multidimensional scaling (NMDS) of the unweighted (presence-absence) and weighted (relative abundance) UniFrac distance. This metric uses overlap in branch lengths to estimate phylogenetic distance between pairs of samples (Lozupone and Knight 2005). The NMDS was graphed in R (R version 3.2.1; R Core Team 2015) using ggplot2 (Wickham 2009) and the 'stat_ellipse' function with 95% confidence intervals. We tested for differences in overall microbial community composition across treatments by performing a permutational multivariate ANOVA (perMANOVA) implemented using the Vegan function 'ADONIS' in R (999 permutations; Oksanen et al. 2016). Treatment (shrub, shrub interspace, and shrub removal) was used as the predictor variable, and the elevation was included as 'strata' (a blocking variable), which restricted permutations to within sites

Substrate Induced Respiration

Substrate induced respiration was measured using an LI-8100A (LI-COR Biosciences, Lincoln, Nebraska, USA) automated soil gas flux system and a modified SIR method (West and Sparling 1986). By measuring CO_2 production over time, SIR

estimates potential microbial respiration of those microorganisms that are active and glucose-responsive (Anderson and Domsch 1978), and therefore provides a coarse approximation of a key microbial function. Briefly, 15 g of field moist soil was weighed out into 250 mL jars and 30 mL of a glucose solution (30 g glucose L^{-1} H_2O) was added to each. The jars were sealed with a lid modified with rubber tubing running from inside the jar into the LI-8100A and shaken at 180 rpm for 30 minutes while continuous flux measurements were recorded. After 30 minutes CO_2 flux rates ($\mu mol*kg^{-1}*s^{-1}$) were calculated using LI-COR software.

Microbial Biomass C/N

Microbial biomass C and N were measured using a chloroform fumigation-extraction (CFE) technique (Brookes et al. 1985). Briefly, 5 g of field moist soil from each soil core was separated into paired unfumigated and fumigated samples. Unfumigated samples were extracted immediately with 25 ml of 0.5 M K_2SO_4 by shaking for 1 hour on an orbital shaker and then filtering through a 1.2 µm glass fiber filter (Thomas C5500, Thomas Scientific, Swedesboro, NJ). Filtered samples were frozen at -20 °C for subsequent analysis of dissolved organic C and N. Fumigated samples were weighed out into 250 ml Erlenmeyer flasks, exposed to 2 ml of ethanol-free $CHCl_3$, capped, and incubated for 24 hours at room temperature. Following incubation, caps were removed and flasks were vented for 30 minutes under a fume hood, and the soil was extracted with 25 ml of 0.5 M K_2SO_4 as described above. Unfumigated and fumigated extracts were shipped overnight on dry ice to the EcoCore Analytical facility at Colorado

State University, Fort Collins, CO, for analysis of total organic carbon (TOC) and nitrogen (TON) on a Shimadzu TOC-L autoanalyzer (Shimadzu Scientific Instruments, Inc., Carlsbad, CA.). Total organic C and N were calculated as $mg*L^{-1}$ for each sample using the unfumigated values. We calculated microbial biomass C and N by subtracting organic carbon/nitrogen from the unfumigated samples by their paired fumigated samples, and by dividing the subsequent C- and N-flush by kEC and kEN coefficients of 0.45 and 0.69, respectively (Wu et al. 1990, Joergensen and Mueller 1996). Results are expressed as mg C, $N*kg\ soil^{-1}$, where average site/treatment values of gravimetric water content (GWC) were used to calculate soil dry weight because GWC was measured on only three replicates per treatment (i.e. under and outside shrub) at each elevation.

Soil Abiotic Properties

Soil abiotic properties including VWC and pH were measured at the same time and sampling location of each soil core. VWC (%) was measured to 12 cm depth using a Campbell Scientific HS2 Hydrosense II probe (Campbell Scientific, Logan, UT, USA) and pH was measured by mixing soil from 10 cm depth in a sterilized cup with DI water and using an Extech PH100 ExStik pH meter (Extech instruments, Nashua, NH, USA).

Total (bulk) soil C and N was determined by combustion using a Flash EA1112 combustion soil analyzer (Thermo Fischer Scientific, Waltham, MA, USA) in the Environmental Sciences Research Laboratory at the University of California Riverside. Total C and total N values correlated strongly with the unfumigated values of TOC and

TON by treatment (under and outside shrubs), and therefore we used only TOC and TON values for this study (Pearson's r= 0.834 TOC and TC) and 0.908 (TON and TN).

Mycorrhizal Analyses

Roots of the dominant alpine bunchgrass (*Koeleria macrantha)*, and cushion plant (*Eriogonum ovalifolium)* were separated from intact soil and then 0-2 mm diameter roots were rinsed with distilled water and cleaned with fine brushes to remove soil particles. These roots were then cleared in a 10% KOH solution for 10 minutes at 60°C and stained in a 5% ink-vinegar solution following the methods of (Vierheilig, Coughlan, Wyss, & Piché, 1998). Arbuscular mycorrhizal fungi (AMF) colonization was quantified at 40× magnification using the magnified intersections method (McGonigle et al. 1990). We used a Bayesian multiple regression model with a varying-intercept (Gelman and Hill 2007) to determine the effects of sagebrush cover (target plant growing under shrub or outside) and elevation (3100m, 3500m, or 3800m site) on AMF colonization. The response variable was average percent mycorrhizal colonization in the roots of each species. The regression intercepts varied by treatment (outside or under sagebrush) and elevation, and bulk soil C and N were also included as covariates. Non-informative priors were used for each parameter, and significance of each variable was determined by calculating the probabilities that posterior parameter distributions did not overlap zero (see below for additional detail on this approach).

Structural Equation Modeling

We used structural equation models to test and quantify the hypothesized connections between sagebrush expansion and soil biotic and abiotic parameters and processes (Fig.1.1a, b). Structural equation modeling is a useful approach for disentangling complex sets of direct and indirect interactions (Grace et al. 2010) but remains relatively underutilized in soil ecology (Eisenhauer et al. 2015). We developed models based on *a priori* understanding about the functional relationships between soil variables in this and other ecosystems and used the broad metamodel (Fig 1.1a) to structure our specific hypotheses (Fig. 1.1b).

In light of the large quantity and complexity of microbial sequencing data, we developed four separate models in order to best capture and incorporate different hypotheses about how microbial community structure was affected by sagebrush. These models quantified the microbial community as: (i) diversity (Shannon diversity index), (ii) richness (number of OTUs), (iii) composition (first axis of weighted NMDS), (iv) ratio of oligotrophic abundance to copiotrophic abundance (for description see sequence analyses). Models were fit in a Bayesian framework using the R2jags package (Su et al. 2015) in R version 3.2.2 (R Core Team 2015). Non-informative priors were used on all parameters, and models were checked for convergence using visual assessment and the Gelman-Rubin diagnostic on three independent chains with sufficient burn-in periods discarded. Model structure was similar to that in Grace (2014) except mixed models were used to allow for different effects of shrubs across elevations and random effects were

used to account for the replicate soil cores in which microbial communities were characterized at each location. Model code is available upon request. Statistical significance and strength of relationships within the models were assessed using the posterior parameter distributions of intercepts and slopes of mixed models describing each connection in Fig. 1.2a-d. Each variable was standardized before analysis in order to facilitate comparison of estimated path coefficients. We used the posterior distributions of each parameter to calculate the probabilities that it was different from zero, and three probability levels are reported (85, 90, and 95%) probabilities (Fig. S1.1), respectively, that the relationship is different from zero). Because the effect of shrub cover in this study was a categorical test (samples were taken from underneath shrubs, shrub interspace and in shrub removal plots), these effects were calculated in the model as the difference between intercept terms that were allowed to vary by treatment. Therefore, the assessment of strength and statistical significance of shrub effects were based on the posterior distribution of the difference between shrub and shrub interspace intercepts calculated within the model.

Results

Molecular Analyses

At an even sequencing depth of 41,010 reads, our efforts yielded an average of 7,462 OTUs per sample (standard deviation ± 714 OTUs). Overall, these OTUs belonged to 46 phyla, 161 classes, 311 orders, 495 families, and 822 genera. Actinobacteria (20.6 ± 5.1 %), Verrucomicrobia (17.1 ± 3.9 %), Proteobacteria (15.8 ± 2.5 %), Acidobacteria (13.4 ± 1.6 %), and Planctomycetes (10.0 ± 1.5 %) were the dominant bacterial phyla,

together accounting for more than 75% of sequences across all samples (76.9 ± 14.5%; Fig. 1.3). Within the Actinobacteria, the Actinomycetales order was most prevalent (comprised 40 ± 2 % of the Actinobacterial sequences) followed by the Gaiellales (21 ± 2 %) and Solirubrobacterales (19 ± 1 %). The Verrucomicrobia genus, *DA101*, was the most abundant genus recovered with an overall relative abundance of 14.0 ± 4 %.

Marked differences in microbial community structure were observed among shrub, shrub interspace and shrub removal treatments. Across all elevations, microbial communities underneath shrubs were more diverse (greater number of observed OTUs and higher Shannon diversity) than those in shrub interspace ($P < 0.05$; Fig. 1.4a, b). At the lowest elevation site, shrub-associated microbial communities were also more diverse than in areas where shrubs had been removed ($P < 0.05$; treatment x elevation interaction $P < 0.05$; Fig. 1.4a, b). In addition, microbial community composition was significantly affected by treatment (unweighted UniFrac $R^2 = 0.07$, $P < 0.001$, Fig.1.4c; weighted UniFrac $R^2 = 0.15$, $P < 0.001$, Fig. 1.4d). Shrub and shrub interspace plots harbored distinct microbial communities (unweighted UniFrac $R^2 = 0.04$, $P < 0.001$, Fig.1.4c; weighted UniFrac $R^2 = 0.14$, $P < 0.001$, Fig. 1.4d), as did shrub and shrub removal plots (unweighted UniFrac $R^2 = 0.05$, $P < 0.001$, Fig.1.4c; weighted UniFrac $R^2 = 0.12$, $P < 0.001$, Fig. 1.4d). Although the unweighted UniFrac metric also revealed significant differences in microbial composition between shrub interspace and shrub removal plots ($R^2 = 0.04$, $P = 0.02$), when the relative abundances of taxa were taken into account interspace-associated microbial communities were similar in composition to those

microbial communities from shrub removal plots (weighted UniFrac, $R^2 = 0.04$, $P = 0.26$; Fig. 1.4c, d).

Treatment-induced shifts in community composition were accompanied by changes in the relative abundance of some dominant taxa. For example, Beta-proteobacteria consistently increased in shrub plots compared to both shrub interspace and shrub removal plots ($P < 0.01$). However, changes in the relative abundances of particular phyla did not always occur in the same direction at each elevation. Actinobacteria decreased with shrub removal compared to shrub interspace plots at 3100 m elevation ($P < 0.01$; Fig. 1.3), whereas this phylum increased with shrub removal compared to interspace and shrub plots at 3800 m elevation ($P < 0.05$; treatment x elevation interaction $P < 0.001$). Similarly, Verrucomicrobia was unaffected by treatment at 3100 m elevation ($P > 0.05$) but decreased with shrub removal compared to shrub interspace plots at 3800 m ($P < 0.05$; treatment x elevation interaction $P = 0.05$; Fig. 1.3). However, after taking into account multiple comparisons, many of these significant trends disappeared; only the effect of treatments on Beta-proteobacteria remained statistically significant.

Mycorrhizal Analyses

Arbuscular mycorrhizal fungal percent root colonization of the two species, *Koeleria macrantha* and *Eriogonum ovalifolium,* was highly variable within sites. The only significant effect of shrub cover was a tendency for greater AMF colonization in plants growing underneath shrub canopies compared to outside for *E. ovalifolium* at the

high elevation site (0.943 probability that AMF colonization was greater underneath shrubs).

Structural Equation Modeling

Parameter estimates from each of the structural equation models are most easily visualized in Fig 1.2, S1.1 and table S1.1. Here we summarize the primary results related to the hypothesized relationships in Fig. 1.1b. Sagebrush cover was strongly associated with increased microbial diversity and richness (Fig. 1.2a, b), and with microbial community composition (Fig. 1.2c) at all elevations (Fig. S1.1). Sagebrush cover significantly increased TOC and TON (hereby referred to as *soil* organic C [SOC] and *soil* organic N [SON]), at the low elevation site and VWC at the high elevation site (Figs 1.2 and S1.1). SOC was also increased under sagebrush canopies at the high elevation site in one version of our model (Fig 1.2d). SON was positively related to SIR and pH was positively associated with microbial diversity and richness. Volumetric water content and SON had inverse relationships with microbial diversity and SON alone was inversely related to microbial richness. Volumetric water content also had a positive relationship with SIR in one version of our model (Fig 1.2d). When considering links between microbial community structure and function, microbial diversity and oligotrophic: copiotrophic ratios were positively related to SIR at the high elevation sites only. Although not directly included in the SEM, microbial biomass C and N were also higher under sagebrush canopies than outside ($P < 0.001$).

Discussion

In this study we examined the below-ground impacts of a native species expanding its range over the last 50 years in the White Mountains of California (Kopp and Cleland 2014). Our approach utilized a structural equation modeling framework, in which a priori hypotheses (Fig. 1.1b) of relevant direct and indirect relationships between sagebrush cover and soil abiotic and biotic variables were tested. Although most measurements displayed variability among sites, several trends were strong across the entire sagebrush expansion chronosequence suggesting consistent impacts on soil microbial community structure and function. In addition, a sagebrush removal experiment showed that microbial community structure can return to pre-shrub composition relatively quickly (<5 years), demonstrating compositional resilience of the microbial community.

Changes in Soil Microbial Communities with Shrub Expansion

We observed a strong influence of sagebrush establishment on soil bacterial and archaeal community diversity (Shannon's index), OTU richness, and overall community composition. Specifically, microbial communities were consistently more diverse and had higher richness under sagebrush canopies than outside, a finding that is congruent with prior research on woody shrub encroachment (Wallenstein et al., 2007; Hollister *et al.* 2010; Yannarell *et al.* 2014). This trend held true across all elevations. We hypothesized that microbial community diversity, richness and composition would be altered by sagebrush, potentially due to a higher diversity and altered abundance of litter

sources (shrub, grass, cushion) entering the soil environment (Hooper et al. 2000). Studies suggest that litter sources can shift microbial biomass, community composition and structure by increasing substrate variability and diversity of chemical compounds, and that this can vary through stages of decomposition (Meier and Bowman 2008, Chapman and Newman 2010, Chapman et al. 2013). An analogous explanation could be posed regarding the diversity of below-ground inputs, both in regards to root exudates and senesced root litter (De Deyn et al. 2008). Although not directly included in the SEM, microbial biomass C and N were also higher under sagebrush canopies than outside, suggesting that sagebrush establishment promotes higher total microbial biomass in soils ($P < 0.001$) in addition to altering composition.

In addition to microbial diversity and overall biomass, the relative abundance of particular microbial functional groups provides an ecologically relevant way to organize and draw inferences on complex molecular data (Fierer et al. 2007). While sagebrush cover was not directly related to oligotrophic: copiotrophic ratios in the structural equation models, abundances of particular phyla did vary with shrub cover including an increase in Beta-proteobacteria in shrub soils and shifts in Actinobacteria and Verrucomicrobia with shrub removal. The increase in Proteobacteria in shrub soils is consistent with Wallenstein *et al.*2007 who found increased Proteobacteria in arctic shrub soils, suggesting that these bacteria thrive in C and nutrient rich soils under shrub canopies, and exhibit copiotrophic attributes (Fierer et al. 2007, Wallenstein et al. 2007). In addition, there was a strong negative relationship between oligotrophic: copiotrophic ratios and SIR at high elevation site, consistent with glucose induced respiration rates

reported for copiotrophic microorganisms in other studies (Blagodatskaya et al. 2007, Hopkins et al. 2014). Therefore, these findings suggest that shifts in functional groups may significantly alter microbial activity which has important implications for the cycling of C in areas of recent sagebrush expansion (Metcalfe *et al.* 2011).

It is also important to note that the impacts of sagebrush expansion on soil fungal community composition were not directly tested in this study, although fungi are represented in several measured components of our system including microbial biomass C/N, mycorrhizal colonization, and substrate induced respiration. These measurements showed varying levels of response to sagebrush presence. In particular, mycorrhizal fungal colonization was higher for cushion plants (*E. ovalifolium*) growing under sagebrush canopies than outside at the high elevation site only. We did not see a similar trend for the grass species (*K. macrantha*), or at lower elevation sites, suggesting high species and site level specificity in mycorrhizal responses to shrub encroachment. Soil fungi are known to be affected by and have significant feedbacks on plant community composition and performance, including positive and negative PSFs of fungal symbionts and pathogens (Kulmatiski et al. 2008, Maron et al. 2011, Hilbig and Allen 2015). Therefore, determining changes in the composition of both free living and symbiotic fungal communities will be important for a complete understanding of the impacts of native range expansion on soil microbial community structure and function.

Mechanisms by Which Encroachment Alters the Soil Community

We expected one way that sagebrush would influence microbial diversity is by modifying soil pH. Indeed, woody shrubs can alter pH (Buyer et al. 2016), which is one of the most important factors affecting soil bacterial community structure as many microorganisms have narrow pH niches for growth (Fierer et al. 2007, Lauber et al. 2009, Rousk et al. 2010). As predicted, soil pH strongly influenced microbial diversity and richness; however, sagebrush cover had no significant relationship with pH at any elevation. Soil pH also showed no clear trend across the elevation gradient suggesting that there is high within-site variability in pH in this ecosystem. Although soil pH had strong effects on the microbial community, and sagebrush had no detectable impact on soil pH, sagebrush cover had approximately 2-5 times stronger effects on microbial community structure than pH. This suggests that abiotic factors such as pH cannot fully explain the differences we observed in microbial communities in areas of sagebrush expansion.

We found evidence of altered soil nutrient levels underneath sagebrush with important cascading effects on microbial communities. In our study, soil organic C and N content were significantly higher under sagebrush canopies than in shrub interspace at low elevation sites, and SOC was slightly higher at the high elevation site in one version of the model. This local enrichment under shrubs is known as the "island of fertility" effect and can be caused by many factors including accumulation of litter, trapping of airborne nutrients, and reduced runoff under shrub canopies (Schlesinger et al. 1996,

Ridolfi et al. 2008). This phenomenon is particularly important in dryland ecosystems, which is likely why we saw this effect most strongly at the low elevation sites, where annual precipitation is significantly lower (Schlesinger et al. 1996). Furthermore, SON was positively related to substrate induced respiration rates, suggesting increased potential for microbial decomposition of soil organic matter under sagebrush. This contradicted our predictions that sagebrush would have a dampening effect on SIR due to microbial acclimation to lower litter quality (Cornelissen et al. 2007). Finally, unlike the effects of sagebrush on microbial diversity, SON showed a consistently negative relationship with microbial diversity and richness, suggesting that sagebrush may have indirect effects on soil microbial community composition via changes in soil nutrients.

By altering water use and shading, shrubs can influence the amount of water that is available for nutrient diffusion and microbial use in the soil (Gómez-Aparicio et al. 2005, Huxman et al. 2005). In our models, soil moisture (VWC) was associated with decreased microbial diversity, which is consistent with the idea that in dry soil conditions, increased heterogeneity of microsites and spatial isolation of soil pores may promote microbial diversity and species coexistence (Frey 2007). However, the impact of sagebrush on soil moisture varied by elevation; at high elevation sites, where sagebrush is most recently established (i.e. <50 years), soil moisture was significantly higher under sagebrush canopies. These patterns are consistent with shrubs physically trapping snow under their canopies at high elevations, thereby increasing snowpack depth and delaying snowmelt (Leffler and Welker 2013). Research from arctic and alpine systems has suggested that snow trapped under shrubs may insulate soils and further stimulate winter

microbial activity and nutrient breakdown (Weintraub and Schimel 2005, Leffler and Welker 2013), although we only observed a positive impact of VWC on substrate induced respiration (SIR) in one version of our model. VWC is known to be highly spatially and temporally variable, and the impacts of woody plants on hydrologic cycles can be strongly influenced by climate (Bradford 2014); therefore, continuous measurements over time would be important in order to fully tease apart the net effects of sagebrush presence on soil water status. As climate continues to warm, the impacts of shrub expansion on soil moisture will likely become even more important at high elevation sites as snowpack levels diminish and hydrologic cycles become increasingly altered (Callaghan et al. 2011).

Although not measured in this study, sagebrush is known to produce litter volatiles including terpenes, jasmonic acids, and several other categories of secondary metabolic chemicals which may have direct or indirect influences on soil microbial communities (Weaver and Klarich 1977). Sagebrush has also been shown to have allelopathic effects on seed germination of heterospecific plants (Karban 2007), which may alter the soil microbial community associated with particular plant species. In general, plant volatiles such as those present in sagebrush litter can deter or attract different soil fauna to litter food sources (Austin et al. 2014), increase or decrease microbial respiration (Weaver and Klarich 1977, Asensio et al. 2012), alter microbial growth and nitrogen mineralization (Asensio et al. 2012), and disrupt plant-mycorrhizal associations (Nilsson et al. 1993, Wardle et al. 1998), however the net impacts of these of plant volatiles on soil microbial communities are still poorly understood.

Shrub removal, microbial resilience, and legacy effects

In addition to the observed changes in soil microbial communities due to sagebrush encroachment, we also found evidence that these changes are reversed with the subsequent removal of shrubs. Shannon diversity, OTU richness and overall microbial composition (based on weighted UniFrac metric) at 3100 m elevation differed significantly between shrub and shrub removal plots but were not different between interspace and shrub removal plots. This suggests a potentially high level of microbial resilience or ability to return to a "pre-disturbance" condition over short time scales (<5 years) (Shade *et al.* 2012; Allison & Martiny 2008). Our findings also suggest that, in contrast to other studies, long term microbial legacy effects of shrub expansion may not persist (Throop and Archer 2007). Although few studies have targeted microbial resilience after shrub removal, Shade et al. (2012) concluded that only 13-15 % of studies testing resilience after a disturbance reported a reversal or return to pre-disturbance composition of soil microbial communities. One recent study focused on shrub thinning in a Namibian savannah found results similar to ours (Buyer *et al.* 2016), indicating that microbial communities may respond quickly to, but also recover quickly from, woody shrub encroachment. This type of compositional resilience, which could be leveraged for climate mitigation and habitat restoration, is likely promoted by rapid growth rates of bacteria and archaea, opportunistic species, flexibility in substrate use, and/or dispersal from neighboring sites (Allison and Martiny 2008, Shade et al. 2012). Identifying which of these mechanisms contributes to microbial resiliency after shrub removal and measuring the rates at which microbial communities return to their previous composition,

will allow for improved predictions of how microbially mediated ecosystem processes will respond to global change (Allison and Martiny 2008, Shade et al. 2012).

Conclusions

Overall, we observed stronger effects of sagebrush on soil communities at high and low elevation sites versus middle elevation, which is consistent with research suggesting that changes in vegetation dynamics are likely to be strongest at the leading and trailing edges of range expansions (Svenning and Sandel 2013). However, while we aimed to control for variation in site level environmental conditions - including parent material, aspect, and plant community composition - we cannot fully isolate the chronosequence of sagebrush expansion from climatic changes along the altitudinal gradient. Specifically, precipitation increases with elevation and temperature decreases, as is common for alpine ecosystems. Nonetheless, the influence of sagebrush on microbial communities was strong across all elevations, and microbial community composition responded similarly post sagebrush removal at the two opposing ends of our elevation gradient, suggesting a causal link between these factors.

While it is known that plant community composition is a major driver of soil microbial community composition (Wardle et al. 2004), specific mechanisms for how climate and land use driven changes in plant community composition will affect soil microbial communities have remained elusive (Classen et al. 2015). We believe that coupling modern next generation sequencing with soil biotic and abiotic measurements in a structural equation modeling framework offers exciting opportunities for disentangling

the complex network of plant-soil interactions. Through this framework we have uncovered interesting connections between plant range expanders, abiotic soil parameters, and the structure and function of soil microbial communities. In particular, our results show that sagebrush can have strong direct effects on soil microbial community structure including increased diversity and richness and altered community composition. The SEM approach revealed that sagebrush expansion can have important indirect effects on microbial communities by creating changes to soil nutrients and moisture. Teasing apart direct and indirect pathways of plant impacts on below-ground ecosystem function is a critical, yet unresolved area of ecological research. With additional studies, this approach could provide a more complete and predictive understanding of the impacts of native range expansions on terrestrial ecosystems.

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Tables

Table 1.1 Site level soil characteristics for each elevation. Values shown are mean of all samples with standard deviation in parentheses. Middle elevation sites did not include shrub removal soils.

Elevation (m)	TOC (mg/L)	TON (mg/L)	VWC (%)	pH	microbial biomass N (mg N/kg soil)	microbial biomass C (mg C/kg soil)	CO2.flux (umol/kg/s)
3100 (N=15)	1.7 (0.69)	0.18 (0.061)	1.9 (0.83)	6.2 (0.36)	11 (7.5)	170 (90)	0.097 (0.072)
3500 (N=10)	2.5 (0.42)	0.29 (0.041)	5.3 (0.93)	5.9 (0.33)	25 (10)	320 (90)	0.2 (0.1)
3800 (N=15)	2.6 (0.7)	0.34 (0.091)	8.1 (2.2)	6.1 (0.54)	36 (20)	480 (190)	0.22 (0.14)

Figures

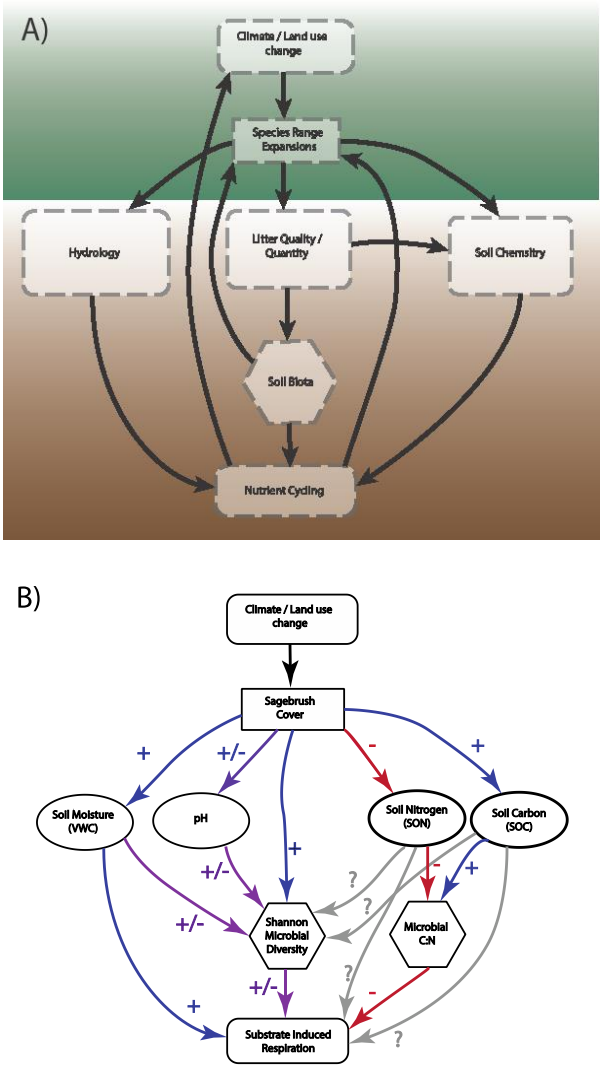

Fig. 1.1 Conceptual diagrams of plant range expansion impacts on soil and ecosystem processes. A) Broad structural equation meta-model outlining general relationships among above- and below-ground processes affected by species introductions and range expansions; B) Specific parameters measured in our research ecosystem in California, with hypothesized relationships (blue=positive; red=negative; purple & grey =unknown) tested in structural equation model. Elliptical nodes represent abiotic variables and square and polygon nodes represent biotic variables in the system.

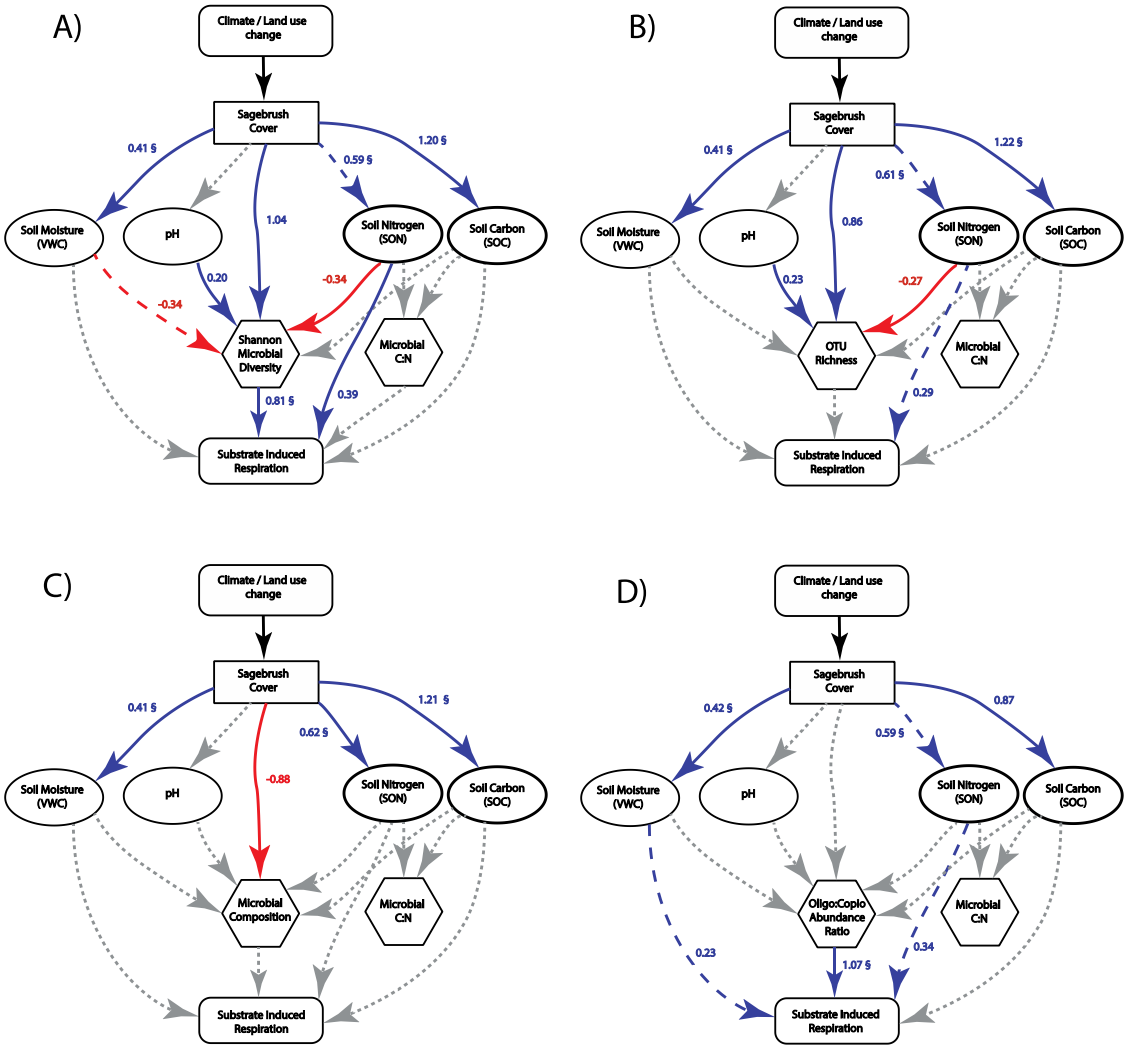

Fig. 1.2 Structural equation modeling results for A) microbial diversity, B) microbial richness, C) microbial composition, and D) oligotrophic:copiotrophic ratios. Line color denotes the direction of each relationship, with positive relationships in blue, negative relationships in red, and grey lines for relationships not significantly different from zero. Solid lines represent > 90% probability that a parameter estimate was different from zero, while dashed lines had ≥ 85% probability of being non-zero, and dotted lines (in grey) had < 85% probability of being non-zero. Results which were significant across all elevations show the average parameter estimate value for the three sites and elevation specific results are noted by § with the strongest elevation result displayed (further details in results and Fig.S1.1). Elliptical nodes represent abiotic variables and square and polygon nodes represent biotic variables in the system. Note that the top relationship between climate / land use change and shrub cover was not explicitly tested in this study.

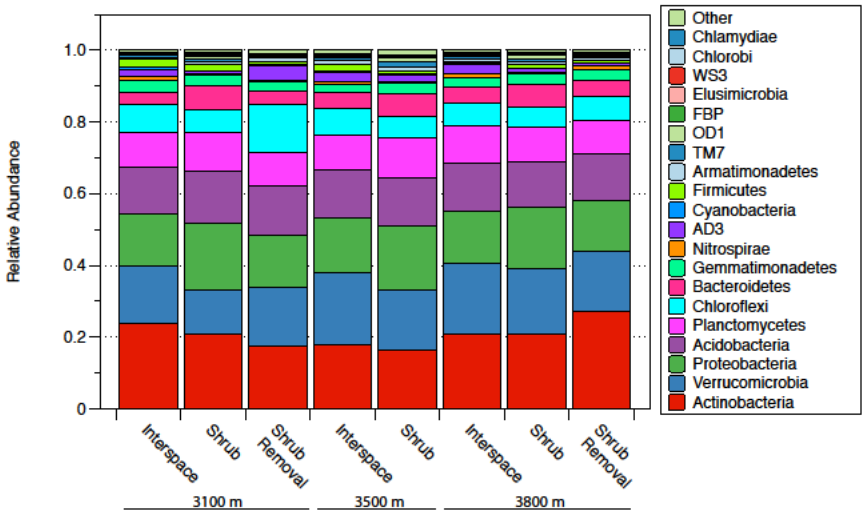

Fig. 1.3 Relative abundances of bacterial and archaeal phyla averaged across replicates (N = 9-10 per treatment) from under (shrub) and outside (interspace) sagebrush canopies, and from shrub removal plots at 3100 m, 3500 m (no shrub removal) and 3800 m elevation sites.

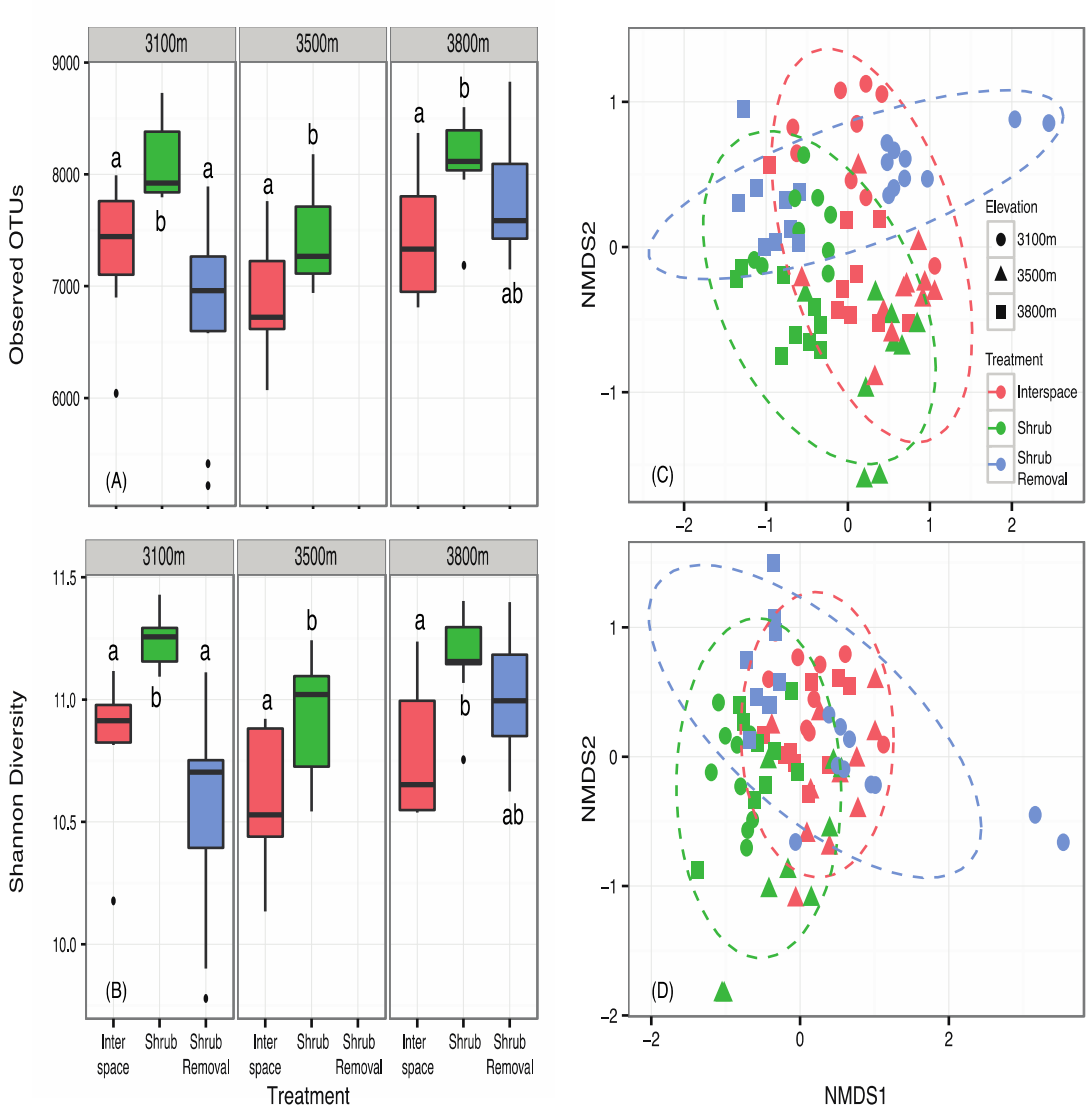

Fig. 1.4 Influence of treatment (interspace, shrub, shrub removal) on alpha diversity and overall community composition across all three elevations. (A) Number of observed OTUs, (B) Shannon diversity index, (C) NMDS (nonmetric multidimensional scaling) of unweighted UniFrac metric, (D) NMDS of weighted UniFrac metric. In (A) and (B), different letters denote significant differences ($P < 0.05$) among treatments within an elevation. In (C) and (D), each symbol corresponds to a sample collected from a particular elevation, and each color corresponds to a treatment. Points that are close together represent samples with similar bacterial and archaeal community composition, and the dashed ovals represent 95% confidence intervals of sample ordination grouped by treatment. The stress value for the unweighted (C) and weighted (D) metrics were 0.08 and 0.09, respectively. Shrub removal treatments were not sampled at 3500 m elevation.

Supplemental Tables and Figures

Table S1.1 Parameter estimates for structural equation models i-iv corresponding to path coefficients in figure 1.2 a-d. This table corresponds to the figure S1.1, but here are given mean, standard deviation (SD), 95% credible intervals, and probabilities (Pr) that each parameter was not different from zero, calculated from the posterior distributions of parameters.

	Shannon Diversity				OTU Richness				NMDS1				Olig:Copio Abundance			
Parameter	**Mean**	**SD**	**95% Credible Interval**	**Pr**	**Mean**	**SD**	**95% Credible Interval**	**Pr**	**Mean**	**SD**	**95% Credible Interval**	**Pr**	**Mean**	**SD**	**95% Credible Interval**	**Pr**
SOC on CN	-0.23	0.235	(-0.697,0.232)	0.164	-0.24	0.237	(-0.711,0.247)	0.158	-0.23	0.239	(-0.701,0.236)	0.166	-0.23	0.237	(-0.69,0.231)	0.162
SON on CN	0.14	0.227	(-0.31,0.582)	0.276	0.14	0.226	(-0.298,0.583)	0.265	0.14	0.23	(-0.314,0.578)	0.277	0.13	0.228	(-0.328,0.579)	0.283
PH on Microbes	0.20	0.129	(-0.0466,0.458)	0.064	0.23	0.134	(-0.0319,0.503)	0.042	-0.04	0.158	(-0.329,0.284)	0.401	-0.09	0.139	(-0.375,0.175)	0.255
SOC on Microbes	-0.01	0.189	(-0.377,0.365)	0.472	0.04	0.19	(-0.344,0.429)	0.410	-0.01	0.214	(-0.44,0.412)	0.474	0.20	0.208	(-0.211,0.6)	0.171
SON on Microbes	-0.34	0.201	(-0.732,0.0578)	0.047	-0.27	0.207	(-0.659,0.158)	0.095	-0.04	0.244	(-0.518,0.42)	0.434	-0.12	0.223	(-0.561,0.333)	0.289
VWC on Microbes	-0.34	0.3	(-0.927,0.241)	0.126	-0.24	0.335	(-0.868,0.49)	0.236	0.04	0.396	(-0.796,0.762)	0.455	-0.22	0.314	(-0.86,0.411)	0.238
Shrubs on Microbes (Low elev)	1.03	0.512	(0.0328,2.06)	0.022	0.87	0.512	(-0.146,1.86)	0.045	-1.19	0.559	(-2.3,-0.116)	0.017	-0.24	0.513	(-1.26,0.772)	0.323
Shrubs on Microbes (Mid elev)	1.09	0.491	(0.119,2.06)	0.013	0.80	0.485	(-0.116,1.81)	0.049	-0.85	0.508	(-1.85,0.136)	0.046	-0.37	0.471	(-1.28,0.557)	0.218
Shrubs on Microbes (High elev)	1.01	0.435	(0.205,1.89)	0.010	0.91	0.453	(0.0649,1.84)	0.022	-0.60	0.487	(-1.58,0.332)	0.109	-0.18	0.442	(-1.08,0.637)	0.341
Shrubs on pH (Low elev)	0.46	0.686	(-0.905,1.83)	0.253	0.42	0.68	(-0.93,1.75)	0.266	0.45	0.675	(-0.865,1.79)	0.255	0.44	0.696	(-0.983,1.8)	0.263
Shrubs on pH (Mid elev)	-0.12	0.678	(-1.44,1.21)	0.432	-0.12	0.683	(-1.49,1.19)	0.430	-0.14	0.689	(-1.55,1.22)	0.418	-0.12	0.675	(-1.46,1.19)	0.431
Shrubs on pH (High elev)	-0.34	0.677	(-1.66,0.961)	0.309	-0.33	0.67	(-1.62,1.01)	0.312	-0.35	0.673	(-1.68,0.937)	0.302	-0.34	0.68	(-1.65,0.98)	0.308
CN on Respiration	0.00	0.161	(-0.308,0.328)	0.497	0.00	0.164	(-0.309,0.327)	0.491	0.02	0.16	(-0.298,0.328)	0.461	-0.01	0.156	(-0.309,0.304)	0.467
Microbes on Respiration (Low elev)	-0.05	0.273	(-0.606,0.486)	0.431	-0.06	0.316	(-0.69,0.584)	0.428	0.10	0.201	(-0.281,0.511)	0.315	0.13	0.557	(-0.832,1.37)	0.408
Microbes on Respiration (Mid elev)	-0.02	0.492	(-0.975,1.01)	0.483	-0.24	0.549	(-1.39,0.874)	0.331	-0.12	0.624	(-1.5,1.03)	0.422	-0.26	0.778	(-1.91,1.28)	0.370
Microbes on Respiration (High elev)	0.81	0.58	(-0.39,1.95)	0.081	0.58	0.737	(-0.894,1.95)	0.217	-0.34	0.726	(-1.67,1.03)	0.321	1.07	0.574	(0.178,2.33)	0.031
SOC on Respiration	0.11	0.252	(-0.39,0.593)	0.326	0.14	0.256	(-0.375,0.645)	0.290	0.21	0.24	(-0.266,0.692)	0.187	0.01	0.255	(-0.488,0.509)	0.491
SON on Respiration	0.39	0.293	(-0.161,0.985)	0.090	0.29	0.281	(-0.263,0.856)	0.150	0.24	0.26	(-0.28,0.755)	0.181	0.34	0.295	(-0.242,0.904)	0.125
VWC on Respiration	-0.05	0.265	(-0.58,0.453)	0.420	0.01	0.316	(-0.609,0.608)	0.490	0.10	0.274	(-0.471,0.622)	0.352	0.23	0.215	(-0.182,0.667)	0.148
Shrubs on SOC (Low elev)	1.20	0.481	(0.203,2.14)	0.006	1.22	0.486	(0.319,2.16)	0.006	1.21	0.485	(0.242,2.19)	0.006	1.23	0.492	(0.303,2.21)	0.006
Shrubs on SOC (Mid elev)	0.38	0.496	(-0.591,1.35)	0.219	0.39	0.487	(-0.567,1.38)	0.213	0.39	0.49	(-0.557,1.34)	0.211	0.39	0.483	(-0.525,1.35)	0.209
Shrubs on SOC (High elev)	0.49	0.492	(-0.473,1.47)	0.162	0.49	0.48	(-0.433,1.42)	0.154	0.50	0.501	(-0.517,1.48)	0.159	0.51	0.489	(-0.455,1.48)	0.147
Shrubs on SON (Low elev)	0.59	0.472	(-0.342,1.52)	0.106	0.61	0.483	(-0.342,1.56)	0.103	0.62	0.473	(-0.322,1.6)	0.096	0.59	0.481	(-0.336,1.53)	0.111
Shrubs on SON (Mid elev)	-0.25	0.485	(-1.23,0.72)	0.304	-0.26	0.478	(-1.21,0.645)	0.291	-0.26	0.46	(-1.16,0.641)	0.285	-0.26	0.476	(-1.21,0.646)	0.292
Shrubs on SON (High elev)	-0.23	0.478	(-1.16,0.683)	0.316	-0.20	0.488	(-1.18,0.745)	0.338	-0.22	0.469	(-1.13,0.7)	0.318	-0.22	0.482	(-1.16,0.746)	0.326
Shrubs on VWC (Low elev)	-0.23	0.308	(-0.827,0.383)	0.233	-0.23	0.305	(-0.839,0.368)	0.222	-0.22	0.321	(-0.863,0.412)	0.245	-0.22	0.309	(-0.81,0.397)	0.235
Shrubs on VWC (Mid elev)	0.12	0.309	(-0.472,0.733)	0.349	0.12	0.313	(-0.527,0.732)	0.353	0.12	0.311	(-0.494,0.722)	0.346	0.13	0.311	(-0.491,0.731)	0.344
Shrubs on VWC (High elev)	0.41	0.305	(-0.188,1.01)	0.087	0.41	0.303	(-0.188,1.01)	0.088	0.41	0.309	(-0.203,1.03)	0.093	0.42	0.304	(-0.159,1.05)	0.083

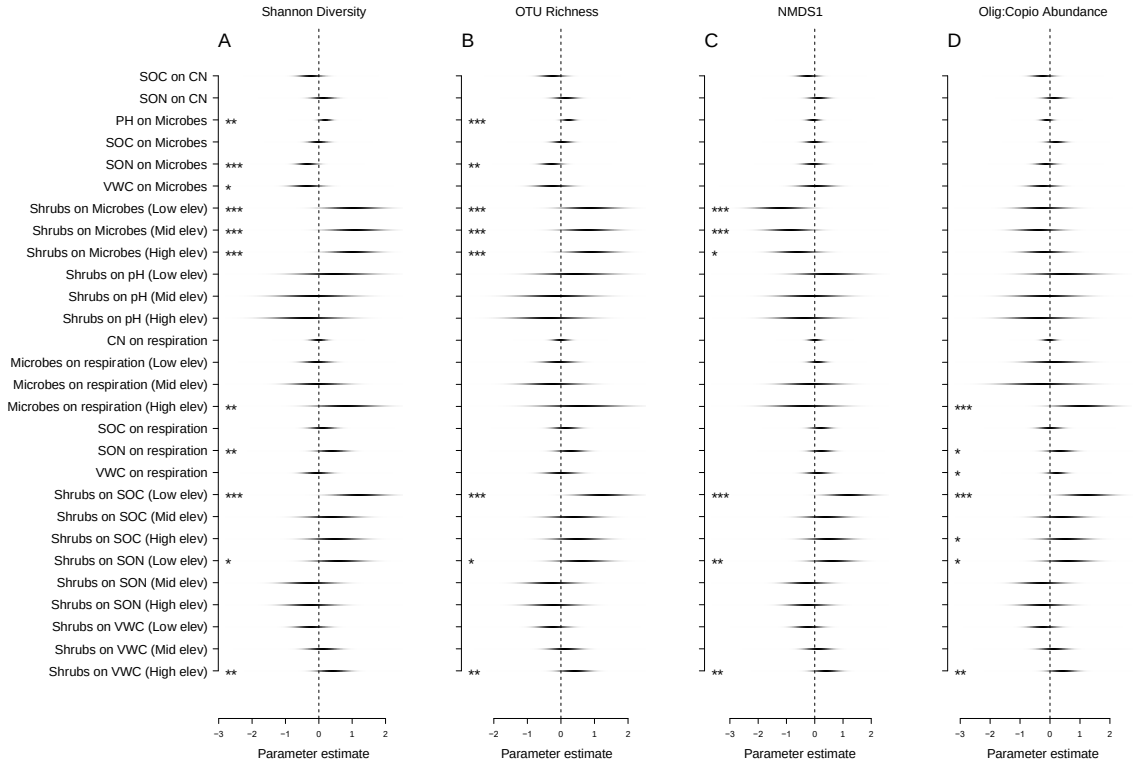

Figure S1.1 Parameter estimates for structural equation models corresponding to path coefficients in fig 1.2 a-d. Posterior probability distributions are plotted using the denstrip package in R (Jackson 2008). We used the posterior distributions to calculate the probabilities that each parameter was different from zero, and three levels of significance are plotted (one, two, and three asterisks denote 85, 90, and 95% probabilities respectively, that the relationship is different from 0). Because the effect of shrub cover in this study was a categorical test (samples were taken from underneath shrubs, from interspaces between shrubs, and in shrub removal plots), these effects were calculated in the model as the difference between intercept terms that were allowed to vary by treatment. Therefore, the assessment of strength and significance shrub effects was based on the posterior distribution of the difference between shrub and no-shrub intercepts. Low, Mid and High elevations correspond to 3100m, 3500m, and 3800m respectively. "Microbes" refers to the microbial response included in each model (e.g. Shannon diversity, OTU richness, Oligotrophic: Copiotrophic abundances, and NMDS1, the first axis of the NMDS analysis of community composition). VWC=Volumetric water content, res=respiration.

Jackson, C. H. (2008) Displaying uncertainty with shading. The American Statistician, 62(4):340-347.

Chapter 2

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

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 1) determine whether the diversity and community composition of soil fungi vary across elevation gradients and to 2) 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 modeling to tease apart effects of the environment and intra-community 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 environmental change.

Introduction

Changes in global climate and land use are having significant impacts on above and belowground organisms worldwide including plants and soil microbes (Wolters et al. 2000). This is particularly true in cold arctic and alpine biomes where warming is occurring at an accelerated pace (Rammig et al. 2010, Pepin et al. 2015). Alpine environments have been relatively poorly studied when considering the impacts of global change on belowground soil organisms (Lazzaro et al. 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 and Margesin 2016). Therefore, it is necessary to improve our baseline knowledge of how soil fungal communities change across elevation and climate gradients in order to 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 et al. 2013). In particular, the relative importance of different mechanisms including soil abiotic conditions, plant associations, and interactions among taxa are difficult to disentangle. Soil fungal diversity often declines with increasing elevation in alpine environments (Schinner and Gstraunthaler 1981, Körner 2003). 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 (Körner 2003, Bahram et al. 2012, Peay et al. 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 and Margesin 2016) or that fungal community composition, but not alpha diversity and richness, vary across elevation gradients (Shen et al. 2014, Lanzén et al. 2016). In general however, it is known that elevation is an important predictor of fungal communities worldwide (Kivlin et al. 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 (Körner 2003, 2007, He et al. 2016). 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 2014), and an 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).

Individual fungal taxa may differ in their responses to elevation gradients due to their environmental tolerances and plant associations. For example, ectomycorrhizal (ECM) and arbuscular mycorrhizal fungi (AMF) decline in diversity at higher elevations because of declines in plant species hosts at high elevation sites (Wu et al. 2007, Bahram et al. 2012, Shen et al. 2014, Tedersoo et al. 2014). However, Dark Septate Endophytes (DSE) maintain high abundances in alpine environments where in general mycorrhizal abundance is low (Körner 2003, Newsham 2011, Schmidt et al. 2012). 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 Archaerhizomycetes 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 et al. 2007). 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, this is likely to have strong impacts on fungal diversity and community structure (Harmon et al. 1986, Bardgett et al. 2005, De Boer et al. 2005, Nielsen et al. 2015). Shifts from herbaceous to woody shrub 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 et al. 2001, Hollister et al. 2010). In arctic tundra, Ascomycota and Chytridiomycota were more abundant in grass tussock soils than shrub soils, while Zygo and Basidiomycete fungi were more abundant in shrub soils (Wallenstein et al 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 and 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 has become increasingly recognized as an important determinant of microbial community structure that is often missing from traditional analyses (Wardle 2006, Little et al. 2008, Cordero and Datta 2016). 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 (Gessner et al. 2010, Bell et al. 2013). 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 et al. 1997, Maestre et al. 2009, Li et al. 2013). 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 et al. 2014, Cavieres et al. 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 aims to 1) determine whether the diversity and community composition of soil fungi vary across elevation gradients and to 2) 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 test three primary hypotheses: (i) Fungal diversity decreases and community composition changes with elevation in alpine soils; (ii) Vegetation more strongly influences fungal diversity and community structure than abiotic soil parameters as soil fungi are closely linked to plant identity; (iii) Interactions among fungal taxa will further shape community structure and positive interactions will dominate negative interactions due to high abiotic stress in alpine soils.

To test these hypotheses, we combine observational field data with a manipulative shrub removal experiment along an elevation transect of alpine shrub expansion. We utilize next generation sequencing and joint distribution modeling in a novel way to tease apart effects of the environment and intra-community interactions on soil fungi.

Methods

Soil Sampling

Soils were sampled in August 2015 in the White Mountains of California, near Crooked Creek (3094 m; 37° 29’ 56” N, 118° 10’ 19” W) and Barcroft (3800 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 327mm at Crooked Creek Station and - 1.7 °C and 456 mm at Barcroft Station (Hall 1991). Sampling took place within a transition zone from sub-alpine sagebrush steppe into alpine fellfields dominated by prostrate cushion plants and perennial bunchgrasses. As described by Taylor (1974) 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 3657 m elevation) contains seventeen plant species with the three most common including *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 of 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: 3100, 3500, and 3800 m (however sagebrush removal plots were only at 3100 and 3800 m elevations). This elevation gradient spans the observed sagebrush range expansion from subalpine (<3500 m) to alpine (>3500m) areas over the last 50 years (Kopp and Cleland 2014). In 1961, A. rothrockii was not present at the 3800 m site and was found in moderate to low densities at the 3500 m site, while the subalpine (3100 m) site had historically high sagebrush cover (Mooney, 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 circular 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 x 10 cm deep) were collected from directly under and outside 5 sagebrush individuals at each elevation site. In addition, two replicate soil cores were taken from five sagebrush removal plots at the low (3100 m) and high (3800 m) elevation sites. For soils characterizing non-shrub 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 of the direct influences of the sagebrush species. For shrub removal plots, only aboveground sagebrush biomass was removed in order to prevent significant disturbance to soil structure. Soil was placed in sterile specimen cups and stored at -80 ◦C prior to analysis.

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 fiber filter (Thomas C5500, Thomas Scientific, Swedesboro, NJ). Extracts were shipped overnight and analyzed 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 et al 1985). We subtracted unfumigated TOC/TON from paired fumigated samples and divided by the kEC and kEN coefficients of 0.45 and 0.69, respectively (Wu et al. 1990, Joergensen and Mueller 1996).

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*) (Schoch et al. 2012, Öpik et al. 2014) and poor mapping to phylogeny (Yarza et al. 2017).

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 diH20. Thermocycler settings were 94ºC for 3 minutes, followed by 35 cycles of 94ºC for 45 seconds, 50ºC for 60 seconds, and 72ºC for 90

seconds, followed by 72ºC for 10 minutes. Forward primers contained unique 12-base Golay barcodes as described in Walters *et al.* (2015); (see also Hamady *et al.* 2008). We then did PCR clean-up using a Nucleo Spin Gel-Extraction kit (Macherey-Nagel GmbH & Co. KG). Purified samples were pooled in equimolar concentrations and sequenced in a multiplexed 2- x 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.

Bioinformatics

Sequences were demultiplexed and processed with the split_libraries_fastq.py from QIIME 1.9.1 (Caporaso et al. 2010). Samples with less than 10,000 reads were removed. 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. Samples were also normalized using cumulative sum scaling (CSS) in the Metagenomeseq (Bioconductor) package in R for analyses of differential abundance of taxa (HMSC analyses; see below).

Statistical analyses

Alpha and Beta Diversity 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 as for soil nutrients, as TOC and TON were correlated across samples ($r=0.56$, $p<0.001$). All 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 development team 2013). 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 co-vary with both elevation and vegetation type, 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 2.1.

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 *et al.* 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 heteroscedascity of predictors using a Breush-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 2.1).

Joint Distribution Models

CSS normalized read abundance of fungal OTUs at different taxonomic levels were analyzed 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 multi-species communities. This approach is increasingly favored for analyzing plant and animal community data but is just beginning to be used for microbial community (sequencing) data (Aivelo and 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 greater than 90% probability of being different from zero ($p < 0.1$). 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 taxa 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 the environmental effects and may be influenced by both direct interactions among taxa and also common responses to unmeasured environmental variables.

Results

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%), Chytridomycota (0.3%), Glomeromycota (0.3%), 0.5% Protists (Cercozoa) and Microsporidia-like organisms (Rozellomycota) (0.5%). Seven percent of the total sequences had no blast hit so taxonomy could not be assigned.

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=0.047; richness: df=32.76, t-value= -2.555, p=0.015). The low elevation (3200 m) site had significantly higher richness than both the middle (3500m) and high (3800m) sites, while the latter two sites were not significantly different from each other (3500 vs. 3200: z value -2.531, p=0.031; 3800 vs. 3200: z value -2.555, p=0.028, Fig.2.1) and Chao1 diversity was slightly lower at the high vs. low elevation site (3800 vs. 3200: z-value= -2.063, p=0.097).

Vegetation type (shrub, outside shrub, 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=0.003, Fig. 2.1) and diversity (df=21.610, t-value= -2.688, p=0.013) and lower richness than shrub removal soils (z-value= -2.345, p=0.049). 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=0.037, Fig. 2.1) 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=0.045, Fig. 2.1).

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 was significantly better than the elevation x vegetation only model (Δ AIC: 22.1 diversity, 19.1 richness, Table 2.1). Additionally, models incorporating all abiotic parameters were significantly better than both elevation and vegetation type only models

for diversity and richness (Δ AIC>>2, Table 2.1). No single abiotic parameter was a significant predictor of alpha diversity alone.

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= 0.001, R^2=0.087; elevation: df=2, F=4.19, p=0.001, R^2=0.125, interaction: df=3, F=1.79, p=0.001, R^2=0.81 respectively; Fig. 2.2). Across vegetation types shrub soil community composition was different from both interspace soil (p=0.001) and shrub removal soils (p=0.001), however shrub removal soil was not different than shrub interspace soils. Across elevations, low elevation soils differed from both middle (p=0.001) and high (p=0.001) elevation soils, and middle soils also differed from high soils (p=0.001)

For abiotic drivers, VWC and TOC were significant predictors of fungal community composition (VWC: df=1 F=4.379, p= 0.001 R^2=0.0703, TOC: df=1, F=2.755, p=0.001 R^2=0.0467) 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= 0.004 R^2=0.0541, TOC int: df=1, F=2.755, p=0.001 R^2=0.0467)

Joint Distribution modeling

Joint distribution models through the HMSC framework 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 and Hanlin 1998), were more abundant in shrub interspace soils (p=0.098). The order Rhizophlyctidales, a soil inhabiting, cellulose-degrading chytrid (Letcher 2008) was more abundant in shrub soils (p=0.0825). The corresponding families Phyllachoraceae and Rhizophlyctidaceae were also more abundant in interspace and shrub soils, respectively (p=0.11, 0.102) although these probability estimates were slightly higher than our proposed cutoff (Table 2.2, S2.1).

Family level models also revealed that the Puccinaceae, a Basidiomycete rust fungal pathogen, and the more abundant in shrub interspace soils (p=0.07), while the Pluteaceae, a family in the Agaricomycota closely related to Amanita, were more abundant in shrub soils (p=0.095). In shrub removal soils, both Lachnocladiaceae and Auriscalpiaceae, two families in the Russulales order of Agaricomycota, had higher relative abundance (p=0.1, 0.08), as well as the Thelotremataceae, a lichenized Pezizomycotina (p=0.07) (Table 2.2, S2.1).

Across elevation, there was an increase in relative abundance for the family Botryobasidiaceae, an Agaricomycete of the Cantharellales order (p=0.054). In addition, this family and closely related Cantharellales (family incertae sedis) increased in relative abundance with higher pH (p=0.05, 0.077) (Table 2.2, S2.1).

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 34-99% of the variation dependent on fungal class with an average of 74%, while core explained between 0.4%-37% of the variance with an average of 10% (Fig. 2.3, Table S2.2). Overall, fixed effects including elevation, vegetation type, and biotic and abiotic soil parameters explained between 1-50% of total variation (Fig. 2.3, Table S2.2). 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 most (~2.7%) and Volumetric water content (VWC) explaining the least (~1%).

Taxa associations

After accounting for fixed effects, there were significant positive and negative associations among individual fungal taxa. At a correlation (r) level of +/- 0.3 or greater, 25 fungal orders showed varying positive and negative relationships (Fig. 2.4). Out of these 25, 10 fungal orders had correlations (r) of +/- 0.4 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 (Fig. 2.4, Table S2.3).

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 stronger 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, and positive interactions 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. Understaing 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.

Alpha and Beta diversity

Fungal diversity, including the Chao1 index and OTU richness, declined with increasing elevation, exhibiting the strongest decline from the subalpine (3200m) to alpine sites (3500, 3800m). 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, Fig. 2.2). These results are in accordance with previous alpine research highlighting that fungal diversity declines with increasing elevation (Körner 2003, Sundqvist et al. 2013). Elevation, in and of itself, is not a mechanistic driver (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). In a previous study at this location, we observed that Shannon diversity was unaffected and OTU richness slightly *increased* with elevation for bacteria and archaea (Collins *et al.* 2016), signifying very different controls over bacterial and fungal alpha diversity across elevation gradients.

Vegetation type and abiotic soil properties also influenced fungal diversity and community composition, and their relative importance differed for alpha and beta diversity. Shrub soils at high elevation sites had lower richness than shrub soils at the low elevation site; 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 more 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, demonstrating that alpine fungal communities can shift rapidly in response to changes in plant communities.

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 their influence via elevation and plant community shifts.

Fungal beta diversity was also regulated by VWC in addition to SOC, but VWC was a stronger 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 decreases in fungal diversity with increased precipitation in a rainfall manipulation experiment in California grasslands. We see similar results for both alpha 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 stable 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 hotspots and diversity of niches, promoting more distinct fungal communities across sites (Frey 2007, Classen et al. 2015).

There is reasonable evidence that fungi are more closely associated with plant species identity than other microbial groups, particularly prokaryotes, which are predominantly regulated by abiotic soil properties (Nielsen et al. 2010, Cassman et al. 2016). 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 prokaryotes (Hammel 1997, De Boer et al. 2005, Thorn and Lynch 2007). Our data partially supported this hypothesis, with vegetation type being a stronger 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 et al. 2016). Trends in fungal diversity and community composition in suggest that shrub expansion may affect 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 thereby impacting fungal community composition in this arid environment. In the same way, alpine cushion plants influence soil fungal communities via enhanced soil moisture and nutrients, as well as buffering changes in soil pH, which then has a stronger

influence on fungal communities in adjacent open soils (Roy et al. 2013). We similarly found that soil pH played a smaller role than water and nutrients in determining fungal community composition, which may reflect a strong influence of plant communities on the abiotic conditions for soil fungi.

Joint modeling of fungal communities

The amount of taxa-specific distribution data generated from sequencing, combined with the joint distribution modeling approach, offer 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 most strongly, taxa 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 and Thorn 2006, Zak et al. 2011) and also include mycorrhizal species. They are important decomposers in cold, dry environments, as has been shown in arctic studies (Ludley and Robinson 2008) and are dominant in forest floor communities (Edwards and Zak 2010). The increased relative abundance of these fungal groups may result from increased woody litter accumulation from shrubs, both above and belowground, may provide important substrate for decomposer fungi, in particular 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 over time 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 bio-control for agricultural weeds (Stubbs and 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 et al. 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 need 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 under-sampling (Paulson et al. 2013) any attempt to estimate true biological abundance from sequence read abundance is imperfect (Weiss et al. 2017). Nonetheless, we use and interpret these differential abundance data in a manner consistent with other recent analyses of the environmental effects on microbial composition across samples (Ghanbari et al. 2017, Timonen et al. 2017).

Variance partitioning

Despite the significant effects of vegetation and elevation 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 propose 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) 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 et al. 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 (Fig. 2.3), in which

individual members are likely to have more conserved environmental responses (Lu et al. 2016).

Taxa 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 et al. 2014, Ovaskainen et al. 2017). As hypothesized, interactions tended to be positive rather than negative (Fig. 2.4), proposing 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 and Zak 2011), and proliferate during and directly after peak plant biomass in alpine soils (Zinger et al. 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 xerophillic Basidiomycete fungi (Zalar et al. 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 xerophillic taxa, in particular ascomycetes. Colonization of a substrate (e.g. leaf, piece of wood) by saprophytic fungal taxa may prevent other fungi from utilitzing 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 either via 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 et al. 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 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.

Comparison across microbial groups

Because soil bacterial and fungal communities are intricately linked and play synergistic roles in decomposition (De Boer *et al.* 2005) and well as interactions with plants in the rhizosphere (Artursson et al. 2006), it 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 linked to 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 et al. 2009, Siles and Margesin 2016) although was not linked to elevation or vegetation type in our system (Collins et al. 2016). This contrasts to the patterns observed in fungal communities in that elevation was a much stronger predictor of alpha diversity and that shrub expansion promoted 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 that 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 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 (Rowe and Lidgard 2009, Nottingham et al. 2016). We found similarly in this study, that sampling location and spatial heterogeneity across the landscape were dominant drivers of soil fungal community structure and composition, and it is likely that increased sampling intensity within 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 have significant influence on abiotic soil properties, seasonal fluctuations in resources can be equally important or stronger predictors of microbial community composition (Shahnavaz et al. 2012, Lazzaro et al. 2015). Further, bacteria and fungi respond very differently to seasonal events including snowpack, snowmelt and peak growing season (Zinger et al. 2009, Lazzaro et al. 2015). In general, annual cycles of biomass, diversity, and turnover of particular taxa are more pronounced for bacteria than fungi, as fungi tend to be more cold tolerant, and can utilize more recalcitrant plant compounds to maintain their biomass under winter snowpack (Zinger et al. 2009, Lazzaro et al. 2015). As inter-annual seasonal variability increases with climate change (Nicholls and 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.

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 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 (Martiny et al. 2006, Kivlin et al. 2011). 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 in a global change context.

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Tables

Table 2.1 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 (PLOTID) was included as a random effect of sampling location within sites. Models are listed in order of best fit using delta AIC. Beta diversity (community composition) was assessed using non-metric multidimensional scaling (NMDS) of the Bray-Curtis dissimilarity metric (Bray) and Permutational multivariate analysis of variance (perMANOVA) via the function 'adonis'. Elevation, vegetation type, 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. Models are listed in order of best fit (R^2 values).

Alpha diversity		**Δ AIC**	**R^2**	**Df**
Full model	lmer(chao1~soil.pH + VWC + TOC + Vegetation*Elevation + (1|PLOTID) lmer(observed_otus ~ soil.pH + VWC + TOC + Vegetation*Elevation + (1|PLOTID)	0 0	0.20 0.273	13 13
Interaction	lmer(chao1 ~ Vegetation*Elevation + (1|PLOTID); lmer(observed_otus ~ Vegetation*Elevation + (1|PLOTID)	22.1 19.1	0.167 0.254	10 10
Abiotic variables	lmer(chao1~soil.pH + VWC + TOC + (1|PLOTID) lmer(observed_otus ~ soil.pH + VWC + TOC + (1|PLOTID)	61.1 57.9	0.152 0.202	6 6
Elevation	lmer(chao1 ~ Elevation + (1|PLOTID); lmer(observed_otus ~ Elevation + (1|PLOTID)	67.2 62.5	0.088 0.154	5 5
Vegetation type	lmer(chao1 ~ Vegetation + (1|PLOTID); lmer(observed_otus ~ Vegetation + (1|PLOTID)	70.4 68.5	0.026 0.038	5 5
Beta diversity			**R^2**	**Df**
Elevation	adonis(Bray ~ Elevation)		0.126	2
Vegetation type	adonis(Bray ~ Vegetation)		0.087	2
Interaction	adonis(Bray ~ Elevation*Vegetation)		0.081	3
Abiotic variables	adonis(Bray ~ VWC*Vegetation, strata= Elevation) adonis(Bray ~ TOC*Vegetation, strata= Elevation) adonis(Bray ~ soil.pH*Vegetation, strata= Elevation)		0.07 (0.054-int) 0.047 (0.046-int) 0.022 (0.038-int)	1 2 1 2 1 2

Table 2.2 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 & 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 & Kirk, 2007)
Auriscalpiaceae	Basidiomycota (Agaricomycetes)	Shrub removal	Saprotrophic, wood decomposition	(E. Larsson & Larsson, 2003)
Thelotremataceae	Ascomycota (Lecanoromycetes)	Shrub removal	Lichenized	(Mangold, Martín, Lücking, & Lumbsch, 2008)
Botryobasidiaceae	Basidiomycota (Agaricomycetes)	High elevation	Wood, litter decomposition	(K. H. Larsson, 2007)

Figures

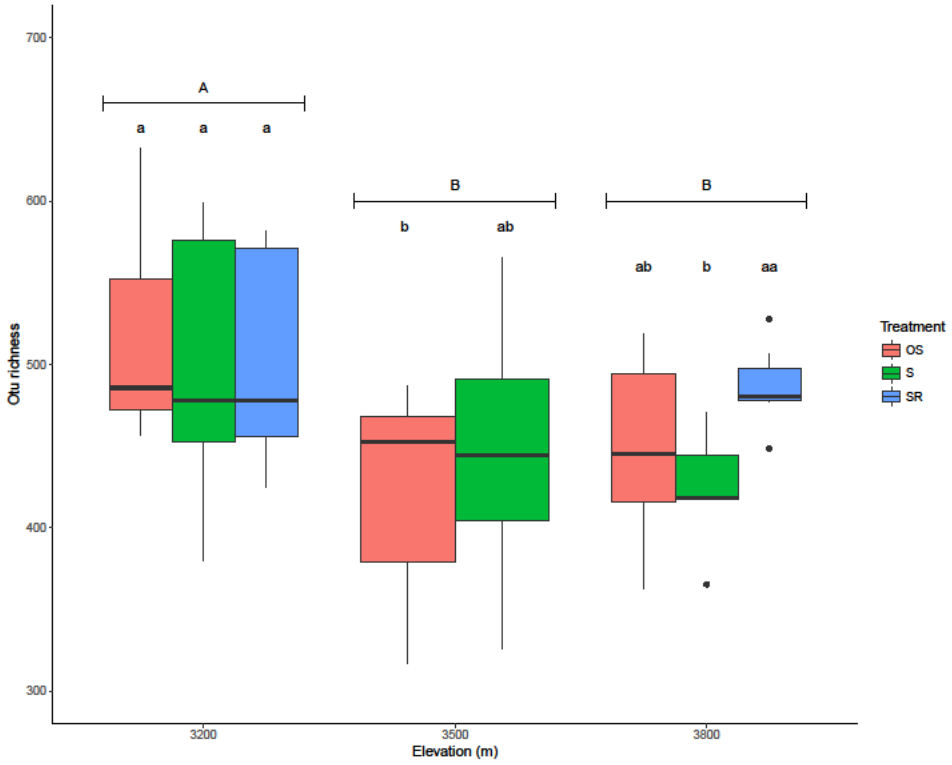

Fig. 2.1 Alpha diversity results for the effect of vegetation type (OS=shrub interspace, S=shrub, SR= shrub removal) OTU richness for alpine soil fungal communities at three elevation sites (3200m, 3500m, and 3800m asl). Capital letters denote significant differences between elevation sites while lower case letters denote significant differences between vegetation types within a site and for the same vegetation type across sites.

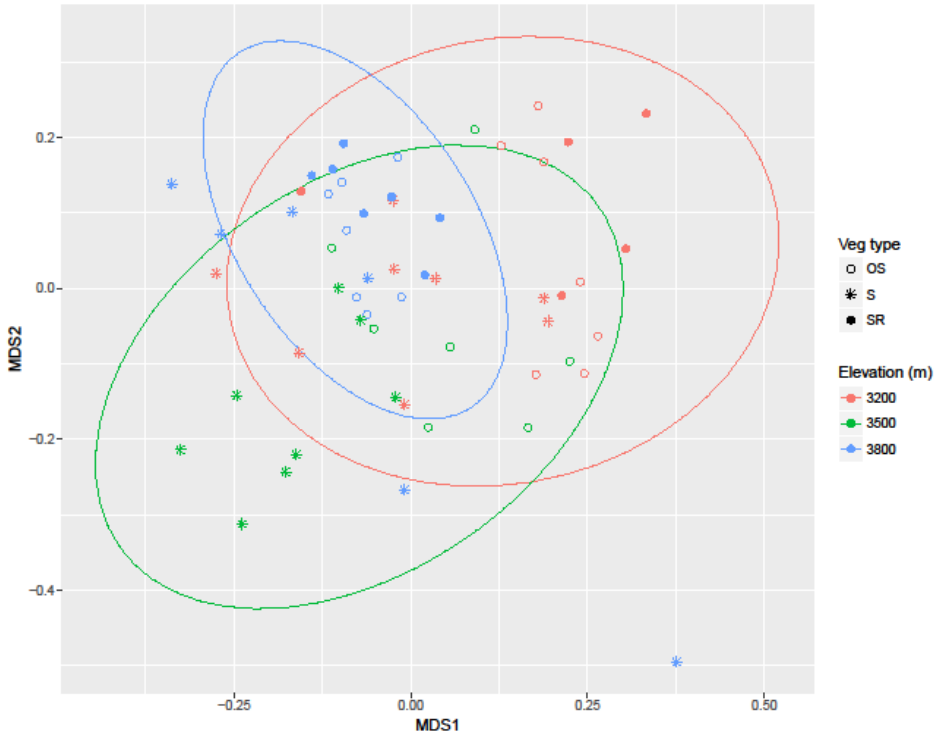

Fig. 2.2 Non-metric multidimensonal scaling (NMDS) of community dissimilarity (Bray-Curtis) of soil fungi (Stress value=0.145). Each point corresponds to a soil sample collected from one of three vegetation types (shape) or elevation sites (color). Points which are close together signify samples with similar fungal community compsoition. Colored ovals represent 95% confidence intervals of sample ordination grouped by elevation.

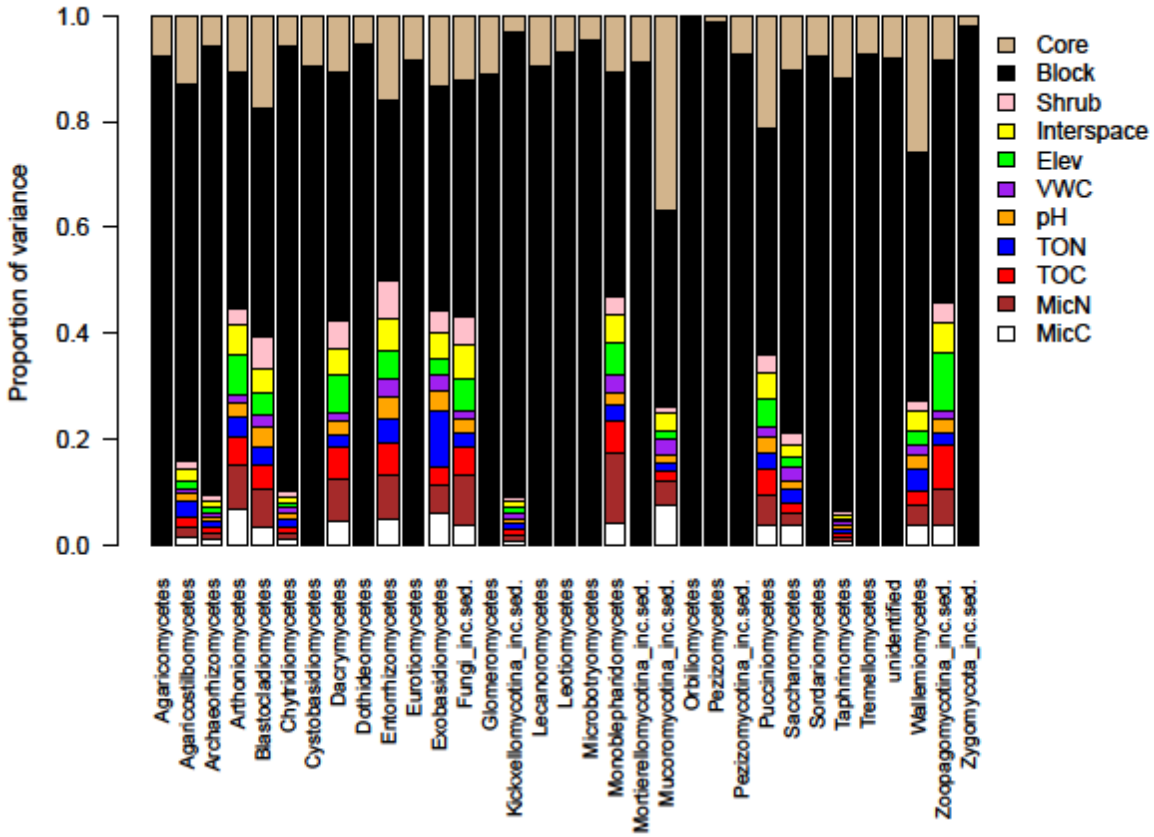

Fig. 2.3 Results of variance partitioning for the variation in fungal relative abundance (at the class level) in response to vegetation type (shrub and interspace), elevation, soil pH, VWC, TOC, TON, and Microbial biomass C and N. Core replicate as well as sampling location ("block") were included as random effects.

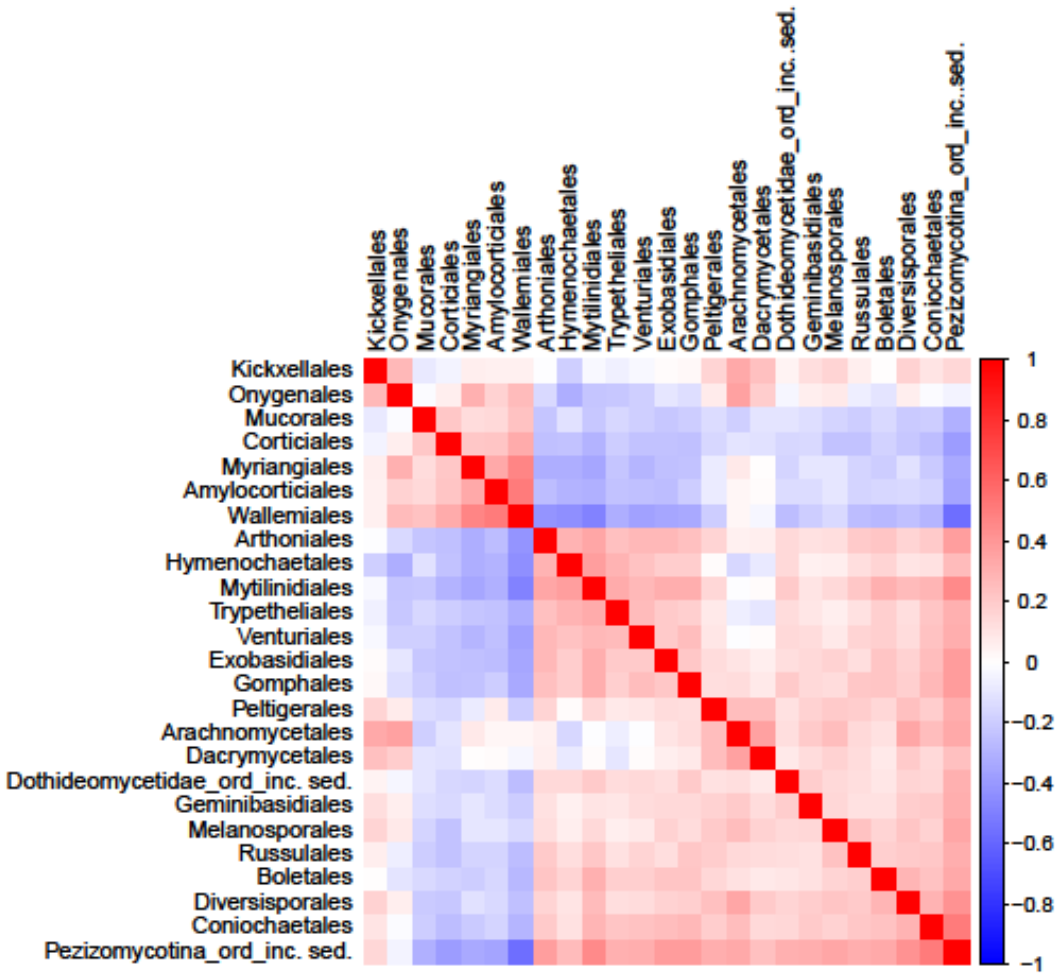

Fig. 2.4 Estimates of associations among fungal taxa (at the order level) based on residual correlations after accounting for fixed predictors. Taxa have a positive (red), negative (blue), or neutral (white) association with other taxa and the strength of this association (Pearson correlation coefficient, r) is depicted by the shade of the color. 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. 85 fungal orders did not have any associations meeting this criterion.

Supplemental Tables and Figures

Table S2.1 Mean and standard deviation (sd) of parameter estimates for the posterior distribution of joint distribution models. Parameters estimate the effects of vegetation type (shrub, shrub interspace, shrub removal) and elevation on the relative read abundance of fungal taxa at the family level, as described in Table 2.2. Significant responses are shown in bold with grey background.

	Elevation		Interspace		Shrub		Shrub Removal	
	mean	sd	mean	sd	mean	sd	mean	sd
Acarosporaceae	0.025426	0.174777	-0.01996	0.145508	-0.0263	0.149327	0.130003	0.193881
Acaulosporaceae	-0.04428	0.131033	-0.05881	0.090242	-0.11477	0.098588	0.048723	0.149667
Agaricaceae	0.038418	0.175785	-0.02645	0.144469	-0.02915	0.153455	0.101943	0.195156
Agaricostilbaceae	0.097001	0.16275	-0.06622	0.121151	-0.01218	0.127852	0.06802	0.190018
Ajellomycetaceae	0.090992	0.158809	-0.0729	0.1245	0.10037	0.130127	0.110909	0.18649
Amanitaceae	-0.1306	0.163763	-0.077	0.131252	-0.00222	0.137081	0.136253	0.198844
Ambisporaceae	-0.01294	0.170932	-0.04661	0.140805	-0.02449	0.149043	0.150778	0.202607
Amniculicolaceae	-0.00557	0.143155	-0.12315	0.105384	-0.02743	0.111817	0.126808	0.165398
Amphisphaeriaceae	0.076955	0.154159	-0.15676	0.12943	-0.10743	0.137231	0.080367	0.193941
Amylocorticiaceae	0.030255	0.102163	0.008364	0.071601	0.05613	0.077115	0.062939	0.133585
Apiosporaceae	-0.12512	0.100097	-0.04478	0.064692	-0.02335	0.069719	0.04078	0.126174
Arachnomycetaceae	0.048575	0.157094	-0.02994	0.120794	0.06809	0.12641	0.051605	0.183375
Archaeorhizomycetaceae	0.038617	0.183119	-0.03509	0.147411	-0.04091	0.145159	0.135612	0.201244
Archaeosporaceae	0.024343	0.161467	-0.02683	0.140108	0.035492	0.136835	0.173414	0.190266
Ascobolaceae	0.036283	0.163663	-0.0039	0.129133	0.056891	0.133027	0.221477	0.184426
Ascosphaeraceae	-0.12829	0.104654	-0.04672	0.067646	-0.02359	0.07335	0.045289	0.13287
Atheliaceae	0.01171	0.172658	-0.10265	0.133899	0.00736	0.141837	0.181396	0.195599
Auriculariales_fam_inc.sed.	-0.02183	0.160454	0.024942	0.125098	0.006176	0.129874	0.132472	0.184116
Auriscalpiaceae	-0.07371	0.124614	-0.12686	0.083897	-0.0447	0.093757	**0.200551**	0.145957
Basidiobolaceae	-0.00249	0.175994	-0.0176	0.136599	-0.05413	0.143588	0.134498	0.197204
Bionectriaceae	-0.01583	0.161092	0.003782	0.12942	-0.04227	0.141253	0.110013	0.199605
Blastocladiaceae	0.071089	0.140045	0.071456	0.096817	0.002596	0.104933	0.04972	0.165015
Bolbitiaceae	-0.07297	0.163039	-0.13914	0.116901	-0.05171	0.119103	0.144414	0.171361
Boletaceae	-0.03096	0.16429	-0.00861	0.130159	-0.09052	0.131869	0.162553	0.195943
Boliniaceae	-0.07735	0.142746	-0.04476	0.108646	-0.0394	0.114439	0.12677	0.152661
Bondarzewiaceae	0.075787	0.133915	0.040658	0.092048	0.092895	0.102979	0.046419	0.154108
Botryobasidiaceae	**0.216389**	0.135042	0.004227	0.09069	-0.09637	0.095669	-0.03254	0.165776
Botryosphaeriaceae	0.038413	0.171792	-0.10837	0.148245	0.013684	0.154044	0.128473	0.19058
Byssolomataceae	0.093349	0.101908	-0.03206	0.06661	-0.05358	0.071901	0.008109	0.124944
Cantharellaceae	-0.03849	0.128539	0.030215	0.090239	-0.02723	0.097647	0.063313	0.154572
Cantharellales_fam_inc.sed.	-0.0398	0.164065	-0.00341	0.127167	-0.04917	0.129859	0.178902	0.192343
Capnodiales_fam_inc.sed.	0.015484	0.177418	-0.01657	0.143325	-0.01224	0.148515	0.129451	0.193061
Catenariaceae	-0.02981	0.147179	0.084002	0.107834	0.030951	0.113543	0.079891	0.169036
Cephalothecaceae	-0.00058	0.164347	-0.04332	0.13637	0.001425	0.142959	0.13741	0.191459
Ceratobasidiaceae	0.012338	0.177693	-0.03542	0.13547	-0.02315	0.149022	0.116957	0.192075
Ceratostomataceae	-0.18056	0.143807	-0.15865	0.102522	-0.19194	0.109773	0.135888	0.166295
Chaetomiaceae	0.04654	0.178301	-0.01221	0.146231	-0.03896	0.146349	0.082126	0.193385
Chaetosphaeriaceae	-0.01113	0.145908	0.019748	0.108779	-0.11758	0.117652	0.197814	0.175312
Chaetothyriaceae	0.093662	0.145747	0.013386	0.109654	0.039137	0.117781	0.140247	0.176179
Chaetothyriales_fam_inc.sed.	0.068989	0.150819	0.07508	0.116122	0.028794	0.121731	0.126024	0.175461
Chionosphaeraceae	0.10893	0.160429	-0.05842	0.134296	-0.07319	0.130976	0.126123	0.194294
Chytridiaceae	-0.10501	0.10102	-0.10685	0.06516	-0.09464	0.069405	0.057715	0.116183
Claroideoglomeraceae	0.093469	0.149512	0.017678	0.113379	-0.01513	0.119949	0.065009	0.16544
Clavariaceae	0.033862	0.171141	-0.06656	0.148085	-0.00828	0.146172	0.122278	0.190295
Clavicipitaceae	-0.04988	0.168917	-0.09649	0.14288	0.03036	0.146174	0.147851	0.188793
Clavulinaceae	0.029949	0.170482	-0.02032	0.145043	-0.02489	0.152624	0.131178	0.198064
Collemataceae	0.052775	0.118215	-0.16684	0.076886	-0.12847	0.085749	0.086506	0.144214
Coniochaetaceae	0.025302	0.173168	-0.04598	0.143825	-0.0149	0.144918	0.098715	0.190692
Coniocybaceae	-0.05547	0.105523	-0.03356	0.066907	-0.00081	0.076296	0.10114	0.131917
Coniophoraceae	-0.01146	0.1443	-0.05059	0.109258	-0.0344	0.113077	0.052028	0.16637
Cordycipitaceae	0.019366	0.172132	-0.01697	0.138856	-0.01067	0.144988	0.151992	0.185705
Corticiaceae	0.117801	0.138119	-0.05054	0.106802	-0.08892	0.110413	0.075364	0.166174
Cortinariaceae	0.177975	0.155611	-0.0064	0.118434	-0.09384	0.125351	0.0625	0.195013
Cucurbitariaceae	-0.06705	0.126416	-0.07099	0.091648	-0.0947	0.097359	0.073009	0.15462
Cunninghamellaceae	0.072443	0.125996	0.095708	0.094251	0.055546	0.096661	0.011329	0.167415
Cyphellaceae	-0.02895	0.163881	-0.0371	0.142533	-0.02075	0.141651	0.143378	0.193445

	Elevation		Interspace		Shrub		Shrub Removal	
	mean	sd	mean	sd	mean	sd	mean	sd
Cystobasidiaceae	0.022179	0.175801	-0.06795	0.138128	0.011502	0.143564	0.092143	0.189868
Cystofilobasidiaceae	0.014148	0.168721	-0.05996	0.135688	-0.05035	0.144605	0.159009	0.197499
Cystofilobasidiales_fam_inc.sed.	0.019509	0.094938	-0.02397	0.067802	0.01	0.070711	0.08678	0.130332
Cyttariaceae	-0.06965	0.144672	-0.08159	0.096303	0.036812	0.104861	0.136387	0.167682
Dacrymycetaceae	0.016615	0.11275	0.059497	0.078591	0.044392	0.084708	0.069543	0.139255
Davidiellaceae	0.011707	0.158477	-0.10424	0.120348	-0.09087	0.127061	0.13004	0.193473
Dermateaceae	-0.06771	0.171895	-0.09503	0.135279	0.041912	0.141619	0.217092	0.192027
Diademaceae	0.011101	0.172948	-0.01151	0.137402	0.040534	0.140742	0.136287	0.196559
Diaporthaceae	0.032312	0.152592	-0.07394	0.113756	0.046582	0.118768	0.165977	0.176968
Didymosphaeriaceae	-0.03583	0.171249	-0.04766	0.136656	-0.02216	0.139294	0.14295	0.196699
Dipodascaceae	-0.00101	0.139294	-0.06976	0.099556	-0.02507	0.102439	0.141567	0.172981
Discinaceae	0.040129	0.114948	0.063793	0.076001	0.016288	0.085303	-0.03149	0.140387
Diversisporaceae	-0.07015	0.163021	-0.03629	0.126479	-0.08149	0.130178	0.087531	0.182597
Dothideaceae	-0.00107	0.153804	0.083291	0.115214	-0.04527	0.123701	0.02538	0.172425
Dothideomycetes_fam_inc.sed.	0.016482	0.169643	-0.03194	0.134874	0.025024	0.144965	0.195653	0.192476
Dothioraceae	0.009521	0.171305	-0.02376	0.146897	-0.03897	0.153462	0.111069	0.195442
Endochytriaceae	-0.06102	0.109308	-0.02468	0.070889	0.021181	0.075716	0.052979	0.140732
Entolomataceae	0.012659	0.178865	0.012343	0.144102	-0.02087	0.146859	0.127392	0.192764
Entorrhizaceae	-0.04755	0.165243	0.064589	0.122109	0.034626	0.125609	0.206399	0.183004
Eremomycetaceae	0.037466	0.125926	-0.04228	0.091073	-0.05581	0.097778	0.11198	0.151042
Erysiphaceae	0.040185	0.134902	0.054881	0.093844	-0.06298	0.104615	0.035697	0.164157
Erythrobasidiales_fam_inc.sed.	-0.04081	0.105198	-0.0605	0.071621	-0.08942	0.076397	0.039288	0.140608
Eurotiomycetes_fam_inc.sed.	-0.00089	0.151434	0.028281	0.11217	-0.0231	0.122239	0.059614	0.175555
Fungi_fam_inc.sed.	0.051097	0.115076	0.063057	0.076653	0.012558	0.083536	0.022235	0.138983
Geastraceae	0.011316	0.175781	-0.0451	0.147738	-0.0339	0.144491	0.130137	0.186541
Geminibasidiaceae	0.035054	0.132851	0.02565	0.095207	0.053912	0.099736	0.097687	0.15991
Gigasporaceae	-0.02518	0.122502	-0.07187	0.087589	-0.05084	0.090694	0.050315	0.148973
Glomeraceae	0.016435	0.177025	-0.02734	0.145501	-0.03949	0.151657	0.110981	0.194274
Glomerellaceae	0.048551	0.170193	-0.03118	0.131959	0.037481	0.140224	0.124863	0.193362
Gnomoniaceae	0.102531	0.101678	0.045501	0.067233	0.06762	0.07321	0.016379	0.125275
Gomphaceae	0.077869	0.143735	-0.00452	0.103314	-0.08927	0.110563	0.036008	0.168564
Gymnoascaceae	0.073781	0.133546	0.014197	0.096756	0.006924	0.101983	0.033689	0.167545
Halosphaeriaceae	0.028347	0.172904	0.017701	0.135348	0.019496	0.141222	0.148146	0.207523
Helotiaceae	0.046391	0.172401	-0.02096	0.140409	-1.48E-05	0.142956	0.117638	0.20272
Helotiales_fam_inc.sed.	0.008647	0.174866	-0.03083	0.145998	-0.03463	0.146931	0.099904	0.198142
Herpotrichiellaceae	0.020558	0.1757	-0.03659	0.145972	-0.00197	0.149995	0.110586	0.19505
Heterogastridiaceae	-0.02499	0.131022	0.026945	0.09773	0.074628	0.100563	0.084336	0.16367
Hyaloscyphaceae	0.001086	0.166645	-0.03389	0.137748	0.026153	0.14479	0.136379	0.205183
Hydnaceae	0.018086	0.172555	-0.00322	0.140656	-0.03383	0.143279	0.127914	0.194035
Hydnodontaceae	0.0136	0.174993	-0.02524	0.1486	-0.05647	0.156487	0.11071	0.192484
Hygrophoraceae	0.037186	0.172797	-0.01011	0.143275	-0.03249	0.147472	0.087907	0.192287
Hymenochaetaceae	-0.03243	0.151586	-0.03527	0.112879	-0.10653	0.116185	0.050063	0.18319
Hypocreaceae	0.004721	0.180466	-0.05715	0.146018	-0.01316	0.149348	0.114634	0.195303
Hypocreales_fam_inc.sed.	-0.03262	0.173428	-0.04142	0.143196	-0.05362	0.145627	0.11662	0.189759
Hyponectriaceae	0.036222	0.122798	-0.06912	0.087124	0.007979	0.088561	0.099407	0.154575
Inocybaceae	0.031993	0.174253	0.040665	0.139282	0.045937	0.146951	0.144378	0.192339
Kickxellaceae	0.075478	0.119413	0.067008	0.080436	0.052689	0.087039	-0.00808	0.142281
Kondoaceae	0.011564	0.148058	0.018395	0.108188	0.058164	0.117178	0.111995	0.161136
Lachnocladiaceae	-0.0605	0.133331	0.029129	0.090183	-0.037	0.096826	**0.196485**	0.154121
Lasiosphaeriaceae	0.00179	0.174037	-0.01461	0.135869	-0.00209	0.141285	0.105373	0.193822
Lecanoraceae	-0.04297	0.171227	-0.01966	0.142713	-0.02392	0.15076	0.116666	0.200713
Lecanorales_fam_inc.sed.	0.013592	0.164467	0.069	0.127061	0.004708	0.133551	0.098938	0.1786
Lecideaceae	0.006631	0.170842	-0.03901	0.144329	-0.01711	0.152343	0.144172	0.196859
Legeriomycetaceae	-0.02388	0.172526	-0.01287	0.146043	-0.00188	0.143896	0.164114	0.192544
Lentitheciaceae	-0.01086	0.171682	-0.07106	0.147168	-0.00351	0.146891	0.124342	0.194102
Leotiomycetes_fam_inc.sed.	-0.00657	0.177467	0.009466	0.145826	-0.04528	0.150127	0.108358	0.191921

	Elevation		Interspace		Shrub		Shrub Removal	
	mean	sd	mean	sd	mean	sd	mean	sd
Leptosphaeriaceae	9.50E-05	0.164642	-0.00975	0.14533	-0.01699	0.146414	0.099392	0.19124
Lichtheimiaceae	0.113396	0.160041	-0.12644	0.116702	-0.27862	0.123838	0.018901	0.186223
Lindgomycetaceae	0.113859	0.152569	-0.06783	0.109005	-0.04177	0.114911	0.191503	0.172351
Lobariaceae	-0.02873	0.173372	-0.06572	0.13612	-0.03186	0.139396	0.153537	0.192463
Lophiostomataceae	-0.00114	0.177853	0.019912	0.138779	0.000481	0.145077	0.159447	0.190786
Lycoperdaceae	0.100793	0.098019	0.019653	0.064944	-0.00711	0.066757	-0.02772	0.12407
Magnaporthaceae	-0.02959	0.180505	-0.04234	0.145628	-0.01274	0.152418	0.165387	0.202166
Malasseziaceae	0.028806	0.098973	0.024707	0.063047	-0.01825	0.07008	-0.03373	0.122795
Marasmiaceae	0.050981	0.105445	0.030667	0.064031	0.055501	0.071439	0.007132	0.127183
Massarinaceae	0.040657	0.174416	-0.00716	0.137	-0.01582	0.142309	0.136047	0.196834
Megalosporaceae	-0.06934	0.160345	-0.02524	0.133597	0.00087	0.143004	0.112941	0.194563
Melanommataceae	0.007884	0.165984	-0.13738	0.134122	-0.06997	0.140157	0.150521	0.187747
Meruliaceae	0.150913	0.123508	0.040352	0.091261	0.026727	0.094371	-0.03775	0.149933
Microascaceae	0.043653	0.178077	-0.07741	0.131811	-0.06096	0.143151	0.148675	0.201198
Montagnulaceae	0.101317	0.172378	-0.05349	0.138536	-0.01954	0.144557	0.075933	0.198238
Morchellaceae	0.050614	0.124254	-0.02877	0.081755	0.060931	0.089279	0.093331	0.147926
Mortierellaceae	-0.00237	0.176593	0.013458	0.14789	-0.0049	0.153374	0.108632	0.189729
Mycenaceae	0.0246	0.16936	-0.06767	0.139221	-0.00829	0.148212	0.134199	0.190389
Mycocaliciaceae	-0.01713	0.16371	-0.00273	0.124841	0.007426	0.133585	0.126059	0.186968
Mycosphaerellaceae	0.011295	0.180861	-0.03977	0.138981	-0.01989	0.148958	0.108964	0.20081
Myriangiaceae	-0.02967	0.138815	0.012771	0.091606	-0.00774	0.09719	0.059679	0.154966
Mytilinidiaceae	-0.00727	0.120274	-0.1012	0.086807	-0.1294	0.096021	0.052175	0.144559
Myxotrichaceae	0.031825	0.175166	-0.07271	0.149149	-0.01159	0.150017	0.140879	0.196231
Nectriaceae	-0.00551	0.17355	-0.02515	0.144891	-0.01715	0.146649	0.118755	0.188978
Niaceae	0.036466	0.14407	0.073159	0.109431	-0.03575	0.110973	0.081733	0.172444
Niessliaceae	0.151545	0.128069	-0.09965	0.087338	-0.07925	0.091294	0.06981	0.152413
Ochrolechiaceae	0.070997	0.171989	-0.06771	0.138115	-0.02556	0.147823	0.118037	0.196979
Olpidiaceae	0.039586	0.159432	-0.01841	0.116621	-0.0251	0.122444	0.071966	0.17563
Omphalotaceae	0.034533	0.161477	-0.13327	0.121856	-0.07925	0.128887	0.17229	0.187518
Onygenaceae	0.132435	0.142342	0.031119	0.106343	0.060686	0.103528	0.038265	0.167206
Onygenales_fam_inc.sed.	-0.00561	0.100971	-0.07325	0.067965	-0.07466	0.072086	0.057753	0.129612
Ophiocordycipitaceae	-0.01354	0.176222	-0.08945	0.144168	-0.0264	0.154085	0.091423	0.194816
Ophiostomataceae	0.026859	0.116573	0.012941	0.080189	-0.03078	0.083112	0.040619	0.139406
Orbiliaceae	0.011991	0.17074	-0.01916	0.146472	-0.0151	0.150144	0.124919	0.197739
Pannariaceae	0.002503	0.172699	-0.04787	0.14032	-0.10016	0.146373	0.124498	0.199527
Paraglomeraceae	0.066101	0.163017	-0.10208	0.13508	-0.07437	0.141035	0.131564	0.179985
Parmeliaceae	0.025391	0.175351	-0.04991	0.147378	-0.0307	0.155298	0.096331	0.197386
Peniophoraceae	0.091769	0.102159	0.04196	0.063824	0.019782	0.066272	0.023299	0.124623
Pertusariaceae	0.015924	0.10975	-0.00884	0.078062	-0.0729	0.087574	0.07104	0.149267
Pezizaceae	0.028532	0.181563	-0.02966	0.13983	-0.02664	0.145111	0.139472	0.191681
Pezizales_fam_inc.sed.	0.031642	0.111023	-0.05041	0.076966	-0.10312	0.080358	0.068228	0.142776
Pezizomycotina_fam_inc.sed.	0.011612	0.173329	-0.07597	0.14711	-0.00574	0.14438	0.105434	0.19517
Phaeosphaeriaceae	0.054237	0.16828	-0.04546	0.143598	-0.03243	0.144276	0.090341	0.200476
Phelloriniaceae	0.010615	0.104652	-0.0443	0.069905	-0.0209	0.076676	0.041515	0.137454
Phyllachoraceae	0.097217	0.099735	**0.077318**	0.064598	0.014725	0.069074	-0.06322	0.120507
Physalacriaceae	-0.09967	0.126447	-0.07937	0.09448	-0.00753	0.099389	0.081345	0.156717
Physciaceae	0.049877	0.164411	-0.03217	0.139057	-0.02876	0.142082	0.12923	0.18836
Physodermataceae	-0.00704	0.097392	-0.07576	0.062834	-0.07218	0.069025	0.028106	0.130957
Pichiaceae	-0.02711	0.095895	0.027996	0.063699	-0.0022	0.069505	0.074502	0.126597
Piptocephalidaceae	0.043247	0.098088	0.006351	0.065571	-0.04448	0.071474	0.061219	0.127809
Plectosphaerellaceae	0.090821	0.15632	-0.02466	0.115832	-0.01651	0.129394	0.141291	0.188845
Pleomassariaceae	-0.00547	0.161197	-0.0422	0.130158	0.034357	0.137026	0.136211	0.187365
Pleosporaceae	0.025431	0.17027	-0.07583	0.143564	-0.01943	0.152238	0.101567	0.191446
Pleosporales_fam_inc.sed.	0.018273	0.172173	-0.02432	0.146264	-0.0171	0.154359	0.104052	0.20774
Pleurotaceae	-0.10734	0.171344	-0.0297	0.136268	-0.08625	0.13972	0.136835	0.187889
Pleurotheciaceae	0.053902	0.119445	0.044236	0.081645	0.028103	0.088699	0.088601	0.147151
Pluteaceae	0.078225	0.128077	0.046208	0.091241	**0.127025**	0.097091	0.045592	0.149827

	Elevation		Interspace		Shrub		Shrub Removal	
	mean	sd	mean	sd	mean	sd	mean	sd
Polyporaceae	-0.05903	0.172627	-0.03084	0.133902	0.018081	0.144512	0.171993	0.194595
Polyporales_fam_inc.sed.	0.051123	0.179451	-0.08819	0.135831	-0.02081	0.14714	0.195186	0.1964
Protomycetaceae	-0.02258	0.173648	-0.08047	0.136726	-0.06176	0.137898	0.108559	0.194165
Psathyrellaceae	0.033913	0.171306	0.023198	0.136988	-0.07726	0.145888	0.154303	0.200222
Pseudeurotiaceae	0.025822	0.169844	-0.05162	0.138012	-0.12596	0.139214	0.116503	0.206343
Pucciniaceae	0.088645	0.11275	**0.101732**	0.070034	0.076548	0.075927	0.01223	0.13919
Pucciniales_fam_inc.sed.	0.01228	0.144164	-0.02942	0.101348	-0.00973	0.10666	0.129271	0.163638
Pucciniomycetes_fam_inc.sed.	0.025217	0.100401	-0.01195	0.067814	0.056177	0.070836	0.076834	0.130931
Pyrenulaceae	0.055614	0.116866	0.030132	0.083607	-0.04147	0.087053	0.056139	0.155167
Pyriculariaceae	0.169817	0.139197	-0.03329	0.099971	-0.04166	0.108265	0.083192	0.167706
Pyronemataceae	-0.00792	0.168062	-0.00439	0.135868	-0.10756	0.145569	0.131349	0.192217
Ramalinaceae	0.085071	0.143485	0.028129	0.106814	-0.00975	0.113097	0.042575	0.165188
Repetobasidiaceae	0.008848	0.155604	0.048469	0.122286	0.0171	0.124335	0.110782	0.177786
Rhizinaceae	-0.03994	0.162882	-0.04486	0.126104	-0.01891	0.132366	0.219067	0.193055
Rhizophlyctidaceae	0.067702	0.1107	0.000801	0.076974	**0.101211**	0.079759	0.068562	0.142979
Rhizophydiaceae	-0.0326	0.140689	-0.04322	0.101907	-0.10697	0.105596	0.055801	0.168249
Rhizopodaceae	0.011388	0.124612	0.017509	0.086881	-0.02408	0.093468	0.002996	0.15415
Rhizopogonaceae	0.017545	0.173413	-0.04113	0.144675	-0.04273	0.144628	0.1276	0.198043
Rhytismataceae	0.038135	0.157802	0.023318	0.119483	0.049543	0.124943	0.149276	0.168415
Roccellaceae	-0.04311	0.113063	-0.01719	0.076221	-0.10828	0.082291	0.010256	0.144218
Russulaceae	-0.01769	0.173155	-0.06606	0.133916	-0.04989	0.141921	0.123518	0.193525
Rutstroemiaceae	0.051186	0.172044	-0.14732	0.142723	-0.06481	0.144664	0.144741	0.20058
Saccharomycetaceae	0.078709	0.115131	-0.03883	0.075225	-0.06982	0.079779	0.047366	0.1344
Saccharomycetales_fam_inc.sed.	0.039337	0.166255	0.045712	0.140879	-0.09402	0.145765	0.165361	0.204654
Sarcosomataceae	-0.03768	0.091915	-0.03443	0.062678	-0.02556	0.067491	0.07243	0.118291
Schizophyllaceae	-0.07261	0.112937	-0.02296	0.074143	-0.04653	0.077897	0.054373	0.140321
Sclerodermataceae	-0.07604	0.115374	-0.07173	0.083534	-0.08478	0.094409	0.142629	0.151475
Sclerotiniaceae	0.005089	0.134996	0.032645	0.095065	-0.03673	0.105261	0.098431	0.159033
Sebacinaceae	0.0466	0.170969	0.022984	0.139818	-0.02019	0.145738	0.133903	0.198078
Sebacinales_Group_B	-0.0253	0.170537	0.017982	0.13777	0.008756	0.145029	0.133513	0.19551
Sordariaceae	0.012982	0.173871	0.00487	0.140366	-0.02657	0.152214	0.119204	0.191372
Sordariomycetes_fam_inc.sed.	0.115325	0.158656	0.08997	0.126394	0.023464	0.132194	0.115193	0.181765
Sordariomycetidae_fam_inc.sed.	0.105209	0.166836	0.017657	0.139414	0.006371	0.14044	0.122186	0.19693
Sporidiobolales_fam_inc.sed.	0.028564	0.176216	-0.02889	0.143265	-0.02289	0.148306	0.105037	0.193895
Sporormiaceae	-0.00316	0.175056	-0.02385	0.144667	-0.00727	0.147066	0.097879	0.19501
Stachybotriaceae	-0.08236	0.098995	0.00142	0.065182	-0.03616	0.073154	0.00622	0.131501
Stephanosporaceae	-0.05912	0.164654	-0.02436	0.12631	0.078415	0.133845	0.134279	0.176053
Stictidaceae	-0.01865	0.163803	0.009418	0.132164	-0.02943	0.136273	0.176714	0.185376
Strophariaceae	0.049111	0.129417	-0.03059	0.089163	0.06997	0.097993	0.080661	0.157154
Suillaceae	-0.02821	0.141998	-0.08383	0.099203	-0.06251	0.106152	0.096779	0.173674
Taphrinaceae	-0.01048	0.145844	-0.06182	0.114396	-0.0873	0.119141	0.112563	0.176506
Teloschistaceae	-0.0625	0.182057	0.023829	0.14187	-0.06237	0.145398	0.108791	0.198737
Teratosphaeriaceae	0.026179	0.176722	-0.02556	0.145049	-0.01213	0.14899	0.106049	0.194777
Tetraplosphaeriaceae	-0.00925	0.133195	-0.03275	0.090648	0.054983	0.095936	0.125401	0.15657
Thelebolaceae	0.101651	0.159417	-0.08032	0.124874	-0.07292	0.128991	0.101593	0.182066
Thelephoraceae	-0.01552	0.167092	0.030347	0.136706	-0.03635	0.138943	0.11662	0.195221
Thelotremataceae	-0.00686	0.114744	-0.05581	0.081238	0.017864	0.085468	**0.209954**	0.143152
Togniniaceae	-0.01939	0.152381	-0.00579	0.121651	0.001944	0.125849	0.093293	0.180641
Tremellales_fam_inc.sed.	0.013618	0.169392	-0.02016	0.140783	-0.02162	0.15143	0.100976	0.200164
Trichocomaceae	0.030409	0.171243	-0.02515	0.145325	-0.03619	0.143736	0.108954	0.206249
Tricholomataceae	0.02541	0.168648	-0.0236	0.146953	-0.02402	0.156632	0.112483	0.193106
Trichosporonaceae	-0.02385	0.175523	0.013346	0.145304	-0.03481	0.147072	0.149436	0.193796
Trypetheliaceae	0.095628	0.112871	-0.03008	0.072825	-0.04306	0.080212	0.042618	0.133335
Tubeufiaceae	0.081537	0.144319	-0.19245	0.112368	-0.00418	0.117445	0.196717	0.17898
Typhulaceae	-0.05959	0.102759	-0.10307	0.070163	-0.10548	0.07352	0.096152	0.132337
Umbelopsidaceae	0.052279	0.160717	0.000663	0.115176	-0.0562	0.122166	0.135604	0.176988
Umbilicariaceae	-0.02619	0.150288	-0.10117	0.115297	-0.04243	0.126028	0.118781	0.181537
unidentified	0.010973	0.180135	-0.02684	0.142302	-0.01918	0.148896	0.102444	0.18913
Valsaceae	-0.05396	0.098004	-0.07767	0.061954	-0.06743	0.065406	0.050249	0.12801
Venturiaceae	0.040686	0.180234	-0.02155	0.144879	-0.0323	0.157676	0.131997	0.203139
Verrucariaceae	0.084816	0.171569	-0.02665	0.132297	-0.06709	0.13957	0.137247	0.193551
Vibrisseaceae	0.074269	0.15259	0.025055	0.122469	0.041895	0.132987	0.130205	0.18397
Wallemiaceae	-0.02833	0.122271	-0.09646	0.090928	-0.01036	0.094655	0.100577	0.159615
Xylariaceae	0.024364	0.163128	-0.12358	0.141141	-0.11205	0.144341	0.136146	0.198646
Xylariales_fam_inc.sed.	-0.04479	0.164805	-0.04643	0.135919	0.023252	0.14236	0.16274	0.198088

Table S2.2 Results of variance partitioning analysis. Proportions of total variation explained for each fungal class corresponding to Figure 2.3

	TOC	TON	soil.pH	VWC	Elevation	Interspace	Shrub	Block	Core
Agaricomycetes	8.79E-06	1.71E-05	1.89E-05	9.50E-06	7.50E-06	7.82E-06	8.23E-06	0.925074	0.074848
Agaricostilbomycetes	0.024626	0.031397	0.030282	0.031166	0.015982	0.0097361	0.014063	0.713594	0.129154
Archaeorhizomycetes	0.013061	0.019902	0.022309	0.010052	0.008094	0.0091075	0.010429	0.848797	0.058249
Arthoniomycetes	0.060833	0.111482	0.112697	0.041388	0.027294	0.0139343	0.077831	0.449262	0.10528
Blastocladiomycetes	0.047788	0.132046	0.072757	0.036537	0.038273	0.0232045	0.040974	0.432136	0.176283
Chytridiomycetes	0.011323	0.023034	0.019628	0.016076	0.011818	0.0093085	0.00987	0.840794	0.058148
Cystobasidiomycetes	0.002209	0.004914	0.004918	0.002068	0.002291	0.0020314	0.002061	0.885003	0.094505
Dacrymycetes	0.050767	0.127091	0.111224	0.024509	0.026328	0.0138866	0.070869	0.467749	0.107576
Dothideomycetes	1.82E-06	3.54E-06	3.58E-06	1.78E-06	1.71E-06	1.45E-06	1.71E-06	0.947572	0.052412
Entorrhizomycetes	0.062043	0.150719	0.111469	0.044573	0.041939	0.0352666	0.052891	0.342348	0.158752
Eurotiomycetes	7.39E-06	1.49E-05	1.39E-05	7.11E-06	6.67E-06	6.50E-06	6.51E-06	0.916465	0.083472
Exobasidiomycetes	0.049847	0.099053	0.09314	0.105282	0.035588	0.0301666	0.029484	0.423297	0.134141
Fungi_inc.sed.	0.065506	0.146632	0.087048	0.026262	0.028961	0.015232	0.063069	0.445428	0.121862
Glomeromycetes	0.000283	0.000562	0.000586	0.0003	0.000267	0.0002355	0.000274	0.886677	0.110815
Kickxellomycotina_inc.sed.	0.009758	0.019222	0.019391	0.00816	0.010671	0.0108125	0.01181	0.880659	0.029515
Lecanoromycetes	5.81E-05	0.000115	0.000117	5.62E-05	4.67E-05	5.03E-05	5.82E-05	0.904173	0.095326
Leotiomycetes	1.99E-05	4.11E-05	4.33E-05	2.12E-05	1.79E-05	1.50E-05	1.97E-05	0.932408	0.067414
Microbotryomycetes	0.000328	0.000661	0.00066	0.00038	0.000329	0.0002848	0.000311	0.950339	0.046708
Monoblepharidomycetes	0.055753	0.160512	0.104194	0.030226	0.025841	0.0312247	0.061542	0.425214	0.105494
Mortierellomycotina_inc.sed.	0.000173	0.000375	0.000372	0.000174	0.000135	0.0001346	0.000153	0.909527	0.088957
Mucoromycotina_inc.sed.	0.034486	0.059829	0.088766	0.016563	0.014481	0.0302535	0.018238	0.369719	0.367665
Orbiliomycetes	0.000224	0.000457	0.000486	0.000227	0.000189	0.0002336	0.000223	0.994026	0.003933
Pezizomycetes	0.001842	0.003342	0.003593	0.001991	0.001736	0.0014513	0.001774	0.971404	0.012867
Pezizomycotina_inc.sed.	0.001504	0.003053	0.002705	0.001273	0.001173	0.0009714	0.001284	0.914416	0.07362
Pucciniomycetes	0.051377	0.089564	0.086194	0.030808	0.030799	0.019221	0.051201	0.426748	0.214088
Saccharomycetes	0.023238	0.045755	0.055953	0.025324	0.016708	0.0242181	0.020702	0.686568	0.101534
Sordariomycetes	3.55E-06	7.15E-06	7.16E-06	3.57E-06	3.44E-06	3.22E-06	3.39E-06	0.922929	0.077039
Taphrinomycetes	0.006774	0.014395	0.013836	0.008553	0.005575	0.0069838	0.006968	0.817501	0.119415
Tremellomycetes	2.46E-05	4.77E-05	5.00E-05	2.67E-05	2.21E-05	2.17E-05	2.51E-05	0.928189	0.071594
unidentified	1.03E-06	2.03E-06	2.01E-06	1.08E-06	9.50E-07	9.16E-07	9.63E-07	0.920548	0.079443
Wallemiomycetes	0.037452	0.059177	0.062658	0.041489	0.026117	0.0205511	0.026206	0.467965	0.258387
Zoopagomycotina_inc.sed.	0.059527	0.103948	0.112755	0.023982	0.02518	0.0170998	0.114313	0.460331	0.082865
Zygomycota_inc.sed.	0.001913	0.003954	0.004251	0.002017	0.001498	0.0013351	0.001931	0.96229	0.020813

Table S2.3. Correlation coefficients (r) corresponding to Figure 2.4.

	Amylocorticiales	Arachnomycetales	Arthoniales	Boletales	Coniochaetales	Corticiales	Dacrymycetales	Diversisporales	Dothideomycetidae	Exobasidiales	Geminibasidiales	Gomphales
Amylocorticiales	1	0.037559036	-0.2583969	-0.15817	-0.16613475	0.228231	0.017071897	-0.148944841	-0.138259739	-0.25956486	-0.130068898	-0.1934396
Arachnomycetales	0.037559036	1	0.05249367	0.114558	0.257194369	-0.108658	0.365598701	0.34113251	0.129414353	0.100694388	0.204185253	0.13049768
Arthoniales	-0.258396891	0.052493665	1	0.225758	0.206435768	-0.246382	0.068308761	0.16321874	0.141768707	0.270612201	0.117579395	0.2430177
Boletales	-0.158169487	0.114557579	0.22575755	1	0.233407984	-0.179938	0.081117757	0.283389971	0.098984879	0.227348797	0.116156776	0.22749242
Coniochaetales	-0.16613475	0.257194369	0.20643577	0.233408	1	-0.25617	0.140034355	0.298665433	0.156038019	0.259498279	0.20092058	0.27287138
Corticiales	0.228230778	-0.108657872	-0.246382	-0.17994	-0.256170415	1	-0.119091552	-0.213972239	-0.157335619	-0.23414494	-0.144075676	-0.2477819
Dacrymycetales	0.017071897	0.365598701	0.06830876	0.081118	0.140034355	-0.119092	1	0.20326709	0.08267168	0.061291323	0.139565374	0.08749034
Diversisporales	-0.148944841	0.34113251	0.16321874	0.28339	0.298665433	-0.213972	0.20326709	1	0.164926139	0.177420177	0.192576548	0.18291553
Dothideomycetidae	-0.138259739	0.129414353	0.14176871	0.098985	0.156038019	-0.157336	0.08267168	0.164926139	1	0.128100113	0.190102941	0.20674166
Exobasidiales	-0.259564856	0.100694388	0.2706122	0.227349	0.259498279	-0.234145	0.061291323	0.177420177	0.128100113	1	0.142855618	0.21697143
Geminibasidiales	-0.130068898	0.204185253	0.1175794	0.116157	0.20092058	-0.144076	0.139565374	0.192576548	0.190102941	0.142855618	1	0.14181891
Gomphales	-0.19343958	0.130497678	0.2430177	0.227492	0.272871376	-0.247782	0.087490343	0.182915525	0.206741657	0.216971425	0.141818908	1
Hymenochaetales	-0.299985601	-0.150627303	0.2964545	0.169094	0.116060416	-0.23706	-0.082850895	0.106727492	0.14640432	0.191435269	0.053420979	0.19016415
Kickxellales	0.055632569	0.339654204	-0.0083424	0.006336	0.106615131	-0.043762	0.246363486	0.174479815	0.047025093	0.01541623	0.124510814	0.02022657
Melanosporales	-0.091682426	0.251689103	0.12671058	0.167082	0.17581766	-0.237755	0.17043414	0.224862907	0.145979497	0.175560284	0.159355361	0.13318992
Mucorales	0.14845302	-0.180127453	-0.2259963	-0.14312	-0.195633475	0.215471	-0.102853717	-0.202536885	-0.102229654	-0.21051831	-0.120912099	-0.191016
Myriangiales	0.334830515	0.089231633	-0.3124083	-0.19929	-0.205299324	0.217106	0.00577474	-0.111990903	-0.164066538	-0.2494864	-0.093966668	-0.236739
Mytilinidiales	-0.308207591	-0.003554884	0.34224976	0.306394	0.286664959	-0.299289	0.0134067	0.267427389	0.20496995	0.317667118	0.107835245	0.3128781
Onygenales	0.173756969	0.362282555	-0.1405133	-0.10428	-0.019592877	0.066621	0.198912728	0.068433022	-0.036805169	-0.09726383	0.065899656	-0.1258175
Peltigerales	0.070989606	0.252801393	0.16078273	0.159746	0.190277536	-0.151751	0.255929585	0.246183922	0.11690237	0.135932722	0.170485297	0.12805042
Pezizomycotina	-0.353825208	0.336430896	0.37360689	0.338895	0.516323072	-0.382026	0.245336862	0.423907158	0.304978087	0.381946882	0.317768879	0.38142971
Russulales	-0.163390957	0.14953903	0.20215766	0.181524	0.213640409	-0.231846	0.131221152	0.205955309	0.128132035	0.12937763	0.114623897	0.2135211
Trypetheliales	-0.232554548	-0.066554679	0.24238961	0.182248	0.221849198	-0.19624	-0.094022183	0.129725547	0.138094097	0.201733546	0.095697309	0.18773764
Venturiales	-0.244979958	-0.0040732	0.27841036	0.183449	0.239850851	-0.236766	0.015274513	0.138892985	0.149402555	0.205102485	0.137464563	0.25090699
Wallemiales	0.516546732	0.034105324	-0.415747	-0.27039	-0.280015846	0.326517	-0.037095451	-0.241288715	-0.255318651	-0.34302459	-0.193525071	-0.3381003

	Hymenochaetales	Kickxellales	Melanosporales	Mucorales	Myriangiales	Mytilinidiales	Onygenales	Peltigerales	Pezizomycotina	Russulales	Trypetheliales	Venturiales	Wallemiale
Amylocorticiales	-0.299985601	0.0556326	-0.091682426	0.148453	0.33483052	-0.30820759	0.17375697	-0.07099	-0.353825208	-0.163391	-0.23255455	-0.24498	0.516547
Arachnomycetales	-0.150627303	0.3396542	0.251689103	-0.180127	0.08923163	-0.00355488	0.36228256	0.2528014	0.336430896	0.149539	-0.06655468	-0.0040732	0.034105
Arthoniales	0.296454499	-0.0083424	0.126710578	-0.225996	-0.3124083	0.342249761	-0.1405133	0.1607827	0.373606885	0.2021577	0.24238961	0.27841036	-0.41575
Boletales	0.169094143	0.0063355	0.167081971	-0.143124	-0.1992905	0.306394318	-0.1042773	0.1597459	0.33889535	0.1815239	0.182248271	0.18344862	-0.27039
Coniochaetales	0.116060416	0.1066151	0.17581766	-0.195633	-0.2052993	0.286664959	-0.0195929	0.1902775	0.516323072	0.2136404	0.221849198	0.23985085	-0.28002
Corticiales	-0.237060089	-0.0437625	-0.23775531	0.215471	0.21710569	-0.29928942	0.06662102	-0.151751	-0.382025699	-0.231846	-0.19623993	-0.236766	0.326517
Dacrymycetales	-0.082850895	0.2463635	0.17043414	-0.102854	0.00577474	0.0134067	0.19891273	0.2559296	0.245336862	0.1312212	-0.09402218	0.01527451	-0.0371
Diversisporales	0.106727492	0.1744798	0.224862907	-0.202537	-0.1119909	0.267427389	0.06843302	0.2461839	0.423907158	0.2059553	0.129725547	0.13889299	-0.24129
Dothideomycetidae	0.14640432	0.0470251	0.145979497	-0.10223	-0.1640665	0.20496995	-0.0368052	0.1169024	0.304978087	0.128132	0.138094097	0.14940256	-0.25532
Exobasidiales	0.191435269	0.0154162	0.175560284	-0.210518	-0.2494864	0.317667118	-0.0972638	0.1359327	0.381946882	0.1293776	0.201733546	0.20510249	-0.34302
Geminibasidiales	0.053420979	0.1245108	0.159355361	-0.120912	-0.0939667	0.107835245	0.06589966	0.1704853	0.317768879	0.1146239	0.095697309	0.13746456	-0.19353
Gomphales	0.190164153	0.0202266	0.133189924	-0.191016	-0.236739	0.312878101	-0.1258175	0.1280504	0.38142971	0.2135211	0.187737643	0.25090699	-0.3381
Hymenochaetales	1	-0.1810675	0.063726893	-0.118992	-0.3118939	0.37347379	-0.3125873	0.0105353	0.264896127	0.1259404	0.294298589	0.2335222	-0.43557
Kickxellales	-0.18106747	1	0.160989842	-0.08756	0.06124942	-0.02459184	0.27312303	0.1619317	0.152355574	0.0635118	-0.05865399	-0.0287744	0.057592
Melanosporales	0.063726893	0.1609898	1	-0.166543	-0.092943	0.147024004	0.08751269	0.2000397	0.34021151	0.2376226	0.068885948	0.0806832	-0.1413
Mucorales	-0.118991928	-0.0875598	-0.166542571	1	0.13966706	-0.21870298	-0.0135878	-0.133708	-0.303204502	-0.192358	-0.15769069	-0.1809377	0.231708
Myriangiales	-0.311893889	0.0612494	-0.092943022	0.139667	1	-0.34481149	0.3065933	-0.074993	-0.333219672	-0.163931	-0.22727515	-0.2875139	0.477219
Mytilinidiales	0.37347379	-0.0245918	0.147024004	-0.218703	-0.3448115	1	-0.2218453	0.1531238	0.450086283	0.2172498	0.326524504	0.27940062	-0.48235
Onygenales	-0.31258733	0.273123	0.087512685	-0.013588	0.3065933	-0.22184528	1	0.0746552	-0.041611693	-0.065141	-0.2118914	-0.185702	0.268367
Peltigerales	0.010535298	0.1619317	0.200039741	-0.133708	-0.0749926	0.153123778	0.07465524	1	0.31343557	0.194796	0.082700508	0.09547332	-0.19663
Pezizomycotina	0.264896127	0.1523556	0.34021151	-0.303205	-0.3332197	0.450086283	-0.0416117	0.3134356	1	0.315742	0.303646383	0.31300219	-0.56089
Russulales	0.12594036	0.0635118	0.237622558	-0.192358	-0.1639307	0.217249804	-0.0651413	0.194796	0.315741987	1	0.114626535	0.16952255	-0.25934
Trypetheliales	0.294298589	-0.058654	0.068885948	-0.157691	-0.2272752	0.326524504	-0.2118914	0.0827005	0.303646383	0.1146265	1	0.26707754	-0.31653
Venturiales	0.233522198	-0.0287744	0.080683202	-0.180938	-0.2875139	0.279400616	-0.185702	0.0954733	0.31300219	0.1695225	0.267077539	1	-0.36365
Wallemiales	-0.435573083	0.0575923	-0.141297029	0.231708	0.47721873	-0.48235439	0.26836673	-0.19663	-0.560892519	-0.259335	-0.31653334	-0.3636457	1

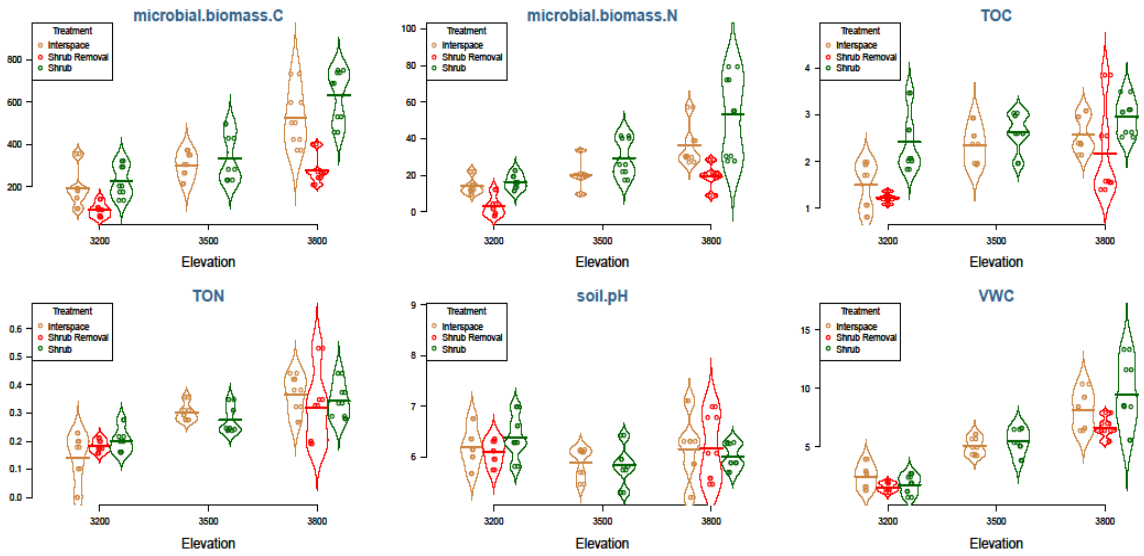

Figure S2.1 Abiotic soil parameters and microbial biomass C and N plotted by elevation and soil treatment.

Chapter 3

Beyond Enemy Release: Multiple microbial mechanisms influence plant-soil feedbacks and range expansion

Abstract

Understanding the complex interactions among plants and soil microbial communities is key to predicting plant species range shifts under a changing climate. Although release from natural enemies (e.g. soil pathogens) has been widely cited as a mechanism leading to range expansion in plants, mutualists and decomposers may also influence plant-soil feedbacks (PSFs) in novel environments. Here, we use a microbially-mediated PSF framework to experimentally test how soil microbial community structure and decomposer traits jointly affect the performance and leaf traits of a range-expanding sagebrush shrub, *Artemisia rockrothii*. We found that the diversity, community composition and relative richness of different functional groups of soil fungi, as well as traits of the decomposer community (extracellular enzyme activity), influenced sagebrush seedling growth through changes in biomass, root: shoot ratios, and leaf traits. Overall, fungal alpha diversity was the single best predictor of plant responses and had both direct and interacting effects with decomposer traits on plant performance and leaf traits. Plant-microbial competition for soil nutrients appeared to be an important mechanism mediating the impacts of fungal diversity and decomposer traits on seedling performance. Finally, fungal indicator taxa suggested that benefits of low pathogen diversity outweighed the costs of decreased mutualist diversity on sagebrush seedling growth in the range-expansion zone. Taken together, our results suggest that multiple microbial

mechanisms have the potential to influence range expanding plant species, and that while enemy release may be an important mechanism, microbes also significantly affect plant performance via competition for nutrients.

Introduction

Changes in climate and land use are creating novel communities of organisms around the globe (Tylianakis et al. 2008). This is due largely to species migrations or range shifts, often upwards in latitude or elevation as they attempt to "track" changes in climate (Parmesan et al. 2003, Valéry et al. 2008). Range shifts are limited by both environmental and biotic filters as well as species dispersal abilities (HilleRisLambers et al. 2012) and many species who attempt to migrate are unable to do so (Zhu et al. 2012). Plant-Soil feedbacks (PSFs) are an important biotic filter which can influence seedling establishment, species coexistence, priority effects and many other processes central to plant community composition and species range shifts (Hodge & Fitter, 2013; Van der Putten et al., 2013). Despite the ubiquity of PSFs, we lack a clear understanding of how different components of the microbial community contribute to PSFs, and how multiple microbial processes may interact to influence PSFs (Kardol, Veen, Teste, & Perring, 2015; Li et al., 2017; Long, Fry, Veen, & Kardol, 2019; Lou et al., 2014). As more plant species migrate in response to global change, knowledge of how the soil microbial community either facilitates or restricts these range shifts is essential (Morrien, Engelkes, Macel, Meisner, & Van der Putten, 2010; van der Putten, Bradford, Pernilla Brinkman, van de Voorde, & Veen, 2016).

PSF research in the context of species invasions and range shifts has often focused on the "enemy release hypothesis," which suggests that PSFs will be less negative for plants growing in 'away' soils due to release from soil pathogens present in 'home' soils (Colautti, Ricciardi, Grigorovich, & MacIsaac, 2004; Engelkes et al., 2008; Keane & Crawley, 2002; Mitchell & Power, 2003; Morrien et al., 2010; Suding et al., 2013; Van Grunsven, Van Der Putten, Martijn Bezemer, Berendse, & Veenendaal, 2010). However, enemy release is only one of a suite of microbial mechanisms that potentially influence PSFs (Mitchell et al., 2006; Morris et al., 2007; Richardson et al., 2000), which can be positive or negative depending on many aspects of microbial community structure and function (Austin, Vivanco, González-Arzac, & Pérez, 2014; Van der Putten et al., 2013). In contrast to 'enemy-release,' a lack of compatible soil mutualists (i.e., mycorrhizal fungi) in the introduced range may result in reduced plant growth (Van der Putten, 2012). For example, a lack of appropriate ectomycorrhizal (ECM) fungi can reduce seedling establishment and growth of invasive pine species (Nuñez, Horton, & Simberloff, 2009). Similarly, arbuscular mycorrhizal fungi (AMF) were less beneficial to *Robinia pseudoacacia* in its expansion range than its historic range, decreasing the overall benefits of soil pathogen release during range expansion (Callaway, Bedmar, & Reinhart, 2011). Ultimately, the relative abundance and richness of soil mutualists and pathogens, as well as the host-specificity of these microbial taxa, will influence the net outcomes of microbially-mediated PSFs on species range shifts.

In addition to their direct positive or negative effects on plants, PSFs can also involve indirect pathways through microbial impacts on nutrient cycling (e.g. microbial

mineralization or immobilization; Knops et al., 2002; Miki, 2012). The role of microbial decomposers (saprotrophs) are particularly under-studied, despite their important influences on plant growth, and may explain a large amount of the variation in PSFs and plant performance (T. Miki, Ushio, Fukui, & Kondoh, 2010). Plant competition with free-living microbial decomposers (saprotrophs) for soil nutrients influences plant growth and resource allocation strategies, particularly in nutrient-limited environments such as alpine, arctic and boreal ecosystems (Schimel & Bennett, 2004). Plants may use multiple strategies to compete with saprotrophic microbes, including altering patterns of root allocation or root functional traits, utilizing mycorrhizal fungi to enhance nutrient uptake and shifting from inorganic to organic nutrient forms (Cantarel et al., 2015; Hodge, Robinson, & Fitter, 2000; Xu, Ouyang, & Cao, 2011). The ability of seedlings to compete with soil microbes in nutrient limited environments will influence the success of species range expansions, particularly for species moving upwards in elevation or latitude.

Although plants and microbes can compete for limiting nutrients, plants also directly stimulate saprotrophic microbes to decompose nutrients into plant available forms (Brzostek, Greco, Drake, & Finzi, 2013; Jacoby, Peukert, Succurro, Koprivova, & Kopriva, 2017). This rhizosphere priming of saprotrophic microbes is closely related to plant signaling with mycorrhizal fungi (Broeckling, Broz, Bergelson, Manter, & Vivanco, 2008), suggesting that plant interactions with free-living and symbiotic microbes are highly linked. Very little work has considered how intracontinental range-expanding plants alter soil decomposer communities and nutrient cycling (but see Manrubia et al.,

2019), or how the functional capacity of decomposer communities may promote or limit the range expansion of these species.

Since identifying causal relationships within a complex set of soil microbe-plant interactions is challenging, microbial traits are a useful tool for isolating the mechanisms of microbial PSFs (Evans et al., 2017; Krause et al., 2014; Legay et al., 2016; Treseder & Lennon, 2015). Specifically, the production of different classes of extracellular enzymes is an important microbial trait that can impact plant growth via changes in decomposition and availability of mineral nutrients for plant growth (T. Miki et al., 2010). Microbial community traits may modulate or enhance the impacts of novel litter chemistry on soil nutrient cycling and plant nutrient benefit (Ke, Miki, & Ding, 2015). For example, invasive species often create positive PSFs through highly decomposable plant litter, but the relative benefit of this strategy can vary significantly based on the nutrient acquisition traits of microbial pathogens and mutualists (Ke et al., 2015) or free-living decomposer microbes (Kardol et al., 2015). Therefore, both the microbial community composition and microbial traits such as extracellular enzyme activity are likely to influence plant growth and resource use during species range expansions.

Lastly, microbially-meditated PSFs may also affect plant functional traits (Cortois, Schröder-Georgi, Weigelt, van der Putten, & De Deyn, 2016; Lau & Lennon, 2011; Xi, Chu, & Bloor, 2018), which can further influence plant performance and ecosystem processes (Van Nuland et al., 2016). For example, plants growing in soil communities conditioned by more resource-conservative plant species can also display more conservative (‘slow’) leaf functional traits (Baxendale, Orwin, Poly, Pommier, &

Bardgett, 2014). This suggests that plant and microbial traits might be coupled, such that microbes with high/low enzyme activity may co-occur with plants possessing similar traits on the fast/slow end of the leaf economic spectrum, and simultaneously influence ecosystem function (Grigulis et al., 2013; Legay et al., 2016). However, the causal direction of this relationship is unclear (plant vs. microbial control), highlighting the need to better understand how soil microbial traits influence plant functional traits and vice-versa.

Here, we assessed how microbially-mediated PSFs influenced a range-expanding sagebrush species (*Artemisia rothrockii* A. Gray (Asteraceae) to better understand the role of PSFs in plant range expansions and to determine how multiple microbial mechanisms simultaneously influence plant performance and functional traits (Fig 1). Specifically, we used a greenhouse experiment to test how soil microbial community structure and decomposer traits jointly affect the performance and leaf traits of *A. rothrockii* seedlings at both the historic and leading edge of this species' range expansion. We focused on soil fungi because they are the major drivers of organic matter decomposition and contain diverse functional groups of mutualists, pathogens, and saprotrophs (Taylor and Sinsabaugh, 2015). We hypothesized that microbial community structure (particularly mutualist: pathogen ratios) would have the strongest effects on seedling performance, whereas microbial decomposer traits would influence seedling traits via specific nutrient economies (i.e. carbon-cycling enzymes influence leaf C traits, and nitrogen-cycling enzymes influence leaf N traits). We further hypothesized that microbial community structure (particularly saprotroph abundance) would strongly

predict microbial decomposer traits. Finally, we hypothesized that seedling performance would be highest in high elevation (expansion zone) and non-shrub (herbaceous, shrub removal) soils due to a decrease in species-specific soil pathogens, as predicted by the enemy-release hypothesis.

Methods

Study Species

Artemisia rothrockii (Timberline Sagebrush) is a California endemic and dominant (sub)shrub species in the White Mountains in subalpine and alpine zones (Rundel et al., 2008, Mooney et al., 1962-described as *A. arbuscula*). *A. rothrockii*'s distribution has been moving upwards in elevation at an average rate of 30 m/decade over the last 50 years (Kopp & Cleland, 2014). This range expansion is likely promoted by changes in climate and land use, including drought, warming temperatures, and grazing cessation, however the influence of PSFs is unknown. Sagebrush is an obligate arbuscular mycorrhizal (AMF) host (Weber, King, & Aho, 2015) and *A. rothrockii* individuals in this range have moderate to high AMF root colonization (~60-80% average, Collins, C.G.- unpublished data). Previous work in this system has shown that soil microbial communities in the expansion zone of *A. rothrockii* differ in both structure and function from those in the historic range (Collins, Stajich, Weber, Pombubpa, & Diez, 2018; Collins, Wright, & Wurzburger, 2016). Specifically, soils under shrubs in the expansion zone harbor higher bacterial and lower fungal diversity, distinct microbial community composition, and higher substrate-induced respiration (Collins et al., 2018, 2016). These patterns suggest that soil microbial communities are strongly tied to plant identity in this

system and may therefore play a significant role in the initial seedling establishment, growth and further range expansion of *A. rothrockii*.

Study Site

Research was conducted at the White Mountain Research Center, located in the White mountains in eastern California and western Nevada, at the western edge of the Great Basin (mean annual temperature -0.4°C; mean annual precipitation 391mm (Hall 1991). Experimental plots were established at both 3100 m elevation and 3700 m elevation, representing the historic (low elevation) and range expansion (high elevation) zones of this species (Kopp and Cleland, 2014). In 1961, *A. rothrockii* was found in moderate to high densities at the 3100 m site and not present at the 3700 m site (Mooney et al., 1962). This shift between subalpine and alpine communities encompasses the transition from sagebrush steppe to true alpine plant communities dominated by prostrate cushion plants and perennial bunchgrasses (P. Rundel et al., 2008; P. W. Rundel, Gibson, & Sharifi, 2005). Species diversity in the White Mountains is fairly low and plant communities across our sampling gradient typically contain between 12 and 17 species including the most common herbaceous species: *Trifolium andersonii*, *Leptosiphon nuttallii*, *Koeleria macrantha*, *Elymus elymoides*, *Carex incurviformis*, and *Eriogonum ovalifolium* (Cheng, 2004).

Field sampling

We conducted a greenhouse experiment with plant and soil material from the White Mountains to better understand how PSFs affect *A. rothrockii* seedlings across its range expansion gradient. In October 2015, approximately 1000 seeds were collected

from 10 mature sagebrush individuals at the low elevation site (historic range) and stored in a desiccator at 4 ˚C for an 11-month cold stratification treatment (Bonner, 2008). In September 2016, soils for use as greenhouse inoculum were sampled from under five sagebrush individuals (< 100 m apart) at each elevation, along with paired soil cores (1.3 cm diameter x 10 cm deep) taken from the shrub interspaces. Shrub interspace cores were taken between 1 and 5 m from the edge of the canopy (based on sagebrush density of the site) in non-shrub, herbaceous plant cover. The corer was sterilized between each sample with a 10% bleach solution to prevent cross-contamination, and two replicate cores were combined into one sample. Soils were sampled in the same location as seeds at the low elevation site, and soils at the high elevation site were collected in 2 areas of recent sagebrush establishment (~200 m apart) as determined by Kopp and Cleland (2014). We also took soil samples using the same coring method from five separate (1 x 1m) plots where sagebrush has been manually removed for 1 year (SR1) and for 5 years (SR5) at both high and low elevation sites. Sagebrush removal plots were established to determine soil legacy effects and previous work showed that after 4 years microbial communities in sagebrush removal plots possessed an intermediate community composition and diversity compared to shrub and non-shrub (herbaceous) soils (Collins et al., 2018, 2016). Therefore, we used these soil inoculum to reflect a microbial community in transition between shrub and herbaceous vegetation types as is likely to occur at different time points of shrub range expansion.

All soil samples were placed on ice in the field and then kept cool in a refrigerator (4 ˚C) for one month prior to use. During this time, soils were sieved through a 2 mm

mesh to remove stones and large plant material. All sampling locations had granitic soils (Colluvium derived from granite) and east-/south-east-facing slopes to control for edaphic and aspect variation. As described in Collins et al. (2016), soil VWC, SON and SOC increase from the low to high elevation sites, however we diluted these abiotic differences in our greenhouse experiment (see below) by adding a small volume of inoculum to sterile background soils (Pernilla Brinkman, Van der Putten, Bakker, & Verhoeven, 2010). All soil samples were kept separate (N=40) to retain the variation in soil microbial communities within and across elevation sites and vegetation types (Gundale et al., 2019) and each sample was split in half with one half sterilized, and the other half live for paired inoculation (Table 1).

Greenhouse experiment

Seeds were surface sterilized with 10% bleach solution and germinated in trays of sterilized soil (autoclaved at 120˚C for 90 minutes). Seedlings were transplanted to larger pots (1600 mL) of sterile background soil after one month and initial seedling height at time of transplanting was used to estimate initial biomass (g) for each seedling via an allometric equation of dry biomass to height, generated from 10 additional seedlings. Background soil in all pots was identical to control for abiotic differences across the soil inoculum types and consisted of a custom mix of equal parts #30 silica sand and peat moss and a 15:10:1 ratio of Dolomite lime (CaMg(CO3)2), Triple Superphosphate (CaH4P2O8), and Potassium nitrate (KNO3) respectively. This closely resembles the granitic soil type where sagebrush grows in the White Mountains, characterized by high percent sand, coarse texture, low organic matter and low water retention (Smithers,

2017). During transplanting, pots were inoculated with 25g (~50 mL, 3% total pot volume) of either sterile or live soil inoculum directly in the rhizosphere zone of the seedling being transplanted. We grew one seedling for each soil sample (paired live and sterile) for a total of 80 seedlings (Table 1) (ISS-MSS design type, Gundale et al., 2019). Seedlings were grown for 4 months from October 2016-February 2017 (one alpine growing season). Greenhouse temperatures ranged from 10°C (low) to 22°C (high) which closely mirror average temperatures during the growing season at these elevations in the White Mountains and supplemental lighting was used in the evenings to extend day length to match the growing season (http://www.wmrc.edu/weather/). Seedlings were watered twice weekly with DI water.

After 4 months, all seedlings were harvested and soils in pots were sieved thoroughly to remove all belowground biomass. During harvest, rhizosphere soil was collected by gently shaking all excess soil from the roots of each plant into a Whirlpak bag, which were then immediately frozen (-20 C) for molecular analyses. Roots were washed in soapy water to remove any remaining soil and all plant material was placed in the drying oven at 60 ˚C for 72 hours and then weighed. Total final biomass (g) for each seedling was calculated by subtracting initial biomass from final biomass. We calculated a PSF value for all seedlings in live inoculum using the equation PSF= {(biomass (g) live soil - biomass (g) sterile soil)/biomass (g) sterile soil}. Biomass in sterile soil was the average biomass of all seedlings from the same soil elevation and vegetation type (i.e. site) as described in (Pernilla Brinkman et al., 2010, FB1 2nd equation). A negative PSF signifies lower growth in non-sterilized (live) soil versus sterilized soil, indicating an

overall negative effect of the soil microbial community. Finally, we calculated seedling root: shoot ratio by dividing the total final belowground biomass (g) by the total final aboveground biomass (g) of each seedling.

Leaf traits

We measured the following leaf functional traits for each seedling: leaf dry matter content LDMC (g/g), specific leaf area SLA (cm^2/g), leaf N (%), leaf C (%), δ13C, and δ15N. During harvest, one average sized leaf was collected from each plant and placed into a coin envelope, weighed within 24 hours on a microbalance for fresh weight (g), and scanned on a flatbed scanner to calculate leaf area (cm^2) using ImageJ software (https://imagej.nih.gov/ij/). Leaves were then placed in the drying oven (60 ˚C for 72 hours) and then weighed for dry weight (g). LDMC was calculated as the ratio of fresh weight (g) to dry weight (g) and SLA was calculated as leaf area (cm^2) to dry weight (g). Leaf chemical and isotope analyses were analyzed on dried leaf material at the University of Wyoming Stable Isotope Facility (Laramie, WY).

Microbial traits (Extracellular Enzyme Assays)

Extracellular enzyme activities were measured on all live soil inoculum (N=40 samples) following a modified protocol Saiya-Cork et al. (2002) as described in German et al. (2011). We measured two common microbial hydrolytic enzymes: Cellobiohydrolase (CBH) and β-N-acetylglucosaminidase (NAG) involved in C and N cycling respectively (Treseder and Lennon, 2015). Microbial substrates were created one week or less before assays. For CBH and NAG assays, 1mM standard solution of 4-Methylumbelliferone (MUB) substrate was made by dissolving 17.6 mg MUB into 1000

mL of MilliQ water on medium high heat and a stir plate. MUB stock solution was diluted to 25uM for use in assays and stored at 4ºC and covered with aluminum foil to prevent light exposure. Fluorescent substrates were created by dissolving 25 mg 4-Methylumbelliferyl β-D-cellobioside (CBH) and 7.59 mg 4-methylumbelliferyl n-acetyl-β-d-glucosaminide (NAG) in 100 mL milliQ water (500 uM and 200 uM, respectively). 50mM sodium acetate buffer was created by dissolving 15.4 g of Sodium acetate in 1L MilliQ water and stored at 4ºC. Buffer pH was adjusted with glacial acetic acid to pH=6 to match the pH of our soils. For all assays, frozen soils were thawed at room temperature from frozen for 1 hour. 1.4 g soil was added to 45.5 mL sodium acetate or maleate buffer and blended on high in a blender for 1 minute and then placed on the stir plate for 5 minutes to create soil slurries. Slurries were loaded into reaction plates within 10 minutes of leaving the stir plate. Black 96 well microplates were loaded by adding appropriate volumes of Sodium acetate buffer, followed by milliQ water and MUB working solution to create MUB standard curves, and finally fluorescent substrates and 200 uL of soil homogenate slurries until all wells contained 250 uL. Soil slurries were loaded with trimmed pipette tips to avoid clogging. Assays were run for 60 minutes and then terminated with 10 uL of 1M NaOH. Fluorescence readings were run on a Promega GloMax Multiplus Plate Reader at 365/450 nm excitation/ emission at the UCR Genomics Core. Enzyme activity (nmol hr^{-1} g^{-1}) was calculated using the following equations:

Activity (nmol hr^{-1} g^{-1}) = {NFU X Buffer vol} / {**ε** X Homogenate volume x 1-hour x soil mass)}

NFU= net fluorescence units = {(Assay-Homogenate control)/Quench coeff.}-Substrate control

Quench Coefficient = **ε** of MUB standard with soil/**ε** of MUB standard with buffer

ε =extinction coefficient=slope of standard curve. MUB std with buffer **ε** = 3049.25

Assay volume =0.25 mL, Homogenate volume= 0.20 mL, Buffer volume = 45.5 mL, soil mass=1.4 g.

Molecular analyses and Bioinformatics

DNA sequencing analyses were conducted on rhizosphere soils from each greenhouse seedling with live inoculum (N=40). We extracted microbial DNA from 0.25 g of soil using a Qiagen DNeasy PowerSoil Kit (Germantown MD, USA) and all DNA extracts were sent on dry ice to Novogene Corporation (Sacramento, CA) for sequencing of the ITS2 region for fungi. Forward and reverse primers ITS3 (5′-GCATCGATGAAGAACGCAGC-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (White et al. 2010) respectively, were used to amplify the ITS2 region. Sample libraries were created using the Illumina TruSeq DNA PCR-Free Library Preparation Kit and sequenced in a multiplexed 2x250 paired end run on the Illumina HiSeq 2500 sequencing platform (San Diego, CA).

Demultiplexed paired-end sequences data were pre-processed by trimming forward and reverse reads to 240 bp (reads length less than 100 bp were dropped), trimming primer sequences, and merging paired-end reads using USEARCH v9.1.13 (Edgar 2010). After pre-processing steps, valid output contained 4,156,070 reads. Quality filtering was proceeded with an expected error less than 0.9 in which 3,656,832 reads passed quality filtering. After pre-processing and quality filtering steps, UPARSE (Edgar 2013) clustering was performed at 97% percent identity to create an Operational Taxonomic Unit (OTU) table which generated 2,797 OTUs. Next, we ran chimera

filtering using VSEARCH (v 2.3.2) (Rognes et al. 2016) which removed 181 reference chimeras. Lastly, taxonomy assignment was run using AMPtk hybrid approach (Palmer et al. 2018) which resulted in 2,470 assigned fungal OTUs and 3,218,660 reads. This output was then rarefied to 21,000 reads per sample with all samples were retained and run through the core_diversity_analyses_py command in QIIME version 1.9.1 (Caporaso et al. 2010). Functional guilds were also assigned using FUNGuild v1.0 (Nguyen et al. 2016) resulting in 749 OTUs with functional assignments.

Fungal Community Structure

We assessed alpha diversity using Chao1 diversity and observed OTU richness outputs from the core diversity analyses in QIIME with vegetation type and soil elevation as predictor variables. Beta diversity (community composition) was assessed using Principal coordinate analysis (PCoA) of the Bray-Curtis dissimilarity metric with the function 'cmdscale' and Permutational multivariate analysis of variance (perMANOVA) with the function 'adonis' in the R package vegan (Oksanen et al. 2016; 999 permutations). Vegetation type and soil elevation were included as predictor variables and checked for within group heterogeneity using the vegan functions 'betadisper' and 'permutest.' Beta diversity was plotted using ggplot2 (Wickham 2009) and the 'stat_ellipse' function in R with 95% confidence intervals. For functional group structure, we compared the relative read abundance of saprotrophs, pathogens, and mutualists in each soil sample as determined by FUNGuild assignments. We first filtered FUNGuild assignments, removing all taxa that were at the confidence level of 'possible' and retaining only assignments that were at the confidence level of 'highly probable' and

‘probable. All unique OTUs assigned to trophic modes beginning with “Saprotroph” (2 total) were counted for saprotroph richness (SAP), all unique OTUs assigned to functional guilds containing “Plant Pathogen” (10 total) were counted for pathogen richness and unique OTUs assigned to mycorrhizal guilds (7 total) were counted for mutualist richness. We then calculated a mutualist to pathogen richness ratio (M:P) for each soil sample.

Structural equation Modeling

We tested the relationships between all microbial parameters (community structure and decomposer traits) and plant responses (performance and leaf traits) using piecewise structural equation modeling in ‘piecewiseSEM’ package in R (Lefcheck 2016). We tested all relationships indicated by solid arrows in Figure 3.1 using the function ‘psem’. Dotted arrows represent inferred relationships for discussion only. To simplify SEM structure, we only included Chao1 for alpha diversity and PSFs for plant performance as these were strongly correlated with observed OTUs and total biomass, respectively. We ran separate SEMs for each plant response (PSF, Root: Shoot, Leaf C: N, δ13C, δ15N, SLA, and LDMC) with each microbial trait (NAG, CBH) with a basic model structure as follows:

Plant response ~ microbial trait + community structure (chao1 + pcoa1 + m:p rich + sap rich)
Microbial trait ~ chao1 + pcoa1 + m:p rich + sap rich + soil elev + veg type
Chao1 ~ m:p rich + soil elev + veg type
M:P rich ~ sap rich + soil elev + veg type
Pcoa1 ~ soil elev + veg type
Sap rich ~ soil elev + veg type

We also tested for an interaction between microbial traits and fungal diversity (NAG/CBH x Chao1) on plant responses and retained this structure when it improved model predictions without reducing fit (AIC). All data were standard normalized prior to running SEMs to allow for comparison among path coefficients. Response variables were tested for normality using a Shapiro-Wilks test and log transformed as necessary for linear models (lm). Generalized linear models (glm) were used with either a Gamma or Quasipoisson distribution to accommodate non-normally distributed variables (Distributions listed in Table 3.2). In piecewise SEM, tests of directed separation are used to determine if there are missing paths (unspecified relationships) within the causal network (Lefcheck 2016). If missing paths were indicated as significant ($\alpha<0.05$), we updated the model with these predictors and re-ran the SEM. We used Fisher's C value to test for Global Goodness of fit for each SEM ($\alpha>0.05$). Finally, R^2 values were calculated for each component model within each SEM.

Indicator Species Analysis

We used indicator species analysis to identify specific fungal taxa underlying the community structure data within our SEMs. Specifically, we determined which fungal taxa characterized soils from different elevation and vegetation types using the function 'mulitpatt' in the 'indicspecies' package in R (Cáceres and Legendre 2009). We calculated Indicator Values ($Indval_g$) based on species (OTU) abundance and considered indicator taxa significant at $\alpha=0.05$ based on permutation tests (N=999) as described in Dufrêne and Legendre (1997). We report indicator taxa at the finest taxonomic resolution

possible and group results by functional guilds (saprotrophs, mutualists and pathogens) assigned by FUNGuild within each vegetation type and soil elevation.

Results

Greenhouse experiment

Total seedling biomass (final - initial) (g) was lower for seedlings in live versus sterile soil inoculum overall, suggesting that the sterilization treatment was effective, and microbes had an overall net negative effect. Plant performance differed by soil elevation and vegetation type with higher PSFs in high elevation soils ($p=0.042$) and marginally higher PSFs and lower root: shoot ratios in shrub soils ($p=0.065$, 0.059) (Fig. S3.3). However, SEM results suggested that the influence of soil elevation and vegetation type on plant performance occurred through changes in the microbial community (see below).

Fungal amplicon sequencing

Sequencing of the ITS2 region for soil fungi resulted in 3,218,660 reads and 2470 fungal OTUs assigned taxonomy. 430 OTUs were assigned to the Kingdom level only. The remaining OTUs belonged to the following Phyla: Ascomycota (57%), Basidiomycota (13%), Glomeromycota (2.8%), Rozellomycota (2.5%), Mortierellomycota (2.4%), Chytridomycota (1.7%) and other rare taxa (2%). The top 5 most abundant fungal classes were the Pezizomycetes, Eurotiomycetes, Dothideomycetes, Leotiomycetes, and Sordariomycetes (Fig. 3.2). FUNGuild assignments were made for 749 OTUs spanning 82 guilds and 8 trophic modes.

Alpha diversity (Chao1) averaged 665.96 +/-156.85 and average richness (OTUs) was 406.05 +/- 105.54, and low elevation soils had higher diversity and richness

($p<0.001$). Vegetation type significantly affected diversity and richness ($p<0.05$), with SR1 soils having the highest diversity and richness (Fig S3.2). Beta diversity was different between soil elevations ($p<0.001$) but not vegetation type ($p=0.105$) (Fig S3.1). Indicator species analysis resulted in 96 taxa clustered by soil elevation (Table S3.1) and 31 taxa clustered by vegetation type (Table S3.2). Indicator taxa high a higher species richness of soil mutualists and plant pathogens in low versus high elevation soils, with 12 plant-pathogen and 16 AMF indicator taxa in low elevation vs 6 plant-pathogen and 4 AMF indicator taxa in high elevation soils.

Structural equation modeling

SEMs revealed that PSF, root: shoot, LDMC and leaf C/N were the plant responses best predicted by microbial community structure and decomposer traits. All microbial parameters differed between either soil elevation, vegetation type or both (Table 3.2, Fig 3.3). Specifically, Chao1, saprotroph richness and mutualist: pathogen richness were higher at low elevation, while saprotroph richness was also higher in SR1 soils. PSFs were negatively associated with fungal diversity (Chao1) while root: shoot ratio was positively associated with fungal diversity. LDMC increased with fungal diversity, decreased with M:P richness and was negatively associated with the first axis of fungal community composition (Pcoa1). Both decomposer traits (NAG and CBH) had positive relationships with leaf C/N and there was a negative interaction between fungal diversity (Chao1) and NAG on leaf C/N. Finally, NAG and CBH were higher in high elevation and shrub soils. Soil elevation and treatment were not significant predictors of any plant response when included in the full microbial model.

Discussion

How soil microbial communities influence the establishment and spread of intracontinental range expanders is still a topic of uncertainty, including the cumulative effects of microbial symbionts, pathogens and decomposers (van der Putten et al., 2016). Here we demonstrate how soil microbes can mediate the performance of a range expanding shrub species via multiple co-occurring processes. We find that PSFs operate both via fungal community composition and free-living decomposer traits and that both influence growth and intraspecific leaf trait variation within *A. rothrockii*. In addition, we find considerable evidence that plant-microbial competition for soil nutrients is an important mechanism influencing sagebrush seedling growth, belowground resource allocation, and leaf chemistry. This competition becomes less severe at a lower level of fungal diversity and increased richness of mycorrhizal taxa. Finally, rather than having its range expansion driven solely by "enemy-release," *A. rothrockii* appears to be influenced by the overall composition and diversity of soil fungi and the relative species richness of both mutualist and pathogen communities. Together, these results suggest several factors could limit sagebrush seedling establishment and further range expansion into high elevation ecosystems and that unpacking the "black-box" of microbially-mediated PSF dynamics will be crucial for understanding how plant species will respond to novel soil microbial communities in the context of species range shifts and global change.

Fungal community structure

We found support for the overall hypothesis that both microbial community structure and microbial traits will influence seedling performance and seedling leaf traits.

Chao1 diversity had a direct negative influence on seedling performance (PSFs) and altered aboveground vs. belowground biomass allocation patterns in seedlings. Overall, we found that seedlings growing in soils with high fungal diversity were more negatively affected by live soil inoculum and had higher root biomass vs shoot biomass. Additionally this was paralleled by a positive relationship between LDMC and fungal alpha diversity, suggesting that increases in soil fungal alpha diversity were correlated with lower growth, increased belowground biomass for resource acquisition and less aboveground biomass for photosynthesis and leaf traits which reflect a conservative growth strategy and higher investment in structural vs photosynthetic leaf tissue (Kazakou, Vile, Shipley, Gallet, & Garnier, 2006).

One potential explanation for these patterns is that greater fungal diversity within the rhizosphere may increase plant-microbial competition for resources, which may be particularly influential for plant performance at the seedling stage. This idea is further supported by the fact that higher root: shoot ratios of seedlings, but negative PSFs, as fungal diversity increased. Thus, seedlings are increasing allocation to roots to compete with soil fungi for belowground resources, but this comes at the cost of reducing photosynthetic capacity. Competition between plant roots and soil microbes in the rhizosphere is common (Kuzyakov & Xu, 2013; Schimel & Bennett, 2004) and can be severe in nutrient limited alpine environments (Jonasson, Michelsen, Nielsen, & Callaghan, 1996; Körner, 2003). Direct uptake of organic soil nutrients is one plant strategy to combat microbial immobilization of necessary nutrients (Lipson & Monson, 1998). Previous work has shown that other *Artemisia* species have a high capacity for

direct uptake of organic nutrients in alpine soils (Miller & Bowman, 2003) which would be supported by the increase in root: shoot biomass of sagebrush seedlings (Lloret, Casamovas, & Penuelas, 2002). Although increased fungal diversity can also increase overall rates of nutrient mineralization, studies have shown that decomposition potentials become saturated at certain levels of diversity, after which additional fungal taxa do not yield increased nutrient availability for plant growth (Gessner et al., 2010; Nielsen, Ayres, Wall, & Bardgett, 2011).

While higher soil fungal diversity was associated with lower seedling performance and more conservative leaf traits, this was not the case for all fungal functional groups. Mutualist: Pathogen richness was negatively correlated with LDMC, suggesting that seedlings may shift to more resource acquisitive leaf traits in soils with more mutualists relative to pathogens. This partially supports our hypothesis that M:P richness would increase plant performance, but only indirectly via sagebrush leaf traits instead of direct PSFs. Soil mutualists, particularly mycorrhizal fungi, can be critical for seedling growth, nutrient uptake and pathogen defense (Van Der Heijden & Horton, 2009) and are an important component of a plant's ability to compete with saprotrophic soil microbes for nutrients (Schimel & Bennett, 2004). Diversity within rhizosphere AMF communities can thus lead to enhanced nutrient uptake and plant growth, particularly for taxa from divergent clades because they can utilize different physical portions of the rhizosphere (Maherali & Klironomos, 2007). Although we did not directly measure AMF colonization in roots, over 95% of the sequences included in the 'mutualist' functional

group were AMF, confirming that they were the appropriate symbiont type for A. rothrockii.

Few PSF studies have examined the influence of microbial communities on both plant growth and functional traits simultaneously, however Cortois et al., (2016) showed that plant species with 'fast' traits such as high specific root length (SRL) experienced more negative PSFs. Consistent with this result, we found that seedlings with 'slow' leaf traits, such as higher LDMC and C/N, had more positive PSFs, but that these relationships were mediated by fungal diversity (Chao1). In contrast, we found that increased relative richness of mycorrhizal vs pathogenic fungi (M:P ratio) in soils was associated with 'fast' traits such as lower LDMC, which suggests that increased richness in AMF taxa and/or reduction in pathogen richness may help to ameliorate the negative competitive effects of the soil fungal community as a whole.

Microbial decomposer traits

Microbial decomposer traits were predictive of seedling leaf traits as we hypothesized but were related to both the plant C and N economy, as CBH and NAG simultaneously influenced the leaf C/N ratio. This suggests that overall decomposer activity, rather than specific enzymes, is most influential on plant nutrient uptake and resource allocation. Specifically, when there was higher CBH and NAG activity, leaf C/N was also higher, showing that increased microbial enzyme production lead to more conservative leaf traits. The positive relationship between CBH and leaf C/N reflects parallel strategies for enhanced C accumulation, which points to plant-microbial competition.

CBH is an extracellular enzyme that degrades cellulose and plant cell-wall materials, which is often the first step in soil organic matter (SOM) decomposition and largely controls litter decay rates in soils (Edwards, Upchurch, & Zak, 2008). This enzyme is present in nearly all fungal lineages, particularly filamentous fungi (Lee Taylor & Sinsabaugh, 2015), and in some bacterial phyla including Firmicutes, Proteobacteria and Actinobacteria (de Menezes et al., 2015). The "microbial N mining" hypothesis, states that because microbes have much lower C:N ratios than plants, soil microbes may use energy from labile C sources (e.g. cellulose) to breakdown more recalcitrant C sources (e.g. lignin) to acquire needed N (Craine, Morrow, & Fierer, 2007). Observed increases in CBH activity may therefore be precursors to microbial N mining to meet their higher N requirements, which is supported by the high correlation between CBH and NAG activity (Pearson's r=0.84). NAG is a chitinase involved in the breakdown of chitin-rich microbial products in SOM, such as bacterial and fungal necromass and soil arthropods (Gaderer, Seidl-Seiboth, de Vries, Seiboth, & Kappel, 2017) used to acquire N, to modify their own cell walls, and to attack other fungi (Lee Taylor & Sinsabaugh, 2015). Therefore, a high level of CBH and NAG activity in the soil inoculum may suggest increased competition with plants for soil organic C and especially organic N, providing a clear mechanism for lower levels of N relative to C in seedling leaves (Dunn, Mikola, Bol, & Bardgett, 2006). As described above, rapid microbial absorption of N has been shown to occur in alpine grasslands as they are highly nutrient-limited ecosystems with low soil organic matter (Song et al., 2007).

However, the level of microbial competition for soil resources may be contingent upon the diversity of the microbial community, as suggested by the negative interaction between NAG and fungal diversity (Chao1) on leaf C/N. Lower leaf C/N reflects less structural and more photosynthetic investment in leaves and is associated with faster plant growth (Grassein, Till-Bottraud, & Lavorel, 2010). A negative interaction between extracellular enzyme activity and fungal diversity on leaf C/N suggests that at as fungal diversity increases the effect of enzyme activity decreases. This suggests a threshold a threshold of fungal diversity above which additional enzyme activity does not increase competition with seedlings for soil nutrients. While we did not find that fungal community structure predicted decomposer traits directly as we hypothesized, the interaction between fungal diversity and enzyme activity on seedling leaf chemistry suggests an important link between microbial community structure and function on sagebrush growth and nutrient acquisition.

Indicator species and range expansion

The hypothesis that sagebrush performance would be highest in non-shrub and high elevation soils was partially supported by the higher PSF values in high elevation soils, but they were marginally higher in shrub than non-shrub soils (Fig S3). However, SEM results suggested that the effects of soil elevation and vegetation type were mediated by changes in the soil community, any primarily fungal diversity, in high elevation soils. Overall, PSFs were highest where fungal diversity was low, which is likely driven by both decreased pathogens and decreased competition with soil microbes for belowground resources. In a previous study of this system, we also found that soil

fungal diversity was lower in high elevation (range expansion) soils, particularly under sagebrush canopies (Collins et al., 2018). This suggests that the microbial communities in our field soil inoculum were relatively stable over 4 months of seedling growth and that the decreased fungal diversity may lead to improved sagebrush seedling growth in situ, as was observed in the greenhouse.

In addition, our results partially support the 'enemy release hypothesis' whereby plant species migrating into novel environments gain a fitness advantage by escaping species-specific pathogens in their home range (Van Grunsven et al., 2007). Indicator species analysis showed that low-elevation soils had twice the number of plant pathogen indicator taxa as compared to high-elevation soils (Table S1) and that these taxa were representative of more diverse lineages including five fungal orders: Pleosporales, Helotiales, Venturiales, Hypocreales, and Spizellomycetales. Closely related fungal taxa within these orders are known to be important fungal pathogens for *Artemisia tridentata* across the Western US (Welch & Criddle, 2003), however it is unknown whether there are species-specific fungal pathogens for *A. rothrockii*.

On the other hand, indicator species analysis also showed that high elevation soils also had much lower diversity of AMF taxa than low elevation soils, and that these were mostly generalist AMF taxa (i.e. Glomeraceae) (Maherali & Klironomos, 2007). Mutualist to pathogen richness was also lower in the high elevation soils (Figure 3). In comparison to soil pathogens, mycorrhizal fungi, and specifically AMF, tend to be much less plant host-specific (Davison et al., 2015). It is thus plausible that decreased mycorrhizal richness in the expansion zone has a lower cost to seedling performance than

the benefit of reduced pathogens, a proposed mechanism facilitating both inter- and intracontinental range expansion (van der Putten et al., 2016). While diversity within AMF communities can certainly provide enhanced plant nutritional benefits, sagebrush seedlings in high elevation soil inoculum maintained sufficient growth with fewer, more generalist mycorrhizal taxa. Previous research found similarly that *Artemisia tridentata* seedlings produced more shoot biomass when inoculated with 'generalist' Glomus species compared to 'more specialized' Acaulospora or Scuttelospora species (Klironomos, Ursic, Rillig, & Allen, 1998), suggesting that sagebrush may not be strongly impacted by AMF diversity, at least at the seedling stage.

By growing seedlings in monoculture, we isolated the effects of soil microbial communities on sagebrush performance, however plant competition can also mediate the outcome of microbially-mediated PSFs, particularly during range expansions (Allen, Meyerson, Flick, & Cronin, 2018; Kulmatiski, Heavilin, & Beard, 2011). Cardinaux et al. (2018) simulated range shifts by combining high- and low-elevation alpine plant species and soil biota and found that benefits of novel soil inoculum were only present in the absence of competition (i.e. grown in monoculture) and disappeared when plants were competing against multiple species. Therefore, to fully consider how soil microbes will affect sagebrush range expansion, further experiments are needed that included competition with other plant species.

Conclusions

Understanding the complex interactions between plants and soil microbial communities is critical for predicting the causes and consequences of species range shifts,

particularly in rapidly-changing alpine environments (Pepin et al., 2015). While soil enemy release is the most common hypothesis, many other microbial processes (e.g. mutualism, decomposition, competition) also have direct implications for species range expansion. Here we find that multiple microbial mechanisms influenced the overall performance, biomass allocation patterns and leaf functional traits of a range expanding sagebrush species. While there was indeed partial support for enemy release, our results suggest that microbes significantly affect plant performance via competition for nutrients. Our research highlights that plant-microbial competition is an important but often overlooked mechanism influencing seedling establishment and growth with important implications for species range shifts in an era of global change.

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Tables

Table 3.1 Soil sampling and greenhouse experimental design.

Vegetation type	Non-shrub (H) (herbaceous)	Intact shrub (S) (*A.Rothrockii*)	1-yr shrub removal (SR1)	5-yr shrub removal (SR5)
Elevation	3100m (low) + 3700 m (high)	3100m (low) + 3700 m (high)	3100m (low) + 3700 m (high)	3100m (low) + 3700 m (high)
Inoculum	Live + sterile	Live + sterile	Live + sterile	Live + sterile
# Seedlings	5 x 2 elevation x 2 inoculum= 20	5 x 2 elevation x 2 inoculum= 20	5 x 2 elevation x 2 inoculum= 20	5 x 2 elevation x 2 inoculum= 20

Table 3.2 Results of all piecewise structural equation models with significant predictors at alpha<0.05. Categorical predictors display result of pairwise contrast with 3100m elevation and herbaceous (H) soil as intercepts.

Response	**Predictor**	**Estimate**	**Std. error**	**p-value**	**R^2**	**DF**	**Distribution**
PSF	Chao1	-0.68	0.33	0.046	0.29	34	Gaussian
Root: Shoot	Chao1	0.77	0.35	0.033	0.20	34	Gaussian
LDMC	Chao1	1.12	0.49	0.029	0.37	33	Gaussian
LDMC	PCoA1	-0.41	0.16	0.015	0.37	33	Gaussian
LDMC	M:P	-0.36	0.15	0.021	0.37	33	Gaussian
C/N	CBH	1.21	0.54	0.033	0.19	33	Gaussian
C/N	NAG	1.45	0.58	0.015	0.19	33	Gaussian
C/N	NAG*Chao1	-0.4	0.18	0.035	0.19	33	Gaussian
M:P	Elevation 3700m	-0.25	0.12	0.035	0.27	34	Quasipoisson
SAP	Elevation 3700m	-0.29	0.085	0.002	0.45	35	Quasipoisson
SAP	Vegetation SR1	0.28	0.11	0.019	0.45	35	Quasipoisson
Chao1	Elevation 3700m	-0.74	0.28	0.015	0.37	34	Gaussian
PCoA1	Elevation 3700m	0.12	0.03	<0.001	0.32	35	Gamma
CBH	Elevation 3700m	1.27	0.37	0.002	0.39	31	Gaussian
CBH	Vegetation S	0.85	0.40	0.044	0.48	31	Gaussian
NAG	Elevation 3700m	1.23	0.35	0.001	0.48	31	Gaussian
NAG	Vegetation S	1.05	0.37	0.008	0.48	31	Gaussian

Figures

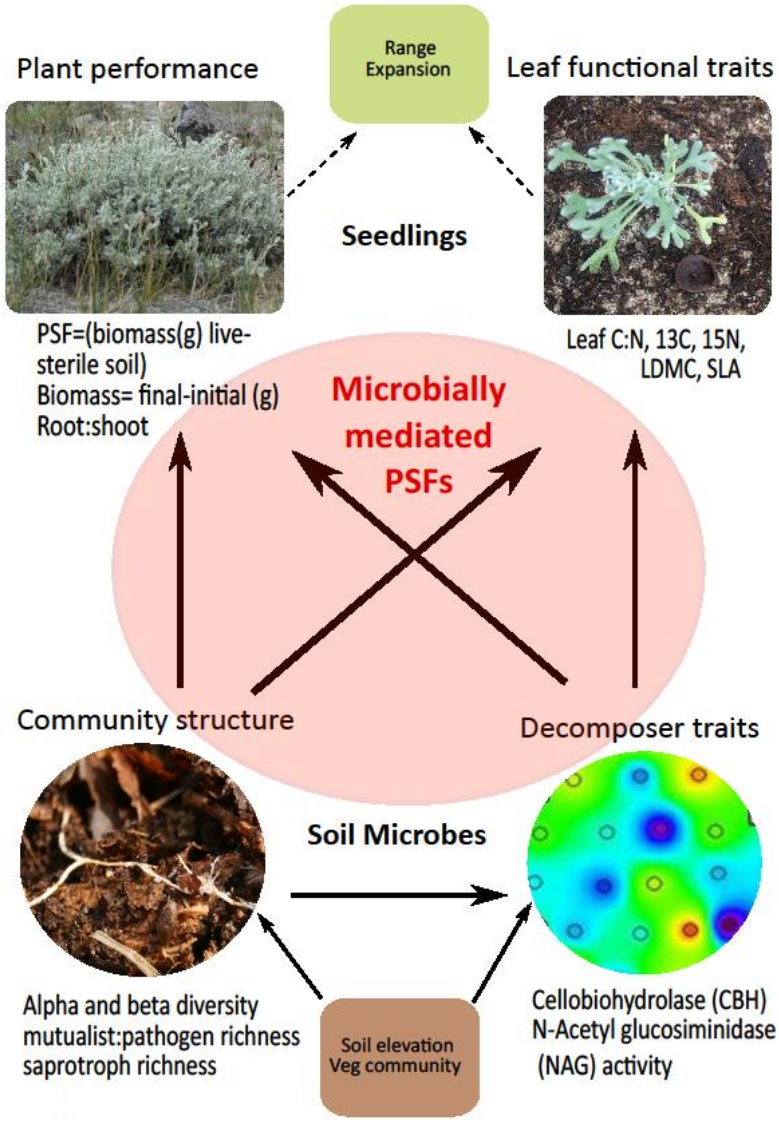

Fig. 3.1. Conceptual diagram of proposed links between microbial community structure of soil fungi and microbial decomposer traits with sagebrush seedling performance and leaf functional traits. Images- bottom left: photograph by Sten Porse 2008, licensed under a CC Attribution-Share Alike 3.0 Unported, 2.5 Generic, 2.0 Generic and1.0 Generic license; bottom right: Colin R. Jackson, 2019-personal correspondence; top left: Jeffrey M. Diez 2018, top right: Courtney G. Collins 2017.

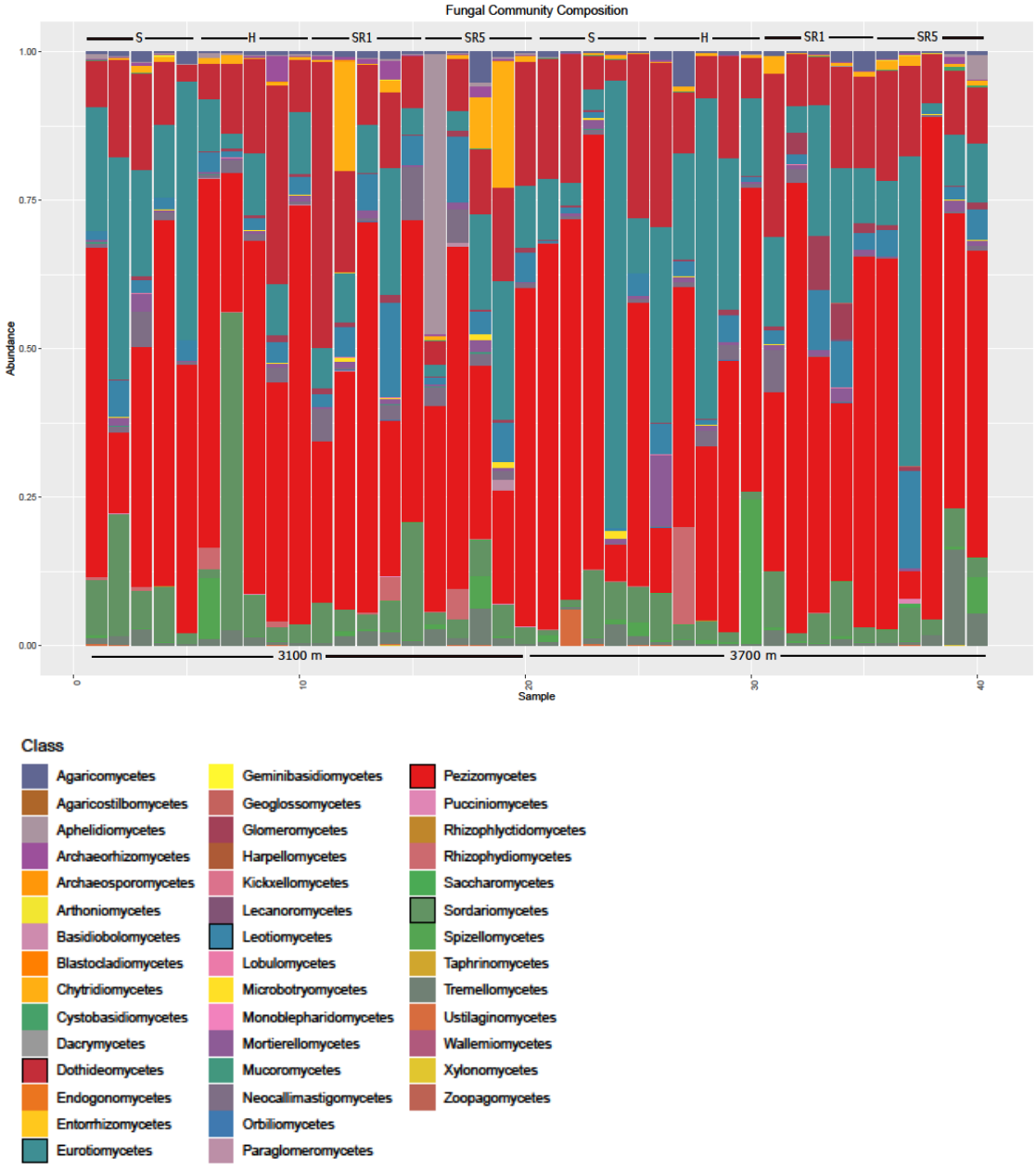

Fig. 3.2. Relative read abundance of soil fungi in each soil sample at the class level. Labels above and below bars designate Elevation (3100, 3700m) and Vegetation type (shrub (S), herbaceous (H), shrub removal 1-year (SR1) and shrub removal 5-year (SR5)) of each soil sample. Colors signify fungal classes.

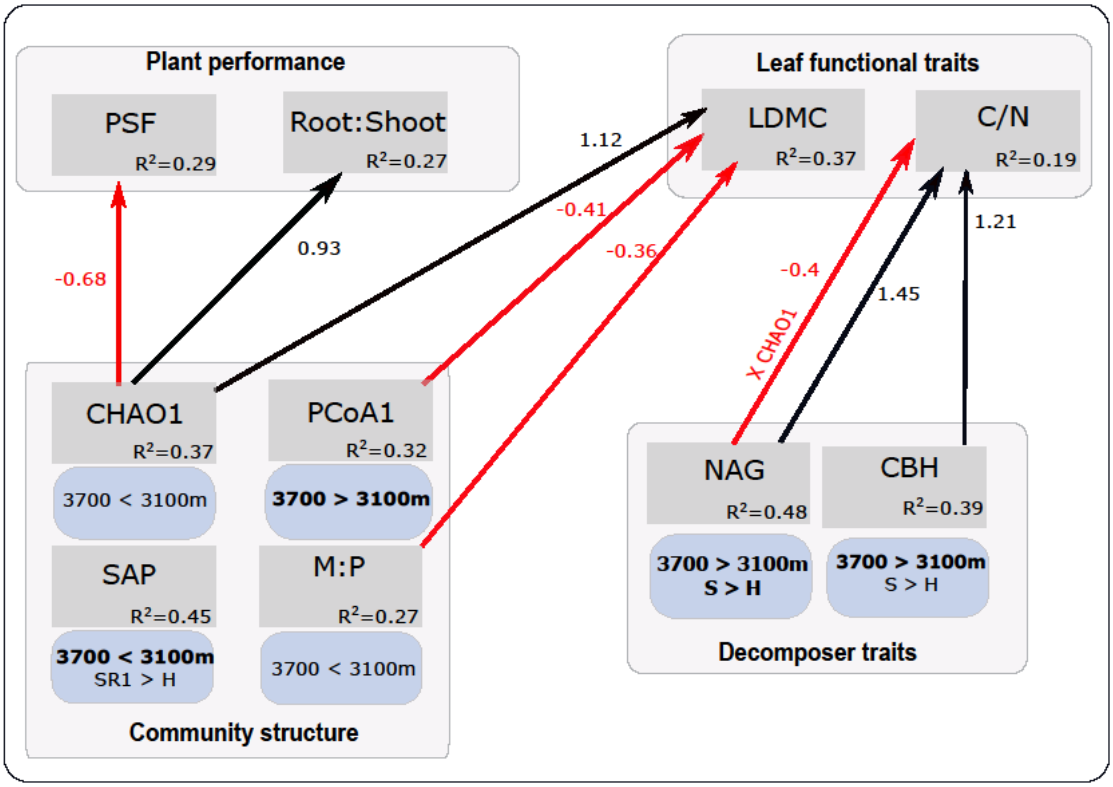

Fig. 3.3. Combined results of significant predictors for all piecewise SEMs as reported in Table 3.2. Continuous variables are shown in grey boxes and categorical variables (soil elevation and vegetation type) are shown in blue ovals. Numbers on arrows signify the slope estimate for each linear predictor within SEMs, red arrows are negative and black arrows are positive. All arrows and regular text in blue ovals are significant at α=0.05 and bold text in blue ovals is significant at α=0.01. R^2 for the component models are shown within the boxes of each response variable.

Supplemental Tables and Figures

Table S3.1 Results of indicator species analysis for soil elevation.

Indicator taxa	Taxonomic level	Trophic Mode	Guild	stat	P value	Soil elev
Helotiaceae	Family	Pathotroph	Plant Pathogen	0.873	0.001	3100
Curvularia	Genus	Pathotroph	Plant Pathogen	0.84	0.003	3100
Clonostachys rosea	Species	Pathotroph	Plant Pathogen	0.835	0.001	3100
Acicuseptoria rumicis	Species	Pathotroph	Plant Pathogen	0.93	0.001	3100
Cylindrocarpon	Genus	Pathotroph	Plant Pathogen	0.826	0.01	3100
Curvularia	Genus	Pathotroph	Plant Pathogen	0.865	0.003	3100
Spizellomyces	Genus	Pathotroph	Plant Pathogen-Undefined Parasite-Undefined Saprotroph	0.748	0.003	3100
Paraphoma chrysanthemicola	Species	Pathotroph	Fungal Parasite-Plant Pathogen-Plant Saprotroph	0.889	0.001	3100
Paraphoma radicina	Species	Pathotroph	Fungal Parasite-Plant Pathogen-Plant Saprotroph	0.826	0.014	3100
Mycena alba	Species	Pathotroph	Leaf Saprotroph-Plant Pathogen-Undefined Saprotroph-Wood Saprotroph	0.608	0.014	3100
Venturiaceae	Family	Pathotroph	Endophyte-Plant Pathogen-Undefined Saprotroph	0.96	0.001	3100
Venturiaceae	Family	Pathotroph	Endophyte-Plant Pathogen-Undefined Saprotroph	0.884	0.003	3100
Preussia	Genus	Saprotroph	Dung Saprotroph-Plant Saprotroph	0.937	0.001	3100
Sordaria	Genus	Saprotroph	Dung Saprotroph-Wood Saprotroph	0.772	0.007	3100
Rutstroemia	Genus	Saprotroph	Plant Saprotroph	0.896	0.001	3100
Hyaloscyphaceae	Family	Saprotroph	Plant Saprotroph-Wood Saprotroph	0.818	0.019	3100
Archaeorhizomyces	Genus	Saprotroph	Soil Saprotroph	0.882	0.011	3100
Archaeorhizomyces	Genus	Saprotroph	Soil Saprotroph	0.876	0.002	3100

Archaeorhizomyces	Genus	Saprotroph	Soil Saprotroph	0.887	0.001	3100
Archaeorhizomyces	Genus	Saprotroph	Soil Saprotroph	0.881	0.003	3100
Archaeorhizomyces	Genus	Saprotroph	Soil Saprotroph	0.891	0.001	3100
Archaeorhizomyces	Genus	Saprotroph	Soil Saprotroph	0.764	0.011	3100
Archaeorhizomyces	Genus	Saprotroph	Soil Saprotroph	0.8	0.023	3100
Archaeorhizomyces	Genus	Saprotroph	Soil Saprotroph	0.75	0.005	3100
Archaeorhizomyces	Genus	Saprotroph	Soil Saprotroph	0.667	0.024	3100
Archaeorhizomyces	Genus	Saprotroph	Soil Saprotroph	0.628	0.03	3100
Paraconiothyrium fuckelii	Species	Saprotroph	Undefined Saprotroph	0.646	0.017	3100
Sporormiella	Genus	Saprotroph	Undefined Saprotroph	0.856	0.001	3100
Cladophialophora	Genus	Saprotroph	Undefined Saprotroph	0.949	0.001	3100
Tetracladium	Genus	Saprotroph	Undefined Saprotroph	0.892	0.002	3100
Neosartorya	Genus	Saprotroph	Undefined Saprotroph	0.923	0.001	3100
Corynespora	Genus	Saprotroph	Undefined Saprotroph	0.932	0.001	3100
Cladophialophora	Genus	Saprotroph	Undefined Saprotroph	0.798	0.021	3100
Lophiostoma compressum	Species	Saprotroph	Undefined Saprotroph	0.784	0.019	3100
Tetracladium	Genus	Saprotroph	Undefined Saprotroph	0.761	0.028	3100
Strumella	Genus	Saprotroph	Undefined Saprotroph	0.592	0.005	3100
Sagenomella striatispora	Species	Saprotroph	Undefined Saprotroph	0.766	0.001	3100
Eucasphaeria capensis	Species	Saprotroph	Undefined Saprotroph	0.608	0.035	3100
Rhizophlyctis	Genus	Saprotroph	Undefined Saprotroph	0.592	0.013	3100
Eucasphaeria capensis	Species	Saprotroph	Undefined Saprotroph	0.582	0.025	3100
Hypoxylon rutilum	Species	Saprotroph	Undefined Saprotroph	0.745	0.003	3100
Clavaria	Genus	Saprotroph	Undefined Saprotroph	0.627	0.028	3100
Scolecobasidium fusiforme	Species	Saprotroph	Undefined Saprotroph	0.589	0.017	3100
Sporormiella intermedia	Species	Saprotroph	Undefined Saprotroph	0.845	0.02	3100
Crocicreas	Genus	Saprotroph	Undefined Saprotroph	0.669	0.024	3100

Leohumicola	Family	Saprotroph	Undefined Saprotroph	0.709	0.013	3100
Infundichalara microchona	Species	Saprotroph	Undefined Saprotroph	0.6	0.035	3100
Pyrenochaeta	Genus	Saprotroph	Undefined Saprotroph-Wood Saprotroph	0.778	0.015	3100
Atractospora decumbens	Genus	Saprotroph	Wood Saprotroph	0.689	0.033	3100
Pichia	Genus	Saprotroph	Undefined Saprotroph	0.548	0.05	3100
Clavariaceae	Family	Saprotroph	Lichenized-Undefined Saprotroph	0.5	0.046	3100
Hygrocybe helobia	Species	Saprotroph	Undefined Saprotroph-	0.5	0.048	3100
Cuphophyllus virgineus	Species	Saprotroph	Undefined Saprotroph	0.564	0.037	3100
Paraglomerales	Order	Symbiotroph	Arbuscular Mycorrhizal	0.917	0.001	3100
Glomeromycetes	Class	Symbiotroph	Arbuscular Mycorrhizal	0.906	0.001	3100
Glomeromycota	Phylum	Symbiotroph	Arbuscular Mycorrhizal	0.789	0.032	3100
Diversispora	Genus	Symbiotroph	Arbuscular Mycorrhizal	0.845	0.012	3100
Claroideoglomus claroideum	Species	Symbiotroph	Arbuscular Mycorrhizal	0.862	0.001	3100
Glomeromycetes	Class	Symbiotroph	Arbuscular Mycorrhizal	0.75	0.036	3100
Glomeraceae	Family	Symbiotroph	Arbuscular Mycorrhizal	0.76	0.013	3100
Archaeosporaceae	Family	Symbiotroph	Arbuscular Mycorrhizal	0.905	0.001	3100
Claroideoglomus	Genus	Symbiotroph	Arbuscular Mycorrhizal	0.87	0.001	3100
Scutellospora	Genus	Symbiotroph	Arbuscular Mycorrhizal	0.626	0.014	3100
Glomerales	Order	Symbiotroph	Arbuscular Mycorrhizal	0.548	0.017	3100
Scutellospora	Genus	Symbiotroph	Arbuscular Mycorrhizal	0.694	0.007	3100
Claroideoglomus	Genus	Symbiotroph	Arbuscular Mycorrhizal	0.558	0.042	3100
Diversisporaceae	Family	Symbiotroph	Arbuscular Mycorrhizal	0.602	0.017	3100

Glomeromycetes	Class	Symbiotroph	Arbuscular Mycorrhizal	0.717	0.017	3100
Diversispora	Genus	Symbiotroph	Arbuscular Mycorrhizal	0.705	0.008	3100
Rhizopogon	Genus	Symbiotroph	Ectomycorrhizal	0.692	0.006	3100
Cantharellaceae	Family	Symbiotroph	Ectomycorrhizal	0.627	0.015	3100
Cenococcum	Genus	Symbiotroph	Ectomycorrhizal	0.739	0.048	3100
Oidiodendron griseum	Species	Symbiotroph	Ericoid Mycorrhizal	0.7	0.022	3100
Oidiodendron truncatum	Species	Symbiotroph	Ericoid Mycorrhizal	0.683	0.022	3100
Meristemomyces frigidus	Species	Pathotroph	Animal Pathogen-Plant Pathogen-Undefined Saprotroph	0.831	0.021	3700
Oleoguttula	Genus	Pathotroph	Animal Pathogen-Plant Pathogen-Undefined Saprotroph	0.666	0.034	3700
Stemphylium	Genus	Pathotroph	Plant Pathogen-Wood Saprotroph	0.656	0.022	3700
Cephalosporium	Genus	Pathotroph	Plant Pathogen-Wood Saprotroph	0.841	0.002	3700
Leptosphaeriaceae	Family	Pathotroph	Plant Pathogen-Wood Saprotroph	0.64	0.018	3700
Pseudopithomyces chartarum	Species	Pathotroph	-Plant Pathogen undefined Saprotroph	0.655	0.042	3700
Preussia funiculata	Species	Saprotroph	Dung Saprotroph-Plant Saprotroph	0.855	0.004	3700
Gliomastix	Genus	Saprotroph	Undefined Saprotroph	0.82	0.002	3700
Sarocladium strictum	Species	Saprotroph	Undefined Saprotroph	0.793	0.012	3700
Westerdykella centenaria	Species	Saprotroph	Undefined Saprotroph	0.724	0.032	3700
Lachnum	Genus	Saprotroph	Undefined Saprotroph	0.793	0.01	3700
Strumella	Genus	Saprotroph	Undefined Saprotroph	0.687	0.037	3700
Rhizophlyctis	Genus	Saprotroph	Undefined Saprotroph	0.613	0.046	3700
Mucor	Genus	Saprotroph	Undefined Saprotroph	0.57	0.026	3700
Cistella	Genus	Saprotroph	Undefined Saprotroph	0.865	0.003	3700
Orbilia auricolor	Species	Saprotroph	Wood Saprotroph	0.657	0.01	3700

Diversisporales	Order	Symbiotroph	Arbuscular Mycorrhizal	0.851	0.001	3700
Glomerales	Order	Symbiotroph	Arbuscular Mycorrhizal	0.571	0.034	3700
Funneliformis mosseae	Species	Symbiotroph	Arbuscular Mycorrhizal	0.683	0.03	3700
Glomeromycetes	Class	Symbiotroph	Arbuscular Mycorrhizal	0.653	0.032	3700
Cortinarius alnobetulae	Species	Symbiotroph	Ectomycorrhizal	0.759	0.046	3700
Inocybe maculipes	Species	Symbiotroph	Ectomycorrhizal	0.608	0.013	3700

Table S3.2 Results of indicator species analysis for vegetation type.

Indicator taxa	Taxonomic level	Trophic Mode	Guild	Stat	P value	Veg type
Penicillium citrinum	Species	Pathotroph	Plant Pathogen	0.745	0.027	H
Claroideoglomus	Genus	Symbiotroph	Arbuscular Mycorrhizal	0.582	0.027	H
Ustilago	Genus	Pathotroph	Plant Pathogen	0.727	0.008	S
Venturia	Genus	Pathotroph	Plant Pathogen	0.632	0.012	SR1
Pestalotiopsis microspore	Species	Pathotroph	Plant Pathogen	0.645	0.004	SR1
Drechslera poae	Species	Pathotroph	Plant Pathogen	0.548	0.05	SR1
Gaeumannomyces caricis	Species	Pathotroph	Plant Pathogen	0.548	0.05	SR1
Pezicula ericae	Species	Pathotroph	Endophyte-Plant Pathogen	0.667	0.027	SR1
Sporormia	Genus	Saprotroph	Dung Saprotroph	0.548	0.05	SR1
Hyaloscyphaceae	Family	Saprotroph	Plant Saprotroph-Wood Saprotroph	0.747	0.001	SR1
Archaeorhizomyces	Genus	Saprotroph	Soil Saprotroph	0.73	0.017	SR1
Geomyces	Genus	Saprotroph	Soil Saprotroph	0.765	0.001	SR1
Paraconiothyrium fuckelii	Species	Saprotroph	Undefined Saprotroph	0.695	0.034	SR1
Cladophialophora	Genus	Saprotroph	Undefined Saprotroph	0.758	0.022	SR1
Mariannaea elegans	Species	Saprotroph	Undefined Saprotroph	0.625	0.032	SR1
Gyoerffyella	Genus	Saprotroph	Undefined Saprotroph	0.632	0.019	SR1
Clavulinopsis helvola	Species	Saprotroph	Undefined Saprotroph	0.588	0.035	SR1
Phlebiella	Genus	Saprotroph	Undefined Saprotroph	0.566	0.046	SR1

Thelebolus	Genus	Saprotroph	Endophyte-Undefined Saprotroph	0.727	0.007	SR1
Diversisporales	Order	Symbiotroph	Arbuscular Mycorrhizal	0.719	0.039	SR1
Glomerales	Order	Symbiotroph	Arbuscular Mycorrhizal	0.657	0.013	SR1
Suillus luteus	Species	Symbiotroph	Ectomycorrhizal	0.622	0.028	SR1
Cenococcum geophilum	Species	Symbiotroph	Ectomycorrhizal	0.765	0.009	SR1
Tylospora fibrillosa	Species	Symbiotroph	Ectomycorrhizal	0.637	0.023	SR1
Leptosphaeria doliolum	Species	Pathotroph	Plant Pathogen	0.566	0.037	SR5
Cephalothecaceae	Family	Saprotroph	Fungal Parasite-Wood Saprotroph	0.648	0.032	SR5
Archaeorhizomyces	Genus	Saprotroph	Soil Saprotroph	0.548	0.05	SR5
Hygrocybe acutoconica	Species	Saprotroph	Undefined Saprotroph-Undefined Biotroph	0.609	0.027	SR5
Glomeromycetes	Class	Symbiotroph	Arbuscular Mycorrhizal	0.548	0.05	SR5
Glomeromycetes	Class	Symbiotroph	Arbuscular Mycorrhizal	0.548	0.05	SR5

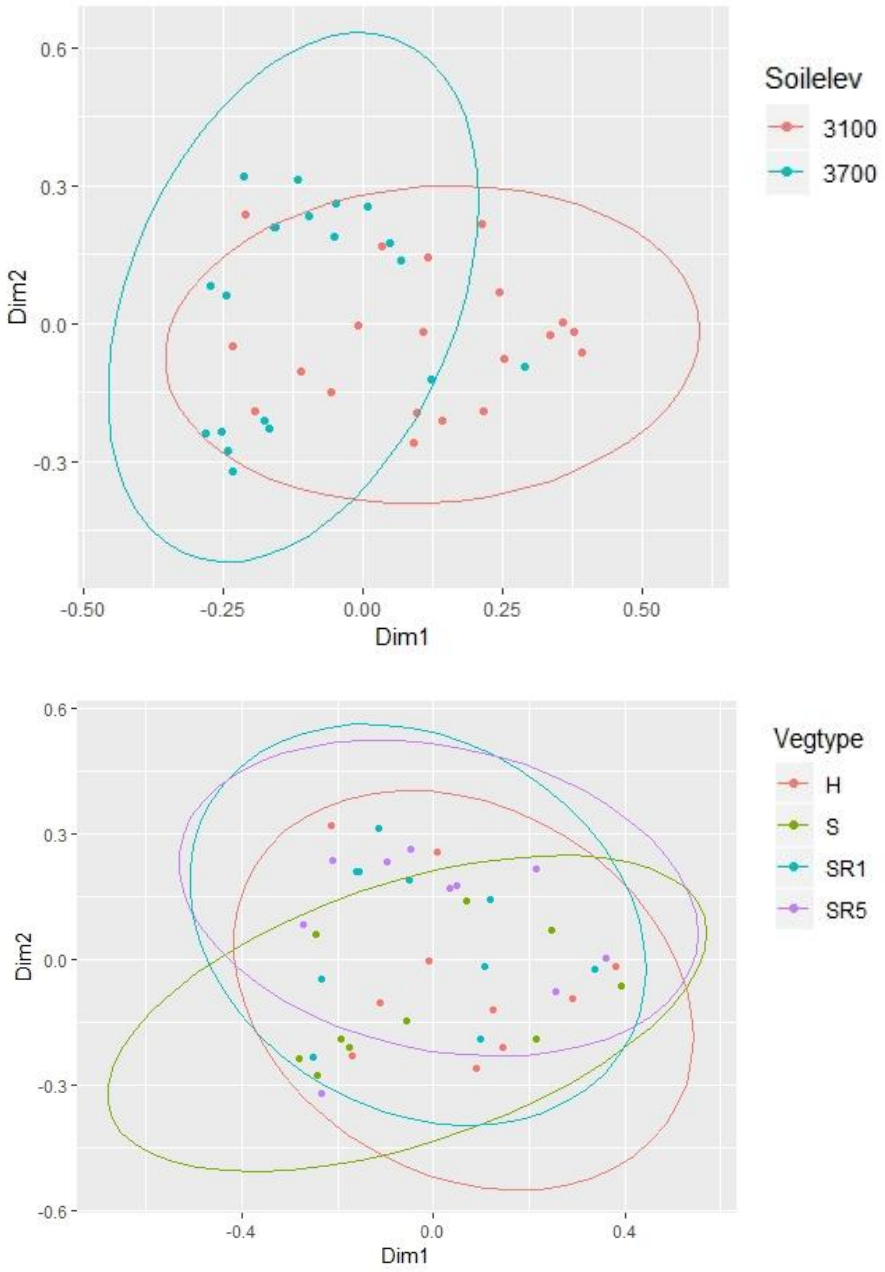

Fig S3.1 PCoA plots of the Bray Curtis distance plotting by soil elevation and vegetation type.

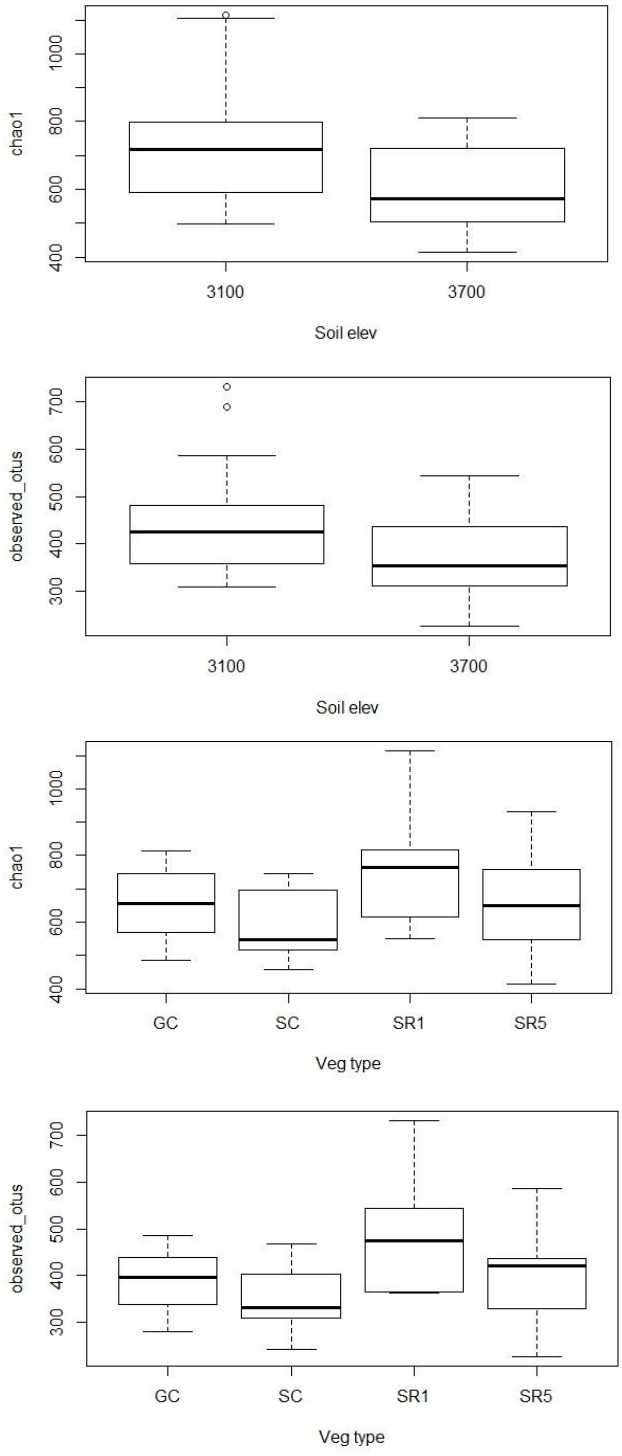

Fig S3.2 Fungal alpha diversity (chao1, OTU richness) plotted by soil elevation and vegetation type.

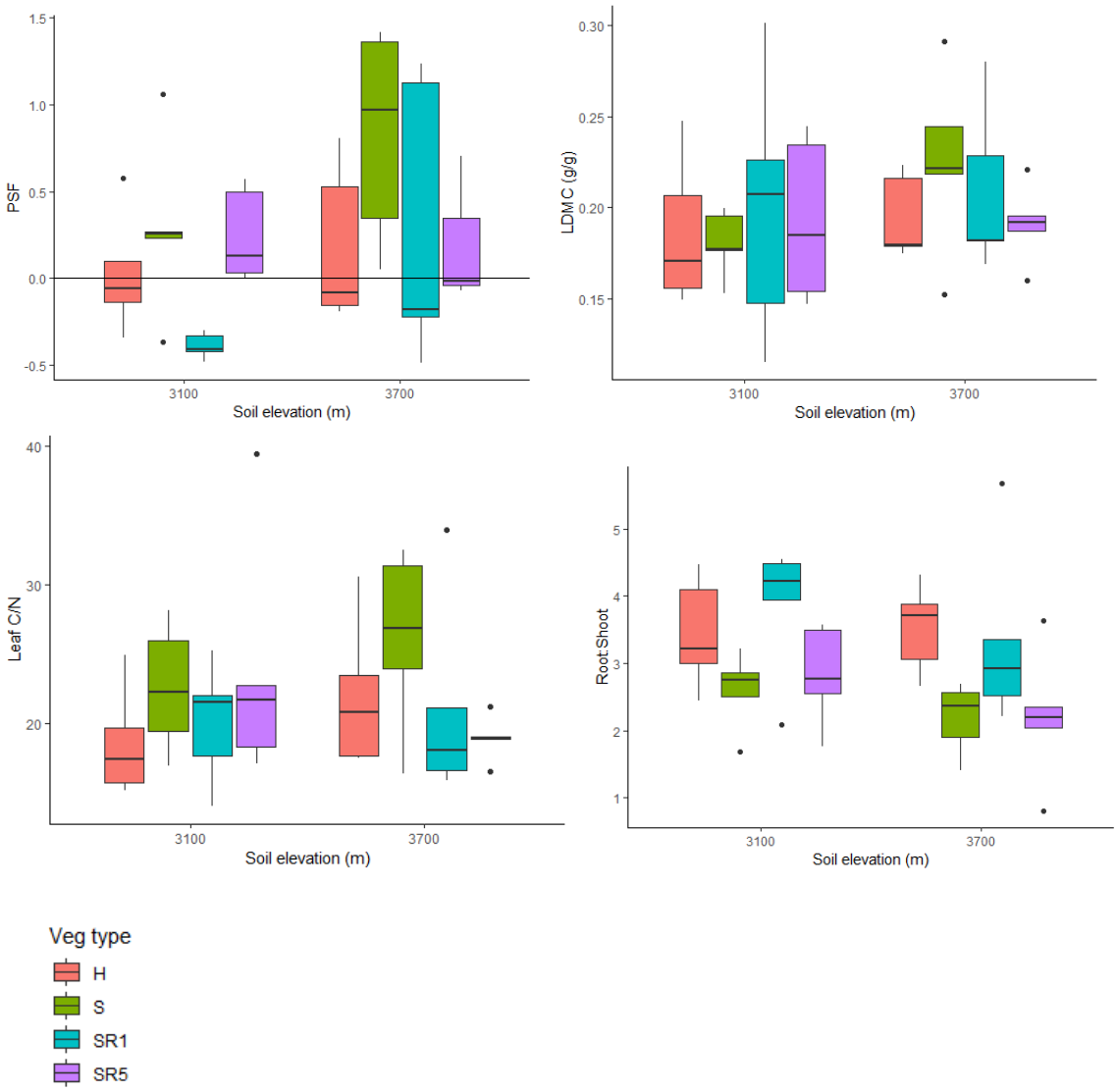

Fig S3.3 Seedling performance and leaf traits from greenhouse experiment plotted by soil elevation and vegetation type.

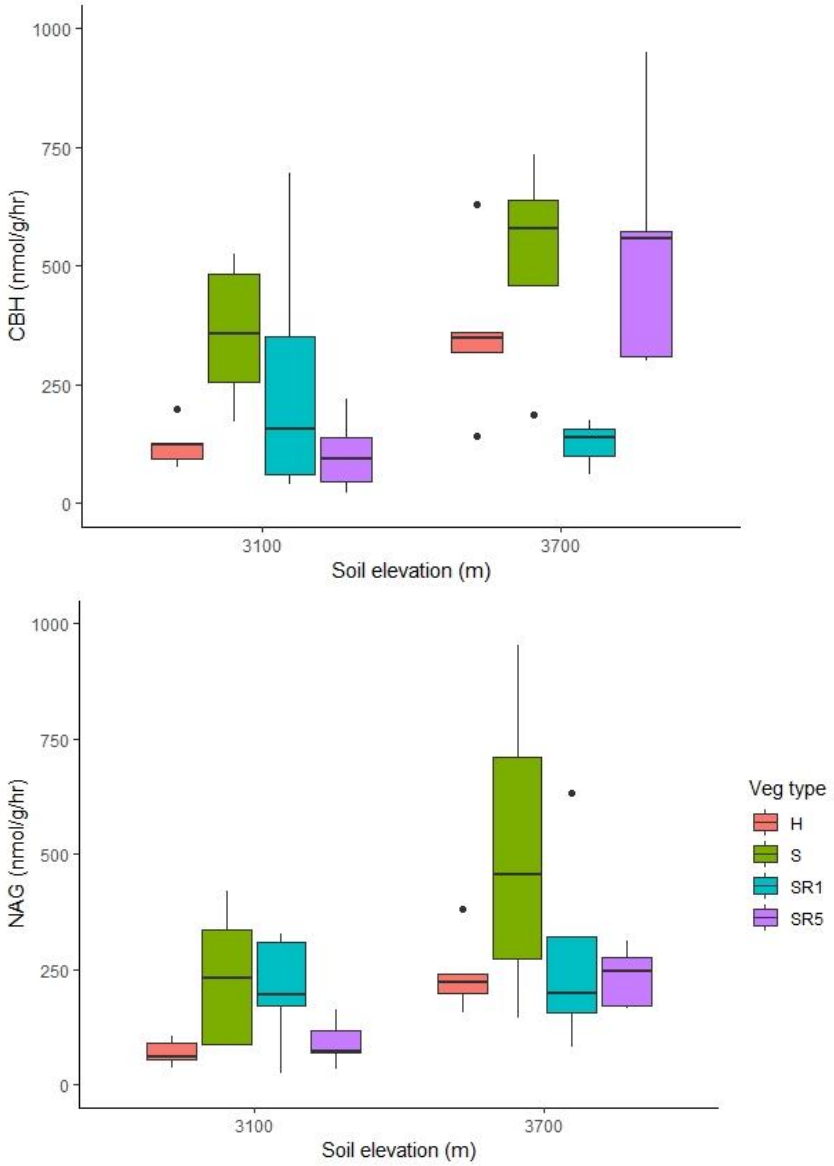

Fig S3.4 Microbial traits from greenhouse inoculum plotted by soil elevation and vegetation type.

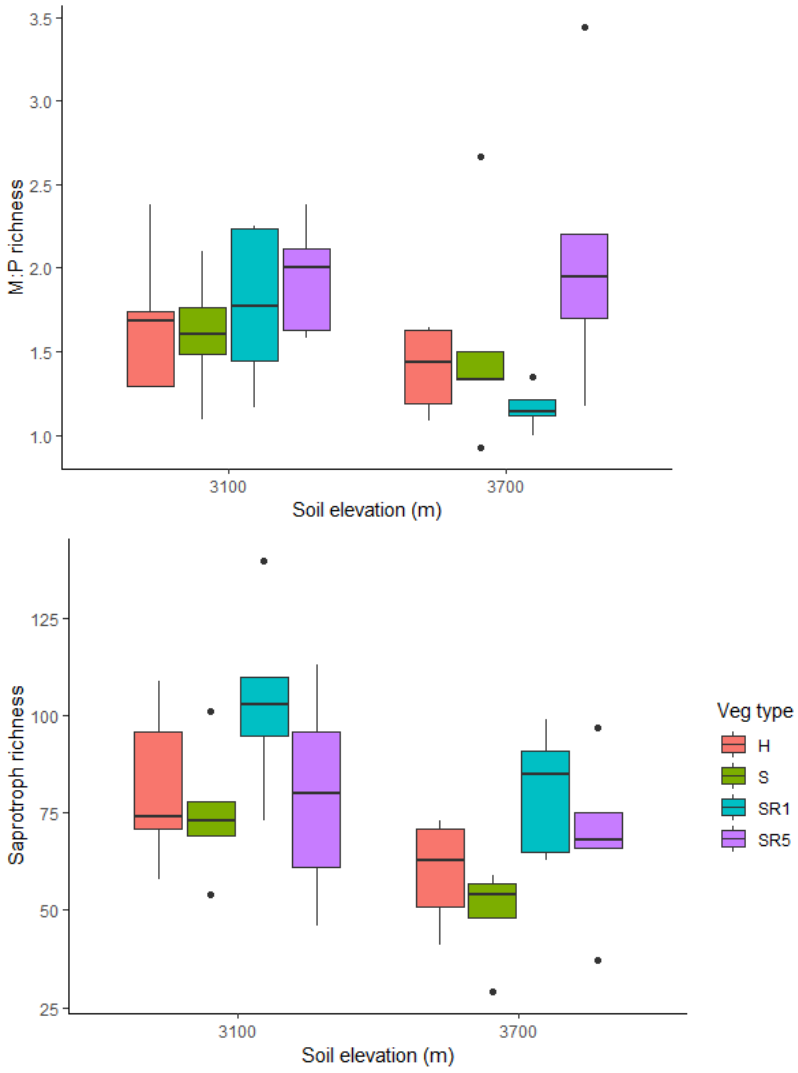

Fig S3.5 Functional group OTU richness plotted by soil elevation and vegetation type.

Chapter 4

Plant-soil feedbacks and facilitation influence the demography of herbaceous alpine species in response to woody plant range expansion

Abstract

Multiple interacting global change drivers are influencing plant population dynamics and species coexistence. Plant species migrations, or range shifts, in response to changing climate is one such driver. Range shifts can create novel competitive interactions for resident plant species and have indirect effects through modifying the local environment including resource pools, microclimate conditions and interactions with soil organisms. These changes can have important implications for community assembly, population dynamics and resident plant fitness. Here we test the impacts of competitive interactions and plant-soil feedbacks (PSFs) of a range expanding sagebrush species (*A. rothrockii*) on the demography and population lambdas of two dominant alpine plant species (*Koeleria macrantha* and *Eriogonum ovalifolium*). We created experimental field plots where aboveground biomass of sagebrush and herbaceous species were manually removed to simulate different competitive and PSF scenarios. We measure demographic rates of *K. macrantha* and *E. ovalifolium* for 4 years in these plots and use integral projection modeling to predict changes in plant population dynamics. We find that sagebrush has an overall net negative effect on herbaceous plant demography, primarily due to negative PSFs for plants growing in sagebrush conditioned soil. However, these negative soil effects are partially buffered via facilitation effects for herbs growing under or nearby sagebrush canopies. These patterns were consistent for both

species we modeled, however the demographic vital rates (growth, flowering, seed production) of *E. ovalifolium* were more sensitive to changes in plant cover and community composition across our experimental plot treatments. Survival had the highest contribution to plant fitness of all vital rates, and we find that in sagebrush increases the sensitivity of herbaceous plant fitness to changes in survival (mortality events). Identifying the major drivers of plant population dynamics and species interactions remains an important and unresolved question in ecology. PSFs are as an important mechanism influencing plant species interactions, yet the majority of PSF research has made little direct connection between plant population dynamics and PSFs *in situ.* We believe that utilizing a field-based approach, focusing on multiple components of plant fitness, is an important next step in understanding the role of PSFs and species interactions in a rapidly changing world.

Introduction

Identifying the major drivers of plant population dynamics and species interactions remains an important and unresolved question in ecology (Sutherland et al. 2013). Classic coexistence theory states that the balance between inter- and intraspecific competition through resource partitioning and negative density dependence will determine plant species assemblages and population densities (Callaway et al. 1997, Chesson 2000, Mangan et al. 2010, Piao et al. 2014). Global change can alter species coexistence patterns through multiple, often interacting drivers. For example, increased inter and intra-annual climate variability may increase (or decrease) niche overlap among some species, while invasive species taxa may competitively exclude rare or functionally

similar native taxa (Valladares et al. 2015). Global change is also driving many native plant species to shift their geographic distributions often moving upwards in elevation or latitude to attempt to 'track' changes in climate or water availability (Parmesan 2006, Kelly and Goulden 2008). These range shifts also create novel species interactions which have important implications for plant community assembly, population dynamics and plant fitness (Caplat et al. 2013).

Competitive interactions between local and range expanding plant species will influence both the ability of the novel species to successfully establish and impacts on community and ecosystem processes after establishment (Körner et al. 2008, Fadrique and Feeley 2016). Successful range shifts may require strong competitive abilities, while lack thereof may limit a species' ability to colonize a new area (Krapek and Buma 2018, Neuschulz et al. 2018). In fact, novel plant competitors were equally or more influential than warming on plant performance in plant community transplants across an alpine elevation gradient (Alexander et al. 2015). Incorporating the influence of novel competitors on the demographic vital rates of resident plant species has been proposed as valuable approach to predict plant population and community responses to species range shifts (Alexander et al. 2016).

In addition to novel competitive interactions, species range shifts may have indirect effects on resident plants through altering the local environment or trophic interactions. For example, newly established plant species may modify local resource pools, microclimate conditions, densities of species-specific herbivores or pollinators and interactions with soil organisms (Tylianakis et al. 2008, Wardle et al. 2011). Plant-soil-

feedbacks (PSFs) are plant-induced changes to the soil which feedback to affect conspecific or heterospecific plant performance (Van der Putten et al. 2013). PSFs can play an important role in shaping plant species interactions and maintaining species coexistence (Bever et al. 1997, 2012, Bever 2003). Non-native invasive species often create negative PSFs for resident plant species which further promote their invasion including reducing the diversity of mycorrhizal fungi or soil mutualists (Hawkes et al. 2006), enhancing native soil pathogens (Eppinga et al. 2006) or selecting for microbes which preferentially degrade their own litter (Austin et al. 2014). Because of the similar role that PSFs play in native range shifts and non-native species invasions (Engelkes et al. 2008, Morrien et al. 2010, Van Grunsven et al. 2010), it is likely that range shifting plant species also create negative PSFs for co-occurring plant species in their new range, however to our knowledge this has not been directly tested.

PSFs can alter many components of the plant life cycle, including growth, survival, and reproduction and can vary in strength depending on plant population size, however the majority of PSF research has only considered effects on plant growth or biomass (Hovatter et al. 2013, Dudenhöffer et al. 2017). For example, seed germination may be limited by species-specific pathogens, particularly in close proximity to conspecific individuals (Mangan et al. 2010) and flower production can be enhanced by spatial heterogeneity of PSFs (Burns et al. 2017). Additionally, PSFs may have contrasting responses across distinct phases of the plant life cycle such as increased growth or vegetative biomass but decreased seed germination or flowering (Mehrabi et al. 2015, Dudenhöffer et al. 2017) having an overall neutral effect on plant fitness. Therefore,

considering all demographic life stages simultaneously is needed for a complete picture of how PSFs influence plant fitness and population dynamics (Dudenhöffer et al. 2017).

Range expanding species who are functionally dissimilar to the native plant community may create especially strong PSFs. Changes in the amount or chemistry of leaf and root litter entering soil organic matter pools, changes to soil hydrology via rooting depth and structure, or association with novel microbial mutualists or pathogens are all mechanisms by which range expanding species can modify the soil environment (Klironomos 2002). For example, Mesquite trees expanding into desert grasslands associate with N-fixing bacteria and have deep taproots, thus altering soil nutrient pools, microbial communities and water availability for resident grasses (Wilson et al. 2001). Novel secondary compounds in litter of range expanding species can also alter interactions of other plants with mycorrhizal fungi and free-living soil microbes (Weaver and Klarich 1977, Nilsson et al. 1993, Wardle et al. 1998), creating potentially positive or negative PSFs. Understanding both direct and indirect effects of species range shifts and their relative influence on local plant communities and the environment will be necessary to predict long term responses of the ecosystems which these species invade

In the White Mountains of California, climate and land use change has led to an upward range expansion of a dominant subalpine shrub species, *Artemisia rothrockii* (Rothrock's sagebrush) into alpine grasslands over the last 60 years (Kopp and Cleland 2014). This range expansion has coincided with decreased abundance of a native bunchgrass (*Koeleria macrantha*) and cushion plant (*Eriogonum ovalifolium*), however the mechanism(s) of these species' declines are unknown (Kopp and Cleland 2014). We

sought to determine whether direct competition with sagebrush or indirect soil effects of sagebrush, a form of apparent competition, were mechanisms underlying the decline in abundance of *K. macrantha* and *E. ovalifolium* in the White Mountains.

Specifically, we asked: Does sagebrush expansion influence the demography of native alpine plant species in the White Mountains? Are sagebrush influences on demography and population lambdas via direct competition and/or apparent competition via PSFs? If so, what are the relative strengths of these mechanisms? We hypothesized that sagebrush creates negative PSFs for *K. macrantha* and *E. ovalifolium,* which manifest in decreased demographic vital rates and lambdas for plants growing in sagebrush soil. Inducing negative PSFs is a common mechanism by which non-native invasive plants gain a competitive advantage over resident species (Suding et al. 2013), and we extend this line of reasoning to a native range expanding species. We predict that the negative effects of PSFs will be stronger than the effects of direct competition with sagebrush because competitive interactions can be weak or shift to facilitation in stressful abiotic conditions such as alpine environments (Callaway et al. 2002, Maestre et al. 2009).

Methods

Study Species

This study takes place across a gradient of *A. rothrockii* range expansion from subalpine (<3500 m) to alpine (>3500 m) zones that has been documented in in the White Mountains of California over the last 50 years (Kopp and Cleland 2014). In 1961, *A. rothrockii* was not present at the 3800 m site, was found in moderate to low densities

at the 3500 m site, and high densities at the 3100 m site (Mooney et al. 1962, Kopp and Cleland 2014). Therefore, this gradient spans from the 'historic range' of A. rothrockii at low elevations to the leading edge of the 'expansion range' at high elevations where *A. rothrockii* transitions from an almost continuous population to isolated patches.

Sagebrush is known to strongly compete with herbaceous plant species for water and nutrients, particularly phosphorus, across the inter-mountain west (Robertson 1947, Caldwell et al. 1985, Fowler 1986, Ryel et al. 2004) In addition, aboveground sagebrush removal led to the re-establishment of herbaceous cover (including *K. macratha* and *E. ovalifolium)* after 4 years in the White Mountains suggesting potentially high levels of interspecific competition (Kopp and Cleland 2018). Sagebrush establishment also alters soil microbial community structure and function, including the diversity and community composition of soil bacteria and fungi, substrate induced respiration (CO_2 flux) and extracellular enzyme activity (Collins et al. 2016, 2018, unpublished data).

These changes are likely to have important feedbacks on herbaceous plant species by altering the relative abundances of microbial taxa such as species-specific soil mutualists and pathogens. In addition, secondary compounds in sagebrush litter may alter how herbaceous plants (grasses, forbs etc.) interact with mycorrhizal fungi and free-living soil microbes (Weaver and Klarich 1977, Nilsson et al. 1993, Wardle et al. 1998). These indirect soil effects of sagebrush on alpine plant growth, a form of apparent competition, may be as strong or stronger than the direct effects of competition with sagebrush (Allen et al. 2018).

Experimental design

We monitored populations of *K. macrantha* and *E. Ovalifolium* at three elevations: 3100m, 3500m, and 3800m. In July 2015, we established 30 experimental blocks, each with four 0.5 x 0.5 m plots (treatments) and each block was repeated 5 times for each species at each elevation (site). Each plot has one of the following 4 treatments: shrub competition, shrub removal, herbaceous competition and herbaceous removal (Fig.1). Shrub plots (competition and removal) were selected where individuals of *K. macrantha* and/or *E. ovalifolium* were growing directly under or very nearby (<0.25m) a sagebrush canopy. Herbaceous (competition and removal) plots were selected in the interspaces of sagebrush between 1 and 5 m away from the nearest shrub canopy, based on the sagebrush density at each site. For competition plots, the entire plant community was left intact. For shrub removal plots, aboveground sagebrush biomass was removed by cutting down stems at the base. For herb removal plots, aboveground biomass of all non-target herbaceous plant species was removed by manually clipping with scissors. For both removal plots, only aboveground biomass was removed to prevent significant disturbance to soil structure, however biomass removal did result in significant bare ground within the plots. All treatments were maintained annually, and any regrowth trimmed back. Differences between competition and removal treatments estimate the effects of sagebrush and herbaceous competition, while differences between the removal treatments (shrub vs herbaceous) estimate the effects of sagebrush plant-soil feedbacks (PSFs) (Fig.1).

Demographic measurements

Within each of the 4 plots (treatments) in all blocks, we tagged up to 5 adult individuals, depending on species density at the site, of either *Koeleria macrantha* or *Eriogonum ovalifolium* and took initial demographic measurements in July 2015. For *K. macrantha*, plant area was calculated by multiplying height of the tallest leaf (cm) by width of the tussock (cm). For *E. ovalifolium*, plant area was calculated through digital image analysis. Photos of each individual plant were taken with a ruler for scale in the field and were then analyzed in ImageJ (Version 1.51 J8) based on the methodology of Jarou (2009). For both species, we measured flowering status (Y/N), and number of inflorescences of each flowering individual. Seed production per inflorescence was calculated as a single value for each species on 100 additional inflorescences which were counted in the laboratory using a dissecting microscope to ensure seed maturity/viability. Beginning in 2016, mortality was also recorded as alive (Y/N) for each individual. Plots were re-sampled yearly in mid-July to early August (depending on snow melt) and all measurements taken for three subsequent years (2016, 2017, 2018).

Recruitment probabilities were estimated using seed germination trials for each species. In September 2017, mature seeds from both species were collected from 10 individuals at each elevation. Seeds were placed in 12 x 12 cm mesh bags and then deployed in the field by fixing them to the upper soil surface using metal stakes. Each bag contained 10 seeds and for each species, 12 bags were deployed at each elevation site, 6 under sagebrush canopies and 6 in shrub interspace. Bags were collected in mid-July 2018, and total number of germinated seeds in each bag were recorded. Probabilities

were calculated as the total of germinated seeds/sum seeds deployed. Due to low overall germination, probabilities were calculated as one value for each species and were not separated by elevation or treatment. In addition, due to low germination percentages and slow growth of alpine plants, we were unable to measure recruit sizes in the field. We estimate recruit size distribution from the seedling dataset of Chu and Adler (2014) for *K. macrantha.* For *E. ovalifolium,* due to the lack of available information on this species, we simulate seedling size data based on the smallest 2.5% of adults in our dataset, which produced a size distribution of 0.001 to 2.5 cm^2 and a mean of 0.6 cm^2.

Population modeling

We calculated demographic vital rates (growth, survival probability, flowering probability, and seed production) on N=229 and 224 individuals with 916 and 896 observations for *K. macrantha* and *E. ovalifolium* respectively. We used linear and binomial mixed effects models in the R package 'lme4' (Bates et al. 2014) with fixed effects of size and treatment and random effects of elevation and sampling year. We tested pairwise differences between treatments using a Tukey test in the 'glht' function of the R package 'multcomp' (Hothorn et al. 2008). Due to model overfitting, only elevation was included as a random effect in the *Koeleria* survival model and only year was included as a random effect in the *Eriogonum* growth model. Size was logged in all models for normality and seed number was logged to transform from count data to continuous. Germination probability was estimated as a single value for each species based on germination trials and recruit size was estimated using an intercept only linear model for the Chu and Adler and simulated datasets respectively.

We used integral projection modeling to calculate population growth rates (lambdas) for each species and partitioned these lambdas using treatment specific model coefficients as well as an overall coefficient for all data (each species separately). To calculate 50% confidence intervals, we bootstrapped lambda estimates for each treatment 1000 times with replacement. We ran sensitivity and elasticity analyses on growth, survival, and probability of reproduction to determine the sensitivity of lambda to changes in specific vital rates. Finally, we calculated the relative difference in lambda values between treatments to estimate the effects of competition and PSFs on lambda and bootstrapped these contrasts 1000 times with replacement to calculate 50% confidence intervals. We used the following 'a-priori' contrasts: Sagebrush Competition: SC-SR, Herbaceous Competition: HC-HR, Sagebrush PSF: SR-HR. For all IPM analyses, we used modified R code from the "Simple Deterministic IPM" (Ch 2) and "Prospective Perturbation Analysis (Ch 4) of (Ellner et al. 2016) (R core team 2015).

Our primary objective with the population models was to assess the relative effect of sagebrush presence on multiple phases of the plant life cycle simultaneously rather than to calculate precise population growth rates. Thus, we consider lambda to be an estimate of the relative fitness of our species of interest among the different plot treatments, not necessarily a prediction of changes in their population size over time

Results

Vital rate models

For *E. ovalifolium*, treatment was a significant predictor of growth, flowering probability and seed production. HC plots had faster growth and higher flowering

probability than both SC and SR plots (growth: est=-0.19, -0.21 p=0.006, 0.003; p.flow: est= -0.98, 0.91, p<0.001) and HR plots had higher seed production than SC plots (seed: est=-0.5 p= 0.002) (Fig 4.2 a-c). For *K. macrantha*, treatment was a significant predictor of seed production only, with HC plots having significantly higher seed production than SC plots (est= -0.2649, p=0.012) (Fig 4.2d).

Lambdas

Lambdas values were higher overall for *E. ovalifolium* than *K. macrantha.* Across treatments, HC and HR plots had higher median lambda values than SC and SR plots for both plant species (Fig 4.3a). For the a-priori contrasts, herbaceous competition had a mostly neutral effect on lambda for both species and was slightly higher for *E. ovalifolium*. Shrub competition had a positive effect on lambda for both species but was slightly higher for *K. macrantha.* Shrub PSFs had strong negative effects on lambda for both species but were more negative for *K. macrantha* (Fig 4.3b).

Sensitivity and Elasticity analyses

Lambdas were most sensitive and elastic to changes in survival and growth and least sensitive to changes in probability of reproduction for both *K. macrantha* and *E. ovalifolium*. Sensitivity and elasticity of lambda varied by treatment across all vital rates. Also, sensitivities and elasticities were very similar for survival, while elasticities were much lower than sensitivity for p. reproduction and growth.

For Survival, SR plots had the highest sensitivity and elasticity for *E. ovalifolium* and the highest elasticity for *K. macrantha* while HR plots were most sensitive. For *Koeleria*, lambdas were most sensitive/elastic to survival of individuals in the most common size

classes (~z(log) 4-5) with a shift in sensitivity to survival of slightly larger individuals in HR plots. For *Eriogonum,* lambdas were most sensitive/elastic to survival of individuals in lower size classes for SC and SR plots (~z(log) 2) and survival of higher and most common size classes (~z(log) 3-4) for HR and HC plots, respectively (Fig 4.4 a, c).

For Probability of reproduction, HC plots had the highest sensitivity and elasticity for *K. macrantha* and highest elasticity for *E. ovalifolium* while HR plots were most sensitive. For *Koeleria*, lambdas were most sensitive/elastic to survival of individuals in the most common size classes (~z(log) 4) however contribution to lambda was very low (<0.1%). For *Eriogonum,* sensitivities/elasticities varied across size classes by treatment, and had a significantly higher contribution to lambda than *Koeleria*, but still relatively small (<2%) (Fig 4.4 b, d).

For Growth, sensitivities were highest in SR plots and lowest in HR and HC plots for *Koeleria* and *Eriogonum* respectively, however elasticities did not noticeably vary by treatment (Fig 4.5). Lambdas were approximately 2x more sensitive to changes in growth for SR plots than HC or HR plots and max sensitives occurred in transitions between medium (~z(log) 2-4) to high size classes (~z(log) 6-7) for both species (Fig 4.5).

Discussion

Understanding the broader implications of species range shifts will be crucial as climate change continues to promote differential species migration and novel species interactions (Midgley et al. 2007). In this study, we sought to tease apart the potential mechanisms by which a range expanding sagebrush species, *Artemisia rothrockii,* affected the growth, survival, and reproduction of two herbaceous alpine plant species.

We tested the mechanisms of direct sagebrush competition and apparent competition via sagebrush PSFs and find that sagebrush has an overall net negative effect on the population lambdas of herbaceous species, primarily due to negative PSFs for plants growing in sagebrush conditioned soil. However, these negative soil effects are partially buffered via facilitation effects for herbs growing under or nearby sagebrush canopies. Overall these results show for the first time, using a manipulative field experiment and demographic modeling, that shrubs may have both positive and negative impacts on herbaceous plant demography due to distinct aboveground and belowground mechanisms.

Vital Rates and Lambdas

A. rothrockii affected the demography and population lambdas of both *E. ovalifolium* and *K. macrantha* across its range in the White Mountains of California. Specifically, plants growing in competition with and in sagebrush conditioned soils exhibited slower growth, reduced probability of flowering and lower seed production than those growing in herbaceous dominated soils. These effects yielded overall population lambdas that were highest in herbaceous competition and herbaceous removal plots and lowest in shrub competition and shrub removal plots respectively. This supports our hypothesis that sagebrush would have a negative effect on the demography of native alpine plants, and these patterns were consistent for both species of interest, suggesting the strong influence of sagebrush on herbaceous plant fitness in this system.

In contrast to growth and reproduction, we found no significant effect of treatment on survival for either plant species, which is not surprising given that overall mortality

was low across our observation period (~2% *Koeleria*, ~2.5% *Eriogonum*) and was only observed in the second and third observation years (2017, 2018). This can be attributed to the fact that alpine plants are often slow growing, long lived, and well adapted to stressful abiotic conditions (Körner 2003). Nevertheless, survival contributed significantly to the overall lambda values of both species (see Elasticity/Sensitivity analyses).

Of our two species of interest, vital rates of *E. ovalifolium* were more affected by treatment, including significant effects on growth, probability of flowering and seed production, compared to only seed production in *K. macrantha* (Fig 4.2). This may be caused by differences in the life history characteristics of these species. *E. ovalifolium* is a slow growing, long lived, cushion plant with an estimated life span ranging from 30-100+ years for closely related species (Anderson 2006). Comparatively, *K. macrantha* is a perennial bunchgrass with an average lifespan between 7-10 years (Dixon 2000). *Koeleria* is solely wind pollinated, while *Eriogonum* is wind pollinated, but also largely insect and bird pollinated, and produces many fewer seeds per inflorescence. Finally, *Koeleria* senesces most of its aboveground biomass annually, while *Eriogonum* retains green leaves throughout the winter. However, overall population lambdas were consistently higher for *Eriogonum* than *Koeleria,* suggesting that through a more conservative growth strategy, this species can maintain consistent growth despite fluctuations in vital rates.

Previous research has shown multiple effects of shrubs on resident herb performance as changes in woody plant cover can affect the resource allocation patterns of herbaceous plants, including shifts between reproductive and vegetative growth (Archer et al. 2017).

Similar to our findings, an herbaceous forb species growing in association with four Mediterranean montane shrubs had reduced reproductive output including lower number of seeds and reproductive stems, and lower infructescence volume compared to individuals growing in open areas (Macek et al. 2016). On the other hand, a dioeceous grass species had increased growth, flowering and survival when growing nearby *Mulinum* shrubs in Chilean grasslands, but this benefit was only present in female individuals (Graff et al. 2018). Competition between herbs growing underneath shrub canopies may also influence the relative positive or negative effects of shrub presence, leading to site and species-specific differences in interactions among plant functional types (Schöb et al. 2013, Armas et al. 2008). Overall, changes created by shrubs may impact multiple components of the plant life cycle, including growth, survival, and reproduction, and these components can have divergent responses which together determine the net outcomes for population growth.

Change in Lambdas

As indicated by the shrub competition contrast, lambdas were slightly higher in sagebrush competition than removal plots, suggesting weak facilitation by sagebrush. Facilitation is common in alpine areas, particularly for nurse plants such as shrubs, which can improve microclimatic conditions for herbaceous plants growing beneath their canopies (Schweiger et al. 2015). Nurse plant facilitation of herbs commonly occurs through enhanced resources such as water and nutrients and by buffering effects of extreme temperatures, wind or snow in the understory (Körner 2003). Indeed, *A. rothrockii* has increased soil moisture and higher soil organic matter content below its

canopies as compared to shrub interspace areas in the White Mountains (Collins et al. 2016). Despite this apparent facilitation effect, lambda values for shrub plots were still much lower than herbaceous plots regardless of treatment (competition or removal), suggesting that the potential benefits do not outweigh the costs of growing in association with this shrub species, consistent with previously observed declines of our species of interest in areas of shrub expansion (Kopp et al. 2014).

On the other hand, the effect of herbaceous competition on lambda was neutral, and herbaceous competition and herbaceous removal plots had more similar lambda values than shrub competition and shrub removal plots. This suggests that plant species identity is an important determinant of species interaction in this system. Additionally, removal of aboveground biomass of neighboring individuals did not significantly improve lambdas for herbaceous plants, implying weak interspecific competition or moderate facilitation between herbaceous species in this system. Recent work has shown that in alpine environments, facilitation intensity may increase with functional dissimilarity among species, particularly with increased cold stress (Gallien et al. 2018). This suggests that species of different functional types (e.g. shrub with herbs) would have stronger facilitation than species of the same functional type which is consistent with the patterns we observed.

The effect of shrub PSFs on lambda was negative, implying that in the absence of competition, plants growing in shrub conditioned soils had lower fitness than those growing in herbaceous conditioned soils. This supports our hypothesis that the effects of sagebrush PSFs on lambda would be more negative than the effects of sagebrush

competition. PSFs are therefore a potentially strong form of apparent competition by which sagebrush negatively impacts resident plant species and facilitates its own establishment and growth in new areas. This phenomenon has been observed in numerous non-native plant invasions (Suding et al. 2013), but to our knowledge, has yet to be tested in native species range shifts. Due to our experimental design however, we can only speculate whether the PSFs of sagebrush on native plant species are due to changes in soil microbial communities, abiotic soil conditions, or both.

One potential PSF mechanism is through secondary compounds (e.g. terpenes, jasmonic acid) in aromatic shrubs such as *Artemisia* can be leached into the soil through leaf litter and via root exudates and have strong negative effects on both conspecific and heterospecific plant growth, metabolism, and seed germination (Weaver and Klarich 1977, Kelsey et al. 1978, Karban 2007). These classes of chemicals can also strongly influence soil microbial community structure and function including microbial biomass C and N, respiration, nitrogen fixation, soil faunal substrate choice, and mycorrhizal networks of co-occurring plant species (Weaver and Klarich 1977, Weston and Putnam 1985, Wardle et al. 1998, Asensio et al. 2012, Austin et al. 2014). For example, organic compounds in the dwarf shrub *Empetrum hermaphroditum* greatly reduced Ectomycorrhizal infection of root tips and mycorrhizal uptake of soil Nitrogen for pine seedlings (Nilsson et al. 1993). Labile C in these compounds may also stimulate free-living (saprotrophic) microbial growth and nutrient immobilization, thus increasing resident plant-microbial competition for limiting soil nutrients. This was proposed as a mechanism by which *Betula, Empetrum,* and *Cassiope* shrub species inhibited the growth

of nearby graminoid species in arctic soils (Michelsen et al. 1995). Finally, soil invertebrates can select food resources based on olfactory cues and chemical compounds in litter (Austin et al. 2014) and thus plants with complex litter chemistry may effectively 'select' for specialized decomposer communities. Therefore, via secondary chemicals, sagebrush may similarly alter soil food webs, decomposition, and plant-microbe competition in ways that enhance their own growth and nutrient acquisition to the detriment of co-occurring herbaceous plant species.

Although it is possible that some belowground competition may still occur between herbaceous and shrub roots after aboveground shrub removal, we expect these interactions to be minor and short term, while soil legacy effects left by sagebrush can last many years after shrub removal or death (Collins et al. 2016, 2018). Therefore, the demography patterns observed in shrub removal plots are very likely attributable to sagebrush soil conditioning rather than remnant belowground competition.

Elasticity/Sensitivity Analyses

We used sensitivity and elasticity analyses to understand which demographic vital rates contributed most significantly to the observed patterns in population lambdas and how robust lambda values were to changes in vital rates. Survival had the highest impact on population lambdas of all vital rates (elasticity/sensitivity values ~0.35-0.4) and sensitivity and elasticity peaked at similar values, showing that lambdas are sensitive to changes in both the total number *and* the relative proportion of individuals surviving within the population. Additionally, survival elasticities peaked at a smaller size class for *E. ovalifolium* highlighting that survival of seedlings and small plants was more

influential for overall population growth in the cushion plant but survival of medium to larger sized plants was more important for the grass. In a similar study, Li et al. (2011) found that survival had the highest elasticities of all demographic rates for an *Artemisia* shrub species and that elasticities varied among three different sand dune habitat types.

Growth had the second largest impact on lambda (elasticity values ~0.22-0.3), and for both species, SC and SR plots were more sensitive to changes in growth than HC and HR plots. This proposes that population lambdas of both herbaceous species may be more sensitive to changes in growth in the presence of sagebrush or in sagebrush conditioned soils, however, this pattern was stronger in *K. macrantha,* likely due to faster growth and shorter lifespan of this species.

Finally, probability of reproduction had a very minor influence on lambda (elasticity values ~0-0.015) and was significantly more important for *E. ovalifolium* than *K. macrantha.* Again, this reflects differences in the life history characteristics of these species in that *Eriogonum* produces fewer seeds per inflorescence and has a more complex pollinator strategy, making reproduction a more important component of its overall population growth.

Conclusions

Alpine plants thrive in complex landscapes where small changes in microclimate, resource availability and species interactions and can have large influences on fitness outcomes (Körner 2003). The movement of woody shrubs upwards in elevation is occurring in alpine ecosystems across the globe due to climate and land use change. This phenomenon requires further study of the long-term implications for herbaceous

vegetation including the persistence of many rare and endemic plant species. Here we find that shrubs have both positive and negative impacts which operate simultaneously and buffer the net outcomes on herbaceous plant lambda values.

First shrubs facilitate herbs growing under or nearby their canopies, likely through enhanced resources such as water and nutrients and by shielding effects of extreme temperatures, wind or snow. At the same time, shrubs cause negative effects on herbaceous plants through PSFs predictably from sagebrush litter chemistry influences on plant growth and soil microbial community structure and function. While the raw values of lambda differed among plots treatments for *K. macrantha* and *E. ovalifolium*, the effects of sagebrush competition and PSFs on lambda were both positive and negative, respectively, for both species. In addition, despite positive and negative effects modulating each other, the net effects of sagebrush on lambdas were significantly more negative than the effects of herbaceous competitors. These results provide potential mechanisms for the observed declines in cover of *K. macrantha* and *E. ovalifolium* in areas of sagebrush expansion.

The majority of PSF research has been carried out in controlled environments, particularly greenhouse experiments, while little direct connection has been made between plant population dynamics and PSFs *in situ* (Kulmatiski and Kardol 2008, Kulmatiski et al. 2008). In addition, while conceptually well developed, attempts to disentangle the effects of direct competition and PSFs on species coexistence have been rare (Bever et al. 1997, Revilla et al. 2013). In this study, we use an experimental field approach combined with population modeling to determine how PSFs and competitive

interactions influence plant demography during species range expansions. We believe this approach is an important next step in understanding plant community dynamics in a rapidly changing world.

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Figures

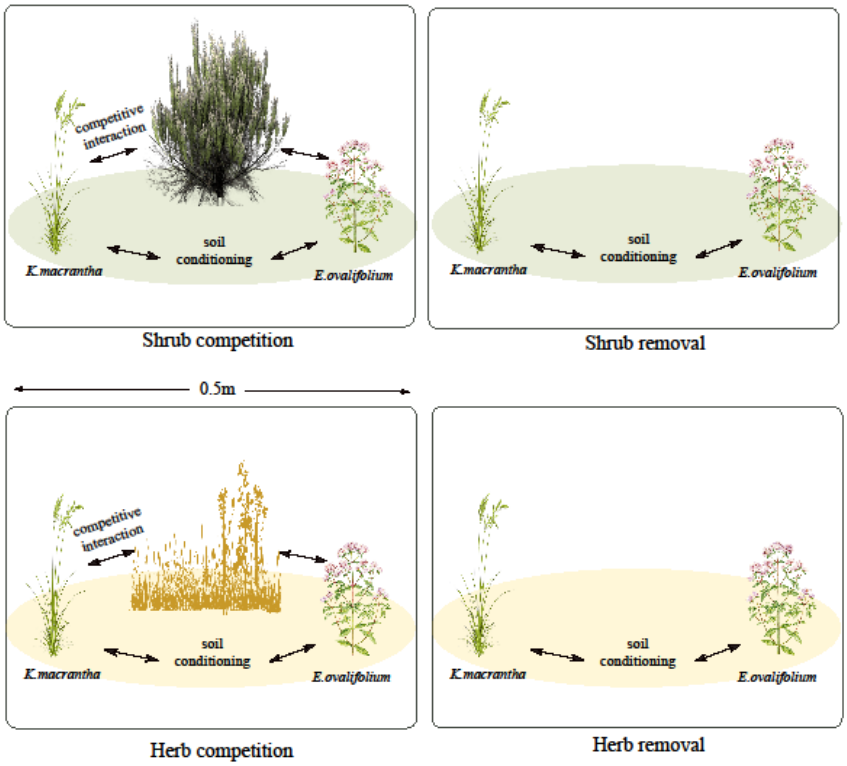


Fig 4.1 Experimental plot design for field demographic measurements. Green circles represent sagebrush soil conditioning and yellow circles represent soil conditioning by the herbaceous community.

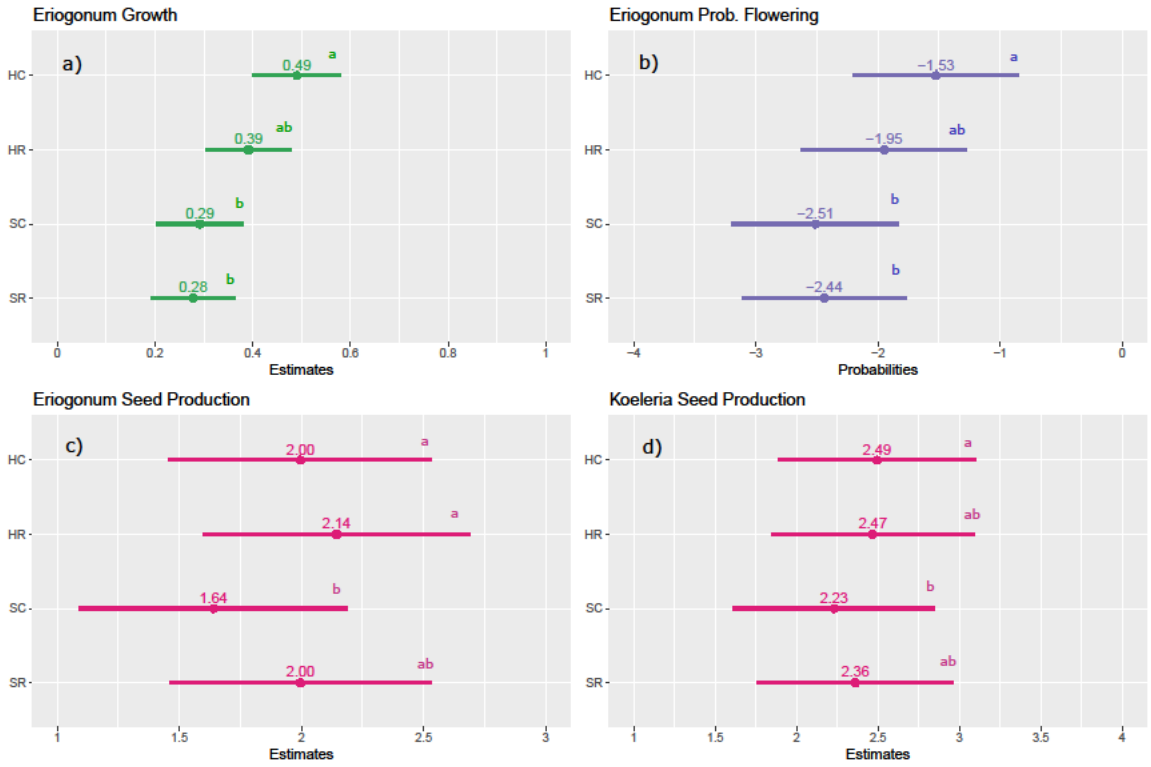


Fig 4.2. Treatment effects on demographic vital rates for *E. ovalifolium* (a-c) and *K. macrantha* (d). Treatments are shown on the y-axis and numbers are point estimates for each model coefficient with lines showing standard error. Letters denote significant differences among treatments as determined by the Tukey test. Models where treatment was not a significant predictor of vital rates are not shown.

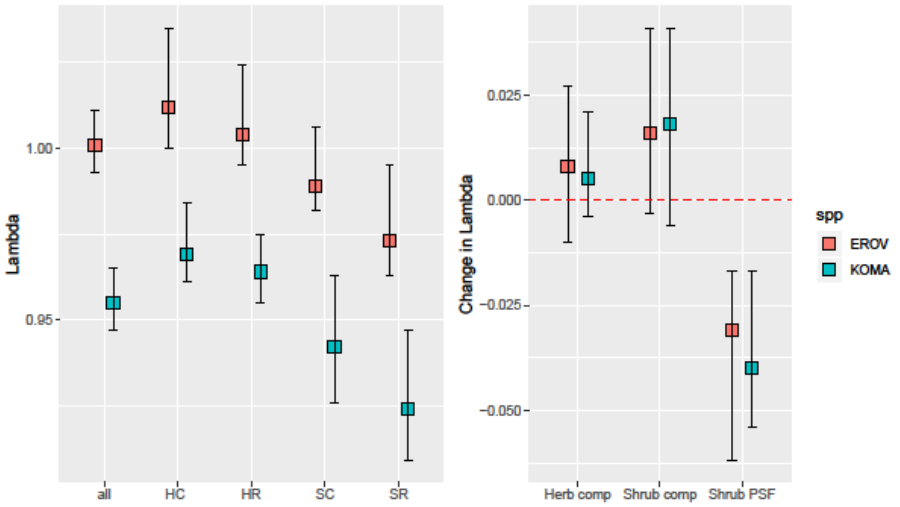
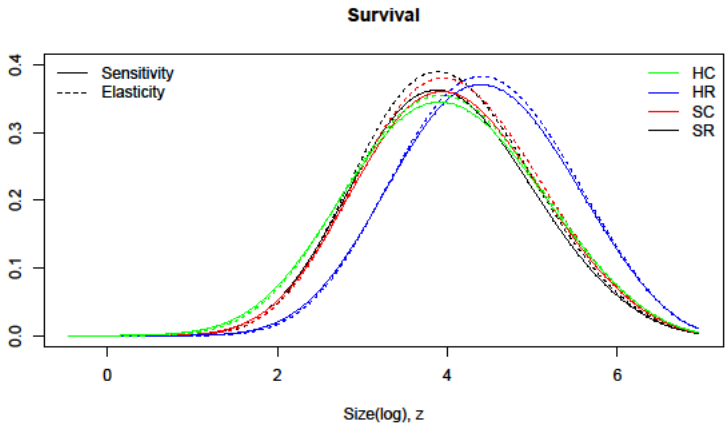


Fig 4.3. Population lambdas (left panel) plotted for all individuals by treatment (HC-Herbaceous competition, HR-Herbaceous removal, SC-Shrub competition, SR-Shrub removal) and Change in lambda (right panel) plotted for each a-priori contrast (herb competition: HC-HR, shrub competition: SC-SR, shrub PSF: SR-HR). Colors denote species and colored boxes show the 'true' lambda value for each IPM or contrast, while error bars show 25th and 75th percentiles of confidence intervals from bootstrapping.

a)


Survival
Sensitivity
Elasticity
HC
HR
SC
SR
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0.2
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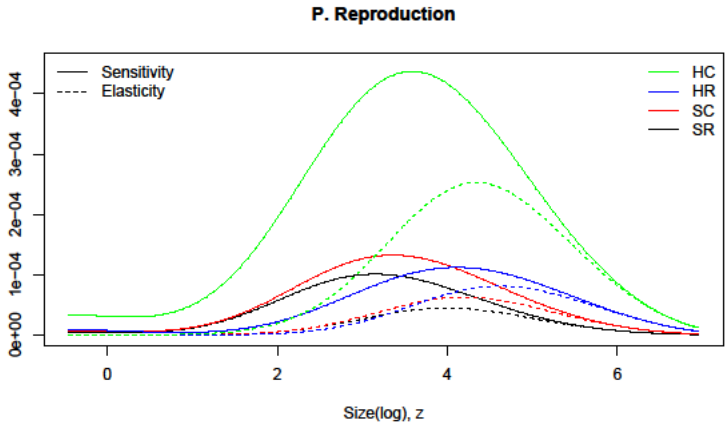
0.4
0
2
4
6
Size(log), z

b)


P. Reproduction
Sensitivity
Elasticity
HC
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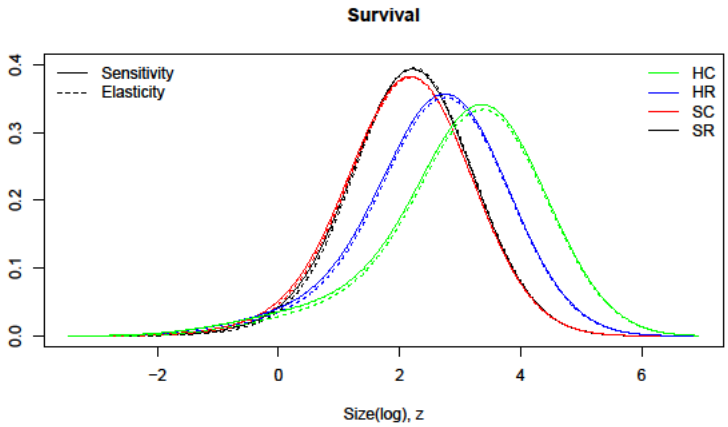
Size(log), z

c)



d)

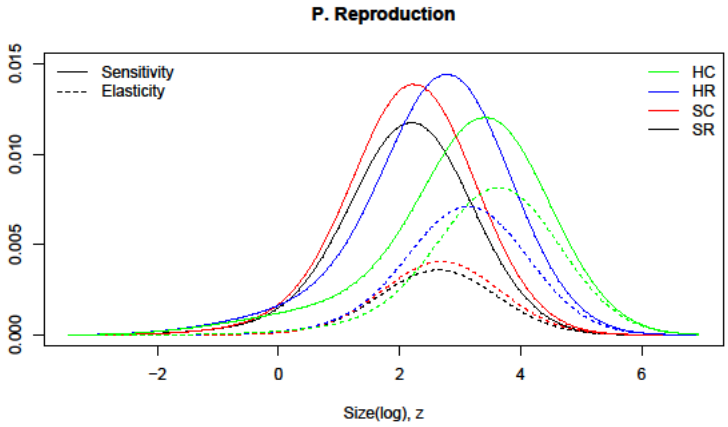
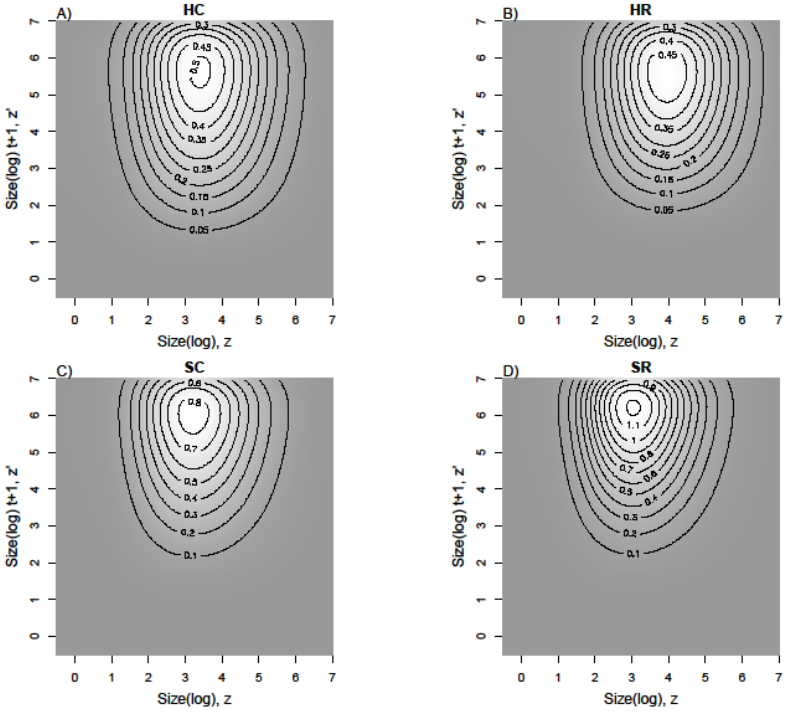


Fig 4.4. Sensitivity and elasticity analyses for survival and probability of reproduction for *K. macrantha* (a, b) and *E. ovalifolium* (c, d). Line colors indicate different plot treatments (HC-Herbaceous competition, HR-Herbaceous removal, SC-Shrub competition, SR-Shrub removal).

a)



b)

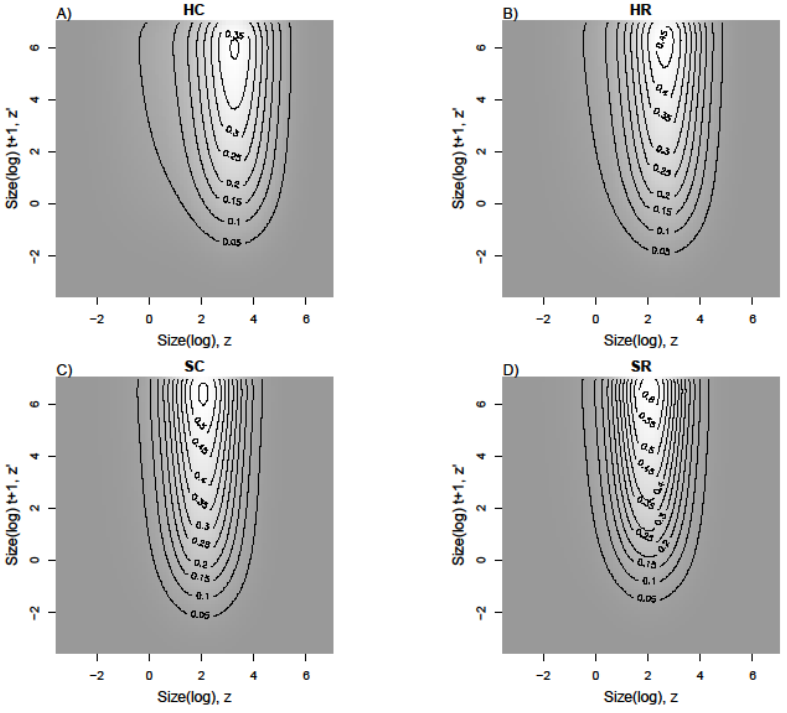


Fig 4.5 Contour plots of sensitivity analyses for growth of *K. macrantha* (top panels) and *E. ovalifolium* (lower panels). Contour lines show sensitivity values at each size transition and colors shift from grey to white as sensitivity increases.

Conclusion

Synthesis and Scope

My dissertation research has examined the cascading effects of shifting alpine plant communities on ecosystem properties, plant species interactions, and plant-soil feedbacks. More specifically, I tested how woody plant range expansion was both influenced by and affected soil microbial communities and belowground ecosystem function across diverse alpine habitats, and how these changes feedback to affect other alpine plant species. I studied a woody shrub, Timberline Sagebrush (*Artemisia spp*.) which is moving upwards in elevation and encroaching into alpine grasslands in the White Mountains of California. I determined that shrub expansion increased bacterial diversity, soil moisture, soil organic matter (SOM) pools and microbial biomass C, which all led to increased microbial respiration (CO_2 flux) (Ch1). Shrub encroachment did not strongly influence overall fungal diversity but increased the abundance of fungal groups including the saprotrophic Agaricomycete fungi, which are important wood and litter decomposers (Ch2). Soil microbial communities influenced both the performance and leaf functional traits of sagebrush seedlings through changes in microbial diversity and extracellular enzyme activity which suggested strong plant-microbial competition for soil nutrients and through a reduced richness of soil pathogens in the range expansion zone (Ch 3). Finally, sagebrush reduced the overall fitness of two co-occurring herbaceous plant species through negative plant-soil feedbacks which overwhelmed the abiotic facilitation effect of sagebrush presence aboveground (Ch 4). My work is among the first to asses both fine and broad scale impacts of a native range expanding plant species on

soil microbial community structure and function and to measure the feedbacks of these changes on both conspecific and heterospecific plant performance.

Understanding the impacts of climate driven plant range shifts both aboveground and belowground is an important task, and California provides an ideal "natural laboratory" in which to examine the impacts of climate driven plant range expansions with steep altitudinal and environmental gradients over short distances. I utilized this natural laboratory and took a chronosequence approach in my research using "elevation for latitude" and "space for time" continuums in which to predict longer term community and ecosystem responses to global change (Körner 2003). My research is unique in its dual focus on the impacts of alpine shrub expansion on processes occurring both aboveground (plant species interactions and demography) and belowground (soil hydrology, nutrient cycling, and soil microbial community structure and function). My work has a strong theoretical grounding in ecosystem, community, and population ecology, and I leveraged a broad toolbox of empirical techniques including field and greenhouse studies, plant demographic monitoring, functional trait analyses, and next generation sequencing. I incorporated advanced statistical techniques such as structural equation modeling, integral projection modeling and joint species distribution modeling into my research to link ecological processes across scales from the microbial to ecosystem level.

This dissertation addresses several important knowledge gaps and unanswered questions in ecology across multiple ecological subdisciplines, including many of the "100 fundamental ecological questions" as defined by Sutherland et al. (2013). First, an

important knowledge gap in microbial ecology is understanding the link between microbial community structure and function. I address this by combining next generation sequencing data for bacterial and fungal community structure with measurements of microbial activity including substrate induced respiration (CO_2 flux) and extracellular enzyme activity of cellulose (C) and chitin (N) degrading enzymes. I find that changes in bacterial diversity and functional group ratios (Oligotrophic: Copiotrophic bacteria) influence microbial respiration and find an interaction between fungal diversity and enzyme activity on seedling leaf chemistry. These results show that microbial community structure can be predictive of microbial function, particularly diversity and microbial functional groups, and that microbial community structure and function can interact to influence plant growth and nutrient acquisition.

A second knowledge gap in plant ecology is understanding the relative importance of biotic vs. abiotic feedbacks between plants and soil for influencing plant growth. I approach this by measuring both biotic (microbial) and abiotic (soil organic C,N, volumetric water content (VWC), pH) soil components and use structural equation modeling to tease apart the relative influence of sagebrush range expansion on both biotic and abiotic factors. I also use a greenhouse experiment controlling for differences in abiotic soil components to determine the biotic (microbial) soil feedbacks on sagebrush seedling performance from soils across the range expansion gradient. I find that sagebrush increases VWC and soil organic C and N and that these changes have important influences on soil microbial community structure and function. I also find that while controlling for differences in abiotic soil conditions, microbial communities

influence plant growth through changes in diversity, extracellular enzyme production, and mycorrhizal to pathogen richness, which collectively control the relative strength of competition between seedlings and free-living soil microbes for limiting soil nutrients.

A third knowledge gap in population ecology is quantifying the relative importance of direct vs. indirect interactions in determining the effect of one species on others. I investigate this through long term plant demographic surveys and targeted species removal in the field to estimate the direct influence of sagebrush competition and the indirect influence of sagebrush soil feedbacks on herbaceous plant species fitness. I combine demographic vital rates into plant population models to determine both how the different components of the plant life cycle (growth, survival, and reproduction) respond to direct and indirect interactions with sagebrush and how these vital rates differentially contribute to overall plant fitness (species lambdas). I find that the sagebrush has negative indirect influences via soil feedbacks and positive direct influences via facilitation on herbaceous plant fitness, and that the negative indirect effects overwhelm the positive direct effects creating an overall net negative impact of sagebrush on two herbaceous alpine plant species.

Finally, this research has important implications for conservation and management in alpine ecosystems which are currently under great threat by global change drivers including warming, drought, grazing and land use changes. According to the UNESCO Mountain Biosphere Research initiative (2003) approximately ¼ of the world's terrestrial land area and 75% of the world's countries contain mountainous regions, from which over 2 billion people depend on for food and water resources. Soils

are also the largest terrestrial carbon (C) sink and microbial communities strongly impact ecological processes that control the balance of CO_2 between soils and the atmosphere. Given these compelling statistics, understanding how global change will alter and alpine ecosystems is critical. My research explores important trends including native range shifts and shrub expansion, which are occurring in alpine environments across the planet. My research also tested the impacts of shrub removal, an important real-life management strategy for restoration of alpine plant communities, on soil microbial communities, biogeochemistry, and the growth of other alpine plant species.

Future directions

A future direction for these research questions would be to scale up globally to understand the influence of woody plant range expansions on soil microbial communities in alpine environments worldwide. Testing the generality of these effects across different climates, abiotic soil conditions, woody plant species, plant functional traits, and mycorrhizal types among other potentially influential factors, would be needed to understand how the results of this dissertation fit into the larger scope of alpine woody plant encroachment. Understanding whether our findings are specific only to sagebrush range expansion in the White Mountains or whether certain patterns are inherent to the process of woody plant range expansion itself would be particularly valuable. In addition, finding easily measurable traits or life history characteristics of woody plant species which are predictive of how soil microbial community structure and function will respond to woody plant encroachment would prove extremely useful for future ecosystem management and global climate modeling efforts. Predicting the effects and feedbacks of

climate-driven changes in plant community structure on soil microbial communities is a critical, yet understudied component of ecological research with immense potential feedbacks to ecosystem function and the global climate system.

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