THE IMPORTANCE OF BIOTIC INTERACTIONS UNDER GLOBAL CHANGE IN THE ALPINE

by

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ABSTRACT

The response of organisms to a rapidly changing environment depends not only on the abiotic conditions they experience, but also their biotic interactions. Here I examine the role of biotic interactions in shaping species responses to global change in an alpine ecosystem. First, I use a long-term experiment to address whether plant communities mediate soil microbial response to simulated nitrogen deposition where I find a decoupling of the plant and microbial communities such that the soil microbial community shifts under nitrogen independently of directional shifts in the plant community. Then I characterize how plant-microbial interactions shape the composition of microbes that live in the roots of alpine plants migrating uphill into previously unvegetated areas by examining the effects of alpine plant migrant density and resultant changes in soil properties. I find that bacterial and fungal root endophytes were only weakly shaped by environmental variables shifting with climate change and that the overall explained variation in community composition was low. Next, I present work from a plant community survey to demonstrate that morphology of a habitat-forming species drives differences in beta diversity but not richness. I then use a manipulative experiment to assess how a habitat-forming species informs the uphill movement of a subalpine plant where I find that the habitat-former increased survival. Finally, I assess the implications of

habitat-forming species for associated taxa under climate change in a conceptual paper that focuses on the roles of facilitation, connectivity, and heterogeneity.

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CHAPTER I

INTRODUCTION

Earth's flora and fauna are experiencing unprecedented environmental change hailing from a diverse range of drivers. For example, anthropogenic emissions of reactive nitrogen (N) have dramatically increased the deposition of N in terrestrial and aquatic systems across the globe (Bobbink et al., 2010; Liu et al., 2013). The Earth has warmed by 0.85°C since 1880 and 19 of the hottest years have occurred since 2000 (Lenssen et al., 2019; GISTEMP Team, 2022). Precipitation regimes have shifted across the globe with increasing incidences of drought and deluge (Konapala et al., 2020), as well as both increases and decreases in snowpack and shifts in snowmelt timing (Mote et al., 2005; Adam et al., 2009). Anthropogenic global change is a major threat to biodiversity, resulting in species distributions which are shrinking and shifting (Lenoir et al., 2008; Chen et al., 2011; Vitasse et al., 2021) and the restructuring of communities (Chapin et al., 2000; Sala et al., 2000).

Over the past couple of decades it has been increasingly recognized that the direct effects of global change are not alone in driving organismal response, and that interactions among taxa also play an important role (Brooker, 2006; Tylianakis et al., 2008). Biotic interactions, such as plant-plant interactions, alter a focal organisms' response to global change via shifts in resource (e.g. water and nutrient availability) and non-resource stressors (e.g. temperature). Plants can alter the amount of light received or the exposure to wind of a neighboring species through

its physical structure (Jones et al., 1997) and plants can shift the soil nutrient environment through their uptake and inputs (Steltzer and Bowman, 1998). For example, nitrogen deposition increased the height of a competitive dominant leading it to shade out nearby plants of a smaller stature (Farrer and Suding, 2016). Hence, global change can result in shifts to communities that are otherwise not predictable from the direct effects of global change alone.

While competitive relationships have historically been a focus, the effects of facilitation, particularly those by habitat-forming species, could buffer the effects of climate change (Bulleri et al., 2016). Habitat-forming species are species which create a 3-dimensional structure that modifies resource and non-resource stressors (Jones et al., 1997). Examples include forests, cushion plants, and shrubs. Through their effects on the environment, habitat-formers could mediate a species response to climate change. For instance, plants which offer ameliorated conditions may promote the range expansion of species tracking their climate niche (Batllori et al., 2009). Though the role of habitat-forming species in mitigating the effects of climate change have been investigated in coastal systems (Bulleri et al., 2016), their role in terrestrial systems requires further study.

In addition to plant-plant interactions, plant-microbial interactions could also play a role under global change (Classen et al., 2015). Plants input resources to the soil microbial community via litter and root exudates (Wardle et al., 2004; Ward et al., 2015) while microbes shift the flow of resources through nutrient cycling (Wardle et al., 2004) and via mutualisms which increase nutrient acquisition or

stress tolerance (Smith and Read, 2008; Johnson et al., 2010). For example, shifts in plant community composition under altered precipitation regimes cascaded to modify soil nematode abundance (Kardol et al., 2010). On the flip side, some ectomycorrhizal fungal partners can help pinyon pines contend with drought (Gehring et al., 2017). Hence, many types of biotic interactions, through shifts in resource and non-resource stressors, are important to consider when predicting an organism's response to global change. However, it is often unclear whether buffering or amplifying effects, or no effect, of biotic interactions will occur.

To study the importance of plant-plant and plant-microbial interactions to plant and microbial response to global change in the alpine ecosystem at Niwot Ridge (Colorado Front Range), I use a combination of landscape-level surveys of plant and microbial communities, manipulative experiments that test the effect of simulated nitrogen (N) deposition on soil microbial communities and the effect of shrub presence on the uphill movement of subalpine plants, and a conceptual paper exploring the role of habitat-forming species under climate change.

In Chapters II and III of my dissertation, I focus on the role of plant-microbial interactions in the face of global change. In Chapter II, I examine how the plant community might indirectly shape, through changes in the quantity and quality of plant inputs, soil microbial community response to N deposition. In Chapter III, I characterize how plant-microbial interactions shape the composition of microbes that live in the roots of alpine plants migrating uphill into previously

unvegetated areas by examining the effects of alpine plant migrant density and resultant changes in soil properties.

The final three chapters focus on how plant-plant interactions can shape species responses to climate change. In Chapter IV, I ask how the abiotic context of an area alters shrub effects on the microclimate and herbaceous alpine community, via shifts in shrub morphology, to better predict how shrubs will facilitate plant communities under climate change. In Chapter V, I empirically test whether shrubs enhance the uphill movement of a subalpine plant by acting as stepping-stones. In Chapter VI, I highlight the potential for interactions with habitat-forming species to mitigate the effects of climate change through facilitation, connectivity, and heterogeneity.

CHAPTER II

DO PLANT-SOIL INTERACTIONS INFLUENCE HOW THE MICROBIAL COMMUNITY RESPONDS TO ENVIRONMENTAL CHANGE?

By Laurel M. Brigham, Clifton P. Bueno de Mesquita, Jane G. Smith, Samuel A. Sartwell, Steven K. Schmidt, Katharine N. Suding. *Ecology*, *103*(1), e03554. https://doi.org/10.1002/ecy.3554

Abstract

Global change alters ecosystems and their functioning, and biotic interactions can either buffer or amplify such changes. We utilized a long-term nitrogen (N) addition and species removal experiment in the Front Range of Colorado, USA to determine whether a co-dominant forb and a co-dominant grass, with different effects on nutrient cycling and plant community structure, would buffer or amplify the effects of simulated N deposition on soil bacterial and fungal communities. While the plant communities were strongly shaped by both the presence of dominant species and N addition, we did not find a mediating effect of the plant community on soil microbial response to N. In contrast to our hypothesis, we found a decoupling of the plant and microbial communities such that the soil microbial community shifted under N independently of directional shifts in the plant community. These findings suggest there are not strong cascading effects of N deposition across the plant-soil interface in our system.

Introduction

Global change, such as warming and nitrogen (N) deposition, directly alters plant (Walker and Wahren, 2006; Payne et al., 2017) and microbial communities (Castro et al., 2010; Ramirez et al., 2010). It also influences interactions within and between plants and microbes, resulting in indirect effects (De Long et al., 2016; Shao et al., 2018; Chen et al., 2019). Importantly, these indirect effects may buffer or amplify the impacts of global change on ecological communities (Holling, 1973; Brooker, 2006; Suttle et al., 2007; Tylianakis et al., 2008; Classen et al., 2015; Pires et al., 2018). For example, the negative effects of warming on plants may be buffered if warming also favors the expansion of other plant species that form favorable microclimates (Anthelme et al., 2014), thereby promoting facilitative interactions. On the other hand, altered plant-plant or plant-pathogen interactions may amplify the detrimental effects of warming on plant populations if interactions shift from facilitative to competitive (Olsen et al., 2016) or if disease outbreak is enhanced under the warmer conditions (Burgess et al., 2017). It is vital to quantify the importance of indirect effects in order to better understand how plant and microbial communities are responding and will continue to respond to their shifting environment.

Human-induced perturbations to the global N cycle have increased the reactive N (e.g. NH_3, NO_X) deposited across the globe (Bobbink et al., 2010; Liu et al., 2013). Nitrogen deposition often affects plant communities by altering community composition, reducing species richness, and increasing aboveground

biomass (Bowman et al., 2006; Lebauer and Treseder, 2008; Cleland and Harpole, 2010), and affects soils by reducing pH, increasing nitrate (NO_3^-) and ammonium (NH_4^+) concentrations, decreasing base cations and buffering capacity, and increasing toxic elements such as aluminum (Bowman et al., 2006, 2008; Lieb et al., 2011). While N deposition has declined in some parts of the world due to legislation (Du et al., 2014), the return of ecosystem properties, including plant composition, nutrient cycling, and soil chemical characteristics, to their previous state has been slow (Street et al., 2015; Bowman et al., 2018; Crawford et al., 2020).

Shifts in plant communities do not occur in isolation, and changes in plant composition can cascade to affect soil bacterial and fungal communities. While edaphic properties, such as pH and resource availability, are typically considered the major drivers of soil microbial community distribution (Fierer et al., 2007; Lauber et al., 2009; Tedersoo et al., 2014), there are several ways in which changes in plant community composition can impact microbial communities. Plants influence the soil microbial community through the quantity and quality of resources (e.g. litter, root exudates) they produce (Wardle et al., 2004; Ward et al., 2015), as well as through their effects on other soil physicochemical characteristics (e.g. pH and water holding capacity (Stefanowicz et al., 2018)). Plants can have effects on specific microbial taxa (e.g. through allelopathic compounds; Lankau and Strauss, 2007) and entire communities (e.g. via alterations in nutrient cycling; Rodrigues et al., 2015). Therefore, N deposition can affect the soil microbial community through changes to the soil, such as enhanced nutrient availability or

lower pH (Ramirez et al., 2010; Chen et al., 2019), and through changes to the plant community, through shifts in the biomass or relative abundance of plant species (Suding et al., 2008; Yuan et al., 2016; Zeng et al., 2016; Shao et al., 2018). In particular, changes in dominant plant species are theorized to be a strong driver of belowground shifts because these species represent a considerable amount of community biomass and shape ecosystem and community properties (Grime, 1998; Gaston, 2011). The response of dominant plant species and the overall plant community to N deposition could buffer or amplify the effects of N addition on the soil microbial community, hence making it important to investigate both responses and their interaction.

Our study utilizes an 18-year N addition and co-dominant plant removal experiment in the Front Range of Colorado, USA to ask if and how different dominant plant species shape bacterial and fungal response to simulated N deposition. *Geum rossii* (hereafter *Geum*; a slow-growing rosaceous forb that declines precipitously with N addition (Suding et al., 2008)) and *Deschampsia cespitosa* (hereafter *Deschampsia*; a fast-growing bunchgrass) were annually removed from plots for 18 years. Previous work in these plots has demonstrated that N addition and co-dominant removals impact the remaining co-dominant as well as forbs and graminoids (Suding et al., 2006, 2008). We hypothesize that N addition will increase available soil N, increase the abundance of *Deschampsia*, and reduce the abundance of *Geum* (Suding et al., 2008). We also predict that the removal of *Deschampsia*, as a strong competitor in the system, will result in the

competitive release of *Geum* and other less abundant species. The removal of *Geum*, which some past work indicates as having a facilitative effect on other forbs (Suding et al., 2006), will result in an increase of *Deschampia* and a decline in other forb species. Furthermore, we expect that the removal of *Geum*, whose litter slows N cycling (Steltzer and Bowman, 1998), will have a compounding effect on N addition, causing even greater shifts towards increased N availability and the dominance of *Deschampsia*.

Resultant shifts in the plant community should indirectly shape how the soil microbial community responds to N addition. Specifically, we hypothesize that the co-dominant species will indirectly influence how the soil microbial community responds to N addition by altering the input of plant materials to the soil, but that the two dominant species will have distinct effects due to their different characteristics. This indirect effect could either amplify or counteract the direct effects of simulated N deposition on the soil microbial community. We predict that the presence of *Geum*, which has a high phenolic content and is a driver microbial N immobilization (Steltzer and Bowman, 1998; Bowman et al., 2004), will buffer the effects of N addition by depressing the response of fast growing, copiotrophic and nitrophilic organisms, while the presence of *Deschampsia*, a driver of N mineralization (Steltzer and Bowman, 1998), will amplify the effects of N addition and the response of the microbial community. A lack of an interaction between simulated N deposition and the plant community would suggest that the soil microbial community responds to N independently of the plant community.

Methods

Study Design

We conducted our study at Niwot Ridge in the Front Range of the Rocky Mountains, Colorado, USA (40.05˚N, -105.59˚W) in a long-term N addition and co-dominant species removal experiment initiated in 2001 (Suding et al., 2008). The experiment consists of seven experimental blocks within a ~5 km^2 region on Niwot Ridge, ranging in elevation from 3,397 to 3,544 m a.s.l. Plots (1 m^2) within the seven blocks were chosen such that they were co-dominated (~30% each) by *Geum* and *Deschampsia*. The N (NPK 40-0-0, using urea-N) was added as slow-release fertilizer pellets. Starting in 2001, 144 g of fertilizer/m^2 was applied at the start of each growing season. In 2008, N addition was reduced to 72 g/m^2 and in 2011, to a rate of 25 g/m^2. Previous work with these pellets in our system indicates that the average N inputs were 28.8 g N x m^{-2} x yr^{-1} from 2001 – 2007, 14.4 g N x m^{-2} x yr^{-1} from 2008 – 2010, and 5 g N x m^{-2} x yr^{-1} from 2011 – 2018 (Bowman et al., 1993). The reduction in applied N accounts for the cumulative effects of N addition. All rates of N addition were well above the proposed saturation rate in the area, estimated at 1 g N x m^{-2} x yr^{-1} (Bowman et al., 2006). At Niwot Ridge, wet and dry ambient N deposition is 0.6 g N x m^{-2} x yr^{-1} (Sievering, 2001). Removal of the co-dominant species began in 2001 and was achieved through clipping the aboveground biomass. The removal treatment consisted of *Geum* removal, *Deschampsia* removal, or no removal. Annual clipping was done one to three times throughout the growing season as needed. Plots were trenched to a depth of 10 cm 1

- 3 times per growing season to limit the influence of the co-dominant species growing on the periphery of the plot. By 2008 the average amount of biomass being clipped was around 1.2% of the original biomass removed from the plots, indicating a successful removal of the selected co-dominant species (Figure A2.1). Had we assessed soil microbial community composition in the early years of the experiment when large amounts of biomass were clipped for the removal treatment, we could not have been sure whether interactive effects of the co-dominant removal treatment were a result of the plant community responding to disturbance due to biomass removal or to the absence of the co-dominant plant species. Hence, it was important to wait until the co-dominant biomass removed had tapered off before we could assess the potential indirect effects of our co-dominant plant species on the soil microbial community. Additionally, sampling at this later stage of the experiment ensured that the plant and microbial communities were experiencing a long-term press disturbance, which allows us to better predict their response to chronic N deposition. There was a total of 42 plots (6 treatments across 7 blocks).

Plant community composition data was collected annually from 2002 until 2018 during peak biomass using the point intercept method. Only top hits (the tallest species at each point) were recorded, but species that were present in the plot and not hit were assigned a value of 0.5. Before calculating plant community response to treatment, non-vascular plants as well as rocks and litter were removed from the dataset. Aboveground biomass was harvested in 20 cm x 20 cm square areas within each plot every other year from 2004 to 2018. The collected biomass

was sorted into biomass accumulated that year and litter. The location of the square within the plot was shifted each year to minimize impact.

Soil properties

From 26 July to 3 August 2018, soils were collected by homogenizing ten soil cores per plot taken to depth of 10 cm using a core 2 cm in diameter. Samples were kept on ice while being transported to the lab within 8 hours of collection. A subset was immediately aliquoted for DNA extraction and kept at -20°C for a maximum period of 2 months. Additionally, sieved soil was aliquoted for gravimetric soil moisture, pH, and K_2SO_4 extractions for NH_4^+ and NO_3^-. We measured pH using a SevenCompact pH meter S210 (Mettler Toledo, Greifensee, Switzerland) on soils that had been mixed 2:1 with deionized water and allowed to equilibrate with atmospheric CO_2 for 30 minutes. We extracted N from 10 g of soil using 50 ml of 0.5M K_2SO_4 and shaking for 2 hours at 140 rpm. The following day, this mixture was filtered, frozen, and delivered to the Colorado State University Soil, Water and Plant Testing Laboratory (Fort Collins, CO). Negative values indicate that the N content was below the detection limit and were thus set to 0. All roots collected during the sieving processes were washed over a 250 μm sieve, dried at 60°C for 72 hours, and weighed for root biomass.

Soil sequencing

For the bacterial and fungal community analysis, DNA was extracted from 0.25 g of moist homogenized soil using a Qiagen DNeasy PowerSoil Kit according to the manufacturer's protocol (Qiagen, Hilden, Germany). Extracted genomic DNA

was diluted 1:10 in sterile culture grade water (Sigma-Aldrich Co., St. Louis, MO, USA). PCR was then used to amplify the V4 hypervariable region of the 16S rRNA gene using barcoded 515f and 806r primers and the first internal transcribed spacer region (ITS) of the rRNA gene using barcoded ITS1-F and ITS2 primers to assess the structure of bacterial and fungal communities, respectively (Leff et al., 2017). PCR was conducted with Promega HotStart Mastermix (Promega, Madison, WI, USA) in a 25 μl reaction. The thermal cycling conditions were as follows: 3 min initial denaturation at 94°C, 35 cycles of 45 s denaturation at 94°C, 1 min annealing at 50°C, 1.5 min elongation at 72°C, and a 10 min final elongation at 72°C. Amplicons were purified and normalized with the SequalPrep Normalization Kit (Invitrogen Inc., CA). Samples were then pooled into single 16S and ITS amplicon libraries and sequenced on an Illumina MiSeq2000 (pair-end 2 x 300 bp) at the University of Colorado BioFrontiers Institute (Boulder, CO, USA). Sequences were demultiplexed using idemp (https://github.com/yhwu/idemp/blob/master/idemp.cpp) and sequencing adapters were removed using cutadapt (Martin, 2011). Reads were then processed using the DADA2 pipeline (Callahan et al., 2016). First, reads were quality filtered and dereplicated. Then, exact sequence variants (ESV) were inferred and paired-end reads were merged. Next, chimeras were removed, and taxonomy was assigned using SILVA (Quast et al., 2013) for the bacterial sequences and UNITE (Abarenkov et al., 2010) for the fungal sequences. Two samples from the ITS data were dropped due to low quality reads. The 16S sequences were rarefied to

13,283 sequences per sample and the ITS sequences were rarified to 14,946 sequences per sample.

Data analysis

To test for effects of the dominant plant species and N addition on soil properties and aspects of the plant community, we ran linear mixed effects models with block as a random effect and N addition, dominant plant removal, and their interaction as fixed effects (function 'lmer,' package *lme4*; Bates et al., 2015). When required, variables were log or square-root transformed to meet assumptions of normality and homogeneity of variance. Pairwise treatment comparisons were assessed via Tukey's honest significant difference (function 'emmeans', package *emmeans*; Lenth et al., 2018). To test for treatment effects on the plant community composition in 2018, we ran a permutational multivariate analysis of variance (PERMANOVA) on Bray-Curtis dissimilarity matrices calculated on square-root transformed relative abundances with co-dominant plant removal, N addition, and their interaction as predictors (function 'adonis,' package *vegan*; Oksanen et al., 2019). Block was included as 'strata' (a blocking variable), which restricted permutations to within blocks. Plant species present in fewer than 5% of plots (2 plots) were removed from the compositional data prior to the PERMANOVA. We conducted a similarity percentage analysis to identify plant species contributing to treatment differences (function 'simper,' package *vegan*). We expected that both N addition and the co-dominant plant species would have strong effects on soil properties and the plant community as those components of the system are the

pathways through which we would expect buffering or accelerating processes to occur.

To test the responses of the microbial community, we ran two partial distance-based redundancy analysis (dbRDA), described below. Data were relativized and then subset before analysis—we retained ESV with a mean relative abundance greater than 0.05% (e.g. Oliverio et al., 2017), thereby removing rare taxa. Before this filtering there were 6,336 bacterial and 6,199 fungal ESV. To test for the effects of our categorical treatment variables on microbial community composition, we first conducted a partial dbRDA on the bacterial and fungal communities with block as our condition (function 'dbrda,' package *vegan*) and N addition, plant removal, and their interaction as predictors. We then ran a partial dbRDA on both microbial communities with a suite of continuous edaphic and plant-related variables as predictors. The continuous predictor variables of interest were *Geum* and *Deschampsia* relative abundance, forb and graminoid abundance, plant richness, live aboveground biomass, soil NO_3^-, soil NH_4^+, and soil pH (Figure A2.2 for a principal coordinates analysis with these predictors [functions 'cmdscale' and 'envift,' package *vegan*]). Where *Geum* and *Deschampsia* were removed from plots, their relative abundances were set to NA when calculating forb and graminoid abundance. Of *Deschampsia*, *Geum*, forb, and graminoid abundance, only graminoid abundance was retained in the dbRDA due to collinearity ($r > 0.7$). All continuous variables were scaled to have a mean of 0 and a standard deviation of 1. For the dbRDA containing the continuous predictors, we used forward selection to

choose the best model based on adjusted R^2 (function 'ordiR2step,' package *vegan;* Blanchet et al., 2008). P-values were adjusted using a Holm correction to reduce the risk of Type I error resulting from conducting multiple significance tests during forward selection.

We followed up on significant effects of our categorical variables with the nonparametric Kruskal-Wallis test (function 'taxa_summary_by_sample_type,' package *mctoolsr;* Leff, 2017); the Benjamini-Hochberg correction was used to account for the multiple comparisons. We ran the Kruskal-Wallis test at the phylum and ESV level for both bacteria and fungi. We also ran the test at the genus level for fungi to test for effects on the response of arbuscular mycorrhizal fungi (AMF) and dark septate endophytes (DSE), both of which are associated with plant nutrient uptake (Johnson et al., 2010; Newsham, 2011). AMF genera were defined as genera within the phylum Glomeromycota (Schüßler et al., 2001; Tedersoo et al., 2018). The DSE genera were defined as those described in reports of known DSE (Jumpponen and Trappe, 1998; Newsham, 2011). We also looked for AMF and DSE in the compositional data containing rare taxa to more accurately determine the number of detected genera. All statistical analyses and visualizations were performed in R ver. 3.4.2 (R Core Team, 2020).

Results

Both dominant plant removal and N influenced the plant community

The co-dominant plant species had a strong effect on most aspects of the plant community, which was similar in magnitude to the effect of N addition, but

there were no interactive effects on any of the measured plant variables. The removal of *Geum* and the addition of N decreased live aboveground biomass by 34% and 20%, respectively (Table 2.1, Figure 2.1a, Table A2.1). On the other hand, root biomass was 37% higher with N addition (Table 2.1, Figure 2.1b).

As expected, N addition caused *Geum* to decline by 81% while *Deschampsia* increased by 61% (Table 2.1, Figure 2.1c, d). Forbs declined with N addition (Table 2.1, Figure 2.1e), including *Artemisia scopulorum*, *Erigeron simplex*, and *Castilleja occidentalis* ($P < 0.05$). Graminoids increased with N addition (Table 2.1, Figure 2.1d), including *Carex scopulorum* and *Carex nova* ($P < 0.05$).

The removal treatments indicated a general facilitative relationship between *Geum* and other forbs, and competitive relationships between *Deschampsia* and *Geum*. The removal of *Geum* led to a decline in other forbs and an increase in graminoids (Table 2.1, Figure 2.1e, f, Table A2.1). *Deschampsia* abundance increased 52% under *Geum* removal (Table 2.1, Figure 2.1d). On the other hand, the removal of *Deschampsia* led to a decline in other graminoids and an increase in forb abundance (Table 2.1, Figure 2.1e, f, Table A2.1), with a 32% increase in *Geum* abundance under *Deschampsia* removal (Table 2.1, Figure 2.1c).

Nitrogen addition significantly decreased plant species richness from an average of 14 species/m^2 to 12 species/m^2 (Table 2.1, Figure 2.1g), but there was not an effect of removal. As indicated by the PERMANOVA, plant community composition was shaped by both N addition and co-dominant plant removals, with

the latter explaining a greater amount of variation, but there was no interaction (Table 2.1, Figure 2.2a).

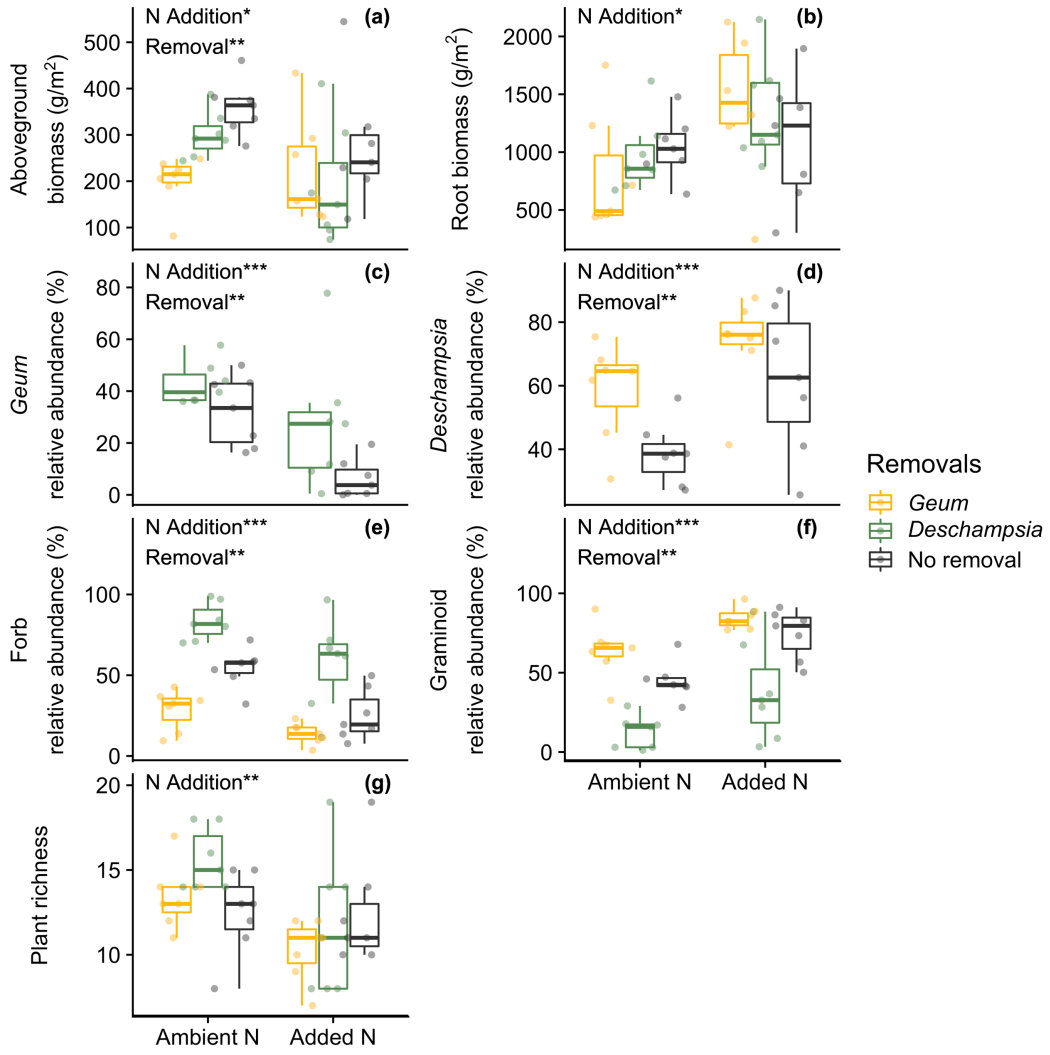

Figure 2.1. Under ambient N, aboveground biomass was lower with *Geum* removal relative to the control (a). Root biomass was higher with N addition (b). *Geum* declined under N addition and increased under *Deschampsia* removal, there was no interaction (c). *Deschampsia* increased under both N addition and *Geum* removal, there was no interaction (d). Forb abundance declined with N addition and *Geum* removal but increased with *Deschampsia* removal (e). Graminoid abundance increased with N addition and *Geum* removal but decreased with *Deschampsia* removal (f). N addition lowered plant species richness (g). Significance codes: "***" $P < 0.001$, "**" $0.001 \leq P < 0.01$, and "*" $0.01 \leq P < 0.05$.

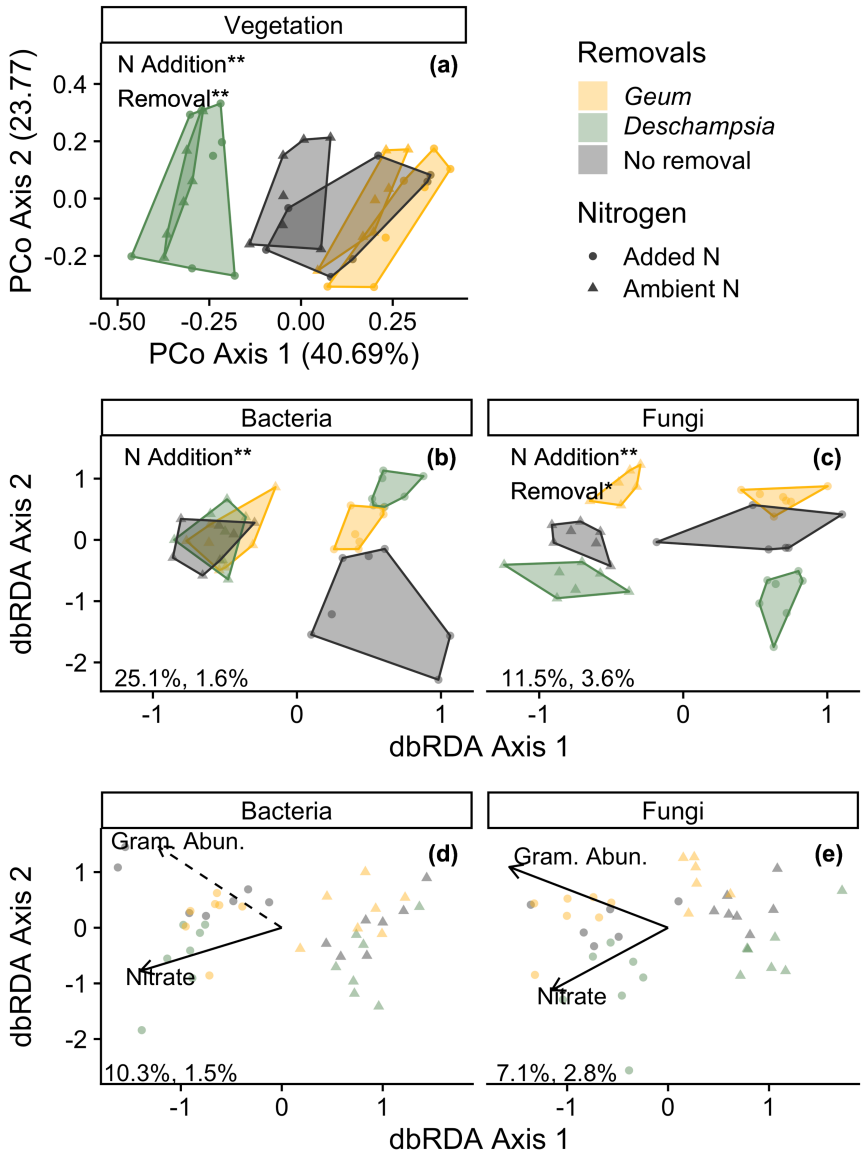

Figure 2.2. Both N addition and dominant plant removal influence plant community composition, but there was no interaction (a). Only N addition shaped bacterial community composition (b) while both N addition and co-dominant plant removal impacted fungal community composition (c). Nitrate was a driver of both bacterial (d) and fungal community composition (e), and the latter was also shaped by graminoid abundance. Percentages in the bottom left corner of each panel represent the variation explained by the first and second dbRDA axes, in that order. The dashed arrow indicates the predictor was not significant ($P > 0.05$). Significance codes: "***" $P < 0.001$, "**" $0.001 \leq P < 0.01$, and "*" $0.01 \leq P < 0.05$.

Dominants plants had little effect on soil properties

Our results showed a weak effect of the co-dominant plant species on soil properties. The soil was acidic with a pH that ranged from 4.4 to 5.1 (mean ± SD; 4.8 ± 0.2). There was an interaction between N addition and co-dominant plant removals such that the soil pH was lower with *Geum* removals under added N relative to ambient N (Table 2.1, Figure 2.3a). As a main effect, nitrogen addition caused a slight decline in pH; the mean pH in N addition plots (4.8 ± 0.2) was 1.3 times more acidic than the pH in the control plots (4.9 ± 0.2) (Table 2.1). The available soil NO_3^- increased by over 1,000% with a mean of 3.5 ± 7.3 μg g^{-1} of soil under N addition (Table 2.1, Figure 2.3b). The available soil NH_4^+ increased by 37% with a mean of 50 ± 16 μg g^{-1} of soil under N addition (Table 2.1, Figure 2.3c). Contrary to our expectations, there was no effect of our co-dominant plants on soil N (Table 2.1). Soil moisture ranged from 18 to 84% (38 ± 18%), but it was not affected by treatment (Table 2.1, Figure 2.3d).

Table 2.1. Effects of N addition, dominant plant removal, and their interaction on soil and plant variables. The Stat column refers to the test statistic (χ^2 for LME, pseudo-F for PERMANOVA). Bolded values highlight significant effects ($P < 0.05$). n = 42 for soils, plants, and bacterial statistics; n = 28 for *Deschampsia* and *Geum* abundance as the respective removal treatments were first subset out (df = 1 for the Plant Removal and Interaction for these two variables).

				N Addition (df = 1)			Plant Removal (df = 2)			Interaction (df = 2)		
Dataset	Response Variable	Model	n	Stat	R^2	P	Stat	R^2	P	Stat	R^2	P
Soils	pH	LME	42	6.0		**0.02**	0.3		0.87	8.4		**0.02**
	Ammonium	LME	42	6.9		**0.01**	3.3		0.19	3.7		0.16
	Nitrate	LME	42	30.0		**< 0.001**	1.2		0.55	0.4		0.82
	Moisture	LME	42	1.2		0.27	1.8		0.41	3.5		0.17
Plants	Aboveground Biomass	LME	42	5.5		**0.02**	9.8		**0.007**	4.8		0.09
	Root Biomass	LME	42	5.5		**0.02**	0.3		0.85	2.3		0.32
	Geum abundance	LME	28	18.8		**< 0.001**	9.4		**0.002**	1.2		0.28
	Deschampsia abundance	LME	28	15.2		**< 0.001**	10.2		**0.001**	0.9		0.35
	Forb abundance	LME	42	25.9		**< 0.001**	79.8		**< 0.001**	1.8		0.42
	Graminoid abundance	LME	42	26.1		**< 0.001**	80.1		**< 0.001**	1.8		0.41
	Richness	LME	42	7.9		**0.01**	4.0		0.14	5.1		0.08
	Composition	PERMANOVA	42	3.0	0.06	**0.003**	6.0	0.23	**0.001**	0.6	0.02	0.40

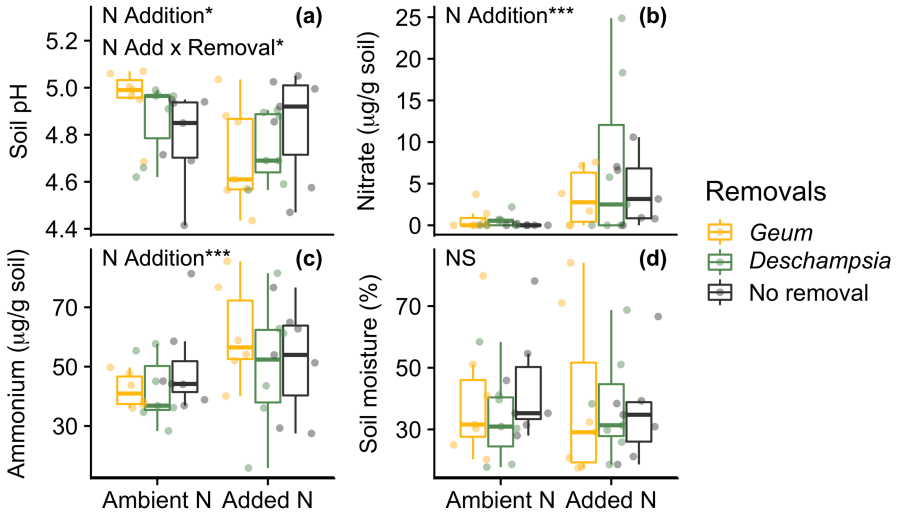

Figure 2.3. Soil pH was lowered by N addition only when the dominant plant *Geum* was removed (a). N addition drove an increase in soil nitrate (b) and ammonium (c). Significance codes: "***" represents $P < 0.001$, "**" represents $0.001 \leq P < 0.01$, and "*" represents $0.01 \leq P < 0.05$.

The bacterial community was not influenced by the plant community, and shifts in plant composition did not mediate bacterial response to N

A total of 407 bacterial ESV were left following the removal of rare taxa, with a mean richness of 231 ± 28 ESV per sample. At the phylum level, we found that Acidobacteriota (percentage of sequences: 27%), Proteobacteria (22%), and Chloroflexi (16%), dominated the bacterial communities. The class Acidobacteriae constituted 93% of the Acidobacteriota reads, Gammaproteobacteria and Alphaproteobacteria represented 66% and 34% of Proteobacteria reads, respectively, and Ktedonobacteria comprised 84% of Chloroflexi reads.

Contrary to our hypothesis that the plant community would mediate bacterial response to simulated N deposition, we found no interaction between N addition and plant removal (Table 2.2). Only N addition alone (Table 2.2, Figure 2.2b), driven by increased soil NO_3^- (Table 2.3, Figure 2.2d), shaped the soil bacterial community. The relative abundance of 11 bacterial phyla differed significantly between ambient and added N plots ($P < 0.05$, Figure 2.4), including Acidobacteriota (declined 28% under N, Figure 2.4a), Verrucomicrobiota (declined 53% under N addition, Figure 2.4j), and Bacteroidota (increased 89% under N addition, Figure 2.4c). There were 182 out of 407 bacterial ESV that were significantly affected by N addition with 77 ESV increasing with added N (including 20 ESV only detected in N addition plots (Table A2.2)) and 105 declining with added N (including 3 ESV not detected in N addition plots (Table A2.2)).

Table 2.2. N addition significantly impacted both the bacterial and fungal communities. Fungal communities were additionally shaped by the co-dominant plant removal treatment. Bolded values highlight significant effects ($P < 0.05$).

	Bacterial Community			Fungal Community		
Predictor Variable	df	F-value	P-value	df	F-value	P-value
N Addition	1	21.2	**0.001**	1	6.8	**0.001**
Plant Removal	2	0.93	0.44	2	1.7	**0.02**
Interaction	2	0.88	0.53	2	0.90	0.66

Table 2.3. Nitrate and graminoid abundance were the continuous predictors selected via model selection from a suite of continuous predictors (nitrate, ammonium, pH, root biomass, aboveground biomass, and plant richness). Available nitrate significantly impacted both the bacterial and fungal communities. Fungal communities were additionally shaped by graminoid abundance. Bolded values highlight significant effects ($P < 0.05$).

	Bacterial Community			Fungal Community		
Predictor Variable	df	F-value	P-value	df	F-value	P-value
Nitrate	1	5.0	**0.02**	1	2.3	**0.04**
Graminoid Abundance	1	2.27	0.40	1	3.0	**0.04**

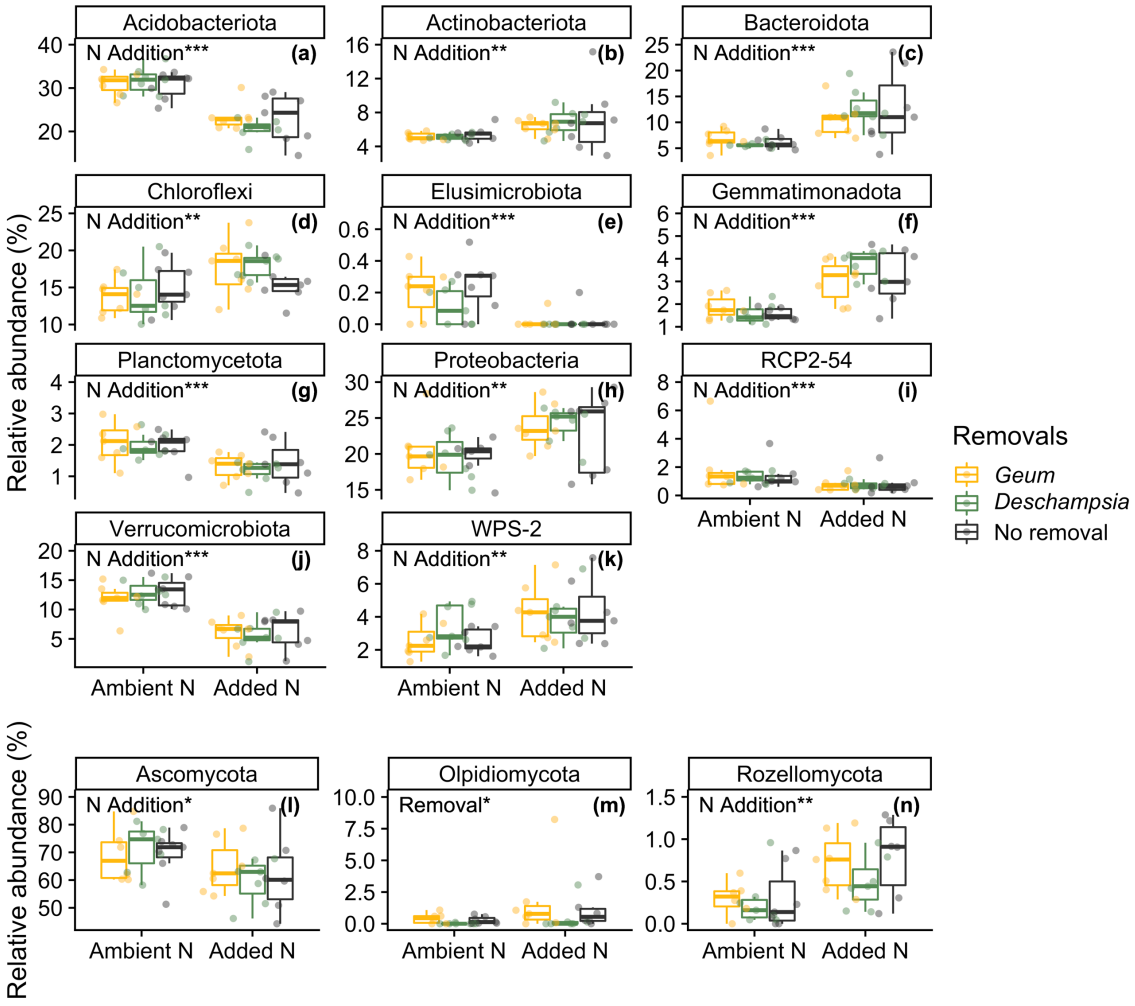

Figure 2.4. There were 11 bacterial phyla that differed significantly between N addition and control plots (a - k), two fungal phyla that different significantly between N addition and control plots (l and n), and one fungal phylum that was affected by dominant plant removal (m). Significance codes: "***" $P < 0.001$, "**" $0.001 \leq P < 0.01$, and "*" $0.01 \leq P < 0.05$.

The fungal community was influenced by the plant community, but shifts in plant composition did not mediate fungal response to N

A total of 340 fungal ESV were left following the removal of rare taxa, with a mean richness of 126 ± 14 ESV per sample. We found that that Ascomycota (percentage of sequences: 66%) and Basidiomycota (17%) dominated the fungal communities. Leotiomycetes and Archaeorhizomycetes made up 49% and 25% of

Ascomycota reads, respectively, and Agaricomycetes made up 76% of Basidiomycota reads.

In contrast to the bacterial community, the fungal community was shaped by both N addition and plant removal, but contrary to our predictions, there were no interactive effects (Table 2, Figure 2.2c). These effects were driven by soil NO_3^- and graminoid abundance (Table 2.3, Figure 2.2e). Under N addition, the relative abundance of Ascomycota decreased by 11% ($P = 0.003$, Figure 2.4l) while the relative abundance of Rozellomycota increased by 140% from 0.28% to 0.69% ($P = 0.01$, Figure 2.4n). Twenty-seven out of 340 fungal ESV were significantly impacted by N addition with 17 of those ESV experiencing an increase in relative abundance (including one ESV only detected in N addition plots which belonged to *Pseudogymnoascus destructans*) and 13 experiencing a decline in relative abundance ($P < 0.05$). There were no phylum-level differences in fungal composition between plots without co-dominant plant removals and plots with *Geum* removal. However, the relative abundance of Olpidiomycota declined 61% and 80% in *Deschamspia* removal plots relative to plots without co-dominant plant removals and plots with *Geum* removal, respectively ($P < 0.05$, Figure 2.4m). There were three AMF genera detected in the dataset subjected to the relative abundance threshold and 14 in the dataset containing rare taxa, but only one genus (an unidentified genus in the Ambisporaceae family) had significantly lower relative abundance under added N, declining by 64% from 0.39% to 0.14%. We found seven DSE genera in the dataset subjected to the relative abundance threshold and 16 in

the dataset containing rare taxa, but only one genus (*Leptodontidium*) saw significantly lower relative abundance under added N, declining by 60% from 0.80% to 0.32%.

Discussion

Understanding the cascading effects of the plant community on soil microbial communities under simulated N deposition is important for parsing apart the drivers of microbial community change resulting from this chronic stressor. While it is widely thought that biotic communities may have feedbacks that can amplify or buffer the effects of external drivers (reviewed by Tylianakis et al., 2008), our results suggest little evidence for these interactions between taxa at our site. The long-term application of N (at a level meant to saturate the system) and removal of co-dominant species represents a press disturbance where communities are set onto a new trajectory rather than being allowed to return to their pre-disturbance state. This design allowed us to assess how the alteration of plant communities under this disturbance may influence the assembly of the soil microbial community. After 18 years of press disturbances our results demonstrate that though co-dominant plants influenced aboveground biomass and plant community composition, the plant community response to N did not mediate how the soil microbial community responded to simulated N deposition. Instead, bacterial communities were altered only by N addition while fungal communities were affected by both N addition and co-dominant plants, but without an interaction. This suggests that the altered

quality and quantity of plant inputs as a result of N addition were not as important as external changes to resource availability in shaping soil microbial community response to N deposition.

In line with our hypothesis, we saw strong effects of 18 years of co-dominant plant removals and N addition on plant community composition and productivity. The plant removal treatments effectively eliminated *Deschampsia* and *Geum* where intended and resulted in alterations to aboveground biomass and plant community composition. The removal of each co-dominant species released from competition the functional type of the remaining co-dominant, primarily driven by an increase in abundance of the dominant itself, with both *Geum* removal and N addition increasing *Deschampsia* and overall graminoid abundance, as hypothesized.

In contrast to our hypothesis, we detected no effect of the co-dominant plant removal on N availability. That the presence of *Geum* or *Deschampsia* did not influence soil N availability was surprising given that previous work demonstrated strong effects of these two co-dominant species on nutrient cycling (Steltzer and Bowman, 1998). However, Steltzer and Bowman (1998) documented nutrient cycling in patches where each of the co-dominant species were separately highly abundant; they appear to play a smaller role in nutrient cycling when they are part of more diverse plant communities, as is the case in our plots (~30% initial abundance of each of the co-dominant species).

We anticipated that the removal of these co-dominant species would alter soil N processes by altering the input of litter that slows (*Geum*) or hastens

(*Deschampsia*) N cycling, thereby establishing a plant-microbial interaction which might mediate microbial response to N. In contrast to our hypothesis, we found similar responses of the soil microbial community to N despite shifts in the abundance of the co-dominant species and other aspects of the plant community, such as root biomass, aboveground biomass, and forb and graminoid abundance. This suggests that N acted directly on the soil bacterial and fungal communities rather than acting indirectly through buffering or amplifying effects of the plant community. The response of the microbial community generally indicated an environment that was more resource rich, which aligns with our finding of higher N availability and a significant effect of NO_3^- availability on the soil bacterial and fungal communities. The relative abundance of copiotrophic taxa, which thrive in resource rich environments, such as Bacteroidota (Fierer et al., 2007), increased and the relative abundance of taxa on the other end of the spectrum, oligotrophic taxa—such as Acidobacteriota and Verrucomicrobiota—declined (Nemergut et al., 2008; Fierer et al., 2012; Ramirez et al., 2012).

In the fungal community, the response was slightly less predictable. Because NO_3^-availability significantly affected the soil fungal community, we expected a decline in fungi that aid in plant nutrient uptake, such as AMF (Johnson et al., 2010) and DSE (Newsham, 2011), because plants generally devote less photosynthate carbon (C) to such fungi under higher resource scenarios (Read, 1991). The weak response of AMF and DSE genera to N addition may be because N addition led to higher demand for phosphorus, maintaining the need for

relationships with fungi that aid in nutrient acquisition. This possibility is supported by other work at our site which demonstrated that neither AMF nor DSE genera within the roots of *Geum* and *Deschampsia* declined with N addition (Dean et al., 2014) and that the total amount of C allocated to Ascomycota (the phylum containing most DSE) by *Geum* and *Deschampshia* did not decline under N addition (Farrer et al., 2013). It is also possible that the lack of AMF response was due to their overall low abundance and/or because specific mycorrhizal primers were not used, hence limiting our conclusions regarding this group. Altogether, there were large shifts in both the soil bacterial and fungal communities as a result of N addition, which occurred independently of shifts in the plant community.

The lack of mediating effects of the co-dominant plant species on soil microbial response to N may be because the presence of *Geum* or *Deschampsia* did not strongly influence soil N availability or root biomass. Hence, the role of the plant community in shaping soil chemistry and resource availability is likely an important factor that determines whether the plant community mediates soil microbial response to N addition (Yuan et al., 2016; Zeng et al., 2016) or whether N addition acts directly on the soil microbial community (Ramirez et al., 2010; Wardle et al., 2013). While we did not find mediating effects of the plant community on microbial response to N, the presence of the co-dominant plant species did affect the fungal community, driven by the abundance of graminoids, which was positively correlated with the abundance of *Deschampsia* and negatively correlated with the abundance of *Geum* and other forbs. The decrease in Olpidiomycota, which contains

known plant pathogens (Tedersoo et al., 2018), in the *Deschampsia* removal plots may suggest that there are pathogens specific to *Deschampsia* which decline in its absence. These results indicate that plant inputs, such as litter and root exudates, as well as specific plant-microbe associations influenced the soil fungal community though they did not shape how the soil fungal community responded to N addition.

This manipulative experiment demonstrates that the effects of simulated N deposition on the soil bacterial and fungal community were not mediated by the plant community but were instead manifest through increased resource availability. Dominant plant species, despite their impacts on aboveground biomass and plant community composition, neither buffered nor amplified the response of the soil microbial community to N addition. Changes to the soil microbial community occurred independently of directional shifts in the plant community, suggesting there are not strong cascading effects of N addition across the plant-soil interface in our system. More broadly, our results highlight the importance of understanding when indirect effects shape community response to global change in order to improve our ability to predict how biodiversity will respond to change.

CHAPTER III

HOST PLANT RELATEDNESS AND ENVIRONMENTAL DRIVERS SIMILARLY SHAPE BACTERIAL AND FUNGAL ROOT ENDOPHYTE COMMUNITIES THOUGH LITTLE VARIATION IS EXPLAINED

By Laurel M. Brigham, Clifton P. Bueno de Mesquita, Marko J. Spasojevic, Emily C. Farrer, Dorota L. Porazinska, Jane G. Smith, Steven K. Schmidt, Katharine N. Suding.

Abstract

Bacterial and fungal root endophytes are critical for their host plants, yet we know little about whether certain drivers differentially shape root endophytes in these two communities. We investigated the effects of host plant phylogeny, plant neighborhood, space, and abiotic drivers on bacterial and fungal root endophyte communities in alpine plants across a gradient in plant density and richness, snowpack, and soil physical and chemical characteristics. The plant neighborhood and snowpack variables are shifting under a changing climate as plants move upward in elevation and snowpack declines. Including these drivers allowed us to assess how global change might influence root endophyte community assembly. We found that host plant phylogenetic relatedness explained the greatest variation in root endophyte composition, yet the explained variation was low (5%). We detected similar levels of importance of plant neighborhood, space, and abiotic effects on bacterial and fungal root endophyte communities suggesting bacteria and fungi in the root endosphere at our site were similarly shaped by these variables. While

there may be weak effects of those variables shifting with climate change, evidenced by the significant effect but low explained variation, it appears that the bacterial and fungal communities across our gradient are more stochastically assembled.

Introduction

Plant associations with microbes are ubiquitous and those microbes residing inside the root, root endophytes (*sensu* Hardoim et al., 2015), are critical in determining plant health. The root endosphere includes fungi and bacteria, amongst other microbes, that can enhance plant growth (Hardoim et al., 2008), increase access to nutrients (Sevilla et al., 2001; Hurek et al., 2002), and protect against pathogens (Sessitsch et al., 2004; Maciá-Vicente et al., 2008). Additionally, the root endosphere can include taxa classified as pathogens; plants may be asymptomatic (Berg et al., 2005) or experience clear negative effects, such as reduced growth (Junker et al., 2012).

Edaphic variables are typically considered the strongest driver of root endophyte communities. Characteristically important variables include soil type (encompassing a variety of edaphic, and often, geographic differences) (Bulgarelli et al., 2012; Lundberg et al., 2012; Glynou et al., 2018; Xu et al., 2020), nutrient availability (Yeoh et al., 2017), and soil pH (Dumbrell et al., 2010). Edaphic variables can act on root endophyte communities through shifts in soil microbial communities, which serve as a source community (Adair and Douglas, 2017; Papik et al., 2020), or via shifts in plant-microbial interactions. For example, arbuscular

mycorrhizal fungi (AMF) colonization declines under higher nutrient availability (Johnson et al., 2003; Bueno de Mesquita et al., 2018a) while soil pH shapes root architecture (Haling et al., 2010) and thus colonization dynamics.

While the soil environment has often been found to be a key driver shaping root endophyte communities, the effect of plant host has been found to be more variable. Studies comparing plants at the level of cultivars and strains often do not show strong host plant control (Shakya *et al.* 2013; Chen *et al.* 2017; Leff *et al.* 2017, but see Bulgarelli *et al.* 2012; Lundberg *et al.* 2012; Singer *et al.* 2019)) . Comparisons across plant species (Aleklett et al., 2015; Kumar et al., 2017) and at coarser taxonomic levels (Glynou et al., 2018) show greater influence of the host plant, where more closely related host plants tend to have more similar root endophyte communities (Yeoh et al., 2017; Fitzpatrick et al., 2018). One possible way to resolve these differences is through the lens of functional differences across host plants: host plant control may be stronger in systems with more diverse and broader differences in the phenotypic traits and genetic characteristics of the host species. However, in addition to phylogenetic scale driving host plant effects, the spatial scale of the study may overwhelm the host plant signature through increased variability in both space and environmental drivers, making geography an important contextual factor to consider.

Studies assessing which drivers shape root endophyte communities typically focus on either root endophytic bacteria or fungi, hampering our ability to generalize about these different communities. Those studies that do examine both

bacterial and fungal root endophyte communities generally find differences in their drivers. For example, Bickford et al. (2018) found a stronger relationship between abiotic variables and bacterial root endophytes compared to fungal root endophytes. Additionally, different host plant genes, which determined plant morphology and physiology, shaped the diversity of bacteria and fungi in the root endosphere of *Arabidopsis thaliana* (Bergelson et al., 2019). Hence, it is paramount to study bacterial and fungal root endophyte communities concurrently in order to obtain a more complete understanding of root endophyte assembly.

Though less frequently focused on, geography and plant neighborhood may also have variable effects on bacterial compared to fungal root endophyte communities. Geography is often encompassed by the commonly used variable "soil type" though geography itself is not explicitly discussed (e.g. Bulgarelli *et al.* 2012; Lundberg *et al.* 2012). However, geography may have differential effects on bacterial and fungal endophyte communities (Bonito et al., 2014), perhaps due to differences in dispersal limitation. The strength of dispersal limitation on microbes varies by size, where larger organisms, such as many fungi, are typically more affected than smaller organisms, such as bacteria (Schmidt et al., 2014; Li et al., 2020). The composition of the plant neighborhood can also shape root endophyte communities and may do so differentially for bacterial and fungal root endophytes. The plant neighborhood can be defined as the composition, diversity, and density of the plants surrounding the focal host plant species. For example, Lumibao et al. (2020) found that host plant density and canopy cover of baldcypress (*Taxodium*

distichum) was related to root endophytic fungal richness. On the other hand, the presence of a dominant plant species in the plant neighborhood had a negligible effect on root-associated bacteria (Dean et al., 2015), though it had a stronger effect on root root-associated fungi (Dean et al., 2014). These findings highlight the importance of investigating both bacterial and fungal root endophyte relationships with a broad suite of drivers to increase our understanding of how these different communities, which have important implications for plant health, are shaped.

While few studies have simultaneously examined the role of the host plant, environmental drivers, and geography on bacterial and fungal root endophyte communities, we did so by capitalizing on a spatially heterogenous gradient of plant density and richness, snowpack, and edaphic properties (i.e. soil pH, nutrient availability, soil texture). Across this alpine environmental gradient, we also tested the importance of host plant relatedness, as measured by phylogenetic distance, on root endophyte communities. We examined bacterial and fungal root endophyte communities associated with 13 alpine plant species in the Colorado Front Range, using high-throughput marker gene sequencing. Importantly, alpine environments around the globe are experiencing declines in snowpack (Stewart, 2009), which impacts plant growth rate, phenology, physiology, and reproduction (Winkler et al., 2018) because snowpack meltwater supplies much of the growing season soil moisture (Williams et al., 2009). Additionally, climate warming can result in plant species moving uphill (Lenoir et al., 2008; Chen et al., 2011), which can drive

changes in both edaphic properties (Bueno de Mesquita et al., 2017) and plant community composition and interactions (Alexander et al., 2015, 2016).

Because our sampling encompassed a diverse set of plant species across a relatively small spatial scale (greatest distance between plots was 1900 m), we hypothesized that host plant phylogenetic distance, reflecting morphological differences amongst host plants, would be the strongest driver of both bacterial and fungal root endophyte communities (H1). We also hypothesized that abiotic variables would have a stronger influence on bacterial root endophytes relative to fungal root endophytes, reflecting stronger associations between bacteria and abiotic drivers (H2). In contrast, we hypothesized that the plant neighborhood and space would explain more variation in fungal root endophytes than bacterial root endophytes, reflecting the stronger influence of plant neighborhood and dispersal limitation on fungi (H3).

Materials and Methods

Study Site

This study took place on a south-facing slope in the Green Lakes Valley, part of the Niwot Ridge Long Term Ecological Research site, in the Front Range of the Rocky Mountains, Colorado, USA (40° 3′ 11′ N, 105° 37′ 50′ W; Figure 3.1). We resampled a subset of spatially explicit plots established in 2007 (King et al., 2010), with the closest plots 5 m away and the farthest plots ~2 km away from each other. The location of these circular plots (1 m in diameter) ranges in elevation from 3638

to 3870 m a.s.l. The soils are acidic with a pH that ranges from 4.52 to 5.82 (mean ± SD; 5.18 ± 0.32). The study area encompasses moderately vegetated patches of alpine tundra meadow (131 stems m^{-2}) to sparsely vegetated talus slopes (8 stems m^{-2}).

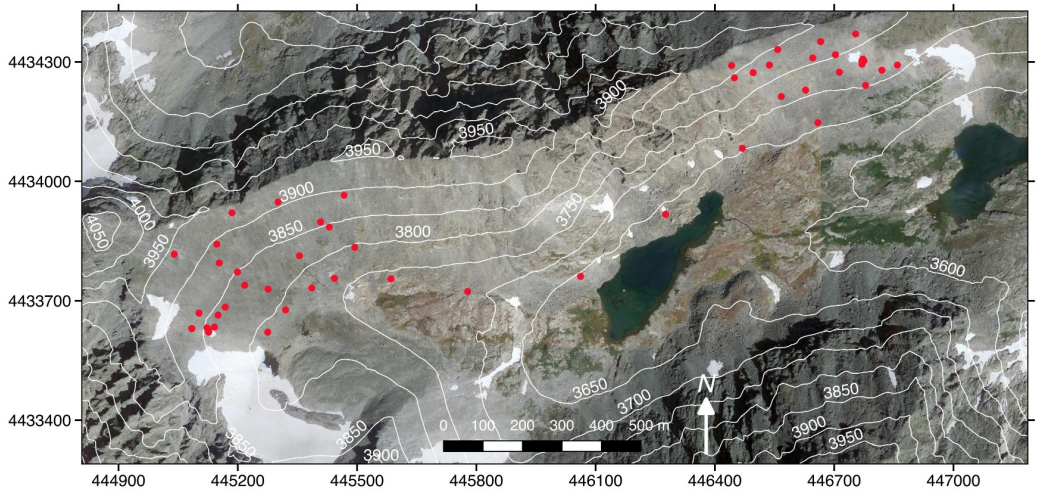

Figure 3.1. Aerial image of the study area, highlighting the location of the 50 plots used in this study (red points) which are arrayed across the Green Lakes Valley in the Front Range of the Rocky Mountains, Colorado, USA. Values along the edge of the image indicate Universal Transverse Mercator coordinates at an interval of 300 m. Values on the map in white indicate elevation in meters.

Environmental and plant characteristics

We assessed the effects of variables which are known to be altered by global change and which could affect root endophyte composition (i.e. snowpack, plant density, plant richness). Though winter precipitation has generally increased in alpine areas (Moberg et al., 2006; Kittel et al., 2015), the warmer temperatures that accompany this shift result in reduced spring snowpack (Stewart, 2009). To assess the role that snowpack plays on root endophyte communities, we estimated mean May snowpack depth at each plot by kriging interpolation of snow depth data from

annual snow surveys (1997 to 2015) conducted at our study site (Bueno de Mesquita et al., 2018a; Farrer et al., 2019). In these surveys, snow depth was manually measured during peak snowpack in May at an average of 483 random locations that were approximately 50 m apart.

Due to climatic changes, tundra plants may expand upward into less vegetated, higher elevation alpine areas. The uphill movement of tundra plants may alter root endophyte communities through increased inputs to the soil (e.g. litter, root exudates; Bardgett and Walker, 2004; Bueno de Mesquita et al., 2019) or shifts in plant interactions (Alexander et al., 2016). For our plant neighborhood metrics, we used plant density, which shapes the quantity of inputs to the soil, and plant richness, which impacts the diversity of plant inputs and is indicative of the potential number of species interactions. We conducted vegetation surveys between 17 August and 4 September 2015. Across our plots, we identified all plants at the species level to estimate richness and plant density was calculated as the total number of stems per square meter across all species.

We also tested the effect of important edaphic properties on root endophyte communities by measuring dissolved organic carbon (DOC), total dissolved nitrogen (TDN), and pH on soils collected 7 – 11 September 2015. We collected three soil cores of 3 cm diameter and 4 cm depth per plot, placed them in a plastic bag, gently homogenized them, and transported them on ice to the lab by the end of the day. Soils were stored at 4˚C for a maximum of one week. Dissolved organic carbon and TDN were measured via soil extractions using 0.5M K_2SO_4 and analyzed using a

Shimadzu total organic C analyzer equipped with a TDN module (Shimadzu Scientific Instruments, Inc., USA) (Porazinska et al., 2018). Soil pH was measured with an Oakton benchtop pH meter (Oakton Instruments, USA) after the addition of 3 ml ultrapure water to 2 g of soil and shaking for 1 h at 175 rpm. In addition to those edaphic properties measured in 2015, soil texture (sand, silt, clay) of the plots was measured by the South Dakota Soil Laboratory (Brookings, South Dakota, USA) in 2008 on soils collected in September of that year (King et al., 2010). While DOC, TDN, and pH were measured in 2015 and soil texture was measured in 2008, one year and eight years before root sampling, respectively, we do not expect that the relative differences in these soil properties across plots were significantly different from those conditions at the time of root sampling because all sampling occurred within the same seasonal time frame, despite the differences in year. See Table 3.1 for the range in plant neighborhood, snow depth, and soil physical and chemical variables.

Table 3.1. The range of values for plant neighborhood, snow depth, and soil physical and chemical variables.

Variable	Unit	Range
Plant Richness	Number	3 - 21
Plant Density	Number per m^2	6 - 131
Mean Snow	cm	69.3 - 340
Sand	%	37 - 87
TDN	micrograms per gram	0.63 - 7.17
pH	pH	4.52 - 5.82

Root endophyte community sampling

To assess the root endophyte communities, we revisited these plots between 15 - 25 August 2016 and harvested individual adult perennial plants of different species (Bueno de Mesquita et al., 2018a). All plants appeared healthy and did not have visible signs of pathogenic infections or herbivory. Plants were collected during the flowering phenophase. To minimize our impact on the populations of these species, only species with more than 5 individuals in a given plot were sampled, and only one randomly selected individual was sampled. Plants were sampled to a maximum of 10 cm depth. Soil was shaken off in the field, plants were placed in plastic bags and transported to the lab on ice. All roots that were < 2 mm in diameter were selected and surface sterilized by rinsing in deionized water, soaking in 70% ethanol for 1 min, soaking in 10% bleach for 1 min, and triple rinsing with sterile deionized water. Samples were then stored in a -70˚C freezer. In the present study, we included only those species that had more than one replicate (Table 3.2), which resulted in 78 sampled plants from 50 unique plots.

Table 3.2. Plant species used in the study, as well as the family and division to which they belong, and their replicate number.

Division	Family	Species	Replicates
Monocot	Poaceae	*Trisetum spicatum*	14
		Festuca brachyphylla	22
		Deschampsia cespitosa	5
		Elymus scriberneri	5
Monocot	Cyperaceae	*Carex pyrenaica*	10
		Carex albonigra	3
		Carex phaeocephala	3
		Kobresia myosuroides	4
Monocot	Juncaceae	*Luzula spicata*	4
Dicot	Asteraceae	*Senecio fremontii*	2
Dicot	Scrophulariaceae	*Besseya alpina*	2
Dicot	Caryophyllaceae	*Silene acaulis*	2
		Stellaria umbellata	2

DNA extraction and analysis

To characterize the bacterial and fungal root endophytes, wet roots were frozen in liquid nitrogen and ground into a fine powder with a sterile mortar and pestle. Roots from each individual plant were handled separately. DNA was extracted from 0.1 g of this powder using the DNeasy Plant Extraction Kit (Qiagen, Hilden, Germany).

We used PCR to amplify the V4 hypervariable region of the 16S rRNA gene using indexed 515f and 806r primers and the first internal transcribed spacer (ITS) region using ITS1F and ITS2 primers, following standard protocols of the Earth Microbiome Project (Amaral-Zettler et al., 2009; Caporaso et al., 2012; Smith and Peay, 2014). All amplified samples were purified and normalized with the SequalPrep Normalization Kit (Invitrogen, Carlsbad, CA, USA), pooled into single 16S and ITS amplicon libraries and sequenced on a MiSeq2000 (Illumina, San

Diego, CA, USA) with pair-end 2 x 300 bp chemistry at the University of Colorado BioFrontiers Institute (Boulder, CO, USA). Data were processed using a combination of UPARSE (Edgar, 2013) and QIIME (Caporaso et al., 2010) pipelines to demultiplex reads, trim reads (230 bp) such that quality scores were > 25, remove singletons, filter chimeras, and pick operational taxonomic units (OTUs) at 97% sequence identity. Using DADA2, we assigned taxonomy with the SILVA database (Quast et al., 2013) and UNITE database (Abarenkov et al., 2010) for bacterial and fungal reads, respectively (function 'assignTaxonomy,' package *dada2*; Callahan et al., 2016). To control for differences in sequencing depth among samples, we rarefied the 16S samples to 4094 reads and the ITS samples to 5238 reads. Relative abundances were calculated by dividing the number of each OTUs' sequence reads by the total number of reads in a sample. Reads examined in downstream analyses were those with a relative abundance greater than 0.05%, thereby removing rare taxa (Oliverio et al., 2017). This was done to focus our analyses on abundant OTUs. Before filtering there were 5561 bacterial and 1555 fungal OTUs and after filtering there were 305 bacterial and 196 fungal OTUs.

Statistical analyses

To assess the relative importance of host plant phylogenetic distance, plant neighborhood, space, and abiotic drivers in shaping the bacterial and fungal root endophyte communities, we conducted a distance-based redundancy analysis (dbRDA) for both bacterial and fungal communities, followed by variation partitioning to determine which variables explained the greatest variation. To

include both spatial and phylogenetic predictors in the dbRDA, we used eigenvector mapping techniques.

To produce eigenvectors to be included in the dbRDA as spatial and phylogenetic predictors, we conducted distance-based Moran's Eigenvector Maps (dbMEM; Dray et al., 2006) and Phylogenetic Eigenvector Regression (PVR; Diniz-Filho et al., 1998), respectively. The dbMEM consists of running a principal coordinate analysis (PCoA) on a truncated Euclidean (geographic) distance matrix constructed from spatial coordinates, with diagonal values that are four times a threshold value (the shortest distance that maintains a connection between all plots [i.e. the longest edge of a minimum spanning tree]) (function 'dbmem,' package *adespatial;* Dray et al., 2020). The matrix is truncated such that distances greater than the threshold value are replaced with four times the threshold. We retained only eigenvectors that corresponded to positive autocorrelation. The PVR consists of running a PCoA on a double-centered cophenetic phylogenetic distance matrix (the pairwise distances between terminal taxa using branch lengths) ('PVRdecomp,' *PVR*; Santos, 2018). We did not have a molecular phylogeny of our plant species and thus subset our taxa from the molecular phylogeny provided by Zanne et al. (2014) (identified to species and sampled at least 3 times) using the software Phylomatic (Webb and Donoghue, 2005). Synthesis-based trees have been shown to be robust for common phylogenetic analyses (Li et al., 2019). Eigenvectors from the geographic and phylogenetic distance matrices represent spatial and phylogenetic relationships among plots and species, respectively, in vector form. The first

eigenvectors represent larger distances amongst plots or species; hence, the first spatial eigenvectors characterize broader spatial patterns and the first phylogenetic eigenvectors characterize divergences closer to the root of the phylogeny (Diniz-Filho et al., 2012; Bauman et al., 2018).

The resulting eigenvectors were subjected to a global test of significance where all eigenvectors from either the dbMEM or PVR were included in a dbRDA ('dbrda,' *vegan;* Oksanen et al., 2019) with bacterial and fungal root endophyte communities (Bray-Curtis dissimilarity matrices using square-root transformed relative abundances) as response variables; the significance of the overall model was tested and an adjusted R^2 was obtained (Blanchet et al., 2008; Bauman et al., 2018). Next, to avoid model overfitting and to enhance predictive power (Gauch, 1993; Bauman et al., 2018), forward selection with double stopping criterion was employed ('forward.sel', *adespatial*, 9,999 permutations); the two criteria are a significance level of 0.05 and the global adjusted R^2 from the aforementioned dbRDA (Blanchet et al., 2008; Bauman et al., 2018). This process resulted in a subset of eigenvectors to be used in downstream analyses, with separate subsets for the bacterial and fungal communities.

Our dbMEM resulted in a total of 12 eigenvectors (MEM; Figure A3.1), during which forward selection retained MEM 4 (the fourth eigenvector) for downstream bacterial analyses and MEM 1, 2, and 4 for downstream fungal analyses. We considered the first four eigenvectors to represent broader spatial patterns (Bauman et al., 2018). To understand the relationship between the

selected spatial variables and environmental heterogeneity, we regressed our subset of eigenvectors against several abiotic and biotic variables (mean May snow depth, soil texture [percent sand], TDN, pH, plant density, plant richness).

Our PVR resulted in a total of 12 eigenvectors (PVR); forward selection retained PVR 1 and 2 for downstream bacterial and fungal analyses. The first phylogenetic eigenvector (PVR 1) corresponded to the split between the functional groups graminoids and forbs, and the second phylogenetic eigenvector (PVR 2) corresponded to splits amongst the graminoids (i.e. between Poaceae, Cyperaceae, and Juncaceae) (Figure A3.2). We hereafter refer to the spatial and phylogenetic eigenvectors as predictors or variables for simplicity.

We ran separate dbRDA for the bacterial and fungal communities to test for the combined effects of host plant phylogenetic distance (our selected phylogenetic predictors from the PVR), plant neighborhood (plant density and plant richness), abiotic drivers (mean May snow depth, soil texture, TDN, DOC, and pH), and space (our selected spatial predictors from the dbMEM) ('dbrda,' *vegan;* Oksanen et al., 2019). The percentage of sand was correlated with both silt and clay content ($r > 0.7$), and so only sand was retained in the model. Additionally, DOC and TDN were correlated ($r = 0.8$) and thus only TDN was retained. We also sought to directly test whether plant traits, a putative driver of the effects of host plant relatedness, shaped root endophyte communities. As we do not have root traits for our species, likely to be the most important traits for root endophyte community assembly, we used leaf carbon (C), a leaf trait that has been shown to significantly shape

rhizosphere communities (Sweeney et al., 2021). Leaf C data were collected at our study site during the summers of 2017 and 2018. We collected one leaf from 7-40 separate individuals depending on the species (all individuals were greater than 1 m apart to ensure that individuals were not clones connected belowground). Leaves were oven dried at 60°C for 4 days. Approximately 10 g of dry material was then shipped to the University of Wyoming Stable Isotope Facility (Laramie, Wyoming, USA) where samples were ground with a steel ball mil and analyzed for %C by weight using a Carlo Erba 1110 Elemental Analyzer (Carlo Erba, Italy) coupled to a Thermo Delta V mass spectrometer (Thermo Fisher Scientific, USA). Species averages were calculated across replicates. Leaf C was correlated with our first phylogenetic variable (describing the split between graminoids and forbs; $r = 0.8$) and was therefore not included in the dbRDA. However, this finding supports the notion that trait variation stemming from host plant phylogenetic distances is a contributing factor to host phylogenetic effects on root endophyte communities. All continuous variables included in the dbRDA were scaled to have a mean of 0 and a standard deviation of 1. The contribution to community variation of the drivers included in the dbRDA (host phylogenetic distance, plant neighborhood, space, and abiotic drivers) was assessed by partitioning ('varpart,' *vegan*). We used a permutation test on our partial dbRDA to determine the significance of testable components ('anova.cca,' *vegan*).

Results

Spatial variables and the environment

All spatial variables were related to a subset of the environmental drivers (Table A3.1, Figure A3.3). Each spatial variable was associated with plant richness and/or density ($P < 0.05$). Two of the broadest spatial variables (MEM 1 and 2) were related to soil chemical properties (soil pH; MEM 1: $P < 0.001$; MEM 2: $P = 0.009$). Additionally, MEM 1 and 4 were explained in part by snow depth (MEM 1: $P < 0.001$; MEM 4: $P < 0.001$) while MEM 2 and 4 were related to soil physical properties (soil texture; MEM 2: $P = 0.03$; MEM 4: $P = 0.02$).

Characterization of root endophyte communities

There was a mean observed richness of 159 ± 30 (mean ± standard deviation) bacterial OTUs per sample. Three of the 16 phyla comprised 75% of the bacterial reads: Proteobacteria made up the bulk of reads (on average, 42% of reads), followed by Actinobacteriota (17%), and Bacteroidota (16%). Alphaproteobacteria and Gammaproteobacteria made up 63% and 37% of Proteobacteria reads, respectively. The class Actinobacteria made up, on average, 92% of the reads in the phylum Actinobacteriota. The class Bacteroidia composed all Bacteroidetes reads.

There was a mean observed richness of 27 ± 8 fungal OTUs per sample. At the phylum level, Ascomycota (on average, 82% of reads) and Basidiomycota (14% of reads) were the dominant fungal phyla. Agaricomycetes made up 96% of Basidiomycota reads while Leotiomycetes and Dothideomycetes made up, on average, 69% and 24% of Ascomycota, respectively. Despite demonstrated root

colonization of these plants, only 1.3% of reads belonged to Glomeromycota (AMF; Bueno de Mesquita et al., 2018a) and this low percentage was not due to the filtering of rare taxa (1.8% of reads when all taxa were considered).

Effects of host phylogenetic distance, plant neighborhood, space, and abiotic drivers on root endophyte communities

All four types of variables analyzed– host phylogenetic distance, plant neighborhood, space, and abiotic effects– significantly influenced both bacterial and fungal endosphere composition (Table 3.3, Figure 3.2). However, together these variables explained only 14% of variation in the bacterial and 11% in fungal root endophyte communities (Figure 3.3).

Table 3.3. Effects of the phylogenetic, plant neighborhood, abiotic, and spatial predictors from the dbRDA. Bolded values highlight significant effects ($P < 0.05$). The first and second spatial variables were not selected for the bacterial community and their absence is represented by a dash. PVR, phylogenetic eigenvectors from Phylogenetic Eigenvector Regression; TDN, total dissolved nitrogen; MEM, spatial eigenvectors from Moran's Eigenvector Maps

	Bacterial Community			Fungal Community		
Predictor Variable	df	F-value	P-value	df	F-value	P-value
Phylogenetic						
PVR 1	1	2.4	**0.004**	1	3.1	**<0.001**
PVR 2	1	3.4	**<0.001**	1	2.4	**<0.001**
Plant neighborhood						
Plant Richness	1	2.6	**0.001**	1	2.3	**<0.001**
Plant Density	1	2.6	**0.001**	1	1.8	**0.004**
Abiotic						
Mean Snow	1	4.4	**<0.001**	1	3.0	**<0.001**
Sand (%)	1	1.4	0.09	1	1.1	0.27
TDN	1	1.0	0.43	1	1.1	0.28
pH	1	2.2	**0.006**	1	1.1	0.34
Spatial						
MEM 1	-	-	-	1	1.8	**0.006**
MEM 2	-	-	-	1	1.9	**0.003**
MEM 4	1	1.9	**0.02**	1	1.3	**0.12**
Residual	68			66		

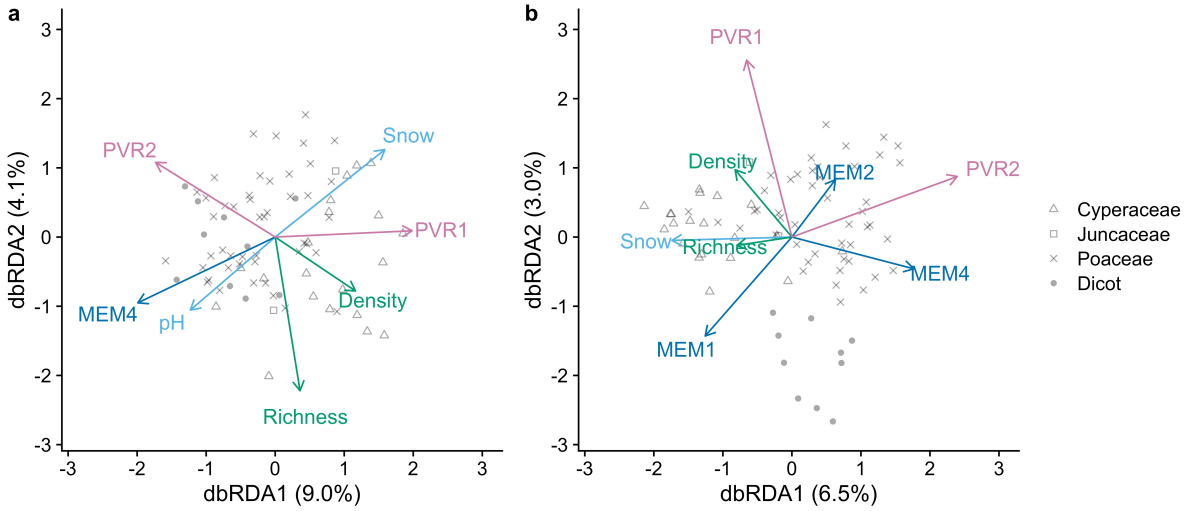

Figure 3.2. dbRDA displaying (a) the bacterial and (b) fungal root endophyte communities and the significant drivers (vectors). The shape of the points on the figure delineate monocots and dicots (relating to PVR 1), where monocots are separated out by family (relating to PVR 2). Only statistically significant variables from the dbRDA are shown. PVR, phylogenetic eigenvectors from Phylogenetic Eigenvector Regression; MEM, spatial eigenvectors from Moran's Eigenvector Maps

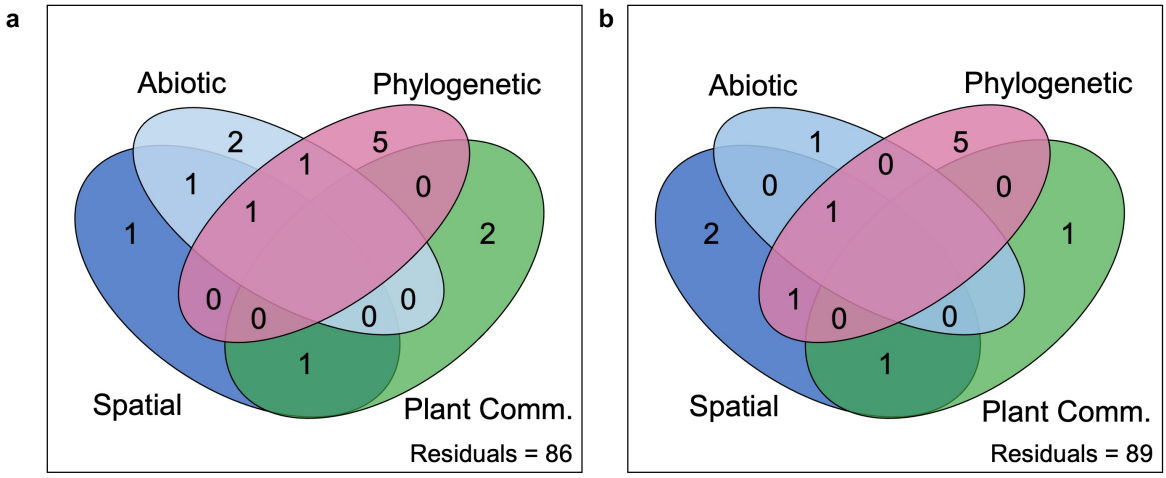

Figure 3.3. Venn diagram displaying the contributions of host phylogeny, plant neighborhood, space, and abiotic predictors in shaping (a) the bacterial and (b) fungal root endophyte communities. The numbers inside the circles indicate the percentage of explained variation and blank spaces indicate values less than zero. All four groups of predictors made a unique and significant contribution ($P < 0.05$).

The unique contribution of host plant phylogeny explained the largest amount of variation for bacterial (5%) and fungal (5%) root endophyte communities.

We found that the separation between functional groups (i.e. graminoids and forbs; bacteria: $F_{1,68} = 2.4$, $P = 0.004$; fungi: $F_{1,66} = 3.1$, $P < 0.001$) as well as differences within graminoids shaped root endophyte communities, where microbial communities in host families Cyperaceae and Juncaceae were more similar to each other than to Poaceae (bacteria: $F_{1,68} = 3.4$, $P < 0.001$; fungi: $F_{1,66} = 2.4$, $P < 0.001$).

The unique contributions of abiotic (2%) and plant neighborhood variables (2%) were the second most explanatory variables for bacterial communities while spatial variables explained the second largest amount of variation in fungal communities (2%). Mean May snow depth was the only abiotic driver that explained variation in both the bacterial ($F_{1,68} = 4.4$, $P < 0.001$) and fungal root endophyte communities ($F_{1,66} = 3.0$, $P < 0.001$). There was an additional effect of soil pH on bacterial communities ($F_{1,68} = 2.2$, $P = 0.006$) but not on fungal communities. All spatial variables included in the models for both bacteria and fungi were significant (Table 3.3). In terms of plant neighborhood effects, plant richness (bacteria: $F_{1,68} = 2.6$, $P = 0.001$; fungi: $F_{1,66} = 2.3$, $P < 0.001$) and plant density (bacteria: $F_{1,68} = 2.6$, $P = 0.001$; fungi: $F_{1,68} = 1.8$, $P = 0.004$) shaped both microbial communities. The adjusted R^2 of shared variation amongst each fraction of variation (phylogenetic, plant neighborhood, spatial, and abiotic) ranged from being negative to 1, indicating little overlap. Permutation tests revealed that each fraction of variation was significant ($P < 0.05$).

Discussion

Understanding the drivers of root endophyte communities is important because of the role these microbial communities play in plant health, and the potential for this community to be altered by global change. We found broad similarities in the factors that shaped the bacterial and fungal root endophyte communities, and that both community types had the greatest amount of variation explained by host plant phylogeny. However, there were only small differences in the amount of variation explained by host phylogeny, plant neighborhood, space, and abiotic effects. Importantly, the total variation explained for both bacterial and fungal root endophyte communities was low, particularly for fungal root endophytes. This low amount of explained variation has been seen before (Queloz et al., 2011; Morris et al., 2013; Glynou et al., 2016; Francioli et al., 2021), and may suggest a central role of stochasticity in determining both bacterial and fungal root endophyte communities.

Community assembly is a balance of deterministic, such as environmental filtering, and stochastic processes, such as drift and priority effects (order of arrival) (Vellend, 2010; Nemergut et al., 2013). It is well known that deterministic processes influence microbial communities, including root endophyte communities (e.g. Schlaeppi *et al.* 2014). Less studied is the role of stochasticity, but work over the past decade has highlighted its importance for microbial community assembly (Caruso et al., 2011; Dini-Andreote et al., 2015; Debray et al., 2021). While not explicitly addressed in our study, the low explained variation in both our bacterial

and fungal root endophyte communities suggests stochasticity is at play. Below we discuss what variation was explained by the drivers we studied and focus on differences detected between bacteria and fungi.

In line with H1, host phylogeny, characterized as variation across functional group (i.e. graminoids, forbs) and within graminoids, explained the greatest amount of variation in both bacterial and fungal root endophyte communities. Interestingly, the same phylogenetic scales were selected for both bacterial and fungal root endophytes, indicating that host phylogeny acted at a similar, coarse scale for both community types. Other work has previously described differences in root endophyte composition across graminoids and forbs (Mommer et al., 2018; Francioli et al., 2020, 2021; Wang and Sugiyama, 2020), where root chemical properties were shown to be an important driver of endophyte communities (Francioli et al., 2021). Other possible drivers include exudate quality and quantity as well as morphological traits, which differ between and within graminoids and forbs (Buttler et al., 2011; Fitzpatrick et al., 2018; Dietz et al., 2020; Williams et al., 2021). Though we were unable to test associations with root traits, we found that leaf C (a trait shown to influence rhizosphere assembly (Sweeney et al., 2021)), was correlated with the phylogenetic variable describing the split between graminoids and forbs. This finding lends support to the notion that broad morphological differences between these divisions may explain differences in their root endophyte communities, though a clear causative link between leaf C and endophytes is beyond the scope of this project. While host phylogeny explained the greatest

amount of variation in both bacterial and fungal root endophyte communities, the variation explained was low indicating that, despite morphological differences between and within functional groups, phylogeny offered little explanatory power.

In partial support of H2, abiotic variables explained a greater amount of variation in bacterial compared to fungal root endophyte communities but the difference in explained variation was low (one percentage point). Previous studies have found edaphic properties, including soil sampling location, resource availability, and pH to be drivers of the bacterial root endophyte communities (Schlaeppi et al., 2014; Chen et al., 2017). Partially in line with that work, soil pH shaped bacterial root endophyte communities, but there was no effect of soil texture or TDN. Soil pH has been shown to be a strong driver of soil bacterial communities (Lauber et al., 2009; Delgado-Baquerizo et al., 2018), suggesting that the effect of soil pH on root endophyte communities may be through its effects on soil microbial communities which can serve as a source community for the root endosphere. Alternatively, soil pH can influence root architecture and therefore shape endosphere membership in this way (Haling et al., 2011). Together, our findings demonstrate an effect of pH in shaping bacterial root endophyte communities (but not fungal root endophyte communities), either through effects on the host plant or through the pool of available colonizers, but to a limited extent.

Snow depth was the sole abiotic driver that shaped both the bacterial and fungal communities. Declines in snowpack, a contributing factor to the uphill movement of alpine plants, can alter plant processes via effects on growing season

timing and length. The effect of snow depth may manifest through its influence on plant and microbial composition (Zinger et al., 2009; Niittynen et al., 2020b), plant processes such as reproduction (Wipf et al., 2009), and biogeochemical cycles (Lipson et al., 1999). Our finding is in line with a study on the biogeography of root endophytic fungi, which similarly found environmental effects driving the distribution of root endophyte communities, including temperature and precipitation metrics (Glynou et al., 2016). These findings suggest that root endophyte communities could be altered under global change via shifts in snowpack, but the effects may be weak, as the amount of variation explained by snowpack was low.

In partial agreement with H3, we found that space explained greater variation in fungal than bacterial root endophyte communities though the difference in explained variation was low (one percentage point). Space, which may act as a proxy variable for multiple types of drivers (including biotic and abiotic) has previously been found to explain variation in both bacterial and fungal root endophyte communities (Glynou et al., 2016; Zhang et al., 2018; Wang et al., 2019) and for bulk soil bacterial communities at our site (King et al., 2010). The spatial extent of our study was small (~3 km^2) compared to studies which encompass large swaths of a country (2.3 million km^2; Wang *et al.* 2019), which may clarify why space explained less variation than has been previously seen. There were more spatial variables selected for fungal endophytes, all representing broad scales, and they explained more variation than for bacterial endophytes. This may be a result of

differences in dispersal capabilities between fungi and bacteria (Schmidt et al., 2014; Li et al., 2020), where fungi are typically more dispersal limited due to their larger size. These findings suggest a role, though small, for spatially structured processes in shaping root endophyte communities, particularly fungal.

In contrast to H3, plant neighborhood explained more variation in bacterial than fungal root endophyte communities. The difference in explained variation, though, was one percentage point. In mountainous ecosystems where climate change is driving alpine plants to move uphill into previously unvegetated regions (Chen et al., 2011; Bueno de Mesquita et al., 2018b), variation in plant density and composition may affect root endophyte communities. While plant richness and density had a significant effect on both the bacterial and fungal communities, these drivers explained more variation in bacterial root endophyte communities. This finding was unexpected, as previous research has demonstrated a stronger influence of plant variables (e.g. biomass, composition, richness) on fungal rather than bacterial root endophyte communities (Dean et al., 2014, 2015) and on soil fungal compared to soil bacterial microbial communities (Sugiyama et al., 2008; Fanin et al., 2019; Chen et al., 2020). Soil microbial communities are a likely intermediary of plant community effects on root endophyte communities; shifts in soil microbial communities occur with alterations in plant density and composition (Knelman et al., 2012; Porazinska et al., 2018; Bueno de Mesquita et al., 2019), with the effects of plants occurring via litter inputs and root exudates (Bardgett and Walker, 2004; Bueno de Mesquita et al., 2019). These shifts in soil microbial

communities could feed back to alter root exudate patterns (Badri and Vivanco, 2009), and hence root endophyte communities. It is also possible that plant density and richness could influence root endophyte communities through plant competitive dynamics, which could then shape root endophyte communities via plant-soil feedbacks (Fitzpatrick et al., 2018). Finally, plant presence shapes the quality of the soil, including the texture and nutrient availability (Bueno de Mesquita et al., 2017), which could alter the relationship between plants and their root endophytes (Yeoh et al., 2017; Xu et al., 2020). However, there was no effect of soil texture or nutrient availability on bacterial or fungal root endophyte communities, suggesting this was not the pathway through which plant density and richness acted in our study. These findings suggest that the uphill movement of plants due to climate change may have a stronger effect on bacterial than fungal root endophytes, but that the overall effect could be low.

Our study provides evidence of the greater relative importance of host plant relatedness compared to space, plant neighborhood, and abiotic drivers in shaping root endophyte communities of a diverse set of plants in a natural setting, but also highlights the little overall explained variation in both bacterial and fungal root endophyte communities in our system. Abiotic drivers explained little variation in root endophyte communities, with no effect of either soil nutrients or soil texture. We detected effects of snow depth and plant richness and density, highlighting potential pathways through which global change may impact root endophyte communities, though the effects may be weak. Bacterial and fungal endophytes

were shaped by a similar suite of drivers, but abiotic drivers were less important for bacterial endophytes than expected and the plant neighborhood was less important for fungal endophytes than expected. Together, these findings demonstrate the weak role played by deterministic drivers in shaping root endophyte communities of alpine plants, the similarity in driver effects on bacterial and fungal root endophytes, and potential effects of global change on root endophytes.

CHAPTER IV

HOW DOES TOPOGRAPHY SHAPE A HABITAT-FORMING SPECIES' INFLUENCE ON DIVERSITY PATTERNS?

By Laurel M. Brigham, Marko J. Spasojevic, and Katharine N. Suding

Abstract

It is well known that environmental heterogeneity has implications for diversity across scales. While environmental heterogeneity is commonly addressed from an abiotic perspective, such as the heterogeneity created by topography, less is known about how abiotic sources of heterogeneity could interact with biotic sources of heterogeneity (e.g. habitat-forming species) to shape diversity patterns. Here we studied whether a habitat-forming species in the alpine, a shrub, responded morphologically to abiotic context (aspect) and whether there were consequences for the microclimate and plant diversity. We found that shrubs grew taller on the warmer, south (Equator)-facing aspect and consequently had stronger effects on minimum temperatures, but that other microclimate characteristics were not altered by aspect or shrub height. While shrubs increased plot-level richness, the difference in richness between paired and open plots did not differ by aspect. On the other hand, the difference in plant composition between paired and open plots was greater on the S-facing aspect where shrubs were taller. These findings indicate that abiotic context had a somewhat minimal effect on the microclimate and diversity patterns fostered by shrubs, suggesting predictable effects of our focal

shrub across the landscape, apart from beta diversity which was enhanced with shrub height.

Introduction

Environmental heterogeneity can drive diversity patterns across a landscape via differentiation of niche space (Chesson, 2000) and opportunities for protection from extreme climate events (Kindvall, 1995). However, most research has primarily focused on abiotic sources of environmental variation and there has been less of a focus on biotic sources of heterogeneity (such as habitat-forming species) and how abiotic and biotic heterogeneity might interact. Habitat-forming species create a three-dimensional biogenic structure that alters the physical environment and can alter resource (e.g. light and water availability) and non-resource stressors (e.g. temperature) (Jones et al., 1994, 1997; Stachowicz, 2001; Ellison et al., 2005). For example, cushion plants have a prostrate, mat-forming morphology which can buffer temperatures and maintain moister soils for plants growing within the cushion compared to conspecifics growing in an adjacent open microsite (Cavieres et al., 2007). Habitat-forming species, by modifying resource and non-resource stressors to which associated taxa respond, should alter diversity across a landscape. If the conditions created within the habitat-forming species patch facilitate a larger number of species, this could enhance patch- or plot-level diversity (Molenda et al., 2012; Ballantyne and Pickering, 2015; Gavini et al., 2020). Additionally, the presence of patches which offer variations in resource and non-

resource stressors could enhance dissimilarities in community composition across a landscape (beta diversity) as habitat-forming species foster species not found in unprotected areas (Wright et al., 2006; Cavieres et al., 2014, 2016; Gavini et al., 2020).

Despite decades of research on habitat-forming species (e.g. nurse plants (Bertness and Callaway, 1994; Stachowicz, 2001; Michalet and Pugnaire, 2016)), less is known about how the abiotic context (e.g. latitude, aspect, elevation, and distance to a body of water) may mediate the impact of habitat-forming species on biodiversity (Jones et al., 2010). By altering the morphology of the habitat-forming species (e.g. height, canopy area, branch density), abiotic factors may alter the effect of habitat-forming species on resource and non-resource stressors and thus diversity. Morphological alterations can occur when the abiotic context shifts optimal growing conditions for the habitat-forming species. For example, a cushion plant (*Arenaria tetraquetra*) in the Sierra Nevada Mountains was larger at higher elevations where it showed enhanced physiological performance compared to lower elevations (Schöb et al., 2013). As a result, the cushion plants at higher elevations increased soil moisture to a greater degree and demonstrated the greatest increase in plant species richness compared to paired open locations (Schöb et al., 2013). Consequently, shifts in the morphology of the habitat-forming species can alter the effects of the habitat-forming species on resource availability and on associated taxa (Jones et al., 2010; Schöb et al., 2013; Bulleri et al., 2016).

Here we ask how habitat-forming shrubs in a temperate montane environment influence diversity of associated plant communities and if that effect is mediated by aspect. Shrubs act as habitat-forming species by altering nutrient and microclimate conditions through litter inputs (Sturm et al., 2005; Brantley and Young, 2010; DeMarco et al., 2014), accumulating snow in the winter thereby increasing early season soil moisture (Liston et al. 2002), and by providing shade and wind protection for plants growing on their lee (Carlsson & Callaghan, 2009). Moreover, shrubs alter both growing season and winter temperatures, buffering growing season temperatures and reducing freeze events (Myers-Smith 2013). Aspect is an important topographical feature which shapes climatic conditions whereby poleward-facing aspects (north, in our study) receive less incoming solar radiation and are therefore cooler and moister (Böhner and Antonić, 2009). Overarching climatic differences between north (N)-facing and south (S)-facing aspects could alter shrub morphology, and thus modify shrub capacity to shift local environmental conditions.

In this study, we investigated the potential interactive effects of aspect (abiotic context), and shrubs (habitat-forming species) on the microclimate and associated plant communities in the alpine tundra. We predict that shrubs will grow larger on the S-facing aspect because shrubs in the tundra are typically limited by cooler temperatures (Myers-Smith et al., 2011; Elmendorf et al., 2012), and that this will increase the capacity of shrubs to alter the microclimate and soil environment on this aspect. Hence, there will be greater differences in resource and

non-resource stressors between open and shrub plots on the S-facing aspect which will intensify the effects of shrubs on plant diversity on the S-facing aspect compared to the N-facing aspect.

Methods

Study Design.

We conducted our study at Niwot Ridge in the Front Range of the Rocky Mountains, Colorado, USA (40.05˚N, -105.59˚W). Niwot Ridge has a short growing season (1- 3 months) with a mean annual temperature of -0.6°C (9.8°C in the growing season) (Jennings et al., 2021) and an average annual precipitation of 1000 mm, with the majority of the precipitation (80%) falling as snow and much of it being redistributed by westerly winds (Litaor et al., 2008). Annual daily wind speeds average 8.5 m s^{-1}, with an average annual daily maximum wind speed of 20.2 m s^{-1} (Morse et al., 2022).

To select the shrubs used in this study, we used a 2 m resolution LiDAR-based digital elevation model to partition our study area into N-facing (aspect less than 67.5° and more than 292.5°) and S-facing (aspect greater than 112.5° and less than 247.5°) aspect layers within QGIS v. 2.18 (QGIS Development Team, 2015). We then applied 300 random points to each aspect and, using overlaid satellite imagery from Google Maps, we noted all points that indicated a shrub. We randomly selected 27 of the points on each aspect that indicated shrub presence and visited these coordinates in the field using a Trimble GeoXT 3000 (Trimble

Navigation Ltd., Sunnyvale, CA), which has sub-meter accuracy. We established a 0.5 x 0.5 m plot on the leeward side of the shrub (*Salix planifolia*) and a paired plot outside of the influence of shrubs around one meter away and in line with the shrub plot. If the coordinates from QGIS did not result in a shrub (misclassification error), we established a plot at the nearest shrub. We ensured that these new shrub locations were within our aspect parameters by taking their coordinates and mapping them in QGIS. This process resulted in 108 plots across a 0.5 km^2 area (2 aspects x 2 plot types x 27 replicates) with the farthest pairs of plots 1200 m away and the closest 5 m away. There was only an 83 m range in elevation across all plots and therefore effects of shrubs and aspect are likely due to those primary drivers rather than elevational differences.

Shrub and plant community data.

To determine whether aspect impacted shrub morphology, we measured the dimensions of each shrub on the lee of which we established plots. The tallest point of each shrub was measured during peak biomass. The maximum width was measured and a secondary width perpendicular to the first was also taken. Across both aspects in our study location, shrubs have been present since at least the 1930s (first high-resolution orthorectified imagery captured at Niwot Ridge) but have experienced infilling in the years since (Figure A4.1). Hence, differences in size are most likely due to differences in growth capacity resulting from abiotic differences across aspect rather than differences in age.

Plant community composition data were collected between 23 July and 7 August 2019 during peak biomass using the point intercept method where all hits were recorded (all species and ground cover classes which touched the intercepting sampling pin). Hits were recorded at 10 cm intervals, resulting in 25 sampling points across each 0.25 m^2 plot. Species that were present in the plot and not hit were assigned a value of 0.5. Before calculating plant community response to shrub presence and aspect, non-vascular plants as well as rocks and litter were removed from the dataset, abundances were relativized, and plant species present in fewer than two plots were removed. Before this filtering there were 71 plant species.

Microclimate data.

We collected soil moisture data twice monthly and continuous soil temperature throughout the 2019 growing season. Between 9 July and 26 August 2019, soil moisture was collected every other week across all plots at a depth of 7.6 cm using the Fieldscout TDR 150 (Spectrum Technologies, Aurora, IL, USA). In addition to average soil moisture across the season, we focus on the first soil moisture measure of the season (9 July 2019), which we refer to as early season soil moisture. This first time point represents soil moisture following the meltout of all plots and incorporates the effect of shrubs on soil moisture as a result of snow accumulation on their lee. iButtons (DS1921G-F5; Maxim Integrated, San Jose, CA, USA) were used to measure soil temperature *in situ* and were buried at a 3 cm depth in the top right corner of plots. To waterproof the iButtons, we sealed them in small, plastic vacuum seal bags and mason's line was tied around the bag to aid in

retrieval. During the summer, iButtons were in the ground between 13 July 2019 and 12 September 2019, logging temperatures every 65 minutes. iButtons in the ground during the summer were used to calculate 95th percentile maximum and 5th percentile minimum soil temperatures (Ashcroft et al., 2012). iButtons were then redeployed on 20 September 2019 for the winter season and collected on 16 June 2020, logging temperatures every 205 minutes and rolling over if the memory became full. We calculated the number of freezing degree days (FDD) and thawing degree days (TDD) using the full range of dates. We calculated FDD as the absolute value of the sum of mean daily temperatures below 0°C and TDD as the sum of mean daily temperatures greater than 0°C across all 330 days of iButton data. Following the summer collection of iButtons, 98 out of 108 iButtons could be located and following the winter collection of iButtons, 94 out of 108 iButtons could be located. There was an overlap of 84 iButtons across which all 330 days were logged, and solely these iButtons were used to measure our cumulative temperature variables: FDD and TDD. Median values were imputed where missing values were present. This was an important step because iButton loss appeared non-random; more iButtons were missing on the N-facing aspect, potentially due to more active marmots in the area (L. Brigham pers. obs.).

Soil collection.

To measure non-climatic effects of shrubs and aspect which may influence plant community composition, we measured total nitrogen (TN) and soil organic matter (OM). On 18 August 2020, we obtained three soil cores of 3 cm diameter and

4 cm depth from each plot, placed them into a plastic bag, and gently homogenized them. Soils were kept on ice and brought to the lab within six hours. In the lab, soils were sieved using a 2 mm sieve, allowed to air dry, and then stored. On 21 June 2021, 10 g of soil was put into an aluminum weigh boat and placed in the drying oven at 100°C for 12 hours. We added 2.5 – 3 g of the oven dry soil to a crucible and placed these crucibles in a 550°C muffle furnace for four hours. We calculated OM with the following equation: (weight of oven dry soil – weight of soil after ignition)/weight of oven dry soil. For TN, air dried samples were crushed with a mortar and pestle and then analyzed in an automated element analyzer at the Research Analytical Laboratory, University of Minnesota in July 2021 (Vario MAX, Elementar, Hanau, Germany).

Statistical analysis.

To assess effects of aspect on shrub morphology, we tested for differences in shrub area and height. To calculate area of the shrub, we assumed the shape of an ellipse and used the following equation: $\pi \times (\text{minor axis} \times 0.5) \times (\text{major axis} \times 0.5)$. We tested for effects of aspect on shrub area and shrub height by running two linear models with aspect as a predictor (function 'lm,' package *stats*). Only shrub height was changed by aspect and shrub height alone was used in downstream analyses.

To determine how aspect and shrub presence interacted to shape the microclimate and soil environment we ran linear models. When required, variables were transformed to meet assumptions of normality and homogeneity of

variance. Pairwise treatment comparisons were assessed via Tukey's honest significant difference (function 'emmeans', package *emmeans*; Lenth et al., 2018). We quantified the difference in microclimate variables that were altered by shrub presence (shrub - open) and tested whether these differences differed by aspect using linear regressions. Where there was a significant difference in the microclimate or soil variables, we determined whether this difference was related to shrub morphology using a linear model with height as a predictor and the value of the environmental variable in the open area as a covariate. These covariates accounted for an effect of aspect on changes in the difference between shrub and open plots that resulted from aspect effects on the open area and were thus not related to shrub morphology.

We then tested for the effects of aspect, shrub presence, and their interaction on plant community metrics. We ran a linear regression on plot-level richness. We ran a distance-based redundancy analysis (dbRDA) on a Bray-Curtis dissimilarity matrix calculated on square-root transformed relative abundances (function 'dbrda,' package *vegan*). We used similarity percentage analysis (function 'simper, package *vegan*) to determine the identity of species which most contributed to dissimilarity among plots on the N- and S-facing aspect. Additionally, we determined species associations with shrub or open plots across both aspects using an indicator species analysis (function 'multipatt', package *indicspecies*; 999 permutations) (De Cáceres and Legendre, 2009).

Next, we tested for differences in shrub effects on richness and beta diversity across aspects. We found the difference in richness between shrub and open paired plots and ran a linear regression to test for an effect of aspect. We also calculated the compositional difference as the Bray-Curtis dissimilarity between paired plots and used this compositional difference as the response in a linear regression to test for an effect of aspect and in a linear regression to test for an effect of shrub morphology. All statistical analyses and visualizations were performed in R ver. 4.1.1 (R Core Team, 2020).

Results

Shrub morphology

Shrub height ranged from 22 to 84 cm with an average value of 53 ± 2 cm (mean ± standard deviation) while shrub area ranged from 1.3 to 9.0 m^2 with a mean of 3.8 ± 0.2 m^2. Shrubs were an average of 16 cm taller on the S-facing aspect (Figure 4.1; $F_{1,52} = 17.94$, $P < 0.001$). Shrub area did not differ across aspects.

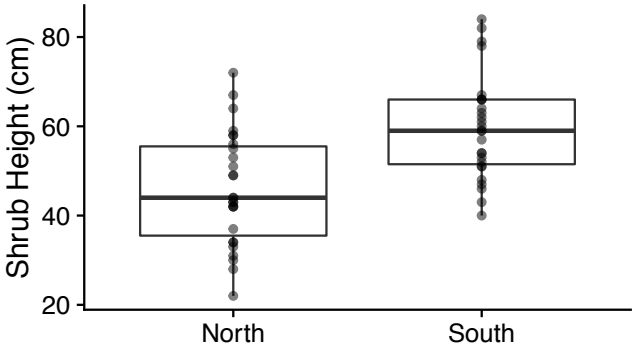

Figure 4.1. Shrubs were taller on the S-facing aspect.

Microclimate and soil environment

Aspect strongly affected the microclimate and soil fertility (Table 4.1). Both OM ($F_{1,104}$ = 46.3, P < 0.001) and TN ($F_{1,104}$ = 39.7, P < 0.001) were significantly higher on the N-facing aspect (Figure 4.2). Early season soil moisture was 70% higher on N-facing aspect (Figure 4.3a; $F_{1,104}$ = 46.4, P < 0.001), and average soil moisture was also higher on this aspect (Figure 4.3b; $F_{1,104}$ = 60.8, P < 0.001). Mean (Figure 4.4b; $F_{1,104}$ = 124, P < 0.001) and maximum temperatures (Figure 4.4c; $F_{1,104}$ = 34.0, P < 0.001) were higher on the S-facing aspect. There were 8.8% fewer FDD (Figure 4.4d; $F_{1,104}$ = 5.12, P = 0.03) and 20% more TDD on the S-facing aspect (Figure 4.4e; $F_{1,104}$ = 53.9, P < 0.001).

Table 4.1. The range of environmental variables across aspects and plot types (mean ± SE), where soils for TN and OM were collected at the end of the 2020 growing season and temperature and soil moisture data were collected across the 2019 growing season.

Variable	Units	North		South	
		Open	Shrub	Open	Shrub
Organic matter	%	34.2 ± 1.5	35.9 ± 1.5	20.9 ± 1.4	19.0 ± 1.2
Total nitrogen	%	1.2 ± 0.05	1.2 ± 0.1	0.8 ± 0.1	0.7 ± 0.04
Early soil moisture	%	34.1 ± 1.8	39.4 ± 1.6	21.1 ± 1.4	22.2 ± 1.3
Mean soil moisture	%	23.0 ± 1.0	24.9 ± 1.0	15.9 ± 0.6	15.5 ± 0.6
FDD	℃ days	985 ± 28.6	849 ± 27.0	902 ± 22.1	770 ± 25.9
TDD	℃ days	988 ± 25.3	912 ± 26.9	1220 ± 21.8	1140 ± 14.2
Mean temperature	℃	10.6 ± 0.2	10.4 ± 0.2	13.5 ± 0.1	12.7 ± 0.2
Max. temperature	℃	23.8 ± 0.9	22.9 ± 0.9	32.1 ± 0.8	28.2 ± 1.4
Min. temperature	℃	3.1 ± 0.1	3.7 ± 0.2	4.1 ± 0.2	5.5 ± 0.2

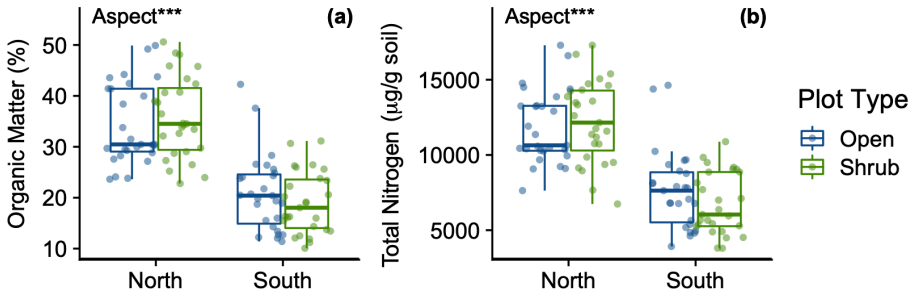

Figure 4.2. Organic matter (a) and TN (b) were higher on the N-facing aspect. Significance codes: "***" P < 0.001, "**" 0.001 ≤ P < 0.01, and "*" 0.01 ≤ P < 0.05.

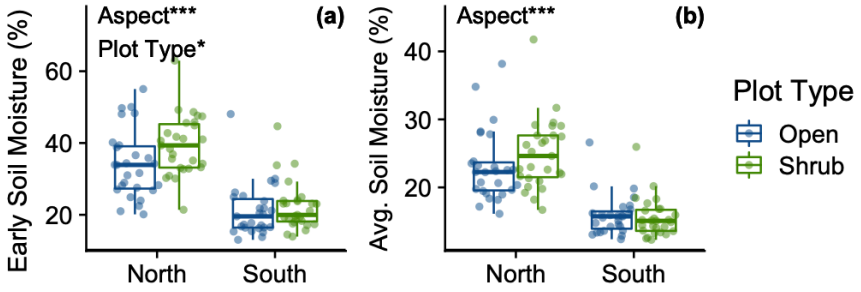

Figure 4.3. Early season soil moisture was greater on the N-facing aspect and in shrub plots (a) while average season soil moisture was only greater on the N-facing aspect (b). Significance codes: "***" P < 0.001, "**" 0.001 ≤ P < 0.01, and "*" 0.01 ≤ P < 0.05.

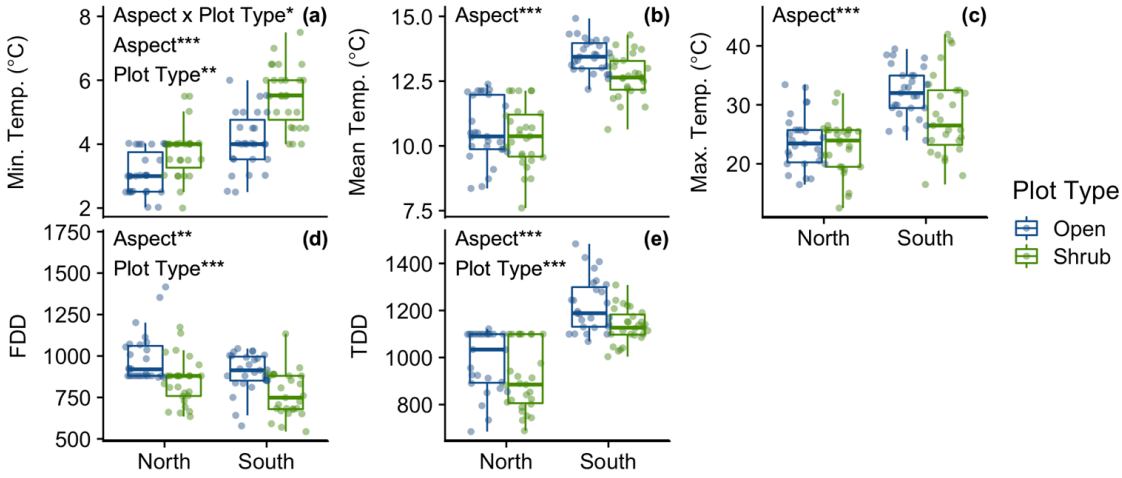

Figure 4.4. Minimum growing season temperatures were higher in shrub plots on the S-facing aspect (a). Growing season mean (b) and maximum temperatures (c) were higher on the S-facing aspect. There were fewer FDD (d) and TDD (e) in shrub plots and on the S-facing aspect. Significance codes: "***" P < 0.001, "**" 0.001 ≤ P < 0.01, and "*" 0.01 ≤ P < 0.05.

Though generally weaker than the effects of aspect, microclimates differed due to shrubs (Table 4.1). Soil moisture was 12% higher in shrub plots early in the growing season, suggesting residual moisture from delayed snowmelt (Figure 4.3a; $F_{1,104} = 4.95$, $P = 0.03$). Shrubs decreased FDD by 14% (Figure 4.4d; $F_{1,104} = 13.6$, $P < 0.001$) and TDD by 7.1% (Figure 4.4e; $F_{1,104} = 5.64$, $P = 0.02$).

There was an interaction between shrub presence and aspect on minimum temperature (Figure 4.4a; $F_{1,104} = 5.54$, $P = 0.02$). Plots on the S-facing aspect and plots on the lee of shrubs had a warmer minimum temperature (Tukey: $P < 0.05$), but shrub presence more strongly increased minimum temperatures on the S-facing aspect compared to the N-facing aspect (Tukey: $P < 0.05$).

In addition to comparing aspect and shrub effects on environmental means, we determined whether aspect influenced the difference in environmental characteristics between paired shrub and open plots. The difference between paired plots demonstrates shrub effects relative to a nearby open tundra reference and was little affected by aspect. There were only effects of aspect on the difference in minimum temperature and early season soil moisture between paired shrub and open plots. The difference in minimum temperature between shrub and open plots was greater on the S-facing aspect (Figure 4.5a; $F_{1,52} = 6.6$, $P = 0.01$) and increased with shrub height (Figure 4.5b; $R^2 = 0.19$, $P = 0.007$). The difference in soil moisture between shrub and open plots it was smaller on the S-facing aspect (Figure 4.5c; $F_{1,52} = 6.4$, $P = 0.01$) and was not shaped by shrub height.

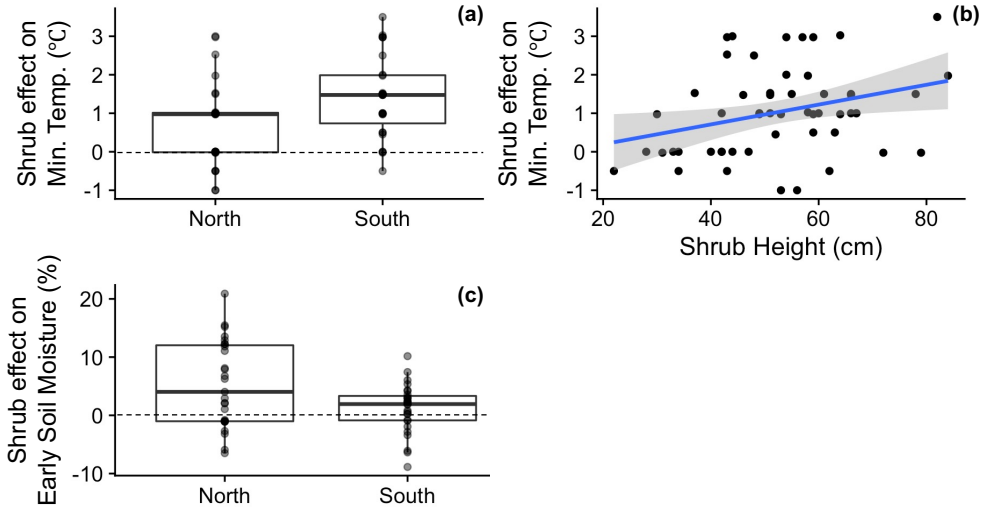

Figure 4.5. The differences between shrub and open paired plots where positive values above the dotted zero line indicate a larger value in a shrub plot compared to an open plot. The difference in minimum temperatures between shrub and open areas was greater on the S-facing aspect (a) and was positively related to shrub height (b). The difference in early season soil moisture between shrub and open areas was greater on the N-facing aspect (c).

Diversity

A total of 57 vascular plant species were detected across both aspects and plot types after the removal of rare species. Rarefied plot-level richness was greater on the S-facing aspect (Figure 4.6; $F_{1,104} = 13.7$, $P < 0.001$) and in shrub plots (Figure 4.6; $F_{1,104} = 5.2$, $P = 0.02$). On the N-facing aspect we detected one species that preferentially associated with shrubs (*Cerastium arvense*; $P = 0.003$) and on the S-facing aspect we detected four (*Sedum lanceolatum*, *Solidago simplex*, *Antennaria media*, and *Poa alpina*; $P < 0.05$). The difference in rarefied plot-level richness between shrub and open paired plots did not differ across aspects.

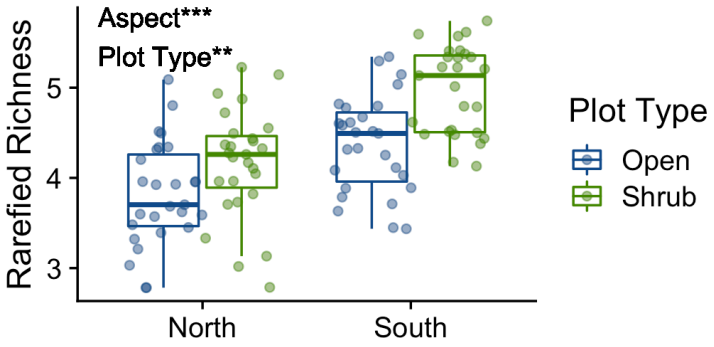

Figure 4.6. Plot-level richness was greater on the S-facing aspect and in shrub plots. Significance codes: "***" $P < 0.001$, "**" $0.001 \leq P < 0.01$, and "*" $0.01 \leq P < 0.05$.

There was an interaction between shrub presence and aspect on plant community composition (Figure 4.7; $F_{1,104} = 2.4$, $P = 0.02$), such that aspect drove a primary separation of community composition which was then differentially altered by shrub presence. The most abundant species per aspect were *Kobresia myosuroides* with an average relative abundance of 27% on the N-facing aspect and *Carex rupestris* with an average relative abundance of 20% on the S-facing aspect. Accordingly, differences in abundance between shrub and open plots were driven by *K. myosuroides* (22% of the variation between plots) on the N-facing aspect and by *C. rupestris* (14% of the variation between plots) on the S-facing aspect. The compositional difference between paired shrub and open plots was greater on the S-facing aspect (average Bray-Curtis distance: 0.51) compared to the N-facing aspect (average Bray-Curtis distance: 0.42) (Figure 4.8a; $F_{1,52} = 5.1$, $P = 0.03$), and compositional difference increased with shrub height (Figure 4.8b; $R^2 = 0.14$, $P = 0.005$).

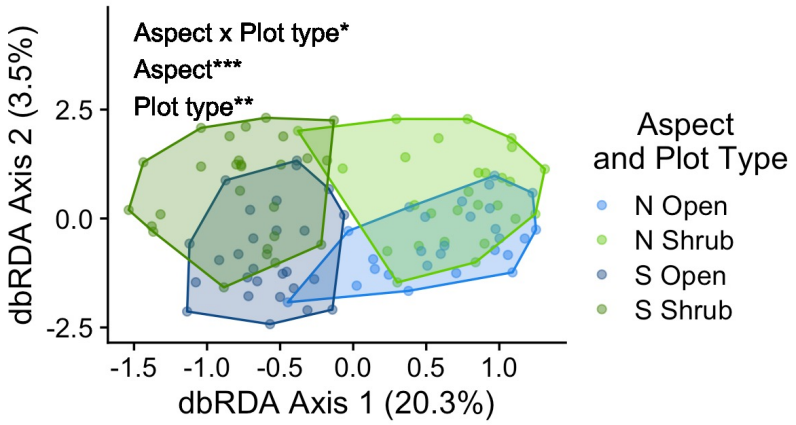

Figure 4.7. There was an interaction between shrub presence and aspect, which shaped plant community composition. Significance codes: "***" $P < 0.001$, "**" $0.001 \leq P < 0.01$, and "*" $0.01 \leq P < 0.05$.

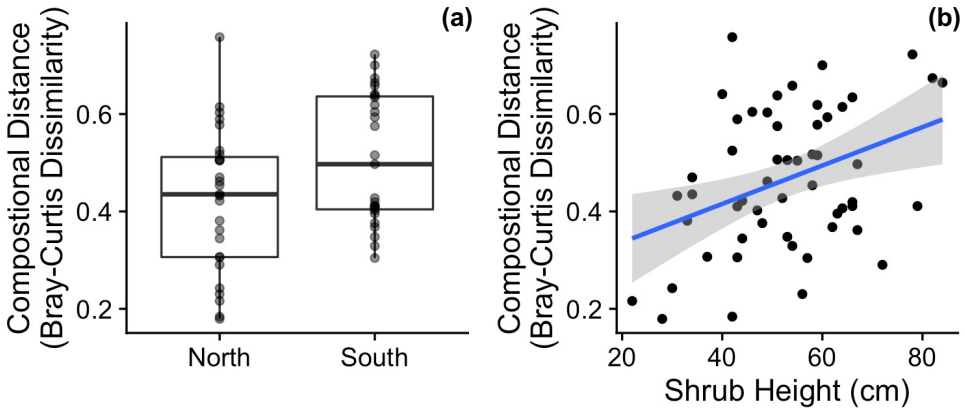

Figure 4.8. The compositional difference between paired plots was greater on the S-facing aspect (a) and was positively related to shrub height (b).

Discussion

Determining how abiotic context shapes the effects of a habitat-forming species is important for predicting their impacts across a landscape. While the notion that abiotic context will alter habitat-forming species effects on the environment and associated taxa is well developed theoretically, it is rarely empirically tested (but see Kleinhesselink et al., 2014; Schöb, Armas, Guler, Prieto, & Pugnaire, 2013). We found that shrubs were taller on the S-facing aspect, as

predicted, and that aspect shaped differences between shrub and open areas for two of the four measured environmental variables altered by shrub presence and plant community composition, but not species richness. Together, these findings suggest that it is important to consider the role that abiotic context will play in shaping shrub effects across a landscape and that the relevance of abiotic context for shrub effects on diversity will depend on a match between those environmental variables that relate to diversity and those environmental variables which show differences between shrub and open areas.

Shrubs shaped early season soil moisture, FDD, and TDD independently of aspect. Fewer FDD with shrubs suggests that shrubs successfully trapped snow and insulated the soil across the range in shrub heights, likely resulting in a more consistent and deeper snowpack throughout the winter season compared to open areas. Following snowmelt, shrubs may also have trapped more outgoing longwave radiation resulting in fewer frost events (Jordan and Smith, 1995). On the other hand, shrubs decreased TDD, indicative of cumulative growing season hours, potentially due to delayed meltout conditions on the leeward side of shrubs, which would additionally explain the higher soil moisture at the start of the growing season. While the independent effect of shrubs on early season soil moisture and TDD was of a lesser magnitude than that of aspect, shrub effects on FDD were of an even greater magnitude than aspect. This suggests that shrubs have the strongest effects on winter thermal conditions and that shrubs, a biotic driver, can shift winter conditions more strongly than aspect, a larger-scale topographic feature.

We found that differences in environmental variables between paired shrub and open areas were generally not altered by aspect. Despite shifts in morphology, shrubs may have had similar effects on the microclimate perhaps because a threshold had been surpassed. For example, shrubs may have accumulated enough snow to insulate the soil across the range of heights we measured, resulting in similar FDD between aspects. The two environmental variables which did demonstrate differences between shrub and open areas across the N- and S-facing aspects resulted from different mechanisms. Canopy-forming species have frequently been found to increase minimum temperatures (Nobel, 1980; Drezner and Garrity, 2003), primarily by trapping outgoing longwave radiation. In our study shrubs may have had a particularly strong buffering effect on minimum temperatures on the S-facing aspect because their taller stature increased the amount of outgoing longwave radiation trapped. On the other hand, shrubs increased soil moisture on the N-facing aspect, where shrubs were shorter and there was no relationship between the soil moisture difference and height. This may have resulted from a synergistic or additive effect of shrubs where the already higher soil moisture of the N-facing aspect was amplified by shrub presence independent of shrub height. These findings suggest that a range of shrub heights will be needed for the full microclimatic effects of shrubs to be realized but that for key variables, such as FDD which shapes exposure to freezing temperatures, shrub presence alone was sufficient for protection.

We detected greater rarefied species richness in the presence of shrubs though the difference in richness between paired plots did not vary across aspects. Hence, across the range of shrub heights, shrubs increased richness of their associated plant community. This result is in contrast to a study which measured facilitation across an elevation gradient and found that cushion plants had stronger facilitative and microclimate effects where they were more compact (Schöb et al., 2013). While shrub morphology varied across aspects, the key difference in our study is likely that microclimate variables which drive richness patterns were not strongly impacted by shrub height. Habitat-forming species facilitate greater species richness where they minimize stressors that relate to diversity (Ballantyne and Pickering, 2015; Cavieres et al., 2016). Hence, a match between those microclimate variables which shift with habitat-former morphology and which shape diversity patterns is important context to consider (Cavieres et al., 2016). Our findings indicate that the effect of shrubs on richness was maintained across a range of heights and thus shrubs should have predictable facilitative effects across our study site.

Aspect mediated shrub effects on plant community composition. Differences in the most abundant species per aspect, which subsequently explained the most variation between open and shrub plots, suggest that the interaction between aspect and shrub presence on community composition was likely driven in part by a filtering effect of aspect on species abundances where shrubs subsequently had different abundances on which to act. These findings suggest that aspect acts at a

broader spatial scale by shaping the filtered species pool (*sensu* Zobel, 2016) while shrubs act more locally by influencing species sorting, which is supported by previous research demonstrating a strong structing effect of aspect on the plant community (Winkler et al., 2016), particularly compared to habitat-forming species (Gracia et al., 2007).

While many studies have compared how habitat-forming species alter community composition, we are the first, to our knowledge, to directly compare how abiotic context and habitat-forming species morphology contributes to compositional difference between paired plots. This distinction allows us to unravel how shrubs shift community composition with respect to the closest open area reference community, yielding a measure of the intensity of compositional shift. We found that on the S-facing aspect, where shrubs were taller, shrubs more strongly shifted community composition from open areas. Because aspect and associated differences in shrub morphology did not result in strong microclimate differences between paired plots, which played out in the equal effect of shrubs on richness we detected across aspects, the greater compositional shift could result from minimum temperatures (whose difference between paired plots increased with shrub height) and/or unmeasured microclimate effects. For example, an important but unmeasured environmental variable is wind speed which should vary by shrub height and shapes wind desiccation of plants (Henry and Molau, 1997). Alternatively, the greater compositional shift with taller shrubs could result from a physical mechanism. Shrubs can act as both seed traps and seed barriers through

their physical structure (Giladi et al., 2013; Filazzola et al., 2019), and this effect on seed movement may be enhanced where shrubs are taller. While many alpine plants are clonal, seed germination in the alpine is more important than once assumed with seedling densities at our field site rivaling rates of seedling establishment from tropical to temperate ecosystems (Forbis, 2003). Hence seed trapping and barrier effects could be an important contributor to the greater compositional shift detected on the S-facing aspect. These findings highlight the importance of considering not only the microclimate consequences of shrub morphology for plant community composition but the direct effects of shrub stature.

The most important consequences of shrub presence include a stronger effect on FDD (a winter variable important for diversity patterns (Choler, 2018; Niittynen et al., 2020a)) compared to aspect, greater richness, and an interaction with aspect to shape composition. When considering shrub morphology, which varied due to aspect, we see that the facilitative effects of shrubs occurred regardless of shrub height but that compositional shifts were stronger where shrubs were taller. Together, these findings suggest that the predictability of shrub effects across a landscape will depend on the diversity metric investigated. While morphology of our focal habitat-former was most likely altered because of physiological performance, differences in habitat-former morphology across a landscape can stem from age (Pugnaire et al., 1996) or genotype (Michalet et al., 2011), highlighting the relevance of considering how the effects of habitat-formers on the microclimate and on plant diversity could differ across a landscape.

CHAPTER V

COULD HABITAT-FORMING PLANTS FACILITATE SPECIES RANGE SHIFTS?

By Laurel M. Brigham and Katharine N. Suding

Abstract

It is well established that species will often be required to shift their distribution uphill to track their climatic niche, but that the pace of climate change may outstrip the pace of their uphill migration. Consequently, microsites promoting conditions favorable for the establishment of the migrating species may facilitate an uphill migration. While topography has been shown to serve this purpose (e.g. Equator-facing slopes), biotic sources of heterogeneity across a landscape, such as habitat-forming species (e.g. trees, shrubs, cushion plants), could also fill this role. However, whether habitat-forming species could act as stepping-stones to facilitate a range shift has been rarely tested. Here we experimentally seeded a subalpine species in the alpine to test how interactions with shrubs and the resident herbaceous community shape its establishment. We found that microsites of higher soil moisture, regardless of shrub presence, increased germination and survival. Shrub-related effects (increased soil organic matter) enhanced survival in the first year. These findings suggest that both shrubs and topographic variability can facilitate the leading-edge migration of a subalpine species by enhancing germination and survival at a critical stage of the seedling's life.

Introduction

Rising temperatures and shifts in precipitation regimes around the globe are causing species distributions to shift poleward and upward in elevation (Parmesan and Yohe, 2003; Lenoir et al., 2008; Chen et al., 2011). High elevation ecosystems are particularly vulnerable to climate change, experiencing rapid warming (Pepin and Lundquist, 2008; Pepin et al., 2015) and resultant declines in snowpack (Mote et al., 2005; Stewart, 2009; Marty and Meister, 2012). Despite strong climatic shifts in montane regions, the response of plant species distributions has been variable with some species even shifting downhill or failing to establish at higher elevations (Frei et al., 2010; Mamantov et al., 2021), indicating nuances in species' responses not encompassed by the macroclimate.

Whether a species is able to shift its distribution upward in elevation can largely depend on seedling establishment (Jackson et al., 2009), the germination and survival of a seedling, which is primarily controlled by the environment experienced by the seedling (the microclimate) rather than the macroclimate (Grubb, 1977). Microclimates can result from abiotic properties (e.g. topography, hydrogeology) and a heterogeneous arrangement of microclimates could alleviate some of the challenges of range shifts by providing suitable conditions for seedling establishment within a relatively short distance (Scherrer and Körner, 2011; Anthelme et al., 2014; Spasojevic et al., 2014; Graae et al., 2018). Importantly, abiotic microclimates are not the only type of microclimate which may impact a species range, though they are the subject of primary study (Hannah et al., 2014).

It has long been known that habitat-forming species, sometimes called nurse plants, promote conditions that facilitate seedling establishment (Bertness and Callaway, 1994; Stachowicz, 2001; Michalet and Pugnaire, 2016). Habitat-forming species create safe sites, offering protection from the elements (e.g. high sun exposure, wind, frost) and enhanced resource conditions (e.g. moisture, soil nutrients), which have been shown to increase establishment (Cavieres et al., 2007). However, the role of habitat-forming species in aiding establishment at and above a species range margin has not been well studied (but see Akhalkatsi et al., 2006; Batllori et al., 2009). Due to the often overlooked role of habitat-forming species in mitigating climate change but their prevalence across a landscape, a better understanding of whether and how the conditions created by habitat-forming species facilitate range shifts is critical.

Though habitat-forming species may affect abiotic conditions that facilitate the establishment of migrating species, interactions with other organisms may alter establishment outcomes. Interactions with herbaceous resident plant species could decelerate the expansion of the focal species through competitive exclusion (HilleRislambers et al., 2013). Alternatively, the resident plant species may contribute to the creation of suitable abiotic conditions and could thus be an additional facilitative interaction to consider (Liancourt and Dolezal, 2020). Belowground, a lack of compatible beneficial microbes in the new area could reduce establishment (Van Der Heijden, 2004). Arbuscular mycorrhizal fungi (AMF) colonize 80% of all terrestrial plant species (Smith and Read, 2008) and can increase

resource uptake and stress tolerance (Smith and Read, 2008; Johnson et al., 2010). However, the predominant fungal association of woody species (which are excellent habitat-forming species) is with ectomycorrhizal fungi (EMF) (Read and Haselwandter, 1981; Tedersoo et al., 2010). Woody species, by hosting predominantly EMF, can suppress AMF through interactions with EMF and high litter inputs (Becklin et al., 2012). Therefore, understanding not only the abiotic conditions under which seedling establishment is facilitated, but also the biotic conditions should improve our ability to predict the parameters of a focal species range shift.

Alpine and subalpine ecosystems in montane regions are ideal to study the role of habitat-forming species, as they are both experiencing an expansion of habitat-forming species and are strongly impacted by climate change. Shrubs, a habitat former, are increasing in abundance and expanding their distribution in alpine areas around the globe (Myers-Smith et al., 2011; Formica et al., 2014; Kopp and Cleland, 2014). While shrubs typically offer buffered abiotic conditions (Myers-Smith et al., 2011; Myers-Smith & Hik, 2013; Pajunen, Oksanen, & Virtanen, 2011; Chapter IV of this disseration), thereby promoting safe sites which can facilitate the expansion of subalpine species needing to move uphill to track their climatic niche, above- and belowground interactions could complicate predictions.

Our study asks how shrub protection, as well competitive and plant-microbial interactions, shape the establishment of a subalpine species in the alpine in the Front Range of Colorado, USA. We experimentally added seeds of a common

subalpine forb, and an alpine grass as a comparison, in areas with shrubs and in open tundra to assess the potential facilitative effect of shrubs. We additionally removed herbaceous neighbors to assess how interactions with tundra vegetation influenced establishment. We hypothesized that shrub presence would be necessary for the establishment of the subalpine species but not the alpine species, and that positive shrub effects would be driven by the safe site conditions promoted by shrubs. We also hypothesized that the facilitative effects of shrubs would outweigh the competitive effects of the resident herbaceous community and the possible reduction in AMF with shrub presence.

Methods

Site and Study Design

We conducted our study in the alpine on an east-facing slope at Niwot Ridge in the Front Range of the Rocky Mountains, Colorado, USA (40.05˚N, -105.59˚W) at an elevation of 3480 m.a.s.l. The study consists of a split-plot design where plots were located in open tundra or on the leeward side of shrubs. One half of each plot was randomly selected to have all biomass removed as a neighbor removal treatment, resulting in subplots that were 50 x 50 cm. This resulted in 28 subplots and 4 treatments: shrub with neighbors, shrub without neighbors, open tundra with neighbors, and open tundra without neighbors. In subplots where neighbors were removed, all aboveground biomass was clipped at the start of the study and removal was maintained throughout the experiment.

Planting protocol

Across these plots we seeded two subalpine species (*Erigeron glacialis* and *Polemonium pulcherrimum*) to determine how shrubs and herbaceous tundra vegetation influenced the establishment of these species. *Erigeron glacialis* and *P. pulcherrimum* are perennial herbs native to the mountains of western North America. These species were chosen because they are common subalpine species and the elevation of the majority of *E. glacialis* and *P. pulcherrimum* occurrence records in the area, 94% and 91%, respectively, (University of Colorado Herbarium, Boulder, CO, USA), were lower than that of the selected planting location (3480 m). *Polemonium pulcherrimum* germinated in one plot, and thus we were not able to include it in our analyses. We additionally seeded an alpine species common in the area (*Deschampsia cespitosa*, perennial bunchgrass) to serve as a baseline alpine plant comparison.

Seeds of all species were collected in August and September of 2019 and stored in coin envelopes at room temperature until cold stratification in early April 2020. To cold stratify seeds, filled seeds were placed onto a moist paper towel, which was folded over and put into a plastic bag. These bags were stored at 4°C for 2 months for those seeds destined for the greenhouse and between 3.5 and 4.5 months for those seeds planted in the field.

Following the first clipping of neighbors, grids were placed in the center of all subplots and affixed using landscape pins. The grids consisted of six columns and five rows of 2.5 cm^2 squares, creating a functional planting space of 75 cm^2. Seven

seeds per species were sewn into 4 wells per planting grid for a total of 28 seeds/species/subplot. Species placement in the grid was determined by randomly generating columns of numbers, which were assigned to species and planted accordingly. In addition to randomly generating seedling locations, we also included four cells for monitoring background emergence across all naturally occurring species (a cumulative area of 10 cm^2 per plot, the same as all experimentally seeded species).

We had anticipated planting seeds in the field in early July 2019, but a late snowmelt year required our planting later in the season. We planted in a stratified manner due to heterogeneity in the timing of standing water dissipation. Therefore, between 18 July and 12 August 2019 we planted seeds as plots became ready. Despite differences in planting date across plots, there were no differences in planting date due to shrub or neighbor presence ($P > 0.05$).

Seedling monitoring

We monitored subplots for seedlings every other week until 20 September 2019 during the first growing season. We monitored background germination during the first germination check of each plot. The following year, we monitored plots for the survival of seedlings. Though toothpicks were placed next to seedlings to indicate emergence in 2019, by the following summer many toothpicks had been lost (likely broken by snow) making it challenging to determine whether seedlings in year two were a result of year one survival or year two emergence. Hence, all

seedlings were combined into a measure of "total survival" which included both categories. In 2020, plots were monitored on 29 June 2020 and 4 August 2020.

We quantified the likelihood of germination using the maximum number (across all monitoring time points) of seedlings that emerged in the first growing season. We quantified first season survival in two ways: 1) proportion of the number of seedlings that germinated in the first year that were alive at the end of the first season and 2) the number of season one survivors. We quantified second season total survival as the proportion of the number of seedlings that germinated across either season that were alive at the end of the second season.

Seedling harvest

Seedlings were harvested on 4 August 2020. Harvested seedlings were kept on ice until we arrived at the lab where the aboveground biomass and roots were separated. The aboveground biomass was dried at 60˚C for 48 hours and weighed.

To determine colonization of the harvested seedlings by AMF, the separated roots were stored in 70% ethanol until staining, done within 2 weeks. Roots were stained with trypan blue using a standard protocol (Schmidt et al., 2008). Briefly, roots were first cleared in 10% KOH for 1 hour at 90˚C, then roots were reacidified in 1% HCl for 20 minutes, and stained overnight in acidic glycerol trypan blue. The next morning, roots were de-stained in acidic glycerol and stored at 4˚C until microscopy. The grid line intersection method was used to determine colonization (McGonigle et al., 1990). Roots were viewed at 200x magnification and 50 intersections with the crosshair on the ocular were made during passes across the

slide at random intervals. At each intersection the presence of AMF structures was recorded. While making 100 intersections is common protocol, the low root material of the seedlings rendered 100 intersections impossible. At times, 25 intersections were necessary. A colonization proportion was calculated for AMF as the number of times out of 50 or 25 intersections that AMF structures were present.

Seedbank

To ensure that our subalpine species were not already present in the seedbank of our experimental area, we grew out soils collected from 23 open tundra and 23 shrub locations. The selected shrubs were adjacent to the experimentally seeded shrubs, and soils were collected on the leeward side. On 23 September 2019 we used a soil knife to collect 2 cubes of soil (5 x 5 x 2 cm) from each location. Soils were put on ice and brought back to the lab where they were stored at 4°C for less than 24 hours. The following day, we sieved soils with 4 mm mesh to remove coarse debris. This mesh size was too large to remove seeds. The soils were homogenized and stored at 4°C until the greenhouse phase. On 18 April 2020 we filled pots (10 x 10 x 10 cm) with Sunshine Mix #3 and added 50 ml of seedbank soil on top (0.5 cm depth of seedbank soil). We monitored for seedlings once per week. Seedlings were marked with a toothpick and allowed to grow until they could be identified to species. Pots were rotated once per week. Seedbank samples were grown out for 11 weeks, at this point no new seedlings had emerged for 2 weeks. We did not detect either of our subalpine species.

Environmental measurements

To determine whether facilitative effects of the shrub were propagated through the microclimate we measured soil temperature and soil moisture. To measure temperature, we deployed one iButton temperature logger (type DS1921G-F5, Maxim Integrated, San Jose, CA, USA) in the rooting zone (3 cm depth) of each subplot. To waterproof the iButtons, we sealed them in small, plastic vacuum seal bags and mason's line was tied around the bag to aid in retrieval. iButtons were first deployed as subplots were seeded; therefore, iButtons were added between 18 July 2019 and 12 August 2019. iButtons were collected on 13 September 2019 and redeployed for winter and the following growing season. All iButtons were then collected on 31 July 2020. iButtons in the ground during the summer were used to calculate an average, 95th percentile maximum, and 5th percentile minimum soil temperature (Ashcroft et al., 2012). We calculated freezing degree days (FDD) as the absolute value of the sum of mean daily temperatures below 0°C across all 272 days of iButton data. We measured soil moisture approximately every other week during the 2019 and 2020 growing season. Soil moisture was taken at a depth of 7.6 cm using a handheld probe (Field Scout TDR150, Spectrum Technologies, Inc., Plainfield, IL). Three measurements were taken at each subplot to achieve an average.

Soil properties

On 4 August 2020, we collected soils for organic matter (OM), total nitrogen (TN), and pH to better understand shrub effects on the soil environment. We

collected three soil cores (10 cm depth, 2 cm diameter) per subplot and we stored the soils on ice until we arrived at the lab. In the lab we sieved the soils using a 2 mm sieve. We then placed duplicate 15 g soil samples into sample cups for soil pH, 10 g of soil into an aluminum weigh boat for OM, and 10 g into an aluminum weigh boat for TN. We measured pH using a SevenCompact pH meter S210 (Mettler Toledo, Greifensee, Switzerland) on the freshly sieved soils which had been mixed 2:1 with deionized water in sample cups and allowed to equilibrate with atmospheric CO_2 for 30 minutes. We placed the weigh boats designated for OM into a drying oven at 100°C for 12 hours. We added 2.5 – 3 g of the oven dry soil to a crucible and placed these crucibles in a muffle furnace set to 550°C for four hours. We calculated OM with the following equation: (weight of oven dry soil – weight of soil after ignition)/weight of oven dry soil. For TN, air dried samples were crushed with a mortar and pestle and then analyzed in an automated element analyzer at the Research Analytical Laboratory, University of Minnesota in July 2021 (Vario MAX, Elementar, Hanau, Germany). Organic matter was correlated with TN($r = 0.97$) and thus only OM was retained in downstream analyses for its ability to describe soil fertility (Oldfield et al., 2018).

Statistical Analysis

To determine the effect of our treatments on establishment of the seeded species we tested for differences in germination, survival, AMF colonization, and final aboveground biomass by running models with shrub and neighbor presence, their interaction, and planting date as predictors, and plot as a random effect due to

the split-plot design. We ran a generalized linear mixed model (GLMM) with a binomial error distribution on a binary germination metric, the proportion of survivors at the end of the first growing season, total survival, and AMF colonization (function 'glmer', package *lme4*); a GLMM with a Poisson error distribution on background germination; and a GLMM with a truncated Poisson error distribution on the number of season one survivors (function 'glmmTMB', package *glmmTMB*). We ran a linear model on aboveground biomass of the survivors at the end of the second season (function 'lmer', package *lme4*). Pairwise treatment comparisons were assessed via Tukey's honest significant difference (function 'emmeans', package *emmeans*; Lenth et al., 2018).

To determine the effect of environmental variables on germination we ran generalized linear models (GLM). We ran GLM with a binomial error distribution on a binary germination metric and a GLM with a Poisson error distribution on background germination. The environmental predictors were average soil moisture and average temperature, shown to be important for germination at our site (Forbis, 2003).

To determine the effect of environmental variables on survival and final aboveground biomass we ran additional models. We ran GLM with a quasibinomial error distribution, because the residuals were overdispersed, on the proportion of survivors at the end of the first growing season and total survival, and a GLM with a truncated Poisson error distribution on the number of season one survivors. We ran a linear model on aboveground biomass of the survivors at the end of the second

season (function 'lm', package *stats*). The environmental predictors for first season survivors were average first season soil moisture, maximum and minimum first season soil temperatures, and OM. The environmental predictors for second season survivors were average second season soil moisture, maximum and minimum second season soil temperatures, OM, and FDD. We included OM in our survival models because of its ability to describe soil fertility, which has been shown to impact survival (Cavieres et al., 2007; Spasojevic et al., 2014) and we included maximum rather than mean temperatures because we expected survival to be better related to extremes (Graae et al., 2009, 2018). When required, GLM were run to test for shrub and herbaceous neighbor effects on environmental variables of interest, with plot as a random effect. All statistical analyses and visualizations were performed in R ver. 4.1.2 (R Core Team, 2020).

Results

Germination

Background and experimental germination were not altered by shrub or herbaceous neighbor presence, but were positively related to soil moisture. The incidence of background germination was similar across shrub and open plots, with an average of 2 seedlings per plot (or 2000 per m^2). Background germination was positively related to average soil moisture in season one, the season during which emergence was monitored (Figure. 5.1a; GLM, $\beta = 0.07$, $P < 0.001$).

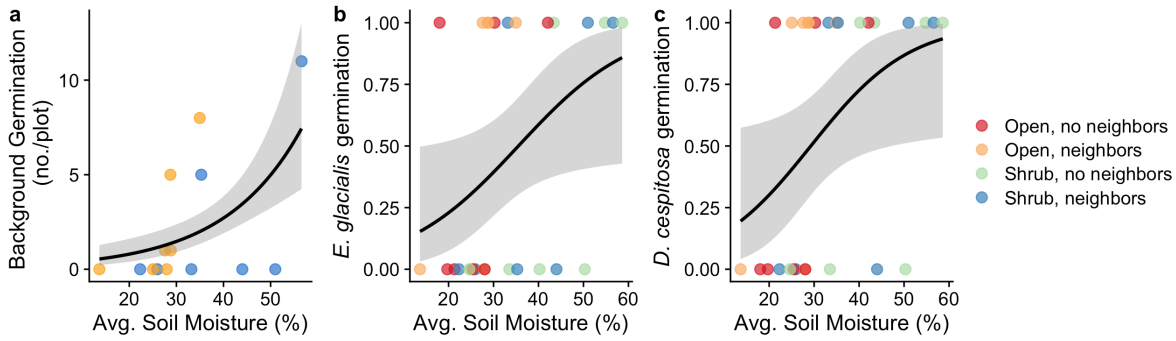

Figure 5.1. Background germination, monitored only in plots where neighbors were not removed, increased with higher average season one soil moisture (a). The likelihood of germination for both experimentally seeded *E. glacialis* (b) and *D. cespitosa* (c) increased with higher average season one soil moisture.

Experimentally seeded *E. glacialis* germinated in 50% of plots (13/28 plots) and, in plots where germination occurred, an average of 30% ± 19% of seeds germinated (Figure 5.2a). The likelihood of *E. glacialis* germination was not shaped by shrub or herbaceous neighbor presence. However, there was a trend of increased *E. glacialis* germination with soil moisture (Figure 5.1b; GLM, $\beta = 0.08$, $P = 0.05$).

Experimentally seeded *D. cespitosa* (the alpine species) germinated in 60% of plots (16/28 plots) and, in plots where germination occurred, an average of 14% ± 14% of seeds germinated (Figure 5.2b). The likelihood of *D. cespitosa* germination was shaped by an interaction between shrub and neighbor presence (GLMER, $\chi^2 = 6.9$, $P = 0.009$) such that that where neighbors were present, *D. cespitosa* had a higher likelihood of germination in open plots (Tukey: $P < 0.05$). There was an additional effect of planting date where *D. cespitosa* seeds planted earlier were more likely to germinate (GLMER, $\chi^2 = 165$, $P < 0.001$). The likelihood of *D. cespitosa* germination was positively related to average first season soil moisture (Figure 5.1c; GLM, $\beta = 0.09$, $P = 0.04$). There was not greater soil moisture in open areas

with neighbors (Tukey: $P > 0.05$), suggesting soil moisture was not the mechanism behind the interaction between shrub and herbaceous neighbor presence on *D. cespitosa* germination.

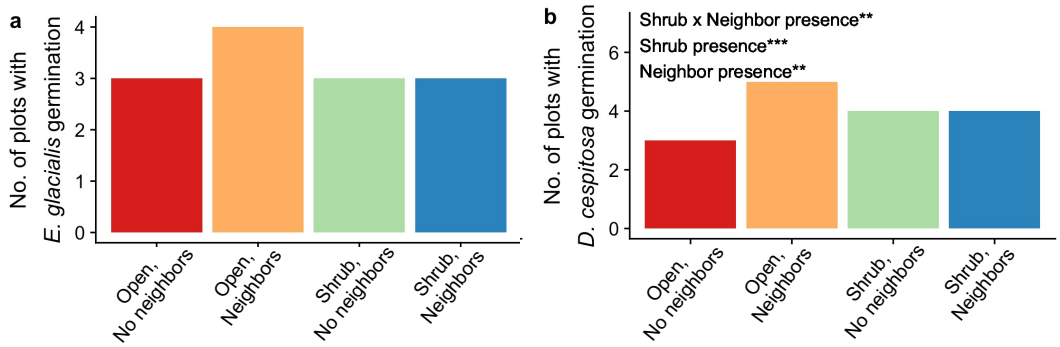

Figure 5.2. The number of plots (out of 28) which demonstrated germination of *E. glacialis* (a) and *D. cespitosa* (b). Significance codes: "***" $P < 0.001$, "**" $0.001 \leq P < 0.01$, and "*" $0.01 \leq P < 0.05$.

First season survival

While the proportion of *E. glacialis* survivors at the end of the first season was only shaped by soil moisture, the number of survivors was increased by shrub effects on soil OM. At the end of the first season, 40% of the *E. glacialis* seedlings that germinated survived per plot (Figure 5.3a). The proportion of *E. glacialis* survivors at the end of the first growing season was not driven by shrub or neighbor presence. There was a marginally significant relationship where the survivorship of *E. glacialis* was positively related to average season one soil moisture (Figure 5.4; GLM, $\beta = 0.13$, $P = 0.08$). While shrubs did not affect the proportion of *E. glacialis* survivors, shrubs enhanced the number of *E. glacialis* survivors (Figure 5.5a; $\chi^2 = 4.5$, $P = 0.03$). Additionally, there was a trend of increased *E. glacialis* survivors with soil OM (Figure 5.5b; GLM, $\beta = 0.09$, $P = 0.08$). Hence, shrub effects on *E.*

glacialis survivors were likely a result of a marginally significant positive shrub effect on soil OM (Figure 5.5c; LMER, $\chi^2 = 3.6$, P = 0.06).

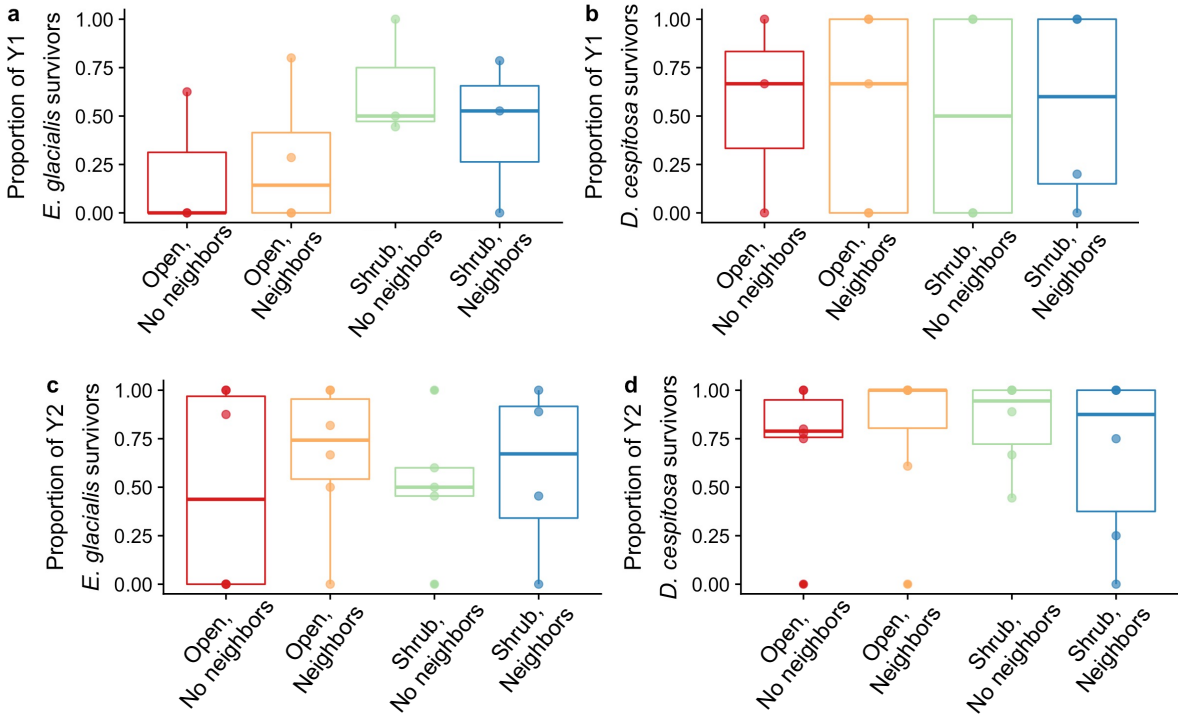

Figure 5.3. The proportion of survivors at the end of the first (a, b) and second season (c, d).

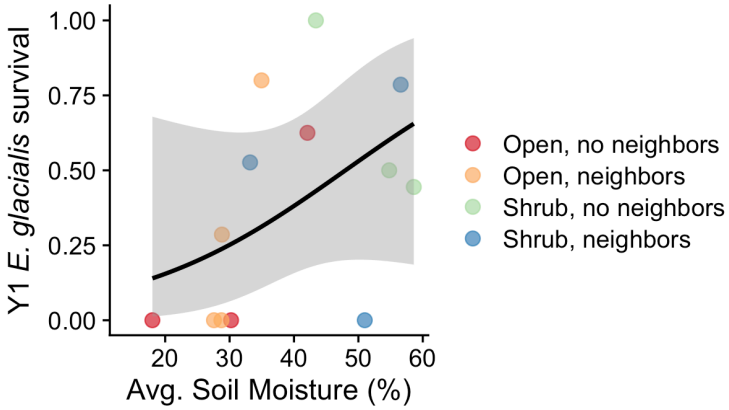

Figure 5.4. The proportion of *E. glacialis* survivors was increased by average season one soil moisture.

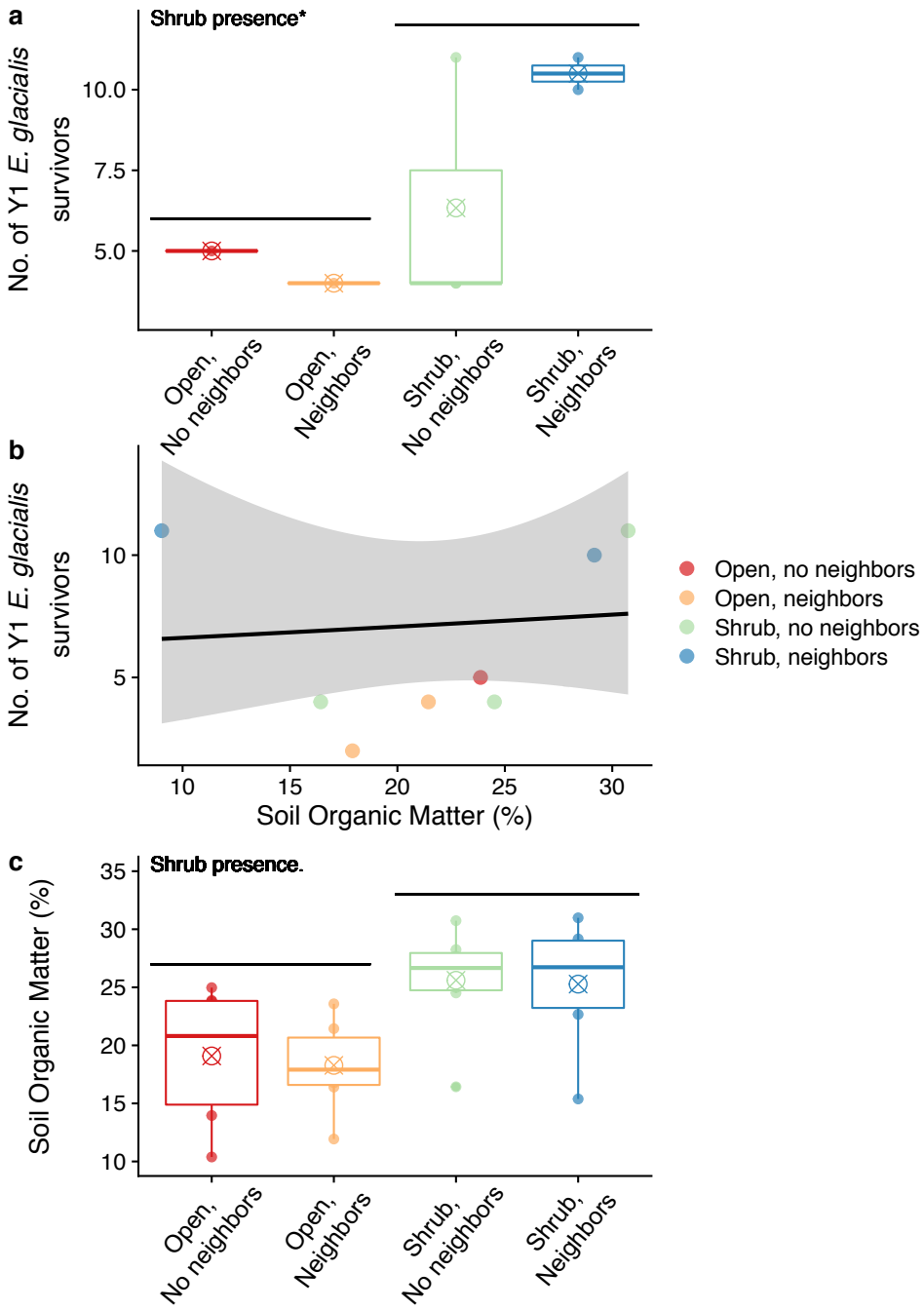

Figure 5.5. The number of *E. glacialis* survivors was increased by shrub presence (a) and showed a positive relationship with soil OM (b), likely driven by the positive effect of shrubs on soil OM (c). Significance codes: "***" $P < 0.001$, "**" $0.001 \leq P < 0.01$, and "*" $0.01 \leq P < 0.05$.

Neither shrub nor herbaceous neighbor presence shaped *D. cespitosa* first season survival though the number of survivors was related to several

environmental characteristics. At the end of the first season, 50% of *D. cespitosa* seedlings survived per plot (Figure 5.3b). Neither shrub nor neighbor presence shaped the proportion of *D. cespitosa* survivors or the number of *D. cespitosa* survivors. The proportion of *D. cespitosa* survivors was not related to any environmental variables though the number of *D. cespitosa* survivors was increased by average season one soil moisture (Figure 5.6a; GLM, $\beta = 0.06$, $P < 0.001$), decreased by minimum temperatures (Figure 5.6b; GLM, $\beta = -1.3$, $P = 0.002$), and marginally significantly decreased by soil OM (Figure 5.6c; GLM, $\beta = 0.08$, $P < 0.06$).

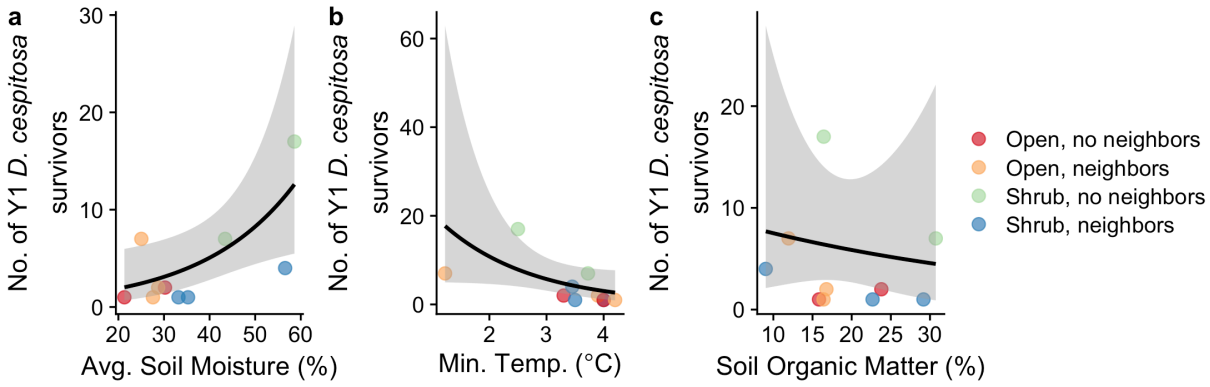

Figure 5.6. The number of *D. cespitosa* survivors was increased by average season one soil moisture (a) and decreased by minimum temperatures (b) and soil OM (c).

Second season survival

Survival at the end of the second season was not shaped by shrub or herbaceous neighbor presence and was not related to environmental characteristics or fungal colonization. The proportion of *E. glacialis* seedlings which survived until the end of the second season was 56% ± 41% (Figure 5.3c). Neither shrub nor neighbor presence explained the combined season one and season two *E. glacialis*

survival, nor any of the environmental variables. The weight of *E. glacialis* survivors at the end of the second season was not shaped by shrub or herbaceous neighbor presence (Figure 5.7a) and was not related to environmental variables. There were no effects of shrub or herbaceous neighbor presence on AMF colonization of *E. glacialis* survivors at the end of the second season. The proportion of *D. cespitosa* seedlings which survived until the end of the second season was 76% ± 35% (Figure 5.3d). Combined season one and season two *D. cespitosa* survival and final aboveground biomass were not related to shrub or neighbor presence (Figure 5.7b), despite an increase in AMF colonization of *D. cespitosa* seedlings where neighbors were removed ($\chi^2 = 17.3$, $P < 0.001$), and were not related to the measured environmental variables.

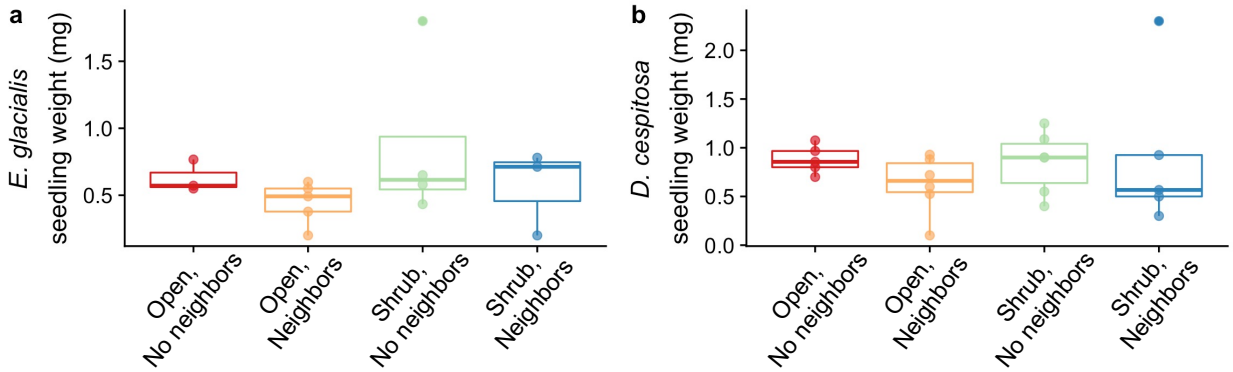

Figure 5.7. The average seedling weight of seedlings collected at the end of the second season for *E. glacialis* (a) and *D. cespitosa* (b).

Discussion

Understanding the effect that habitat-forming species could have on range shifts, including the role of above- and belowground interactions, is important for improving our predictions of how species respond to climate change. We found

partial support for facilitative shrub effects on first season survival of our subalpine species, driven by greater soil OM. We additionally detected positive effects of microsites of higher soil moisture on both subalpine germination and first season survival, indicating the importance of abiotic microclimates for subalpine establishment. Finally, we found no support for competitive effects of the resident herbaceous community on establishment of the subalpine species, nor effects of belowground fungal interactions. These findings suggest that the beneficial effects of shrubs on subalpine establishment at our site will be at the earlier stages of survival and that shrubs may aid a range shift through increased soil fertility.

Our hypotheses that shrub and herbaceous neighbor presence would shape the germination of our experimentally seeded subalpine species, *E. glacialis*, but not our alpine species, *D. cespitosa*, was not supported. There was no effect of shrub or herbaceous neighbor presence on *E. glacialis* germination yet there was for *D. cespitosa* germination. *Deschampsia cespitosa* had a higher likelihood of germination in open plots with neighbors. This finding may suggest that *D. cespitosa* prefers the intermediate shade conditions offered by plots with neighbors but without shrubs whereas light conditions were not as strong a driver of *E. glacialis* germination likelihood. While there were differences in how the two species responded to shrub and neighbor presence, both species responded positively to soil moisture, as did the number of seedlings measured for background germination. These findings are in line with the consistent finding that soil moisture is a strong driver of germination (Cook, 1979; Forbis, 2003) and suggest

that microsite variation in soil moisture conditions, unrelated to shrub or neighbor presence, was important for germination of *E. glacialis*.

Our hypotheses that shrub and neighbor presence would influence the survival of our experimentally seeded subalpine species was partially supported. First season survival of *E. glacialis* was around 40%, which is less than *D. cespitosa* (50%) and less than alpine species measured by Forbis (2003) at our site (80% survival of background seedlings after 50 days). Hence, *E. glacialis* had lower levels of survival compared to those of alpine species persisting in the area. The presence of shrubs and herbaceous neighbors did not alter the proportion of *E. glacialis* survivors, but soil moisture did. Again, this suggests that microsites of higher soil moisture were important for *E. glacialis* establishment in the alpine. On the other hand, the proportion of *D. cespitosa* survivors was not related to any environmental variables, suggesting its ability to survive across the entire range of environmental conditions. The importance of soil moisture for *E. glacialis* germination and survival highlights that though species moving uphill need to track their thermal isocline, those species will also require conditions specific to their germination needs, such as a certain water availability (Hankin and Bisbing, 2021). For instance, Andrus et al. (2018) found that an expected uphill shift of treeline as a result of warming temperatures in the southern Rocky Mountains, USA was stymied by a moisture deficit. In fact, in our study we found no effect of temperature on the germination or survival of the subalpine species, highlighting the important role that water availability may play in range shifts of some species.

In partial support of our hypothesis regarding beneficial shrub effects on the subalpine species, we found a positive effect of shrub presence on the number of *E. glacialis* survivors, shaped by shrub effects on soil OM. Soil OM has previously been found to increase survival, likely because of enhanced soil fertility (Oldfield et al., 2018). For example, Spasojevic et al. (2014) found that the biomass of an experimentally planted species sewn at a higher elevation was increased by soil OM. In contrast to *E. glacialis*, there were no effects of shrub or herbaceous neighbor presence on *D. cespitosa* survival metrics, suggesting that the habitat-forming species had a stronger beneficial effect on the subalpine than the alpine species. While this might suggest that shrubs in the alpine could serve as stepping-stones for the uphill migration of subalpine species, such as *E. glacialis*, the conditions for survival must already be met for the beneficial effects of shrubs to be realized.

We did not detect competitive effects of the herbaceous plant community or the potential for effects of AMF on germination or survival of the seeded species. This might suggest that net facilitative and net competitive interactions, the prevalence of which can shift with resource conditions on a daily basis (Wright et al., 2015), were equally matched resulting in a neutral effect of herbaceous neighbors. Alternatively, competition intensity may have been weak at our moist meadow study site because of a near constant water source for the first part of the growing season, the result of a melting snowbed uphill of the site (Davis et al., 1998). The anticipated belowground effect, AMF colonization, was expected to shape

survival because plant-microbial interactions have been found to be important for range shifts (Van Grunsven et al., 2010; Bueno de Mesquita et al., 2020). However, AMF colonization of *E. glacialis* was not altered by shrub presence and therefore did not contribute to shrub effects on survival.

The total number of survivors at the end of the second season was higher than that of the first. While this number includes combined season one and two survival, the buoyed number aligns with studies finding that first year survival is often the strongest bottleneck (Cook, 1979; Forbis, 2003; Graae et al., 2011). Total survival at the end of the second season could not be explained by shrub or herbaceous neighbor presence, or the measured environmental variables. This suggests that *E. glacialis* survival at the season two timepoint was feasible across the range of environmental conditions measured. Hence, shrub presence may be most important for survival during the first season but not later in the life of the seedling.

Though the conditions for germination were similarly met both for our subalpine species, *E. glacialis*, and our alpine species, *D. cespitosa*, first season survival in the alpine for *E. glacialis* depended on microsites of higher soil moisture and was enhanced by the higher soil fertility offered by shrubs. On the other hand, second season survival was not shaped by the environment for either *E. glacialis* or *D. cespitosa*, suggesting that the additional safe site protection needed by the subalpine species in the first year was not required in the second season. But, because first year survival was the greatest bottleneck, microsite variation and

shrub protection, types of stepping-stones, may be necessary for the uphill movement of *E. glacialis* under current conditions. These findings highlight the important role of microsites for subalpine germination and survival in the alpine while also demonstrating the lack of effects of the resident herbaceous plant community and AMF. Additionally, these findings highlight the importance of a match between the conditions offered by stepping-stones and those required for establishment by a focal species.

CHAPTER VI

COULD HABITAT-FORMING PLANTS BUFFER OTHER SPECIES FROM THE EFFECTS OF CLIMATE CHANGE?

By Laurel M. Brigham and Katharine N. Suding

Abstract

Models utilizing large-scale climate data (macroclimate data) report dramatic range shifts and contractions in the face of climate change. Yet modeling efforts that incorporate smaller scale climate conditions indicate more muted species responses. These discrepancies in species' responses indicates that microclimates, the small-scale conditions experienced by organisms, are more important than the macroclimate for informing a species response to climate change. Habitat-forming species, such as trees, facilitate species via abiotic amelioration; form networks of similar, buffered conditions thereby fostering migration; and add to the heterogeneity of microclimates across a landscape resulting in asynchronous population responses to stressors. These three effects of habitat-formers could mitigate the effects of climate change and are crucial to consider when attempting to predict a species response. To promote further study of the role that habitat-formers may play under climate change, we propose a conceptual framework that highlights the interconnected nature of three fields (facilitation, connectivity, and heterogeneity) with habitat-formers and propose future research methods.

Introduction

Just as we appreciate the shade of a tree on a hot day, the small-scale climatic variations experienced by organisms—microclimates—are more relevant for species' responses to climate change than the macroclimate. While abiotic microclimates (e.g. those driven by topography and hydrogeology) have been the focus of study for decades (Geiger, 1950; Slavich et al., 2014; Lenoir et al., 2017), those driven biogenically by habitat-forming species (Figure 6.1), such as trees, may be as important as abiotic microclimates to species response in this era of rapid climate change (De Frenne et al., 2021). The biogenic microclimate offered by habitat-formers are particularly relevant for climate change because of their prevalence across a landscape, even one that may be topographically homogeneous, and their ability to buffer the conditions experienced by associated taxa from the macroclimate, much like abiotic microclimates. Yet, research indicating the importance of habitat-formers for climate change is fragmented across many fields, including facilitation, connectivity, and heterogeneity (Figure 6.1), lacking a concerted research agenda. Here, we argue that habitat-forming species unite these fields and form a conceptual framework critical to better understanding and managing species' responses to climate change.

Although all species alter their local environment (Clements, 1916), we focus specifically on habitat-forming species which form a structure that can ameliorate stress and promote heterogeneity across a landscape (Figure 6.2). We first present a conceptual framework to integrate fragmented fields where these effects have

traditionally been studied, then examine the importance of the three main components of this integrated framework (facilitation, connectivity, heterogeneity), and end with suggestions for a unified research agenda. Our intention is not to discount the importance of abiotic microclimates, but to highlight the importance of biogenic microclimates so that they may be considered *in tandem*. We focus on terrestrial landscapes but see Bulleri et al. (2018) for a recent review on coastal microclimates.

Habitat-forming species (or habitat-formers). Species which create a 3-dimensional structure that modifies resource (e.g. water) and non-resource stressors (e.g. temperature). Examples include organisms such as cushion plants, shrubs, trees, reef-building corals, and seagrass. Habitat-formers may act as individuals (e.g. a tree) or may act as an aggregated unit (e.g. a forest).
Decoupled conditions. Biogenic microclimates can mitigate the rate of climate change when the microclimate changes negligibly, or at least slower, than the macroclimate. See Lenoir et al. (2017) for methods on calculating the degree of decoupling.
Facilitation. A beneficial interaction which results in enhanced performance of the focal species. We focus here on direct facilitative effects where the facilitator ameliorates abiotic conditions for the focal species.
Connectivity. A network of similar, suitable environmental conditions which facilitates the upward or poleward movement of species in the face of climate change.
Heterogeneity. As opposed to connectivity, heterogeneity provides a variety of environmental conditions which promote different responses to climatic extremes and variability as well as opportunities for movement if habitat preferences shift with climate change.

Figure 6.1. Habitat-forming species and their roles.

Figure 6.2. Habitat-forming species can ameliorate stress (a-c) and, when arrayed patchily across a landscape, habitat-formers can impact connectivity and heterogeneity (c). Densely arranged habitat-forming species (a, c) act at a community-scale and also a play an important, though sometimes inconspicuous role. For example, in (c) not only the conspicuous shrubs (habitat-forming individuals) create a biogenic microclimate, but so does the interstitial grass, depending on the scale experienced by the associated taxa. Photo credits: a) Laurel Brigham, b) Jane Smith, c) Sam Ahler.

New conceptual framework

The fields of facilitation, connectivity, and heterogeneity are united by habitat-formers. An enhanced consideration of the intersection between habitat-formers and these fields will improve our predictions of species' responses to climate change (Figure 6.3). The effects of habitat-formers can occur at the individual level (e.g. a shrub) or the population and community level (e.g. a dense grassland or a forest; Liancourt and Dolezal, 2020), demonstrating the wide variety of habitat-formers and the different scales at which habitat-formers could exert their influence across a landscape.

Under climate change, a species will encounter a new macroclimatic regime, which could cause its distribution to shrink, shift, expand, or stay stable (Lenoir et al., 2008; Chen et al., 2011; Vitasse et al., 2021). In those instances where a species distribution is liable to shrink or shift, perhaps due to limited capacity for

adaptation, the presence of habitat-formers could enhance species persistence and result in different predictions compared to scenarios where habitat-formers are not considered. Shrinkage of a species distribution could be mitigated by microclimate conditions which are buffered, or even decoupled (Figure 6.1), from the macroclimate thereby providing ameliorated conditions (facilitation; Figure 6.3a) or by an asynchronous response of local populations to climate change (heterogeneity; Figure 6.3c). An enhanced ability for a species to shifts its range could prevent range loss (connectivity; Figure 6.3b). Thus, this framework joins an emphasis in the facilitation field on how habitat-formers can ameliorate those variables which are shifting with climate change, in the connectivity field on how habitat-formers can serve as sites for upward or poleward migration, and in the heterogeneity field on how variation caused by habitat-formers could inform habitat availability and metapopulation dynamics under a changing climate.

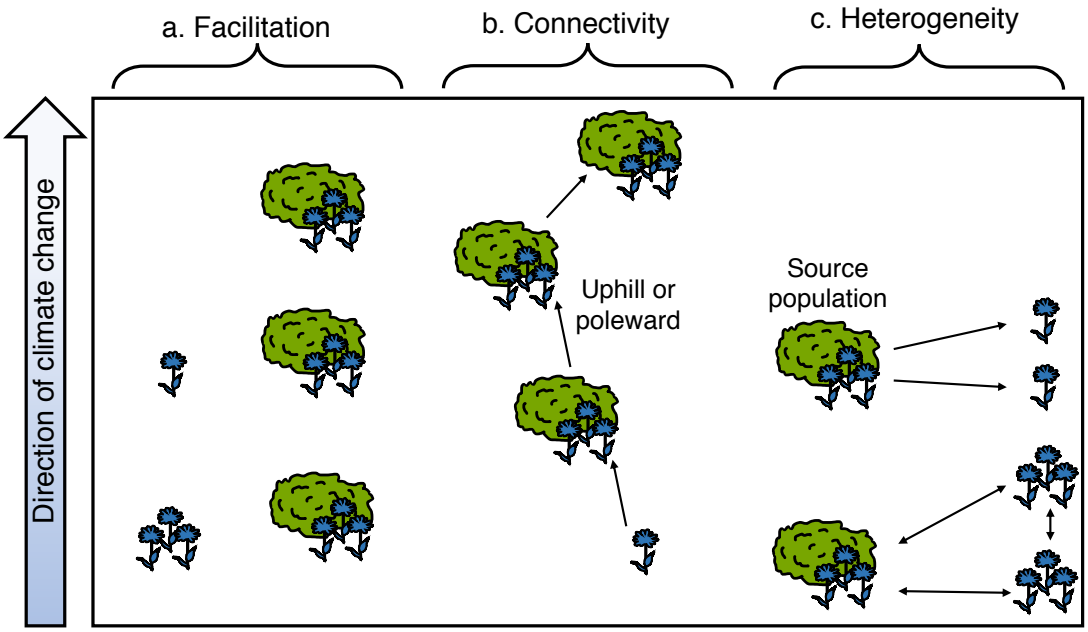

Figure 6.3. Habitat-forming species can enhance persistence under climate change. One way habitat-forming species can shape a species response to climate change is through facilitation, where the drivers of climate change (e.g. warmer temperatures) are ameliorated *in situ*, driven by a buffering or decoupling of the habitat-forming species microclimate from the macroclimate. Connectivity driven by habitat-forming species can foster uphill or poleward migration (the habitat-former provides currently suitable conditions in an otherwise unsuitable matrix [e.g. a warmer microclimate at a cool, high elevation site] and is located at a relevant distance for dispersal). Finally, habitat-forming species can increase heterogeneity across a landscape which increases metapopulation persistence when the habitat-former provides a microclimate that responds less negatively to stressors than microsites without a habitat-forming species. The less responsive microsites then act as source populations for microsites that did not fare well under the stress.

Facilitation

Habitat-forming species facilitate associated taxa through the creation of buffered conditions as a result of their physical structure (Jones et al., 1997). For example, in the Mojave Desert of California, the burrobush shrub (*Ambrosia dumosa*) increased annual seedling survival by buffering temperatures and increasing water availability (Claus Holzapfel and Mahall, 1999). Other work

demonstrates preferential associations with habitat-forming species due to the ameliorated conditions (Westphal et al., 2018).

The importance of facilitation by a habitat-former under climate change depends on the match between the type stressors facing the associated taxa and the type of stressors ameliorated, as well as the degree of amelioration offered by the habitat-former. The magnitude of amelioration offered by the habitat-former is determined by how buffered the biogenic microclimate is from the macroclimate. For example, summer temperature maxima were 5°C cooler and minima were 5°C warmer in woodlands compared to grasslands and heathlands (Suggitt et al., 2011), indicating that woodlands offered more buffered conditions than grasslands and heathlands. Additionally, the rate of climate change can be altered by habitat-forming species if their microclimate is decoupled from the macroclimate (Lenoir et al., 2017). The habitat-former microclimate may be completely decoupled, or it may only be partially decoupled, in which case climate change is slowed but not completely alleviated (Figure 6.4).

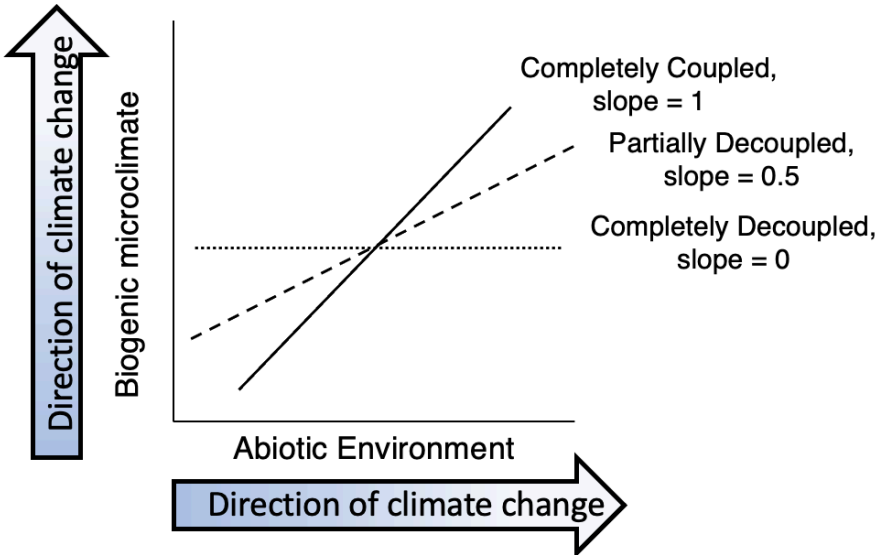

Figure 6.4. Biogenic microclimates have the capacity to mitigate the rate of climate change by promoting conditions decoupled from the macroclimate. A tightly coupled micro- and macroclimate will result in conditions similar to the macroclimate while decoupling will promote conditions which mitigate the rate of climate change.

There is also the possibility that the habitat-former amplifies the effects of climate change. For instance, research shows that when the limitation of a resource stressor (e.g. water availability) is exacerbated by climate change, association with the habitat-former may no longer be beneficial as competitive dynamics start to dominate (Maestre et al., 2009; Michalet et al., 2014a; Butterfield et al., 2016). However, whether the effect of the habitat-former becomes negative can depend on the other benefits provided by the habitat-former. For example, the benefits of shade provided by a canopy-forming species may outweigh the increase in water competition (Chaieb et al., 2021).

On the other hand, if the stressor being exacerbated by climate change is non-resource (e.g. temperature, wind) and the associated taxa remain stressed or are more stressed under climate change then the habitat-former should continue to

facilitate the focal species because the stressor changing is not one for which the habitat-former and focal species are competing. In fact, provided the habitat-former does not experience degradation as a result of the shifting climate (i.e. a morphological change which decreases its capacity to offer a microclimate (Jones et al., 2010)), then climate change should actually increase the potential for amelioration of those stressors if greater decoupling between the habitat-former's microclimate and the macroclimate occurs (Michalet et al., 2014b).

In addition to mitigating exposure and enhancing persistence of native species under climate change, habitat-forming species could provide the same benefits for invasive species. For example, the invasive ripgut brome (*Bromus diandrus*) had higher biomass when growing in conjunction with a native shrub, the California goldenbush (*Ericameria ericoides*) (Kleinhesselink et al., 2014). This suggests that biogenic microclimates could facilitate invasive species in addition to native species, complicating their role across a landscape.

The strongest evidence we have of climatic buffering by biogenic microclimates under climate change comes from forests. Forests have been found to buffer temperatures at a magnitude greater than that of global warming over the past century (De Frenne et al., 2019). This thermal buffering has protected understory species acclimated to cooler temperatures from being replaced by species with a higher thermal optimum (De Frenne et al., 2013; Bhatta and Vetaas, 2016). These studies suggest that including the role of facilitation in a species response to climate change would alter predictions based on macroclimate or topographic

microclimates alone—facilitation should increase persistence by buffering the conditions experienced by the focal species.

Enhanced connectivity

Biogenic microclimates can aid the persistence of species in a region by facilitating shifts in species distributions through increased connectivity between suitable habitats. To track their climatic niche, species may shift their range through establishment in new suitable habitat (Chen et al., 2011). However, if the velocity of climate change (*sensu* Loarie et al., 2009) exceeds the speed of a species range shift, the species will not be able to shift their range fast enough to escape detrimental effects of climate change (Nathan et al., 2011). Microclimates may alleviate some of the challenges of range shifts by providing accessible microsites of suitable abiotic conditions. Corridors of such microclimates can be considered stepping-stones (*sensu* Hannah et al., 2014) because they can increase the velocity of range shifts by providing suitable habitat outside the current range of the species (Lembrechts et al., 2017). For example, at the alpine treeline in northeastern Spain and Andorra, krummholz offered a protected microclimate which enhanced the survival and growth of mountain pine (*Pinus uncinata*) seedlings at their upper range margin, especially during a harsh winter (Batllori et al., 2009). Corridors of these krummholz might then increase the velocity of the mountain pine uphill range shift.

It is important to take a species-specific approach when determining the effects of habitat-forming species on increased connectivity between currently suitable habitat. The biogenic microclimates must be accessible given the migrator's dispersal capabilities (Hodgson et al., 2009). Additionally, provided a focal species can reach a habitat-forming species, it will also be important to consider novel species interactions, which could exclude the focal species from establishing (HilleRislambers et al., 2013; Alexander et al., 2015; Losapio et al., 2021). Hence, for upward or poleward movement of species to be successful, the benefits of niche expansion must exceed the detrimental effects of greater niche overlap with potential competitors in the new areas (Bulleri et al., 2016).

Promotion of heterogeneity

Heterogeneity as a result of habitat-formers occurs at a variety of scales, due to the presence, types, and structure of vegetation (Stein et al., 2014). For example, the presence of trees which dot a savannah and the vertical structure of a forest canopy provide heterogeneity in microclimate conditions at a larger and smaller scale, respectively. The importance of the scale of the heterogeneity depends on the biology of the organism.

As both climatic means shift (IPCC, 2014) and the frequency of extreme climatic events increases (Sillmann and Roeckner, 2008), sources of heterogeneity across a landscape could be important for population persistence by promoting asynchronous responses among local populations (Gilpin and Hanski, 1991) and via

a greater variety of available habitats for colonization. While the patches created by a habitat-forming species may often create a more benign microclimate, this is not a requirement for promoting patchiness. The habitat-forming species need only provide a *different* microclimate in order to create heterogeneity across a landscape, but we will operate under the assumption that habitat-formers with strong climatic buffering foster source populations in the face of climate change. The heterogeneity field should integrate variation caused by habitat-formers in order to better understand metapopulation dynamics under a changing climate.

Heterogeneity provided by habitat-formers could increase metapopulation persistence if the focal species is able to establish in a variety of habitat types which provide different conditions. The effect of a heterogeneous composition of abiotic microclimates on population dynamics under climate change has been well established. For example, topographic variation across habitats conferred population-level resilience of plains grass (*Austrostipa aristiglumis*) to a three-year drought in southeastern Australia, such that terraces and gullies had higher seed production (Godfree et al., 2011) and could therefore act as propagule sources for marginal habitats (rescue effects; Hanski, 1999).

Heterogeneity in biogenic microclimates could also result in asynchronous population responses to climate change. For instance, a bush cricket, *Metrioptera bicolor* (Philippi), experienced increased survival in patches of tall grass relative to short grass during a severe drought, suggesting the capacity for the taller grass community to create a microclimate which mitigated the effect of drought (Kindvall,

1995). Asynchronous responses of local populations to environmental perturbations due to differences in the presence or structure of habitat-formers could buffer the extinction risk of the metapopulation (Gilpin and Hanski, 1991), particularly in the face of extreme events and mean climate change where microclimates respond at different magnitudes and thereby decrease environmental synchrony (Moran, 1953; Hansen et al., 2020). Despite this important possibility, there are few studies which test how heterogeneity provided by habitat-formers alters population dynamics under climate change.

In addition to the heterogeneity provided by biogenic microclimates promoting asynchronous population responses, population persistence will be buoyed if there are a variety of microclimates available for colonizing if habitat preferences change (Davies et al., 2006; Suggitt et al., 2012). For example, while the population dynamics of *M. bicolor* were only assessed in tall and short grass habitats, the author noted the crickets were also found at the edge of nearby pine forest during the drought, a location typically unsuitable for this species (Kindvall, 1995). Hence, heterogeneity in the types of available microclimates, which can be increased by the presence of habitat-formers, could be important for protecting populations from climate change because they buffer populations from extreme events under temporary changes in habitat preference and/or from directional climate change under long-term changes in habitat preference. While the role of local abiotic microclimates in facilitating more local, lateral climatic niche tracking under climate change has been explored theoretically (Graae et al., 2018), modeled

(Luoto and Heikkinen, 2008; Stark et al., 2022), and to a limited extent tested empirically (Suggitt et al., 2018; Virkkala et al., 2020), the role that local biogenic microclimates might play in buffering climate change is still unclear but highly plausible (Anthelme et al., 2014).

Research agenda

An enhanced understanding of how habitat-formers mitigate climate change under the umbrellas of facilitation, connectivity, and heterogeneity is made increasingly possible by an ongoing microclimate revolution. Advanced modeling techniques and technological advances are enhancing our ability to measure the conditions experienced by organisms, which are integral to their response to climate change (Lembrechts and Lenoir, 2020). Over the past decade, advances in technology, such as remote sensing (Zellweger et al., 2019) and data loggers (Wild et al., 2019)), and modeling (e.g. 'Microclimc' R package (Maclean et al., 2021b)) have improved our ability to detect biogenic microclimates and to measure the microclimate conditions at a scale relevant to even the smallest habit-formers and their associated taxa. It should be noted that we are not advocating that only biogenic microclimates be detected, but that they be included alongside abiotic microclimates.

What are the biogenic microclimates present?

To accurately measure biogenic microclimates, remote sensing products, data loggers, and mechanistic models are commonly used. Remote sensing techniques,

like Light Detection and Ranging (LiDAR), can be used to detect habitat-formers at a high resolution (e.g. 50 cm) and record structural characteristics (e.g. height, canopy density) (Lefsky et al., 2002; Schut et al., 2014), which can be used to mechanistically model sub-canopy temperatures (Lenoir et al., 2017). For a more ready-made approach, the 'Microclimc' R package can also be used to mechanistically model sub-canopy temperatures (Maclean et al., 2021b). Alternatively, arrays of microclimate data loggers (data logger examples and a discussion of how to accurately measure microclimates given here, Maclean, Frenne, et al., 2021) can be used in tandem with LiDAR and digital elevation models to statistically model microclimate conditions (e.g. Ashcroft, Gollan, Warton, & Ramp, 2012; Greiser, Meineri, Luoto, Ehrlén, & Hylander, 2018; Vanwalleghem & Meentemeyer, 2009). For smaller scale projects or where less intensive computational methods are desirable, expert knowledge could be used to identify habitat-formers of potential importance and an array of low-cost data loggers could be used to define the biogenic microclimate (Chapter IV of this dissertation).

Though detecting biogenic microclimates is an important part of the process, there is another commonly overlooked consideration—the habitat-former's viability from a longevity, accessibility, and size perspective. Where longer term effects are anticipated, it is important consider whether the habitat-former is itself either resistant or resilient to climate change. Loss or degradation of the biogenic microclimate through declines in the habitat-former (e.g. as a response to climate change or land use change) could result in sudden increases in exposure, declines in

microclimate heterogeneity, and consequences for associated taxa (Ellison et al., 2005; Thomsen et al., 2010). In addition to their resistance to climate change, the provided biogenic microclimates should be accessible and large enough to support a population (Hanski, 1999).

Do the biogenic microclimates facilitate species, increase connectivity, and/or promote meaningful heterogeneity?

We highlight some of the methods available for understanding how habitat-formers, once detected, can mitigate climate change though facilitation, connectivity, and heterogeneity. We additionally highlight areas where, though the methods exist, integration with habitat-formers could be enhanced.

High-resolution microclimate data garnered through mechanistic or statistical modeling enables researchers to model distributions of a focal species and assess the roles of habitat-formers. A determination of currently suitable habitat and future habitat (determined by overlaying species distribution models (SDM) onto future climate conditions) provides species-specific information regarding where habitat-formers currently facilitate species and where habitat-formers enhance connectivity, respectively (Lenoir et al., 2017). It is currently rare to use climate data which account for habitat-formers in SDM, but it is becoming more common as the methods become increasingly accessible to researchers (Lenoir et al., 2017). See Lembrechts et al. (2018) for a detailed review of the methods used to garner microclimate data and incorporate it into SDM. Where there are already sufficient demographic data on the focal species, these data can be used to

parameterize matrix models, and these population models could be linked to output from SDM which were run using microclimate data that included habitat-formers to understand how habitat-formers shape population viability under climate change (Keith et al., 2008; Brook et al., 2009; Franklin et al., 2014). To our knowledge, linking population models with SDM run with microclimate data which accounts for habitat-former microclimates has not been done.

Field studies are a useful way to directly assess the impacts of habitat-formers under climate change. Long-term observations and experimental manipulations have been used to assess how habitat-formers facilitate species under climate change via habitat amelioration (De Frenne et al., 2013; Anthelme et al., 2014; Bhatta and Vetaas, 2016). Seed and transplant experiments (Batllori et al., 2009; Chapter V of this dissertation), as well as correlative studies which measure seedling association (Akhalkatsi et al., 2006; Bonanomi et al., 2021), have been used to determine how habitat-formers foster upward migration. However, to our knowledge, the potential for heterogeneity created by habitat-forming species to enhance local movement under shifts in habitat preference has not yet been investigated. Demographic studies which capture climate extremes or variability, and studies with experimental manipulations, have been occasionally used to determine the role of habitat-formers in shaping metapopulation dynamics under climate change (Kindvall 1995), but should be more extensively conducted. These data could be deliberately collected to include habitats defined by habitat-formers (e.g. Godfree et al., 2011 [topographic example]; Kindvall, 1995), or more generally

across a landscape and related to the structure or presence of nearby habitat-formers via vegetation maps, compositional surveys, or products derived from multispectral aircraft imagery. To our knowledge, very little work has been done to assess how habitat-formers shape metapopulation persistence under climate change (but see Kindvall, 1995). With a common research agenda, one which incorporates the role of habitat-formers under climate change into facilitation, connectivity, and heterogeneity research, we could garner an improved understanding of how habitat-formers shape species' responses.

Conclusion

We urge researchers to consider the role that biogenic microclimates could play in shaping population responses to climate change. Because habitat-forming species, and thus their biogenic microclimates, could be lost or degraded by climate change, it is important to determine when and where their impacts could be relevant and make a concerted effort to protect those habitat-forming species. We highlight three pathways—facilitation, connectivity, and heterogeneity—through which habitat-forming species and their microclimates could buffer other organisms from climate change. The intersection between these fields and habitat-formers holds unique possibilities for climate change mitigation. We do not proffer habitat-forming species as the solution to species extinctions in the face of climate change, but as a short- to medium-term buffer while farther reaching solutions are worked

toward. We highlight the importance of further researching, protecting, and managing habitat-forming species.

CHAPTER VII

CONCLUSIONS

My dissertation demonstrates that 1) soil microbes responded directly to simulated nitrogen deposition, 2) bacterial and fungal root endophytes responded similarly to environmental drivers but that those drivers shifting with climate change, such as plant richness and density at this high alpine site, explained little variation in those communities, 3) aspect altered shrub morphology with consequences for beta diversity but not richness, 4) shrubs enhanced first year survival of a subalpine species via soil fertility suggesting its role as a stepping-stone, and 5) habitat-forming species may mitigate climate change through facilitation, enhanced connectivity, and increased heterogeneity. Together these findings suggest that plant-microbial interactions did not play a strong role in shaping microbial communities in our system, but that plant-plant, specifically habitat-forming species interactions with associated plant taxa, could be important under climate change.

This research refines predictions for the role of biotic interactions in shaping an organisms response to global change (Brooker, 2006; Classen et al., 2015). We found little evidence for the importance of plant-microbial interactions on microbial community response under global change in our system. In Chapter II, we found that only simulated nitrogen deposition increased soil nitrogen availability, rather than feedbacks from plant community composition shifts, and in Chapter II, root endosphere community composition was largely unexplained by our environmental

drivers. Hence, the effect of plant-microbe interactions on microbial response likely depends on the degree to which plants shift resource conditions for microbes relative to the direct effects of global change (Wardle et al., 2004) and the overall importance of deterministic drivers for community assembly (Nemergut et al., 2013).

Consistent with the literature, we found that plant-plant interactions, specifically those interactions with habitat-forming species, should be considered for more accurate predictions of a species or community's response to climate change (Brooker, 2006; Tylianakis et al., 2008; Bulleri et al., 2018). The importance of interactions with habitat-forming species varied somewhat across our two empirical studies where we found weaker effects of shrubs in the more protected, moist meadow area (Chapter V) compared to the more exposed and drier meadow location (Chapter IV), highlighting that the magnitude of habitat-former effects will depend on the degree to which amelioration is required and occurs. Additionally, there must be a match between those conditions which are shifted by the habitat-forming species and those conditions which shape desired outcomes, such as plot-level richness, beta diversity, germination, or survival, for the habitat-former to shape species' responses.

Overall, this thesis contributes to our understanding of the importance of biotic interactions under climate change by highlighting key nuances, including how the importance of a biotic interaction can depend on the stressors experienced by

the focal species or community and the degree to which those stressors are shifted by biotic interactions.

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APPENDIX

CHAPTER II APPENDIX

Table A2.1. Post-hoc Tukey's honest significant difference test P-values for the dominant plant removal treatment.

Dataset	Response Variable	*Geum* removal v. *Deschampsia* removal	*Geum* removal v. No removal	*Deschampsia* removal v. No removal
		P	P	P
Plants	Aboveground Biomass	0.67	**0.01**	0.09
	Forb abundance	**< 0.001**	**0.01**	**< 0.001**
	Graminoid abundance	**< 0.001**	0.06	**< 0.001**

Table A2.2. The mean relative abundance (%) of bacterial ESVs that were only detected in plots with either ambient or added N.

Phylum	Class	Order	Family	Genus	Ambient N	Added N
Verrucomicrobiota	Verrucomicrobiae	Chthoniobacterales	Chthoniobacteraceae	*Candidatus Udaeobacter*	0.11	0
Acidobacteriota	Acidobacteriae	Subgroup 2	NA	NA	0.18	0
Bacteroidota	Bacteroidia	Sphingobacteriales	Sphingobacteriaceae	*Mucilaginibacter*	0.13	0
Acidobacteriota	Acidobacteriae	Acidobacteriales	Acidobacteriaceae (Subgroup 1)	*Granulicella*	0	0.30
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Rhodanobacteraceae	*Rhodanobacter*	0	0.74
Bacteroidota	Bacteroidia	Chitinophagales	Chitinophagaceae	NA	0	0.94
Bacteroidota	Bacteroidia	Chitinophagales	Chitinophagaceae	NA	0	0.20
Gemmatimonadota	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	NA	0	0.34
Actinobacteriota	Actinobacteria	Micrococcales	Microbacteriaceae	*Salinibacterium*	0	0.21
Bacteroidota	Bacteroidia	Chitinophagales	Chitinophagaceae	NA	0	0.62
Proteobacteria	Gammaproteobacteria	Burkholderiales	Nitrosomonadaceae	*Nitrosospira*	0	0.15
Proteobacteria	Alphaproteobacteria	Micropepsales	Micropepsaceae	NA	0	0.12
Proteobacteria	Gammaproteobacteria	Burkholderiales	NA	NA	0	0.23
Actinobacteriota	Actinobacteria	Micrococcales	Microbacteriaceae	*Rathayibacter*	0	0.22
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Rhodanobacteraceae	*Dokdonella*	0	0.11
Acidobacteriota	Acidobacteriae	Acidobacteriales	Acidobacteriaceae (Subgroup 1)	*Granulicella*	0	0.11
Acidobacteriota	Acidobacteriae	Acidobacteriales	Acidobacteriaceae (Subgroup 1)	*Granulicella*	0	0.24
Acidobacteriota	Acidobacteriae	Acidobacteriales	Acidobacteriaceae (Subgroup 1)	*Granulicella*	0	0.11
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Rhodanobacteraceae	*Rhodanobacter*	0	0.11
Bacteroidota	Bacteroidia	Chitinophagales	Chitinophagaceae	NA	0	0.32
Gemmatimonadota	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	NA	0	0.22
Acidobacteriota	Acidobacteriae	Acidobacteriales	Acidobacteriaceae (Subgroup 1)	NA	0	0.17
Bacteroidota	Bacteroidia	Chitinophagales	Chitinophagaceae	NA	0	0.14

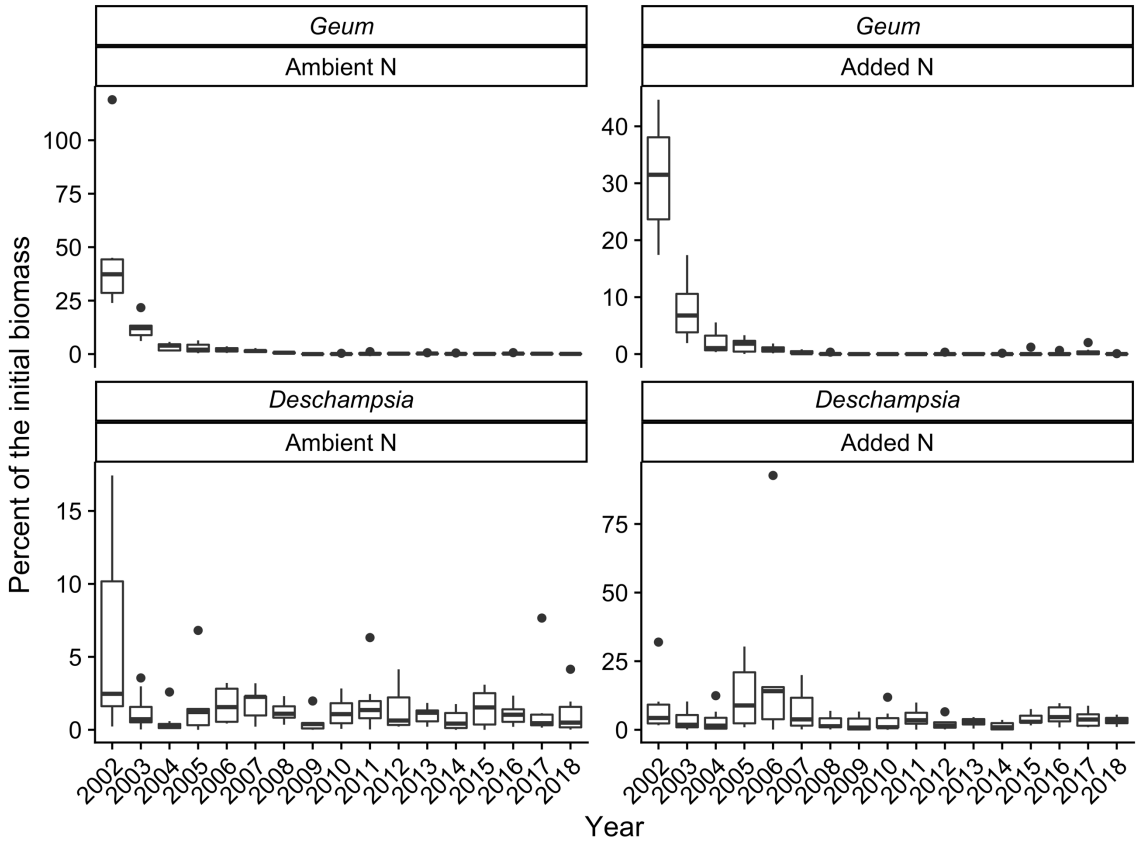


Figure A2.1. The annually clipped biomass for maintenance of the removal treatments, represented as the percentage of biomass initially removed in 2001.

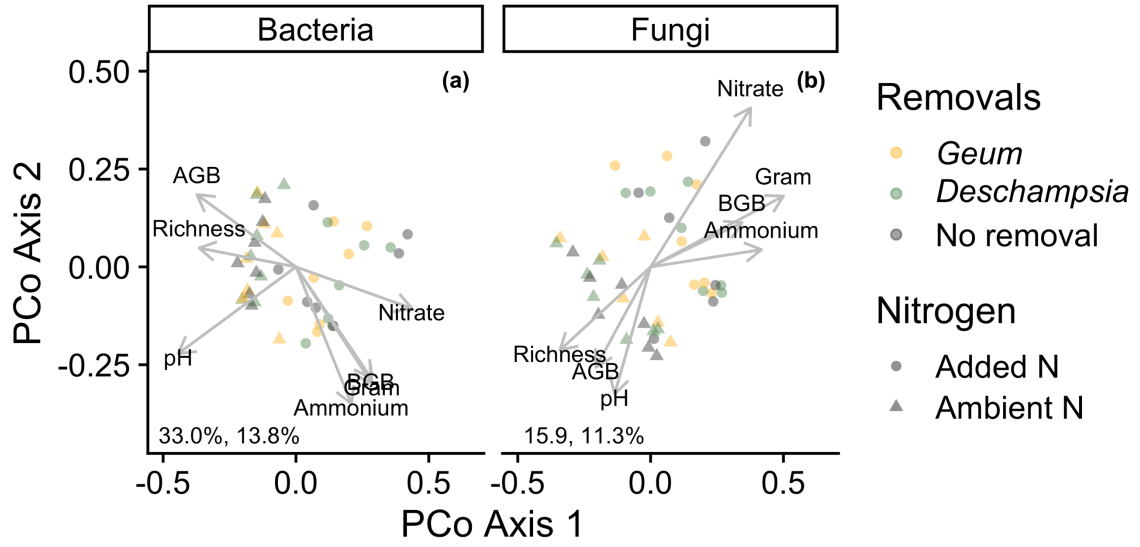


Figure A2.2. A principal coordinates analysis which shows the relationships between the continuous predictors and the bacterial (a) and fungal (b) communities. "AGB" and "BGB" represent aboveground and belowground biomass, respectively, and "Gram" signifies graminoid relative abundance.

CHAPTER III APPENDIX

Table A3.1. Standardized regression coefficients from linear models for each MEM. Significance codes: "***" represents $P < 0.001$, "**" represents $0.001 \leq P < 0.01$, and "*" represents $0.01 \leq P < 0.05$. TDN, total dissolved nitrogen; MEM, spatial eigenvectors from Moran's Eigenvector Maps

	Spatial Variables		
	MEM 1	MEM 2	MEM 4
R^2 =	0.39***	0.24***	0.58***
Plant Richness	0.34**	-0.64***	0.28*
Plant Density		0.47***	-0.39***
Mean Snow	0.40***		-0.60***
Sand (%)		0.25*	0.20*
TDN			
pH	0.46***	0.30**	

Figure A3.1. Map of all dbMEM variables where squares are plots. Black squares are positive values and white squares are negative values. The size of the squares relates to the absolute value of the dbMEM scores where larger squares are larger numbers. Scores which show the greatest numerical difference indicate the spatial scale of the dbMEM eigenvector.

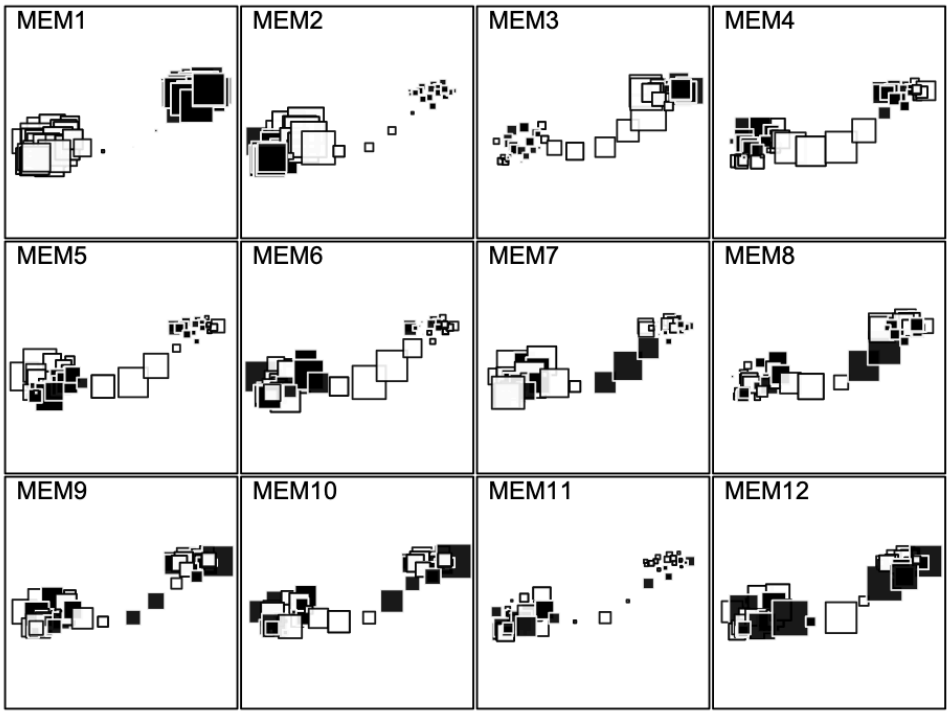


Figure A3.2. Heat map showing the relationship between host phylogeny and the PVR eigenvectors (labeled on top of the heatmap). The color gradient legend indicates numerical similarity of the PCoA axes scores from the PVR, which were scaled to have a mean of 0 and a standard deviation of 1. Axes scores which are more similar to each other are more similar in color. PVR, phylogenetic eigenvectors from Phylogenetic Eigenvector Regression.

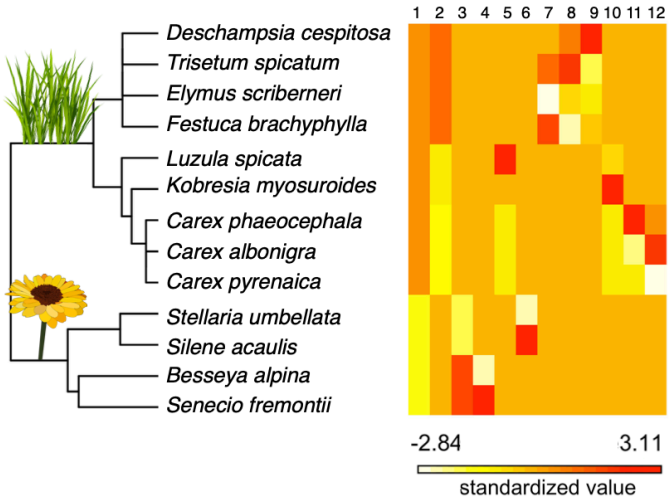
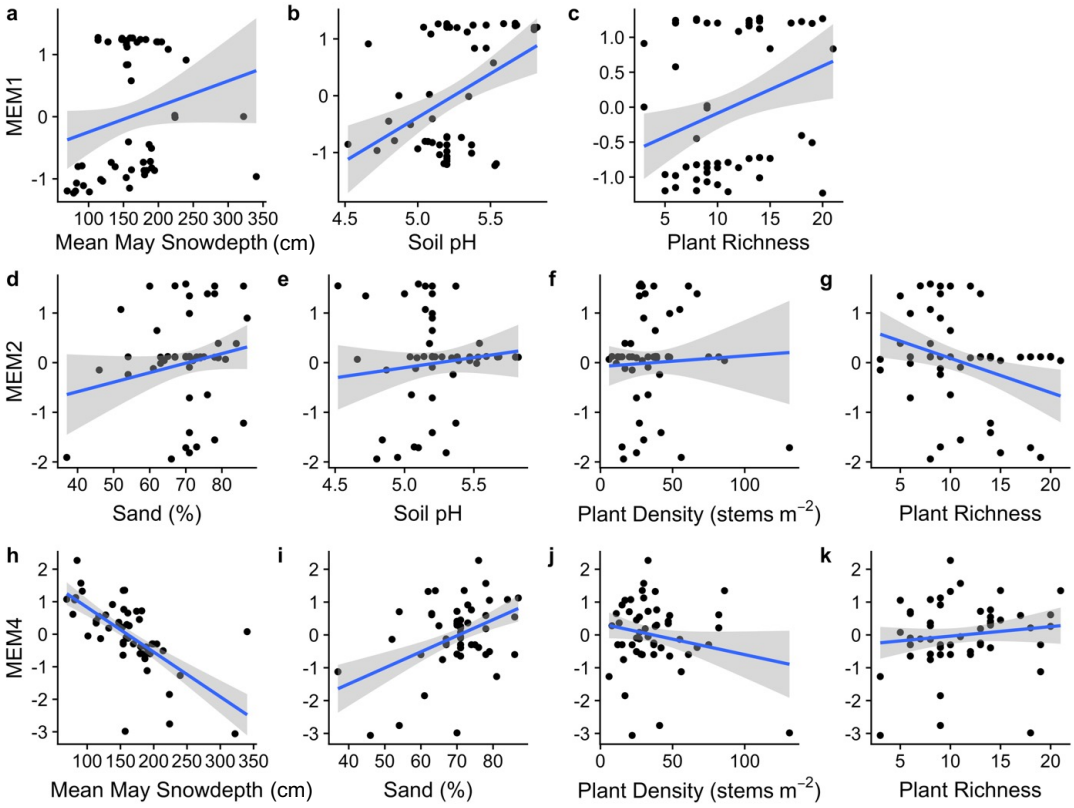


Figure A3.3. All of the selected spatial variables were related to environmental variables. MEM 1 (a - c), MEM 2 (d - g), and MEM 4 (h - k). MEM, spatial eigenvectors from Moran's Eigenvector Maps.



CHAPTER IV APPENDIX

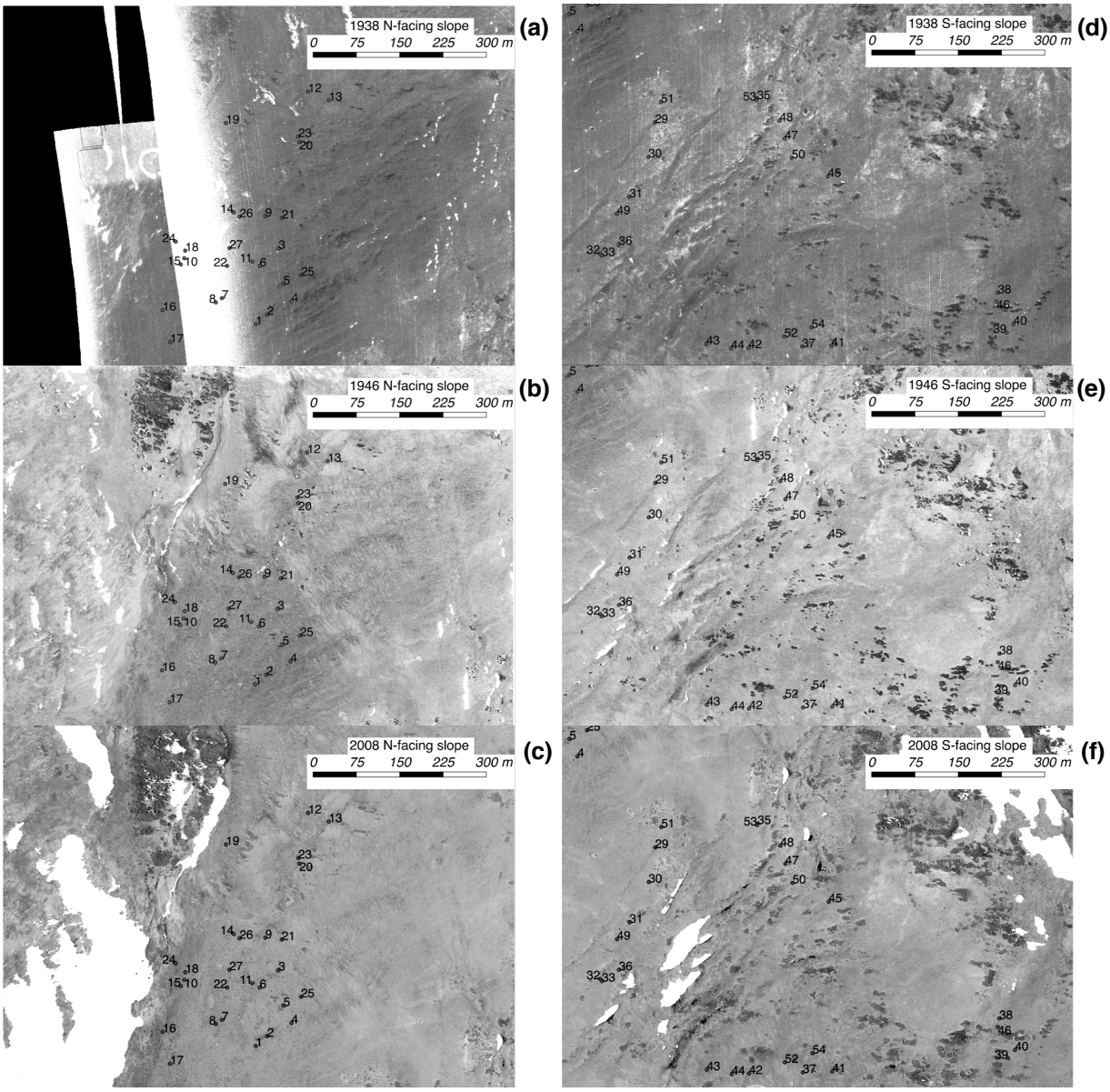


Figure A4.1. A map of the study location where all shrubs used as plot sites are denoted and numbers 1- 27 are shrubs on the N-facing slope and shrubs 28 – 54 are on the S-facing slope. These maps demonstrate that shrubs have been present across both aspects since the 1930s and have infilled over the following years. Hence, differences in shrub heights across aspect are more likely due to differences in growing conditions than age.