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ROOT ASSOCIATED MICROBES: THE MEDIATORS BETWEEN PLANTS AND SOIL

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ROOT ASSOCIATED MICROBES:
THE MEDIATORS BETWEEN PLANTS AND SOIL

by

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DISSERTATION

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DEDICATION

To my children: To Oren and the child I still hope to have. It is for you I have dedicated my studies to the Earth- to know our planet and find ways to protect it, in hopes that there might still be some beautiful and wonderful things left to share with your own children.

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ROOT ASSOCIATED MICROBES:
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ABSTRACT

Globally, increasing human populations have either caused or accelerated several types of environmental change. Symbiotic microbes have powerful effects on plant fitness, yet little study has been done on how microbial-plant relationships are affected by environmental changes. In two different ecosystems I explore how either nitrogen (N) pollution or drought can alter root associated microbe (RAM)-plant relationships using Next Generation Sequencing. In moist-meadow alpine tundra at Niwot Ridge, CO, I examine the relative contribution of host identity, N enrichment, and plant neighborhood on RAM diversity and community composition in two co-dominant plant species; *Geum rossii* and *Deschampsia cespitosa*. In New Mexican piñon-juniper woodlands, I sampled roots from a site in which mass *Pinus edulis* dieback was simulated to mimic the effects of extreme drought events, which are predicted to become more frequent as climate change progresses. I examine the effect of host and neighbor identity, as well as the effect of dead *P. edulis* neighbors, on root associated fungi (RAF) of *P. edulis* and *Juniperus monosperma*. I also compare RAF communities between piñon-juniper woodlands and more arid juniper

savanna, a good proxy for what piñon-juniper woodlands will become should these extreme drought events become more frequent.

I found that biotic assembly mechanisms (plant host and neighborhood) are important to structuring RAM communities in alpine tundra but not in piñon-juniper woodlands. In the arid southwest, abiotic factors appear to be more important in structuring RAF communities, while contrary to previous research, host identity has little effect on RAF community composition. These unusual results could be a product of the more sensitive sequencing methods, or an anomaly caused by the drought experienced by these field sites at time of sampling. In both ecosystems, plant neighborhood influenced RAM (community composition in alpine tundra, diversity in piñon-juniper woodland). In alpine tundra, both host identity and plant neighborhood mediated RAM response to N. These data emphasize the complex feedback systems between environment, plant communities, and their microbes. As a plant community becomes altered in response to the environment, the response of the microbial community to the environment will shift, making plant-microbe dynamics difficult to predict.

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CHAPTER 1

Introduction

Globally, increasing human populations have either caused or accelerated several aspects of environmental change, such as climate change, altered biogeochemical cycles, eutrophication, and anthropogenic disturbance (Vitousek *et al.* 1997). The effects of these environmental changes on vegetation are well studied (e.g. Chapin III 1996; Hansen *et al.* 2001; Clark *et al.* 2007; Kelly and Goulden 2008; Breshears *et al.* 2009), and effects on soil microbes (e.g. Young *et al.* 2004; Swaty *et al.* 2004; Edgerton-Warburton *et al.* 2007; Nemergut *et al.* 2008; Dunbar *et al.* 2012) slightly less studied. In contrast, little research has been done on how microbial-plant feedbacks are affected by environmental change (Klironomos *et al.* 2011).

Symbiotic root associated microbes can affect host fitness through mutualistic or parasitic interactions. They can improve host access to nutrients, improve drought, UV or heat resilience, produce plant growth regulators, provide pest and pathogen resistance, or be pathogenic (Klironomos 2002; Rodriguez *et al.* 2008; Badri *et al.* 2009; Porras-Alfaro and Bayman 2011). Because root microbes affect host fitness, it stands to reason that belowground root microbial dynamics could have significant and lasting effects on aboveground vegetation dynamics.

Root associated microbial communities can be structured by host identity and/or the abiotic soil environment (Hardoim *et al.* 2011). The surrounding plant community also partially determines a focal individual's microbial community (Badri *et al.* 2009; Dean *et al*. 2014a; Dean *et al.* 2014b). Therefore, changes in a

vegetation community could impact host fitness through changes to the microbial community, resulting in a feedback loop that further affects plant community composition (Klironomos 2002). This feedback loop between plant and microbial communities could make the effects of environmental changes difficult to predict.

My dissertation research focuses on characterizing microbial response to changes in the abiotic environment and the vegetation community caused by anthropogenic activities. The treatments I focus on are nitrogen (N) enrichment to explore the effects of N pollution, and plant dieback mimicking those associated with global-change type drought (drought attributed to climate change). I also look at root microbe response to plant competitors in two ecosystems where dominance between two equally dominant plant species shifts as a result of either N pollution or drought. By characterizing microbial response to abiotic change and the nearby plant community, I aimed to contribute to our understanding of plant-microbe-environment interactions, to identify correlations between plant and microbial responses, and to identify microbial taxa that could potentially be driving plant response to environmental changes. The data presented in these chapters identify the most abundant and dynamic microbes in systems undergoing chronic environmental changes. These types of data are critical for directing future experiments on specific microbe-plant interactions that can determine the nature of the relationships between individual microbe and plant taxa.

My research focuses on nitrogen (N) pollution in moist meadow alpine tundra at Niwot Ridge, Colorado (chapters 1 and 2), and global-change type drought in piñon-juniper woodlands of New Mexico (chapter 3). Both systems experience

brief, 2-3 month growing seasons, and both are co-dominated by two plant species that show divergent responses to the environmental changes critically affecting these biomes.

Chapters 1 and 2: Nitrogen deposition has been increasing for several decades at Niwot Ridge, CO due to pollution from the cities of Boulder and Denver. Currently Niwot is receiving 8kg/ha of atmospheric N deposition, which is within the critical load range for alpine, subalpine and arctic systems (critical load refers to the input amount that begins to alter vegetation or microbe composition, or other ecological functions; Bowman *et al.* 2006; Pardo *et al.* 2011).

Moist meadow alpine tundra at Niwot is co-dominated by a rose, *Geum rossii*, and a grass, *Deschampsia cespitosa*. In N enrichment plots, *G. rossii* individuals fail to return in the spring, causing a dramatic decline in the population, while *D. cespitosa* populations increase. Competitor removal experiments have shown that competition does not drive *G. rossii* response to N, as *G. rossii* populations decline in N plots even when *D. cespitosa* is removed (Suding *et al.* 2005, Suding *et al* 2008). The cause of *G. rossii* decline with N is unknown, but we hypothesized that root-associated microbial communities were implicated. We used Next Generation Sequencing to describe the fungal (Chapter 1) and bacterial (Chapter 2) communities in control, N addition and *D. cespitosa* removal treatment plots, implemented in a factorial design.

Chapter 3: Climate change is expected to cause many parts of the world, such as southwestern North America, to become hotter and drier with increased incidences of extreme drought (Leung *et al.* 2004; IPCC 2007; Christensen *et al.* 2007; Seager *et al.* 2007; Weiss *et al.* 2009). Piñon-juniper woodlands cover 19 million ha of the American southwest (Gottfried *et al.* 1995), and have been experiencing extreme drought on and off for centuries (Breshears *et al.* 2005). These woodlands are co-dominated by piñon (*Pinus edulis*) and juniper (*Juniperus monosperma*) trees. Under extreme drought, piñon dies back and juniper remains initially, causing a major restructuring of the vegetation community (Breshears *et al.* 2005; Mueller *et al.* 2005; Breshears *et al.* 2009). In 2009, 16 000 adult piñon trees were girdled in a 4ha area to simulate mass piñon mortality similar to that caused by extreme drought (Eitel *et al.* 2011). We used Next Generation Sequencing to describe the root associated fungal communities of both hosts, and how they were affected by neighbor identity, including dead piñon neighbors. We also described root associated fungal communities in the roots of juniper trees from juniper savanna. Juniper savannas are lower in elevation than piñon-juniper woodland, making them hotter and drier. Additionally no piñon grow there, making them a good proxy of what piñon-juniper woodland might become should these extreme drought events become more frequent as projected (Swaty *et al.* 2004).

This research aims to describe root microbiomes of dominant plants in sensitive ecosystems, and to examine the relative impact of biotic (host and neighbor plant identity) vs. abiotic factors on root associated microbial community

assembly in attempt to understand the effects of different types of global change on root microbes, and to identify microbial taxa that may be important players in vegetation response to environmental change to target for future research. Next Generation Sequencing techniques provide an unprecedentedly detailed view into microbiomes. The research presented here contributes to understanding how root microbial communities are structured, not just at a local, but a global scale as well. By describing the root microbiomes of these plants we are contributing to the world's microbial databases, filling in details regarding which microbes are found in which kinds of biomes. Because of the critical role root microbes play in plant health, this kind of research is critical to understanding the environmental effects of our rapidly changing planet.

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CHAPTER 2

Nitrogen deposition alters plant-fungal relationships: linking belowground dynamics to aboveground vegetation change

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ABSTRACT

Nitrogen (N) deposition rates are increasing globally due to anthropogenic activities. Plant community responses to N are often attributed to altered

competitive interactions between plants, but may also be a result of microbial responses to N, particularly root-associated fungi (RAF), which are known to affect plant fitness. In response to N, *Deschampsia cespitosa*, a co-dominant plant in the alpine tundra at Niwot Ridge (CO), increases in abundance, while *Geum rossii,* its principal competitor, declines. Importantly, *G. rossii* declines with N even in the absence of its competitor. We examined whether contrasting host responses to N are associated with altered plant-fungal symbioses, and whether the effects of N are distinct from effects of altered plant competition on RAF using 454 pyrosequencing. Host RAF communities were very distinct (only 9.4% of OTUs overlapped). N increased RAF diversity in *G. rossii,* but decreased it in *D. cespitosa*. *D. cespitosa* RAF communities were more responsive to N than *G. rossii* RAF communities, perhaps indicating a flexible microbial community aids host adaptation to nutrient enrichment. Effects of removing *D. cespitosa,* were distinct from effects of N on *G. rossii* RAF, and *D. cespitosa* presence reversed RAF diversity response to N. The most dominant *G. rossii* RAF order, Helotiales, was the most affected by N, declining from 83% to 60% of sequences, perhaps indicating a loss of mutualists under N enrichment. These results highlight the potential importance of belowground microbial dynamics in plant responses to N deposition.

INTRODUCTION

Nitrogen (N) emissions are increasing globally due to anthropogenic activities (Vitousek *et al.* 1997, Dentener *et al*. 2006), and N deposition rates in many areas of the world are more than an order of magnitude higher than they

would be in the absence of human activity (Galloway *et al*. 2008). Increased N availability can cause a cascade of effects, including alteration of ecosystem function (Carreiro *et al.* 2000, Knorr *et al.* 2005, Treseder 2008, De Vries *et al.* 2010), shifts in dominance between plant species (Pennings *et al.* 2005, Suding *et al.* 2008), reductions in plant species diversity (Strengbom *et al.* 2003, Bobbink 2004, Suding *et al.* 2005**,** Clark and Tilman 2008), and increased vulnerability of systems to invasion (Bobbink 2004, Cherwin *et al.* 2009).

Traditionally, ecologists have assumed that interspecific competition drives plant community response to N, because species with adaptations for low nutrient availability lose their competitive advantage as nutrient availability increases (Bobbink *et al.* 2010). Recent studies have challenged the traditional assumption, showing that plant community response to N is not due solely to altered plant competitive interactions (Roem and Berendse 2000, Johnson *et al.* 2003, Suding *et al.* 2005), and some studies suggest plant-microbe interactions may play a key role (Johnson *et al.* 2008, Suding *et al*. 2008).

Over the past few decades, Niwot Ridge (Colorado, USA), a Long Term Ecological Research (LTER) site, has experienced a steady increase in N deposition from the cities of Boulder and Denver (Williams *et al.* 1996, Sievering *et al.* 1996). Two co-dominant plant species, *Geum rossii* (Rosaceae) and *Deschampsia cespitosa* (Poaceae), each cover 30% of moist meadow alpine tundra at Niwot Ridge (Suding *et al.* 2008). Long term N fertilization, and *G. rossii* and *D. cespitosa* removal plots established in 2001 show that *G. rossii* declines in N plots whether or not it is in competition with *D. cespitosa*, while *D. cespitosa* abundance increases (Suding *et al.*

2008, Farrer *et al.* 2013). These findings suggest that competition is not the only driver of vegetation community response to N in alpine tundra. We suspect that fungal response to N may drive aboveground plant response.

All plants harbor root-associated fungi (RAF), defined here as any fungi within or in contact with plant roots. Mutualist RAF can increase disease resistance and abiotic stress tolerance, aid in nutrient acquisition, and/or reduce growth of targeted competitor plants (Rodriguez *et al.* 2008, Porras-Alfaro and Bayman 2011). Parasitic RAF can play a major role in plant-soil feedback processes that affect plant abundance (Klironomos 2002). Therefore, how RAF respond to N, e.g. loss or gain of mutualists or parasites, should influence host response to N.

RAF respond in a variety of ways to nutrient enrichment. N enrichment can encourage purely parasitic species (Strengbom *et al.* 2002), or mutualistic infection rates may decline (Yesmin *et al.* 1996, Treseder 2004, Morgan *et al.* 2005), or increase with a parallel increase in parasitic tendencies (Johnson *et al.* 1997**,** Upson *et al.* 2009b). Molecular studies that examine RAF response to N report changes to RAF community composition (Frey *et al*. 2004; Porras-Alfaro *et al.* 2007; Avis *et al.* 2008; Cox *et al*. 2010), but the functional meaning of these community shifts is rarely discussed. Next Generation Sequencing, which uncovers more of the microbial communities in environmental samples than traditional methods, has rarely been used to assess microbial response to N.

At Niwot Ridge, soil fungal communities shifted with N, and community shifts were accompanied by altered soil conditions, such as increased soil lignin content, and altered enzyme activity related to N cycling (Nemergut *et al.* 2008). However,

RAF communities are distinct from soil fungal communities due to the unique environment provided by the rhizosphere (Morgan *et al.* 2005, Porras-Alfaro *et al.* 2011), so may respond independently. Though RAF communities have been described in several plant hosts from Niwot (Mullen and Schmidt 1993, Schadt *et al.* 2001, Schmidt *et al.* 2008), the effect of N on RAF has not yet been examined, and the communities of the two moist meadow co-dominants remain undescribed. Because RAF can directly impact host fitness, their response to N enrichment could be critical to aboveground vegetation dynamics (Klironomos *et al.* 2011).

To determine whether RAF could be associated with plant host response to N, we used barcoded 454 sequencing to characterize the RAF community in *G. rossii* and *D. cespitosa,* and examine community response to N. We hypothesize *D. cespitosa* may benefit from N because it is able to terminate relationships with symbiotic RAF as they become less valuable under nutrient enrichment, resulting in a more flexible RAF community. We predicted that *D. cespitosa* RAF communities would respond more to N addition than *G. rossii* RAF, and that *G. rossii* would be more prone to infection by parasitic and pathogenic species than *D. cespitosa* in N plots. The identity of plant species in a focal individual's neighborhood can have a significant, though often weak, effect on the RAF community of that individual (Bahram *et al.* 2011, Bogar and Kennedy 2013). Because *D. cespitosa* increases in abundance in N plots, shifts of *G. rossii* RAF in N plots could be due to altered *D. cespitosa* abundance rather than to N itself. By removing *D. cespitosa* from some plots, we examined the effect of releasing *G. rossii* from competition on its RAF. We predicted the presence or absence of a primary plant competitor would have

minimal effect on RAF communities compared to the effect of N, and that N would have a distinct effect on RAF from *D. cespitosa* removal. Interactions between N addition and *D. cespitosa* removal would imply that the RAF communities of different host species mediate each other's responses to N enrichment.

METHODS

Field:

The study was conducted in moist meadow alpine tundra on Niwot Ridge, an LTER site located 35 km west of Boulder, CO, in the Front Range of the Rocky Mountains, elevation 3297-3544 m. Winter and summer mean temperatures are -13 °C and 8 °C. Soil is under snow pack 9 to 10 months per year (http://culter.colorado.edu/NWT/site_info/climate/climate.html). The moist meadow is composed of forbes and grasses, dominated by *G. rossii*, a rosaceous forb, and *D. cespitosa*, a tillering bunchgrass (May and Webber 1982).

Plots used for this study are a subset of those established by Suding *et al.* (2008) (coordinates between 40 03 01 N, 105 34 13 W and 40 03 38 N, 105 36 02 W). Briefly, 1x1m^2 plots were set up at seven replicate sites, between 200 and 800m apart, in 2001. We used four treatment plot types per site: N addition, *D. cespitosa* removal, *D. cespitosa* removal + N addition, and control. N has been added annually to N addition plots in the form of urea (at a maximum rate of 28.8 g N m^{-2} y^{-1}, ~40 times natural deposition rates), completely saturating soils. *D. cespitosa* has been removed annually by repeated clipping (hereafter called removal treatment).

Clipping succeeded in killing most of the *D. cespitosa* plants in the removal plots; clipped biomass of *D. cespitosa* in 2008 was only 2% of the clipped biomass in 2001.

In 2008, a *G. rossii* individual (defined by a single aboveground rosette) was uprooted at random from each treatment combination at each site. *D. cespitosa* was collected from N and control plots in a subset of sites. This resulted in a total of 28 *G. rossii* and 11 *D. cespitosa* root samples. Two *D. cespitosa* samples were later excluded from analysis due to extremely different RAF composition, indicative of contamination. Plants were sent to University of New Mexico for storage and processing.

Laboratory preparation:

Root surfaces were washed aggressively with milliQ filtered water and stored at -80 °C. Roots were not surface sterilized because we were interested in both endophytes and fungi associated with the root surface. A mix of small, medium and large healthy looking roots were selected from each plant and combined for DNA extraction. Tissue was lysed with liquid N using a mortar and pestle. DNA was extracted using DNEasy Plant MiniKit (Qiagen). *G. rossii* tissue is high in phenolics, which inhibit polymerase enzymes. To ensure a good extraction product, we checked that each sample could be successfully amplified using ITS1F-4 primers. Extracts that could not be amplified were diluted 1:10 in milliQ filtered water to dilute phenolics, which resulted in successful amplification of all samples. Extracts were sent to Research and Testing Laboratories (RTL) in Lubbock, TX for 454 titanium pyro-sequencing of the fungal ITS region, which has been identified as the

fungal barcode and has been used in multiple environmental studies for its resolution at the species level (Schoch *et al*. 2012), using ITS 1F-4 primers. Fungal libraries were created using a one step PCR with HotStar Taq master mix (Qiagen), and the following thermocycles: initial denaturation at 95°C for 5min, then 30 cycles of 95°C for 30sec, 54°C for 30sec, and 72°C for 1min, and a final extension at 72°C for 10min. Amplification products were pooled to equimolar concentrations and cleaned using Diffinity RapidTip (Diffinity Genomics), and size selected using Agencourt AMPure XP (BeckmanCoulter). Hybridizations, emPCR reactions and sequencing followed manufacturer protocols (454 Life Sciences). Samples were sequenced in 3 runs (on a single region each), each sample within a run had its own 8nt barcode.

Sequence analysis:

We used QIIME 1.7.0 (Caporaso *et al.* 2010) to remove reads with mean quality scores less than 25, and shorter than 150bp. Because current curated databases fail to encompass much of the diversity of fungal ITS sequences recovered from environmental samples, we used the *de novo* method in UCHIME as implemented in QIIME (Edgar 2010, Edgar *et al.* 2011) to identify putative chimeric ITS sequences. Each query was compared to all sequences in the sequence library to identify potential pairs of parents and chimeric 'offspring' via 3-way alignments. A total of 385 chimeras were removed from 140,561 sequences.

After chimera removal, sequences that were 97% similar to each other were clustered into operational taxonomic units (OTU) representative of distinct species

(Nilsson *et al.* 2008) using UCLUST (Edgar 2010) through QIIME. The most common sequence in each cluster was selected as the representative sequence for each OTU. Representative sequences were BLASTed (Altschul *et al.* 1990) in QIIME against the Fungal Metagenomics Project's curated ITS database (University of Alaska, Fairbanks) to assign taxonomy. Our results show that multiple OTUs hit to the same species. If a sequence does not have an identical match in the database, the BLAST method results in hits to the best match available (with e-value < 0.001), so OTUs that obtained the same taxonomy assignment are closely related to each other, but not necessarily the same species.

Data Analysis:

In all analyses we test two models. First, we exclude removal plots and test the effect of host, N addition, and their interaction on various RAF community characteristics, such as diversity, community composition, and relative abundance of individual taxa. Second, we exclude *D. cespitosa* samples and test the effect of N, removal, and their interaction on *G. rossii* RAF community characteristics. Community characteristics may change at some taxonomic levels but not others. Because ITS cannot be used to build accurate phylogenies across the Fungi, the degree of relatedness of affected OTUs is not incorporated into any of our analyses. To assess which taxonomic levels were most affected by host and treatment, we performed analyses at all taxonomic levels.

454 sequencing poorly resolves the exact length of homopolymers, which occasionally results in sequences that diverge more than 3% from the cluster to

which they belong. The resulting singletons (OTUs comprised of only one sequence) are likely to be sequencing artifacts. Because these artifacts are made without site bias, they have no effect on community composition estimates (Kuczynski *et al.* 2010). However, they result in overestimation of species richness (Reeder and Knight 2010). We therefore excluded singletons from our analyses. Doing so also excludes some true members of the rare biosphere, but we are interested in taxa that may impact overall plant fitness so are not concerned with extremely rare taxa.

Alpha diversity was calculated in QIIME v1.7.0 (Caporaso *et al.* 2010) using Simpson's diversity index, Simpson's evenness, and taxonomic richness. There was large variation in sequencing depth among samples. To control biasing effects of sequencing depth on alpha diversity measures, we rarefied samples by sub-sampling to the depth of the most shallowly sequenced sample (557 sequences). Rarefaction curves were not saturated at this depth (Fig. S.F1, Supplementary Material). Alpha diversity measures were calculated on each of 100 rarefactions, and averaged. Type III ANOVA (package nlme, Pinheiro *et al*. 2011, in R, R Development Core Team 2011) was used to analyze effect of host and treatments on RAF alpha diversity.

Redundancy analysis (RDA) and Monte Carlo permutation tests based on Euclidean distances of unrarefied data normalized by sample were used to test treatment effects on RAF community composition (vegan package, Oksanen *et al*. 2011; package nlme, Pinheiro *et al*. 2011). One thousand permutations were used to obtain pseudo-F and P statistics. OTUs present in fewer than three plots were removed from analyses, as we were primarily interested in members of the

community that are ubiquitous and whose presence or absence could be responsible for *G. rossii* decline across N plots (McCune *et al.* 2002). Euclidean distances were chosen because shared absences reduce distance between communities. This is a useful approach to address hypotheses concerning species disappearance as well as invasion with disturbance (Anderson *et al.* 2011). Our hypothesis is that loss or gain of RAF species could be responsible for *G. rossii* response to N, and thus shared losses should be counted as important. Data were log transformed to improve signal from less abundant OTUs in community distance calculations, and site was included as a cofactor in all analyses. SIMPER (PRIMER v6) was used to identify which species contributed most to pairwise distances between host and treatment combinations.

SIMPER can confound mean group distances with within group variability, which causes it to sometimes identify the most variable species rather than the taxa that contribute most to community distances (Warton *et al.* 2012). Additionally, low abundance taxa that contribute little to community distances may be important if they are responsible for disease in the host. We therefore used type III ANOVA (package nlme, Pinheiro *et al*. 2011) on rarefied data to verify SIMPER results and to detect less dominant RAF taxa that shift significantly with host and/or treatment. Taxa in fewer than 3 plots were removed from the analysis to focus on ubiquitous taxa whose presence or absence could be responsible for *G. rossii* decline, and because two replicates are too few to make statistical comparisons. Due to the large number of comparisons, a false discovery rate (FDR, Yoav and Yosef 1995) correction was applied to P-values. Only one taxa was correlated with N

amendments after FDR correction, so we also discuss uncorrected results. Overall communities significantly differed between host and treatment plots, suggesting more than a single OTU is affected by N. Our statistical power may be weak given the small number of replicates (n=7 at most per treatment), and disregarding raw P-values likely results in discarding many true positives. Several OTUs with identical database hits were significantly correlated with N in the same direction when FDR was not applied, strengthening support for the effect of N on those related taxa.

RESULTS

Host RAF communities

After filtering and chimera checking, a total of 104,668 sequences were obtained. 103,169 sequences were from *G. rossii* roots constituting 1,499 OTUs (averaging 3,685 sequences and 118 OTUs/sample; SE = 403.46 and 6.33 respectively) and 19,210 sequences from *D. cespitosa* making up 479 OTUs (averaging 2,134 sequences and 89 OTUs/sample; SE = 279.36 and 8.54 respectively). Only 9.4% of the 785 OTUs found in control plots were shared between hosts.

In control plots, both hosts were dominated by Ascomycota (95% in *G. rossii,* 82% in *D. cespitosa*). In *G. rossii* the next dominant phylum was Basidiomycota (4%), followed by Glomeromycota (1%) and fungi with no BLAST hits or hits to unclassified fungi (1%). *D. cespitosa* roots obtained more hits to unclassified fungi (12%) than Basidiomycota (6%).

G. *rossii* roots were strongly dominated by fungi in the order Helotiales (83%), and more than a third of sequences were unidentified Helotiales. The most abundant orders in *D. cespitosa*, were: Helotiales (30%), Mortierellales (16%), Pleosporales (15%), Hypocreales (7%), and Agaricales (6%). Another 9% of the communities were comprised of unknown orders in Ascomycota. We compared alpha diversity measures between hosts from control plots only. *D. cespitosa* communities were more diverse than those from *G. rossii*, mainly due to greater order evenness, and a trend towards greater richness (Table 1, Fig. 1).

Treatment effects on RAF alpha diversity

There were marginal and significant interactions between host and N on Simpson's diversity from family through phylum, in which N decreased or did not change diversity of *D. cespitosa* RAF, but increased diversity of *G. rossii* RAF. N also tended to decrease richness in *D. cespitosa* but increased richness in *G. rossii* (Table 1).

When the effects of N addition and removal were examined on *G. rossii*, removal did not have a significant effect on alpha diversity on its own, but there were marginal and significant interactions between treatments on taxonomic richness at most taxonomic levels, in which N increased richness in the presence of *D. cespitosa* but decreased it in the competitor's absence. Simpson's diversity at the class and order level also exhibited interactive effects: N increased diversity in the presence of *D. cespitosa*, but decreased diversity in its absence. Overall, N and

removal treatments had the most significant effects on family through class alpha diversity (Table 2).

Treatment effects on RAF community composition

G. rossii and *D. cespitosa* RAF community composition were significantly different at all taxonomic levels (as shown by RDA, Fig. 2a, Table 3). This effect was strongest when communities were described by coarser taxonomic groupings such as phylum, where 35.35% of community variation was explained by host (pseudo-F = 11.69, P_{MC} = 0.001). There were marginal or significant interactions between host and N at all taxonomic levels: N addition caused greater shifts in *D. cespitosa* than in *G. rossii* community composition (Fig. 2a, Table 3). These patterns were consistent when using NMDS plots and permutation of Bray-Curtis distances to identify between group variation (vegan package in R, Oksanen *et al*. 2011) (S.F1, S.T1 Supplementary Material). According to SIMPER analyses, the OTUs that contributed most to N-induced community shifts in *G. rossii* were related to *Articulospora tetracladia* (increased), several *Lachnum spp.* (decreased), *Helotiales spp.* (decreased), *Phialocephala spp.* (increased), and a *Phialophora spp.* (increased) (S.T2 Supplementary material). Most OTUs belonged to the order Helotiales. The OTUs that contributed most to community shifts with N in *D. cespitosa* were identified as *Microdochium spp.* (increased), *Geomyces spp.* (increased), and *Herpotrichia juniperi* (decreased) (S.T2 Supplementary material).

When both treatments were considered, we found N affected *G. rossii* communities at finer taxonomic levels (species through order), and explained up to

10.34% of community variation (at the genus level). Removal also had an effect on community composition, but only at the species and genus level (in the latter it explained 5.62% of community variation, Table 3, Fig. 2b). Significant interactions between treatments were found on Bray Curtis distances at taxonomic levels family, order and class but not on Euclidean distances at any taxonomic level (S.T1). Species that contributed most to community shifts with N addition according to SIMPER were again, *Articulospora tetracladia*, several *Lachnum,* Helotiales and *Phialocephala spp*, and a *Phialophora spp*. Species that contributed most to community shifts with removal also belonged to *Articulospora tetracladia, Lachnum* and unidentified Helotiales *spp*, and *Phialocephala europa* (S.T3 in Supplementary material).

Treatment effects on individual RAF taxa

ANOVA found only 4 OTUs to be significantly affected by N in *G. rossii* and 4 to be significantly affected by N in *D. cespitosa* (Table 4). In *G. rossii* these were identified as close relatives of a *Rhizoscyphus ericae* aggregate spp., *Meliniomyces bicolor*, and two unidentified Helotiales. All declined with N except one unidentified Helotiales. In *D. cespitosa*, affected species belonged to the genera *Geomyces* (positively affected by N), *Gyoerffyella*, *Gibberella*, and *Mortierella* (negatively affected). All *G. rossii* OTUs that responded belonged to the order Helotiales. When tested, the order Helotiales as a whole declined significantly with N, from 83% to 60% of community composition. This was also the only taxonomic group to shift significantly with N after FDR correction. No OTUs related to known pathogens

responded significantly to N in *G. rossii*, and one putative pathogen responded negatively to N in *D. cespitosa* (Table 4).

When the effects of both treatments on *G. rossii* RAF were examined, many more taxa were found to be significantly affected by N, likely due to inclusion of more N vs no N replicates crossed with removal (Table 5). Most taxa affected were also identified as important using SIMPER (Table S.T3). Several putative saprobes increased with N, but these were of low abundance and were not found to be important to community shifts using SIMPER. Dark septate endophytes (DSE) increased with N, whereas several species identified as ericoid mycorrhizal fungi (ERM) decreased with N. Eight OTUs were affected by the interaction between N and removal, responding to N differently depending on the presence or absence of *D. cespitosa*. These were mostly unknown Helotiales or *Lachnum spp*, one was related to a common soil fungi in cold soils, one to an ERM, and one to a potential pathogen.

A number of OTUs related to known ERM decreased with removal. Other than this, no consistent trend could be found between ecological function and removal. Though several OTUs affected by removal had BLAST hits to the same genus or species as those affected by N, several genera were affected by only one treatment type.

DISCUSSION

Here we report substantial host-specific effects of N enrichment on RAF, suggesting that RAF communities may be important drivers of response to N in two dominant alpine tundra plants.

Plant species host different RAF communities

There were substantial differences between the RAF communities of the two hosts under natural conditions, perhaps accounting for their divergent responses to N. *G. rossii*'s RAF community was strongly dominated by the order Helotiales. Helotiales are common root fungi, and include many ERM and DSE (Zijlstra *et al.* 2005, Newsham 2009, Tedersoo *et al.* 2009). ERM are common mutualists in nutrient poor soils (Read 1996), and can provide hosts with resistance to plant-produced phytotoxic tannins and other environmental stresses (Cairney and Ashford 2002, Cairney and Meharg 2003). DSE from the Helotiales are common mutualists in cold-stressed habitats, particularly of Rosaceae plants (Upson *et al.* 2009a, Newsham *et al.* 2009, Newsham 2011). ERM and DSE have both been shown to harvest and provide their hosts with nutrients immobilized in organic matter (which is high in cold soils due to slow decomposition rates) (Read 1996, Caldwell *et al.* 2000**,** Upson *et al.* 2009a).

In this study, many of *G. rossii*'s most abundant OTUs match known DSE and ERM, and more are unidentified Helotiales. Inoculation experiments deducing the function of unidentified Helotiales root isolates from cold or heathland soils suggest many are mutualists, especially when supplied with an organic N source, and are

likely DSE or ERM (Zijlstra *et al.* 2005, Upson *et al.* 2009b, Newsham 2011). A large abundance of this taxonomic group might suggest *G. rossii* RAF specialize in harvesting immobilized nutrients from these high organic matter soils, and/or influence *G. rossii* tolerance to environmental stresses associated with cold climates. Interestingly, *G. rossii* immobilizes N in the biomass of associated microbes and phenolic exudates (Bowman *et al.* 2004, Meier *et al.* 2009). Perhaps *G. rossii*'s RAF community improves uptake of these sequestered nutrients.

D. cespitosa RAF were more diverse than those of *G. rossii*. *D. cespitosa* covers a much broader geographic and environmental range than *G. rossii* (biodiversity occurrence data accessed through GBIF data portal, data.gbif.org), which may be facilitated by its diversified symbiont community. Diverse RAF may provide versatility via resistance to a wider variety of stresses, and access to nutrients from a wider variety of sources.

That the RAF communities of the two hosts differ is not surprising, as other research has found unique fungal communities associated with different host species residing in the same habitat (Upson *et al.* 2009a). More interesting is that the RAF communities from the two hosts differed in their response to N, perhaps due to differences in RAF communities under ambient conditions.

N and plant competition differentially impact RAF communities in two co-dominant plant hosts

N had opposing effects on the RAF from the two hosts, increasing Simpson's diversity in *G. rossii* but decreasing or not changing it in *D. cespitosa*. *D. cespitosa* lost

RAF taxonomic richness under N, while *G. rossii* gained richness. N also caused a massive reduction in the dominant order, Helotiales, in *G. rossii*, contributing to the positive effect of N on *G. rossii* diversity. These results contrast with other studies, which have shown a decrease in fungal biodiversity under elevated N (Frey *et al.* 2004, Lilleskov *et al.* 2008, Avis *et al.* 2008). However, these studies have primarily examined aboveground fruiting bodies rather than DNA from root tissue. Observed aboveground fungal diversity may not correlate with belowground diversity (Gardes and Bruns 1996). Porras-Alfaro *et al.* (2007) found N increased arbuscular mycorrhizal fungal (AMF) diversity using molecular methods, and also attributed this to loss of the dominant AMF species.

When *D. cespitosa* was removed, the alpha diversity trends in *G. rossii* were reversed. The ecological or biological implications of these interactions are difficult to determine. As far as we know, no other study has described such interactions between N and neighbor identity on RAF diversity. These data show that presence of certain plant species can influence the effect of N on a focal individual's RAF diversity.

It has been shown that initial evenness in microbial communities provides resilience and preserves functional stability in the face of environmental stress, because uneven communities depend heavily upon the functional role of the most dominant taxa, which may decline without replacement (Wittebolle *et al.* 2009). Thus the extreme dominance and potential functional importance of Helotiales in *G. rossii* may make *G. rossii* RAF vulnerable to functional disturbance. *D. cespitosa* RAF

diversity may provide functional stability in the face of environmental change due to greater functional redundancy.

D. cespitosa communities were more sensitive to N than were *G. rossii* communities. Perhaps this flexible RAF response contributes to *D. cespitosa*'s ability to adapt to N enrichment. Research suggests hosts that reduce or eliminate infection by mutualists under nutrient elevation avoid parasitism, because hosts that cannot control mutualist infection rates run the risk of being parasitized by their once mutualists (Johnson and Oelmüller 2009). Reduction of mutualists could also explain loss of *D. cespitosa* RAF diversity under N.

That the effect of N was stronger and caused shifts across more distantly related species in comparison with removal, suggests N has an effect on *G. rossii* RAF communities that is distinct from the effect of competitor presence. Additionally, RAF communities under removal treatment diverged from communities under N addition. Significant interactions between treatments were found on Bray Curtis (Table S.T1) but not Euclidean distances (Table 3) between RAF communities, but treatment had similar patterns of effect in both ordinations (data not shown). Shared absences reduce Euclidean distances but are not included in Bray Curtis distances, so these results may indicate that there was a stronger interactive effect on relative abundance of present taxa rather than shared losses.

Many of the OTUs responsible for community shifts with N belonged to the same genera as those responsible for shifts with removal, indicating these genera are generally sensitive to disturbance, but the two treatments sometimes elicited

opposite responses from these sensitive genera, and some genera were affected by only one of the treatments.

Description of RAF taxa affected by N

Most taxa affected by N were putative mutualists and commensals. Many *G. rossii* OTUs that significantly declined with N were assigned to the *Rhizoscyphus ericae* aggregate and one of its sub-clades, *Meliniomyces bicolor* (Hambleton and Sigler 2005). The *Rhizoscyphus ericae* aggregate are mainly ERM (Grelet *et al.* 2009). Yesmin *et al.* (1996) also found N reduced ERM infection rates in a greenhouse experiment. A couple putative DSE from *G. rossii* roots increased with N. Upson *et al.* (2009b) found that DSE in the Helotiales behave as mutualists when supplied with an organic N source, but could become parasitic when supplied with an inorganic N source. Most OTUs affected belonged to the order Helotiales. Indeed, the order Helotiales as a whole declined dramatically with N in *G. rossii* roots. Because Helotiales from roots in cold climates seem important to N uptake (Caldwell *et al.* 2000, Upson *et al.* 2009b, Newsham 2011), it is perhaps not surprising that this group is highly sensitive to inorganic N enrichment. Interestingly, few putative saprobic taxa responded to N, suggesting that the plant species turnover that occurs in N fertilized plots does not trigger increases in RAF decomposers. Despite some taxonomic overlap, several RAF species responded only to N or to removal, again highlighting the distinct effects of N vs. competitor presence.

D. cespitosa OTUs found to be affected by N did not share a common phylogeny or known ecological function. The order Mortierellales declined with N,

which contains mostly saprobes, and includes many genera with the ability of complex organic substrate transformations (Wagner *et al.* 2013), but members of this order were rare.

RAF and host response to N

G. rossii are asymptomatic in N addition plots (Farrer, *pers. comm.*), they simply do not return after a 4 year lag from the start of N addition (Suding *et al.* 2008). Parasites may be depleting C resources required for overwintering. There is substantial evidence that *G. rossii* plants are C limited in N plots, having very reduced C:N ratios, lower nonstructural carbohydrate levels in rhizomes, and fewer preformed leaves (necessary for resprouting in the spring) compared to those in unfertilized conditions (Farrer *et al.* 2013). However, Farrer *et al.* (2013) also shows that parasitism is not occurring in the summer. Schadt *et al.* (2003) found that microbial activity peaks in winter in Niwot alpine tundra soils, and that most of winter microbial biomass is fungal. These fungi would require substantial C sources during the 9-month dormant season when plants are not photosynthesizing.

DSE from the Helotiales can become parasitic when supplied with inorganic N (Upson *et al.* 2009b). *G. rossii* DSE may parasitize their host as inorganic N rises. If this were true, however, we would expect Helotiales relative abundance to increase rather than decline in N plots. Some OTUs related to known DSE did increase with N, but given that other related groups primarily declined, evidence for parasitism is weak. One potentially pathogenic genus increased with N, *Papulaspora spp*. Species in this genus can be either plant or fungal pathogens, making its role in *G. rossii*

fitness unclear here. Winter sampling and fungal quantification methods may provide different insights in this regard.

Members of the Helotiales could also simply be beneficial to *G. rossii*, and their lessened dominance corresponds to reduced *G. rossii* fitness due to loss of associated benefits, such as access to organically bound nutrients (Michelsen *et al.* 1996, Caldwell *et al.* 2000), and resistance to phytotoxic tannins and other harsh environmental conditions (Cairney and Ashford 2002, Cairney and Meharg 2003), services provided by DSE and ERM. This hypothesis is bolstered by Schmidt *et al.* (2004) findings that N amendments selected against microbes that break down phenolics and complex organic matter at Niwot Ridge. We found that putative ERM declined with N. Greenhouse experiments are needed to further assess the role of Helotiales *spp.* in host fitness, but given the extreme dominance of this order in *G. rossii*, and research on similar taxa in similar ecosystems, their presence likely influences *G. rossii* fitness, and they are likely involved in N uptake and/or tolerance to stress associated with cold ecosystems.

Summary

Few studies have employed DNA sequencing to examine the effect of N on RAF community composition. Culture and microscopy based techniques have shown repeatedly that soil fertility drives fungal symbiont abundance, richness, and community composition (Peter *et al.* 2000, Frey *et al.* 2004, Edgerton-Warburton *et al.* 2007, Lilleskov *et al.* 2008, Avis *et al.* 2008, Cox *et al.* 2010). We compared RAF

response to altered plant competition and to N to assess whether similar taxa were affected by both.

We found that N affects RAF differently than does altering competitor abundance. We show for the first time that a host plant that thrives under N enrichment harbors a diverse fungal community that is highly responsive to N relative to the fungal community of a host plant that responds negatively to N. Perhaps a flexible RAF community is key to adapting to nutrient enrichment. We confirm that Helotiales are dominant root symbionts in cold soils, and find they are particularly abundant and sensitive to N in a host plant that is negatively impacted by N enrichment, but less abundant and sensitive to N in a plant that is unaffected by N enrichment. RAF are known to affect plant fitness, so these N induced shifts in RAF community could affect plant fitness. If they do, belowground microbial dynamics are implicated in aboveground plant response to abiotic change. Future research should be directed to better describing these alpine RAF and their interactions with alpine vegetation.

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Data Accessibility: Raw DNA sequences, R code, and OTU tables and other R input files are available on Dryad (doi:10.5061/dryad.sv33f). FASTA and QUAL files can be found at SRA under the study accession SRP049180.

Author Contributions: S.L. Dean conducted laboratory work, bioinformatics, statistical analyses, and wrote the paper. A. Porras-Alfaro did initial sample processing. E.C. Farrer conducted field work and helped with the statistical analyses.. D.L. Taylor contributed to bioinformatics.. A. Porras-Alfaro, K.N. Suding and R.L. Sinsabaugh developed project design and acquired funding. All authors edited and participated in discussions related with this manuscript.

Figures and Tables

Figure 1: Order profiles of the two hosts from control plots.

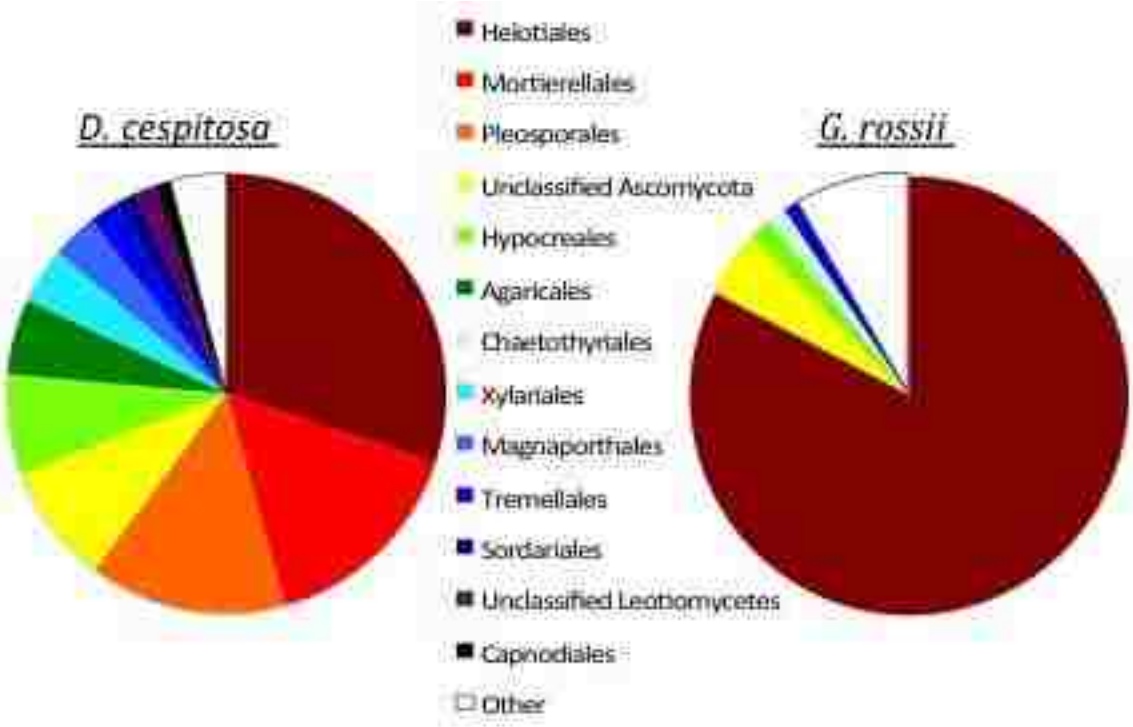


Figure 2: RDA plots of host and treatment effects on RAF communities at the genus level, the taxonomic level at which effects were strongest. **2a:** Axes are constrained by host and N addition. Diamonds indicate N plots, circles indicate no N plots. Dark symbols indicate *D. cespitosa*, open circles indicate *G. rossii*. Significant interaction (pseudo-F = 2.4081, Monte Carlo permutation test P_{MC} = 0.009). **2b:** Axes are constrained by N and removal. Diamonds indicate N plots, circles indicate no N plots. Dark symbols indicate *D. cespitosa* removal plots, open circles received no removal.

Figure 2a

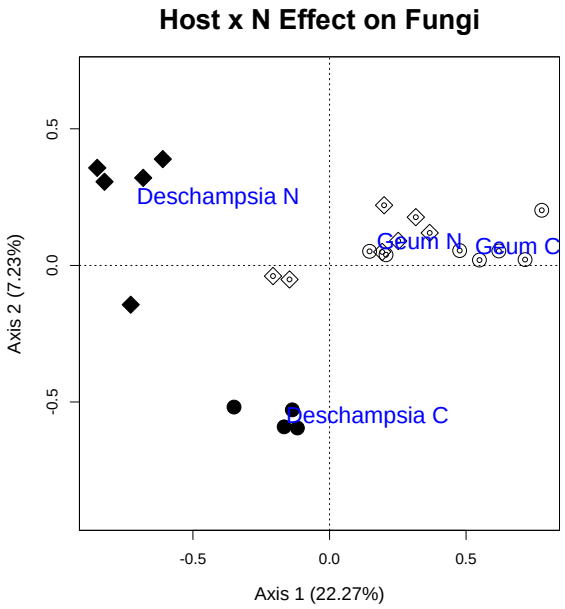
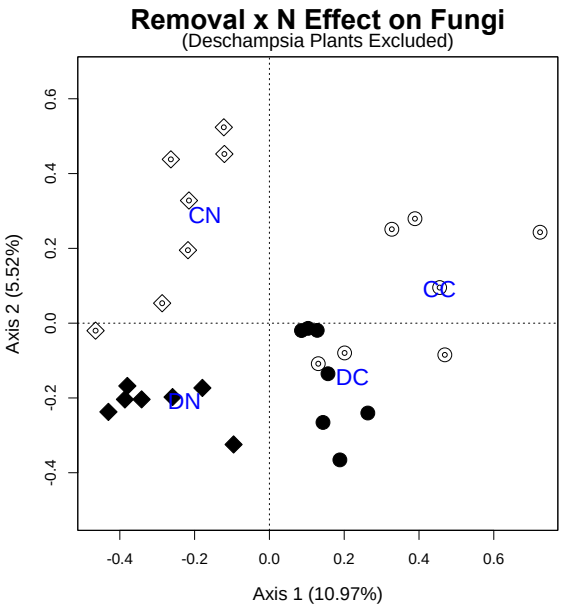
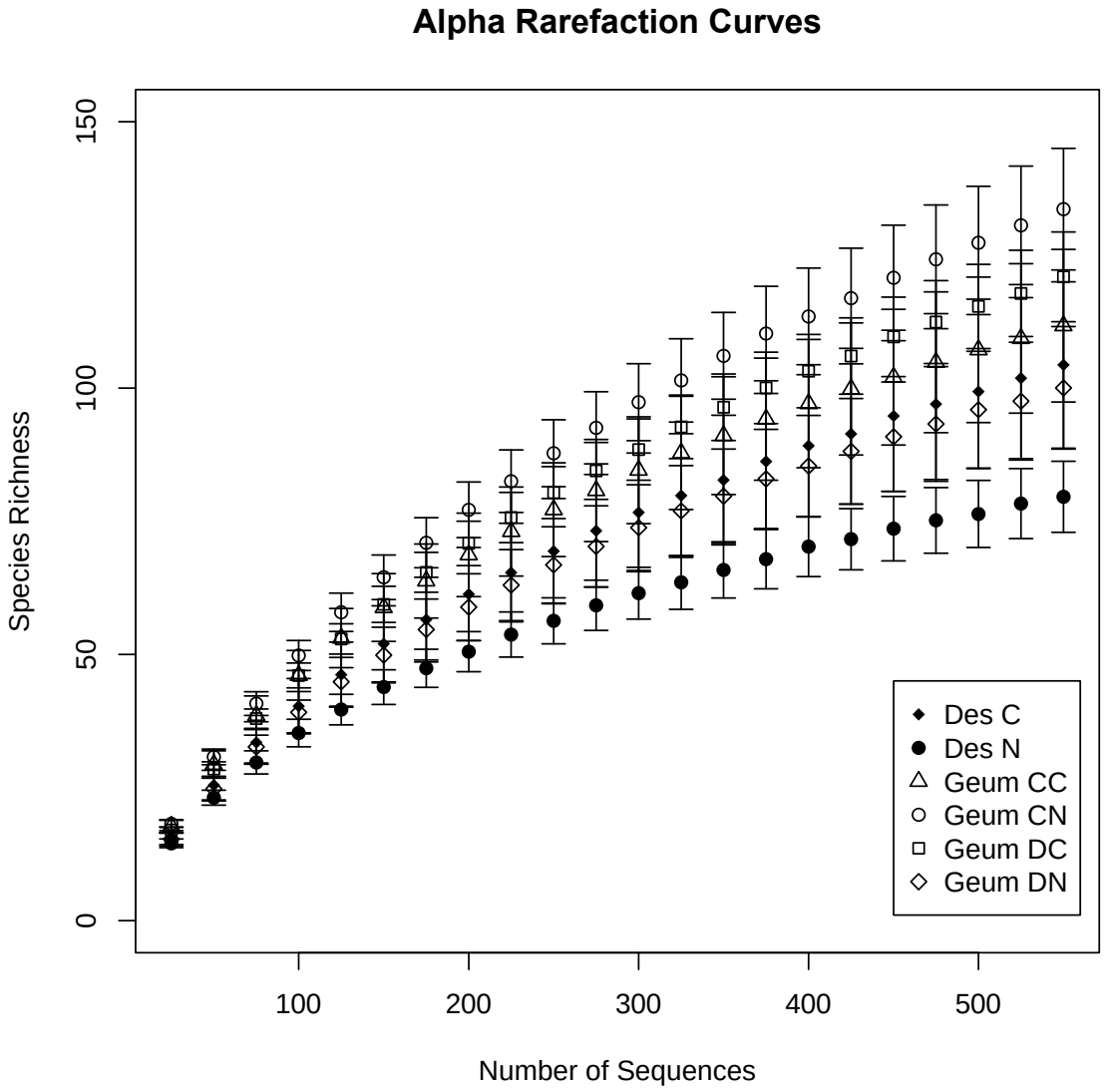


Figure 2b



SF1: Alpha rarefaction curves showing species richness for all host x treatment groups. All samples were rarefied to 557 sequences (the size of the smallest sample). To build rarefaction curves, each sample was rarefied 100 times at each rarefaction depth (25 sequences to 550 sequences with a step size of 25). Mean species richness was calculated for each sample at each sampling depth. These means were used to calculate mean and standard error of richness within each host x treatment group. Bars indicate standard error.



SF2: NMDS plot of Bray-Curtis distances between RAF communities from different hosts and treatments. Communities are described at the genus level to correspond to information in RDA plots. Large symbols indicate *D. cespitosa* communities, small symbols *G. rossii* communities. Diamonds indicate N plots, circles indicate no N. Dark symbols indicate removal, open circles received no removal treatment. Dispersion ellipses are drawn using standard deviation of point scores.

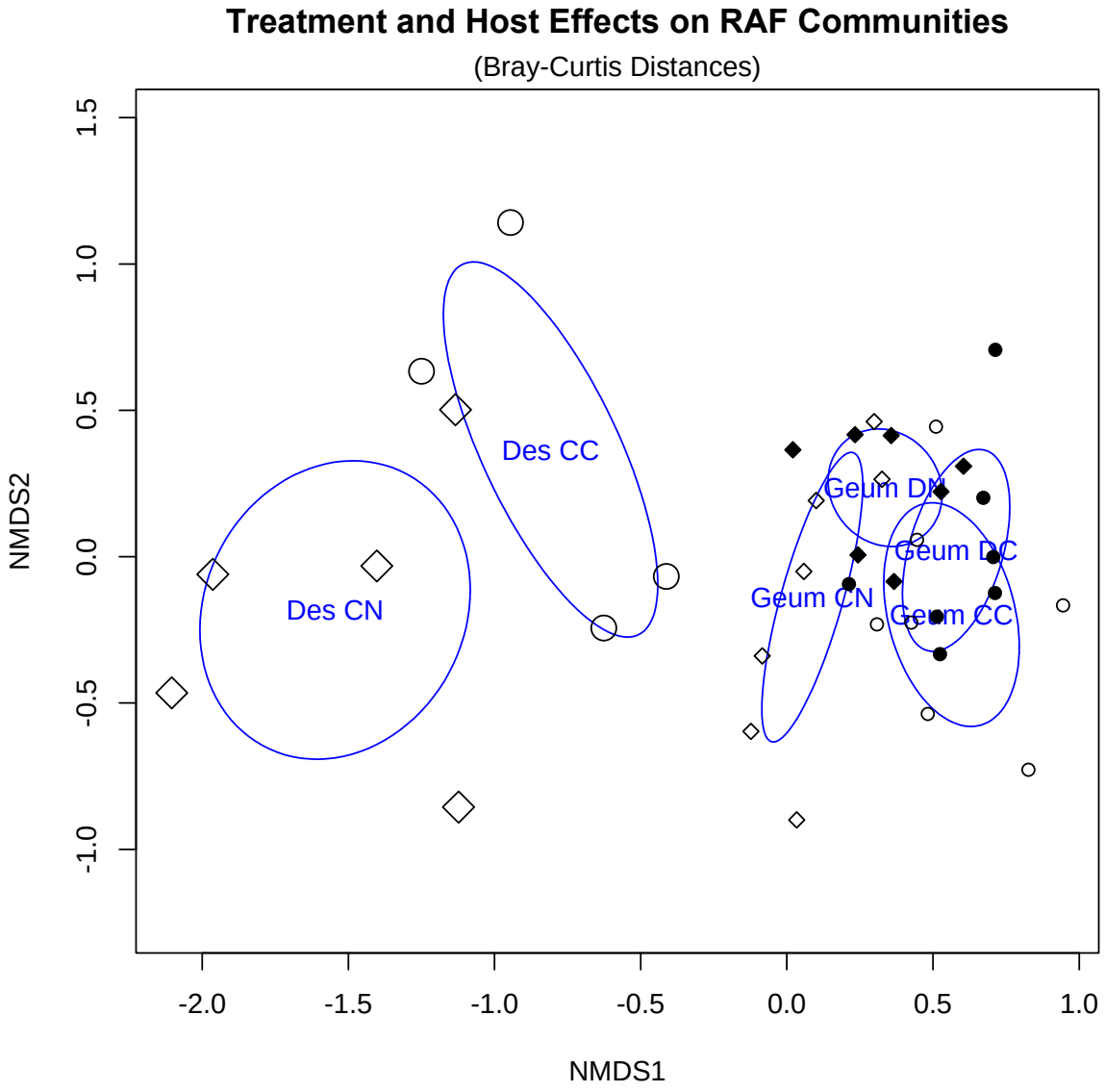


Table 1: Effect of treatment on alpha diversity measures of RAF in *G. rossii* and *D. cespitosa* at each taxonomic level. Diversity measure means for each host x treatment combination, as well as P-values for effect of each treatment and their interaction on alpha diversity, are displayed. Removal plots are excluded from this analysis. DesC = *D. cespitosa* control, DesN = *D. cespitosa* N addition, GeumC = *G. rossii* control, GeumN = *G. rossii* N addition. Marginal and significant effects are italicized, significant effects include an asterisk.

	Means				**Model: host + N + host*N**			**Model: host (control plots only)**
OUT	**DesC**	**DesN**	**GeumC**	**GeumN**	**P(Host)**	**P(N)**	**P(interaction)**	**P(host)**
Simpson's	0.93	0.89	0.94	0.96	*0.05**	0.62	0.23	0.56
Evenness	0.15	0.15	0.26	0.20	*0.06*	0.43	0.45	0.17
Richness	105.30	79.66	112.03	134.45	*0.03**	0.90	*0.08*	0.77
Genus								
Simpson's	0.88	0.80	0.82	0.82	0.46	0.18	0.13	*0.06*
Evenness	0.26	0.22	0.24	0.20	0.52	0.21	0.90	0.61
Richness	34.62	28.18	26.52	33.20	0.68	0.97	*0.09*	0.13
Family								
Simpson's	0.84	0.79	0.69	0.76	*<0.01**	0.84	*0.06*	*<0.01**
Evenness	0.27	0.24	0.21	0.20	*0.08*	0.59	0.82	0.19
Richness	25.72	23.28	18.34	23.26	0.18	0.64	0.18	*0.07*
Order								
Simpson's	0.77	0.78	0.32	0.56	*<0.01**	*<0.01**	*<0.01**	*<0.01**
Evenness	0.29	0.34	0.12	0.15	*<0.01**	0.39	0.89	*<0.01**
Richness	17.39	15.30	13.87	16.79	0.56	0.81	0.16	0.21
Class								
Simpson's	0.73	0.68	0.30	0.49	*<0.01**	0.11	*0.01**	*<0.01**
Evenness	0.42	0.45	0.15	0.2	*<0.01**	0.45	0.82	*0.002**
Richness	9.72	7.56	9.85	10.36	0.12	0.37	0.15	0.92
Phylum								
Simpson's	0.38	0.21	0.08	0.13	*<0.01**	0.25	*0.05**	*<0.01**
Evenness	0.42	0.41	0.34	0.36	0.16	0.92	0.66	0.25
Richness	4.07	3.24	3.45	3.50	0.58	0.23	0.18	0.29

Table 2: Effect of treatment on alpha diversity measures of RAF in *G. rossii* at each taxonomic level. Diversity measure means for each host x treatment combination, as well as P-values for effect of each treatment and their interaction on alpha diversity, are displayed. *D. cespitosa* is excluded from this analysis. CC = control, CN = N addition, DC = *D. cespitosa* removal, DN = *D. cespitosa* removal + N addition. Marginal and significant effects are italicized, significant effects include an asterisk.

	Means				**Model: N + removal + N*removal**		
OUT	**CC**	**CN**	**DC**	**DN**	**P(N)**	**P(removal)**	**P(interaction)**
Simpson's	0.94	0.96	0.94	0.90	0.43	0.12	0.11
Evenness	0.26	0.20	0.16	0.15	0.22	*0.02**	0.51
Richness	112.03	134.45	121.51	100.87	0.94	0.31	*0.08*
Genus							
Simpson's	0.82	0.82	0.77	0.79	0.59	*0.07*	0.74
Evenness	0.24	0.20	0.15	0.21	0.72	0.11	*0.06*
Richness	26.52	33.20	32.99	24.70	0.82	0.77	*0.04**
Family							
Simpson's	0.69	0.76	0.69	0.73	*0.05**	0.67	0.68
Evenness	0.21	0.20	0.16	0.23	0.11	0.84	*0.04**
Richness	18.34	23.26	22.01	17.77	0.89	0.72	*0.08*
Order							
Simpson's	0.32	0.56	0.42	0.40	*0.02**	0.50	*<0.01**
Evenness	0.12	0.15	0.11	0.14	*0.02**	0.38	0.86
Richness	13.87	16.79	17.04	13.25	0.80	0.91	*0.05**
Class							
Simpson's	0.30	0.49	0.40	0.37	*0.08*	0.85	*0.02**
Evenness	0.15	0.2	0.16	0.19	*<0.01**	0.85	0.51
Richness	9.85	10.36	11.09	8.71	0.27	0.81	*0.09*
Phylum							
Simpson's	0.08	0.13	0.10	0.07	0.77	0.58	0.29
Evenness	0.34	0.36	0.29	0.36	0.23	0.47	0.56
Richness	3.45	3.50	4.28	3.29	0.21	0.40	0.17

Table 3: Results of permutation tests on Euclidean distances. "F" indicates pseudo-F. The first model tests the effects of host plant and N on RAF community composition. The second model tests the effect of N and removal on *G. rossii* RAF. Marginal and significant effects are italicized, significant effects include an asterisk.

Host + N + Host x N						
Taxonomic Level	**Host (F)**	**Host (P)**	**N (F)**	**N (P)**	**Host x N (F)**	**Host x N (P)**
Phylum	12.252	*0.001**	1.184	0.295	2.523	*0.093*
Class	9.782	*0.001**	2.502	*0.023**	1.815	0.105
Order	7.247	*0.001**	2.920	*0.011**	2.129	*0.030**
Family	5.584	*0.001**	2.825	*0.003**	2.178	*0.011**
Genus	5.766	*0.001**	2.871	*0.001**	2.252	*0.009**
OTU	3.657	*0.001**	1.919	*0.005**	1.587	*0.033**
N + removal + N x removal						
Taxonomic Level	**N (F)**	**N (P)**	**removal (F)**	**removal (P)**	**N x removal (F)**	**N x removal (P)**
Phylum	0.887	0.428	0.812	0.474	0.708	0.549
Class	1.710	*0.092*	1.865	*0.085*	0.815	0.572
Order	2.215	*0.014**	1.296	0.213	1.140	0.320
Family	1.722	*0.051*	1.085	0.379	1.349	0.164
Genus	3.299	*0.001**	1.786	*0.026**	1.067	0.376
OTU	2.003	*0.001**	1.564	*0.019**	1.068	0.387

Table 4: *G. rossii* and *D. cespitosa* RAF taxa significantly correlated with N addition with type III ANOVA. These data exclude removal plots from *G. rossii* samples. Taxa present in fewer than three plots were removed from analysis. Uncorrected P-values, direction of N effect and relative abundance of the taxa are listed. Taxa significantly correlated with treatment after FDR correction are indicated by an asterisk. Putative ecological function of OTUs correlated with N are included.

G. rossii	**Taxon**	**Effect Direction**	**P**	**Relative Abundance (C)**	**Relative Abundance (N)**	**Ecological Function**
Class	Leotiomycetes	-	0.014	83.87%	66.65%	
Order	Helotiales*	-	<0.001	83.24%	60.27%	
Genus	*Geomyces*	+	0.014	0.11%	2.40%	
	Meliniomyces	-	0.018	7.03%	0.15%	
	Papulaspora	+	0.017	0.00%	0.54%	
	Rhizoscyphus	-	0.023	7.18%	0.57%	
	Unknown Helotiales	+	0.038	4.29%	0.91%	
OTU	Helotiales sp. B1	+	0.002	0.05%	1.67%	Ubiquitous and diverse order
	Helotiales sp .16 MV-2011	-	0.044	6.10%	0.90%	Ubiquitous and diverse order
	Rhizoscyphus ericae aggregate	-	0.039	1.63%	0.05%	Ericoid mycorrhizae
	Meliniomyces bicolor	-	0.024	3.62%	0.00%	Ericoid mycorrhizae
D. cespitosa						
Phylum	Ascomycota	+	0.003	75.13%	87.29%	
Order	Mortierellales	-	0.046	15.63%	8.48%	
Genus	*Mortierella*	-	0.046	18.86%	9.01%	
	Unknown Helotiales	-	0.023	5.78%	1.33%	
OTU	*Geomyces sp.* FMCC-3	+	0.018	0.07%	1.00%	Psychrotolerant soil fungi, Saprobe
	Gibberella sp. PPn9-A Fr	-	0.043	2.51%	0.46%	Potential pathogen
	Gyoerffyella sp. PB1-R3-D Fr.3	-	0.007	0.39%	0.30%	Dark Septate Fungi
	Mortierella sp. W161	-	0.047	1.19%	0.10%	Saprobic

Table 5: *G. rossii* RAF that are significantly correlated with treatment. Taxa present in fewer than three plots were removed from analysis. The direction of treatment effect on each taxon, average relative abundance in each treatment, uncorrected P-values and putative ecological function are listed. Species showing significant interactions between N addition and *D. cespitosa* removal are in bold.

Taxa that shift with N	**Species Name**	**Effect**	**CC**	**CN**	**DC**	**DN**	**P**	**Ecological Function**
Ascomycota								
Leotiomycetes Helotiales Helotiaceae	*Articulospora tetracladia*	-	0.18	0.00	0.00	0.00	0.036	Saprobic
	Meliniomyces bicolor	-	2.03	0.00	0.23	0.06	0.028	Ericoid mycorrhizae
	Meliniomyces bicolor	-	3.14	0.00	0.31	0.10	0.002	Ericoid mycorrhizae
	Rhizoscyphus ericae	-	0.97	0.00	0.03	0.03	0.021	Ericoid mycorrhizae
	Rhizoscyphus ericae	-	1.04	0.04	0.16	0.07	0.031	Ericoid mycorrhizae
	Rhizoscyphus ericae aggregate	-	1.41	0.03	0.63	0.11	0.004	Ericoid mycorrhizae
	Rhizoscyphus ericae aggregate	-	1.22	0.00	0.06	0.03	0.038	Ericoid mycorrhizae
Leotiomycetes Helotiales Hyaloscyphaceae	***Lachnum sp. YM272***	-/+	0.07	0.00	0.00	0.03	0.049	Mostly saprobic, commonly root associated
	Lachnum sp. 252	-	0.08	0.00	0.00	0.03	0.037	Mostly saprobic, commonly root associated
Leotiomycetes Helotiales unknown	***Helotiales sp. 16 MV-2011***	-/+	5.32	0.70	0.95	1.85	0.005	Ubiquitous and diverse order
Leotiomycetes Helotiales incertae sedis	*Phialocephala fortinii*	+	0.00	0.27	0.07	0.04	0.028	Dark Septate Endophyte
	Phialocephala turiciensis	+	0.00	1.25	0.40	0.54	0.033	Dark Septate Endophyte
Leotiomycetes incertae sedis Myxotrichaceae	*Geomyces pannorum*	+	0.00	0.18	0.00	0.00	0.009	Psychrotolerant soil fungi, saprobe
	Geomyces sp. FFI 30	+	0.06	0.99	0.18	0.55	0.025	Psychrotolerant soil fungi, saprobe
	Geomyces sp. FMCC-2	+	0.00	0.11	0.00	0.00	0.008	Psychrotolerant soil fungi, saprobe
	Geomyces sp. FMCC-2	+	0.00	0.25	0.00	0.00	0.013	Psychrotolerant soil fungi, saprobe
Sordariomycetes Hypocreales Hypocreaceae	*Hypocrea rufa*	+	0.00	0.17	0.00	0.04	0.032	Mostly saprobic
Incertae sedis	*Papulaspora sp. MTFD02*	+	0.00	0.40	0.00	0.04	0.012	Potential plant and fungal pathogens
	Papulaspora sp. MTFD02	+/-	0.00	0.16	0.09	0.00	0.049	Potential plant and fungal pathogens
	Spirosphaera beverwijkiana	-	0.57	0.00	0.34	0.00	0.021	Aquatic hyphomycete
	Tetracladium furcatum	+	0.14	0.89	0.13	0.00	0.028	Saprobic
Incertae sedis								
Incertae sedis Mortierellales Mortierellaceae	*Mortierella alpine*	+	0.00	0.14	0.00	0.00	0.009	Saprobic
Taxa that shift with *D. cespitosa* removal	**Species Name**	**Effect Direction**	**CC**	**CN**	**DC**	**DN**	**P**	**Ecological Function**

Ascomycota								
Eurotiomycetes Chaetothyriales Herpotrichiellaceae	*Capronia sp. UBCTRA*	+	0.09	0.00	0.53	0.05	0.030	Root-associated saprobes
	Capronia sp. UBCTRA	+	0.00	0.10	0.24	0.03	0.037	Root-associated saprobes
Leotiomycetes Helotiales Helotiaceae	*Articulospora tetracladia*	-	0.18	0.00	0.00	0.00	0.036	Saprobic
	Meliniomyces bicolor	-	0.22	0.03	0.00	0.04	0.028	Ericoid mycorrhizae
	Meliniomyces bicolor	-	2.03	0.00	0.23	0.06	0.049	Ericoid mycorrhizae
	Meliniomyces bicolor	+/-	3.13	0.00	0.31	0.10	0.002	Ericoid mycorrhizae
	Rhizoscyphys ericae	-	0.97	0.00	0.03	0.03	0.021	Ericoid mycorrhizae
Leotiomycetes Helotiales Hyaloscyphaceae	***Lachnum sp. 252***	+/-	0.08	0.00	0.00	0.03	0.045	Mostly saprobic, commonly root associated
	Lachnum sp. YM272	-	0.07	0.00	0.00	0.03	0.048	Mostly saprobic, commonly root associated
	Lachnum sp. YM272	+	0.00	0.03	0.54	0.00	0.048	Mostly saprobic, commonly root associated
	Lachnum sp. YM272	+	0.55	0.09	1.98	0.16	0.030	Mostly saprobic, commonly root associated
	Lachnum sp. YM272	+	0.00	0.00	0.66	0.00	0.011	Mostly saprobic, commonly root associated
	Lachnum sp. YM272	+	0.00	0.05	0.56	0.00	0.047	Mostly saprobic, commonly root associated
Leotiomycetes Helotiales incertae sedis	*Phialocephala europaea*	-	0.36	0.10	0.00	0.03	0.029	Dark Septate Endophyte
	Phialocephala europaea	-	0.53	0.05	0.03	0.00	0.042	Dark Septate Endophyte
	Phialocephala fortinii	-	0.14	0.03	0.00	0.03	0.044	Dark Septate Endophyte
Leotiomycetes Helotiales unknown	***Helotiales sp. 16 MV-2011***	+/-	5.32	0.70	0.95	1.85	0.008	Ubiquitous and diverse order
	Helotiales sp. 17 MV-2011	-	0.22	0.07	0.00	0.00	0.040	Ubiquitous and diverse order
	Helotiales sp. SC1-1	+	1.74	1.32	7.45	1.84	0.006	Ubiquitous and diverse order
	Helotiales sp. SC1-1	+	0.00	0.00	0.10	0.06	0.046	Ubiquitous and diverse order
Sordariomycetes Diaporthales unknown	*Diaporthales sp. E6927e*	+	0.00	0.03	0.34	0.00	0.039	Includes plant pathogens
Incertae sedis	*Gyoerffyella sp. PB1-R3-D Fr*	+	0.00	0.05	0.17	0.00	0.046	Dark Septate Endophyte
	Gyoerffyella sp. PB1-R3-D Fr	+	0.00	0.05	0.16	0.03	0.046	Dark Septate Endophyte
	Gyoerffyella sp. PB1-R3-D Fr	+	0.00	0.00	0.23	0.10	0.043	Dark Septate Endophyte
	Leptodontidium orchidicola	+	0.00	0.00	0.53	0.03	0.038	Mutualist or parasite

S. T1: Results of permutation tests on Bray Curtis distance matrices. The first model tests the effects of host and N addition on RAF community composition. The second model tests the effect of N and removal on *G. rossii* RAF. Marginal and significant effects italicized, significant effects include an asterisk.

Host + N + Host x N			
Taxonomic Level	**Host (P)**	**N (P)**	**Host x N (P)**
Phylum	*0.007**	0.638	*0.068*
Class	*0.001**	0.124	0.276
Order	*0.001**	*0.020**	*0.034**
Family	*0.001**	*0.045**	0.178
Genus	*0.001**	*0.002**	*0.044**
OTU	*0.001**	*0.012**	*0.047**
N + removal + N x removal			
Taxonomic Level	**N (P)**	**removal (P)**	**N x removal (P)**
Phylum	0.515	0.427	0.156
Class	*0.046**	0.168	*0.049**
Order	*0.003*	0.273	*0.018**
Family	0.125	0.840	*0.035**
Genus	*0.001**	0.166	0.184
OTU	*0.002**	*0.010**	*0.082*

S. T2: SIMPER results, using Euclidean distances, indicating which OTUs are most responsible for RAF community shifts with N for each plant host, and their taxonomic assignments.

OTUs that contribute most to D. cespitosa RAF community response to N	Relative abundance		Av.Sq.Dist	Contrib%
	Des C	Des N		
Ascomycota; Sordariomycetes; Xylariales; n; Microdochium; Microdochium sp. 6/97-20	3.73	19.1	660	26.89
Ascomycota; Leotiomycetes; n; Myxotrichaceae; Geomyces; Geomyces sp. FFI 30	1.74	14.6	406	16.53
Ascomycota; Dothideomycetes; Pleosporales; Melanommataceae; Herpotrichia; Herpotrichia juniperi	11.5	0.00	314	12.80
Basidiomycota; Agaricomycetes; Agaricales; Tricholomataceae; Mycena; Mycena alnetorum	8.67	0.20	226	9.20
Ascomycota; Leotiomycetes; Helotiales; n; Phialocephala; Phialocephala sphaeroides	7.52	0.15	186	7.58
Ascomycota; n; n; n; Tetracladium; Tetracladium furcatum	4.53	5.00	148	6.05
Ascomycota; Leotiomycetes; Helotiales; n; Phialocephala; Phialocephala sp. AU_BD62	1.17	4.17	65.1	2.65
OTUs that contribute most to G. rossii RAF community response to N	**Relative abundance**		**Av.Sq.Dist**	**Contrib%**
	Geum C	**Geum N**		
Ascomycota; Leotiomycetes; Helotiales; Helotiaceae; Articulospora; Articulospora tetracladia	8.02	9.34	195	15.82
Ascomycota; Leotiomycetes; Helotiales; Hyaloscyphaceae; Lachnum; Lachnum sp. YM272	7.66	2.46	160	12.95
Ascomycota; Leotiomycetes; Helotiales; n; Phialocephala; Phialocephala europaea	5.33	7.02	72.9	5.90
Ascomycota; Sordariomycetes; Magnaporthales; Magnaporthaceae; Phialophora; Phialophora sp. olrim753	0.31	4.27	71	5.75
Ascomycota; Leotiomycetes; Helotiales; Hyaloscyphaceae; Lachnum; Lachnum pygmaeum	0.95	4.16	53.4	4.32
Ascomycota; Leotiomycetes; Helotiales; n; n; Helotiales sp. 16 MV-2011	5.78	0.83	51.4	4.16
Ascomycota; Leotiomycetes; Helotiales; n; Phialocephala; Phialocephala turiciensis	0.00	3.04	51.1	4.14
Ascomycota; Leotiomycetes; Helotiales; n; n; Helotiales sp. SC1-1	3.38	1.93	46.4	3.76
Ascomycota; Sordariomycetes; Hypocreales; Ophiocordycipitaceae; Ophiocordyceps; Ophiocordyceps crassispora	0.78	2.87	45.8	3.71
Ascomycota; Leotiomycetes; Helotiales; Sclerotiniaceae; Botryotinia; Botryotinia fuckeliana	1.23	2.90	35.5	2.87
Ascomycota; Leotiomycetes; Helotiales; n; n; Helotiales sp. 859	1.67	2.69	30.8	2.49
Ascomycota; Leotiomycetes; Helotiales; Hyaloscyphaceae; Lachnum; Lachnum sp. YM272	2.18	0.07	28.9	2.34
Ascomycota; Leotiomycetes; Helotiales; Helotiaceae; Claussenomyces; Claussenomyces sp. PDD 95741	2.43	0.00	24.1	1.95
Ascomycota; Sordariomycetes; Xylariales; n; Microdochium; Microdochium sp. 6/97-20	0.00	1.90	22.2	1.80
Ascomycota; Leotiomycetes; Helotiales; n; n; Helotiales sp. MU-2009-3	1.26	1.63	21.9	1.77
Ascomycota; Leotiomycetes; Helotiales; Helotiaceae; Meliniomyces; Meliniomyces bicolor	3.42	0.00	19.7	1.60

Ascomycota; n; n; n; Gyoerffyella; Gyoerffyella sp. PB1-R3-D Fr	0.66	3.12	18.9	1.53
Ascomycota; Leotiomycetes; Helotiales; n; Phialocephala; Phialocephala turiciensis	0.00	2.12	17.5	1.42
Ascomycota; Leotiomycetes; Helotiales; Helotiaceae; Meliniomyces; Meliniomyces bicolor	2.32	0.00	16	1.29
Ascomycota; Leotiomycetes; Helotiales; Hyaloscyphaceae; Lachnum; Lachnum sp. YM272	1.44	0.04	13.5	1.10

S. T3: SIMPER results indicating which *G. rossii* RAF OTUs are responsible for community shifts with N and removal, and their taxonomic assignments. Pairwise comparisons between treatment combinations are shown. CC = control plots, CN = N plots, DC = *D. cespitosa* removal plots, DN = removal + N plots.

OTUs that varied with N addition in plots without removal	**Relative abundance**		**Av.Sq.Dist**	**Contrib%**
	CC	CN		
Ascomycota; Leotiomycetes; Helotiales; Helotiaceae; Articulospora; Articulospora tetracladia	7.16	8.14	171	14.4
Ascomycota; Leotiomycetes; Helotiales; Hyaloscyphaceae; Lachnum; Lachnum sp. YM272	7.35	1.92	158	13.28
Basidiomycota; Tremellomycetes; Tremellales; n; Cryptococcus; Cryptococcus sp. APSS 870	0	4.7	155	13
Ascomycota; Sordariomycetes; Magnaporthales; Magnaporthaceae; Phialophora; Phialophora sp. olrim753	0.24	3.7	51.9	4.37
Ascomycota; Leotiomycetes; Helotiales; n; Phialocephala; Phialocephala europaea	4.78	5.33	46.8	3.94
Ascomycota; Leotiomycetes; Helotiales; n; n; Helotiales sp. 16 MV-2011	5.32	0.7	45.3	3.81
Ascomycota; Leotiomycetes; Helotiales; n; n; Helotiales sp. SC1-1	2.91	1.65	33.6	2.82
Ascomycota; Sordariomycetes; Hypocreales; Ophiocordycipitaceae; Ophiocordyceps; Ophiocordyceps crassispora	0.72	2.44	33.4	2.81
Ascomycota; Leotiomycetes; Helotiales; Hyaloscyphaceae; Lachnum; Lachnum sp. YM272	2.12	0.06	27.9	2.34
Ascomycota; Leotiomycetes; Helotiales; Sclerotiniaceae; Botryotinia; Botryotinia fuckeliana	1.09	2.42	26.1	2.19
OTUs that varied with N addition in removal plots	**Relative abundance**		**Av.Sq.Dist**	**Contrib%**
	DC	DN		
Ascomycota; Leotiomycetes; Helotiales; Helotiaceae; Articulospora; Articulospora tetracladia	7.08	28.2	604	41.55
Ascomycota; Leotiomycetes; Helotiales; Hyaloscyphaceae; Lachnum; Lachnum sp. YM272	12.4	4.07	226	15.55
Ascomycota; Leotiomycetes; Helotiales; n; n; Helotiales sp. SC1-1	8.99	3.49	158	10.84
Ascomycota; Leotiomycetes; Helotiales; n; n; Helotiales sp. SC1-1	7.45	1.84	70	4.81
Ascomycota; Leotiomycetes; Helotiales; n; Phialocephala; Phialocephala europaea	4.41	6.18	46.8	3.22
Ascomycota; Leotiomycetes; Helotiales; n; n; Helotiales sp. SC1-1	2.37	0.11	33.8	2.32
OTUs that varied with removal in plots without N addition	**Relative abundance**		**Av.Sq.Dist**	**Contrib%**
	DC	CC		
Ascomycota; Leotiomycetes; Helotiales; Hyaloscyphaceae; Lachnum; Lachnum sp. YM272	12.4	7.35	270	22.67
Ascomycota; Leotiomycetes; Helotiales; n; n; Helotiales sp. SC1-1	8.99	2.91	176	14.76
Ascomycota; Leotiomycetes; Helotiales; Helotiaceae; Articulospora; Articulospora tetracladia	7.08	7.16	154	12.94
Ascomycota; Leotiomycetes; Helotiales; n; n; Helotiales sp. SC1-1	7.45	1.74	70.6	5.92
Ascomycota; Leotiomycetes; Helotiales; n; n; Helotiales sp. 16 MV-2011	0.95	5.32	44	3.69
Ascomycota; Leotiomycetes; Helotiales; n; Phialocephala; Phialocephala europaea	4.41	4.78	39.6	3.32

Ascomycota; Leotiomycetes; Helotiales; n; n; Helotiales sp. SC1-1	2.37	1.1	36.3	3.04
Ascomycota; Leotiomycetes; Helotiales; Hyaloscyphaceae; Lachnum; Lachnum pygmaeum	2.43	0	28.6	2.4
Ascomycota; Leotiomycetes; Helotiales; Hyaloscyphaceae; Lachnum; Lachnum sp. YM272	0.04	2.12	28	2.35
Ascomycota; Leotiomycetes; Helotiales; n; n; Helotiales sp. SC1-1	1.93	0.49	26	2.18

OTUs that varied with removal in N addition plots	**Relative abundance**		**Av.Sq.Dist**	**Contrib%**
	DN	CN		
Ascomycota; Leotiomycetes; Helotiales; Helotiaceae; Articulospora; Articulospora tetracladia	28.2	8.14	577	43.71
Basidiomycota; Tremellomycetes; Tremellales; n; Cryptococcus; Cryptococcus sp. APSS 870	0.18	4.7	153	11.6
Ascomycota; Sordariomycetes; Magnaporthales; Magnaporthaceae; Phialophora; Phialophora sp. olrim753	0.07	3.7	52.8	4.01
Ascomycota; Leotiomycetes; Helotiales; n; Phialocephala; Phialocephala europaea	6.18	5.33	51.5	3.9
Ascomycota; Leotiomycetes; Helotiales; Hyaloscyphaceae; Lachnum; Lachnum sp. YM272	4.07	1.92	45.2	3.43
Ascomycota; Sordariomycetes; Hypocreales; Ophiocordycipitaceae; Ophiocordyceps; Ophiocordyceps crassispora	1.44	2.44	33.7	2.56
Ascomycota; Leotiomycetes; Helotiales; n; n; Helotiales sp. 859	1.96	2.24	29.1	2.2
Ascomycota; Leotiomycetes; Helotiales; Hyaloscyphaceae; Lachnum; Lachnum pygmaeum	2.04	2.64	28.9	2.19
Ascomycota; Leotiomycetes; Helotiales; Sclerotiniaceae; Botryotinia; Botryotinia fuckeliana	0.06	2.42	26.8	2.03

CHAPTER 3

Assembly Of Root Associated Bacteria Communities: Interactions Between Abiotic And Biotic Factors

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Running Title: Plant-Bacteria Response to N Pollution

ABSTRACT

Nitrogen (N) deposition in many areas of the world is over an order of magnitude greater than it would be in absence of human activity. We ask how abiotic (N) and biotic (plant host and neighborhood) effects interact to influence root-associated bacterial (RAB) community assembly. Using 454 pyrosequencing we examined RAB communities from two dominant alpine tundra plants, *Geum rossii*

and *Deschampsia cespitosa,* under control, N addition, and *D. cespitosa* removal treatments, implemented in a factorial design. We hypothesized that host would have the strongest effect on RAB assembly, followed by N, then neighbor effects.

The most dominant phyla were Proteobacteria (mostly Gamma-proteobacteria), Actinobacteria, Bacteroidetes and Acidobacteria. We found RAB communities were host specific, with only 17% overlap in operational taxonomic units (OTUs). Host effects on composition were over twice as strong as N effects. *D. cespitosa* RAB diversity declined with N, while *G. rossii* RAB did not. *D. cespitosa* removal did not influence *G. rossii* RAB community composition, but *G. rossii* RAB diversity declined with N only when *D. cespitosa* was absent..

We conclude that RAB of both hosts are sensitive to N enrichment, and RAB response to N is influenced by host identity and plant neighborhood.

INTRODUCTION

The root provides unique environments for bacterial growth, with bacteria living inside (endophytic) and on the surface of plant roots. These root associated bacteria (RAB) can improve host fitness in a variety of ways, either by improving access to nutrients, producing plant-growth regulators, improving environmental stress tolerance, preventing pathogen infection or toxin production by pathogens, or inducing host defenses and systemic resistance (Badri *et al.* 2009, Lugtenberg and Kamilova 2009).

Root associated bacterial communities can be structured by host identity, and/or soil environment (Nguyen *et al.* 2003, Köberl *et al.* 2011, Kolton *et al.* 2011, Hardoim *et al.* 2011). Nitrogen (N) enrichment has been shown to alter soil bacterial

community composition, and microbial activity (Compton *et al.* 2004, Schmidt *et al.* 2004, Nemergut *et al.* 2008, Fierer *et al.* 2012), but the root provides a very different environment from bulk soil due to high C availability from root exudates and harbors distinct bacterial communities (Nguyen 2003, Gottel *et al.* 2011). Little research has been done on the effect of N on RAB. A shift in RAB community composition could affect host fitness, especially if involving mutualist/pathogen abundance. Increasing N availability causes shifts in dominance between plant species (Pennings *et al.* 2005, Suding *et al.* 2008), reductions in plant species diversity (Strengbom *et al.* 2003, Bobbink 2004, Suding *et al.* 2005**,** Clark and Tilman 2008), and increased vulnerability of systems to invasion (Bobbink 2004, Cherwin *et al.* 2009). Describing root associated microbial response to N is important to understanding plant community response to N enrichment.

Alpine tundra at Niwot Ridge, a Long Term Ecological Research site in Colorado, USA, has been receiving increased rates of N deposition from the cities of Boulder and Denver over the last few decades (Williams *et al.* 1996, Sievering *et al.* 1996, Suding *et al.* 2008). Moist meadow alpine tundra is co-dominated by a rosaceous forb, *Geum rossii,* and a bunchgrass, *Deschampsia cespitosa*. *Deschampsia cespitosa* expands and *G. rossii* declines in N addition plots, regardless of the presence of the competing co-dominant, suggesting competition alone is not responsible for the contrasting N responses (Suding *et al.* 2008, Farer *et al.* 2013). *Geum rossii* and *D. cespitosa* have different patterns of belowground carbon allocation in N addition plots, particularly to soil bacteria (Farrer *et al.* 2013). Root associated fungi (RAF) of the two hosts are also differentially affected by N at the

same site, those of *D. cespitosa* responding more strongly to N than those of *G. rossii* (Dean *et al.* 2014). Additionally, the presence of *D. cespitosa* mediates how *G. rossii*'s RAF respond to N. Host is the primary influence on RAF composition, with only 9.4% of operational taxonomic units (OTUs) overlapping between hosts. Putative *G. rossii* RAF mutualists declined with N, suggesting that RAF may play a role in contrasting host responses to N (Dean *et al.* 2014).

Here we consider whether RAB are sensitive to soil N in alpine tundra. We used 454 pyrosequencing to examine RAB communities from *G. rossii* and *D. cespitosa* in N addition, *D. cespitosa* removal, and control plots. We did not include *G. rossii* removal treatment because the year we collected samples *G. rossii* was nearly absent from N plots, so *G. rossii* absence is confounded with N enrichment. By removing *D. cespitosa* we aimed to parse the effects of plant competition from N on RAB, and on *G. rossii* decline. We expected RAB dynamics to be similar to the observed RAF dynamics at this site. Specifically, we expected host identity to have the strongest effect on RAB community structure, followed by N effects, then presence/absence of the competitor. We expected *D. cespitosa* RAB to be more sensitive to N than *G. rossii* RAB, and to find interactions between competitor presence and N on the RAB community. Because *G. rossii* declines with N, we expected putative mutualistic RAB to decline in and/or pathogenic RAB to with N in *G. rossii*.

METHODS

Study site and field sampling. The study was conducted in moist meadow alpine tundra on Niwot Ridge, an LTER site located 35 km west of Boulder, CO, in the Front

Range of the Rocky Mountains, elevation 3297-3544 m. Winter and summer mean temperatures are 13 °C and 8 °C. Soil is under snow pack 9 to 10 months per year (http://culter.colorado.edu/NWT/site_info/climate/climate.html).

Plots used for this study are a subset of those established by Suding *et al.* (2008). Briefly, 1x1m^2 plots were set up at seven replicate sites in 2001. We used four treatment plot types per site: N addition, *D. cespitosa* removal, *D. cespitosa* removal + N addition, and control. Nitrogen has been added annually to N addition plots in the form of urea (at a maximum rate of 28.8 g N $m^{-2}\,y^{-1}$, ~40 times natural deposition rates). Exploratory analyses indicate this did not affect pH (S.T6). *D. cespitosa* has been removed annually by clipping; at the time of this study *D. cespitosa* was effectively removed from the plots, with removed biomass in 2008 only 2% of the removed biomass in 2001.

In 2008, a *G. rossii* individual (defined by a single aboveground rosette) was uprooted at random from each treatment combination at each site. *D. cespitosa* individuals were also collected from a subset of control and N plots. This resulted in a total of 28 *G. rossii* and 11 *D. cespitosa* root samples. One *D. cespitosa* sample was later discarded as it possessed an extremely different RAB community, indicating potential contamination. Plants were sent to University of New Mexico for storage and processing.

Laboratory preparation. Because we were interested in all bacteria closely associated with the root, we did not surface sterilize. Root surfaces were washed with tap water until no soil particles remained. Then each root was rinsed 3 times with sterilized milliQ filtered water and stored at -80 °C. A mix of small, medium and

large healthy looking roots were selected from each plant and combined for DNA extraction. Tissue was lysed by grinding with liquid N using a mortar and pestle. DNA was extracted using DNEasy Plant MiniKit from Qiagen, following the instruction manual (QIAGEN, Hilden, Germany). *G. rossii* tissue is high in phenolics, which inhibit polymerase enzymes, thus some samples had to be diluted 1:10 in milliQ filtered water to enable amplification. Extracts were sent to Research and Testing Laboratories (RTL) in Lubbock, TX for 454 titanium pyro-sequencing of the v4 region of bacteria 16S rDNA, using 515F/806R primers (Walters *et al.,* 2011). Prior to sequencing, DNA from each sample was diluted by RTL to 20ng/uL (concentrations checked with a nanodrop spectrophotometer, Nyxor Biotech, Paris, France). Sequence libraries were created using a one step, 30-cycle PCR using a 25uL reaction with Qiagen HotStar Taq master mix (Qiagen Inc., Valencia, CA), and the following thermocycles: initial denaturation at 95 °C for 5min, and then 35 cycles of 94°C for 30s, 54°C for 40s and 72°C for 1 min, and a final extension at 72°C for 10 min. Amplification products were pooled to equimolar concentrations and cleaned using Diffinity RapidTip (Diffinity Genomics, West Henrietta, NY) and size selected using Agencourt AMPure XP (BeckmanCoulter, Indianapolis, IN). Hybridizations, emPCR reactions and sequencing followed manufacturer protocols (454 Life Sciences, Branford, CT). Samples were sequenced in two runs (on a single region each). Each sample within a run had its own 8nt barcode. Sequences with poor tag read quality were excluded from sequence files.

Bioinformatics. Sequences were denoised, filtered, and checked for chimeras using AmpliconNoise (Quince *et al.,* 2009) in QIIME v1.7.0 (Caporaso *et al.,* 2010).

Sequences smaller than 200bp and with an average quality score less than 25 were discarded. AmpliconNoise identifies chimeras using Perseus, which we ran using default parameters. Sequences that were 97% similar to each other were clustered into OTUs representative of distinct species using UCLUST through QIIME. The most common sequence in each cluster was selected as the representative sequence for each OTU. Sequences were BLASTed against the Greengenes reference database (version 12_10, McDonald *et al.,* 2012) to assign taxonomies. The majority of sequences in *G. rossii* belonged to plant chloroplast. These sequences were discarded.

Data Analysis. Distance based redundancy analysis (dbRDA, vegan package, Oksanen *et al*., 2011) was used to ordinate weighted UniFrac distance matrices of unrarified OTU tables, normalized to sample totals (QIIME, Caporaso *et al.,* 2010). Weighted UniFrac incorporates phylogenetic relationships between shared and unshared species into distance calculations. We plotted data in a single dbRDA plot to visualize the relative effects of all treatments on community composition. Permutation tests (capscale, vegan package, Oksanen *et al*., 2011) were performed on distance matrices to determine significance of treatments. Due to the unbalanced design of the experiment, we performed two tests: first, we tested the effect of host and N on RAB (host + N + host x N, excluding *D. cespitosa* removal); second, we tested the effect of N and *D. cespitosa* removal on *G. rossii* RAB (N + removal + N x removal, excluding *D. cespitosa*-host samples*)*.

To determine which individual taxa were affected by host and treatment, we compared the relative abundance of each taxon across hosts and treatments using

type III ANOVA (package nlme, Pinheiro *et al*., 2011). We determined how many and which RAB taxa responded to N in each host separately, excluding data from *D. cespitosa* removal plots to equalize comparisons. We examined which taxa responded differently to N in the two hosts by testing for interactions between host and N (host + N + host x N), also excluding *D. cespitosa* removal plots. We also tested which *G. rossii* RAB taxa responded to N and *D. cespitosa* removal (N + removal + N x removal). Due to the large number of comparisons, a False Discovery Rate (FDR, Yoav and Yosef, 1995) correction was applied to P-values. Few taxa were correlated with any treatment after FDR correction. However, community composition was affected by host and N, indicating individual taxa must be affected. Additionally, some taxa affected by host, N or neighbor prior to FDR correction were abundant and had a large effect size, so we also discuss uncorrected results. Our statistical power is low given the number of replicates afforded in this study, and disregarding raw P-values likely results in discarding many true positives.

We calculated several alpha diversity measures for each sample: phylogenetic diversity, species richness, Simpson's diversity, and Simpson's evenness (QIIME, Caporaso *et al.,* 2010). There was large variation in sequencing depth among samples (*G. rossii*: 125-1905 sequences, *D. cespitosa* 1727-3131 sequences). To control biasing effects of sampling depth on observed diversity we discarded the smallest sample (125 sequences) and performed 100 rarefactions on each remaining sample to 304 sequences (the size of the next smallest sample). Some of the treatments were undersampled. Nevertheless, this depth allowed detection of differences in richness between treatments (S.F1). Alpha diversity

measures were calculated for each sample in each rarefied OTU table, and the values for each sample were averaged across all tables.

We tested the effect of host, N, and *D. cespitosa* removal on RAB alpha diversity measures using type III ANOVA (nlme package, Pinheiro *et al*., 2011) in R (R Development Core Team, 2012). First we excluded the *D. cespitosa* removal plots and tested whether host, N, or their interaction affected diversity (host + N + host x N). Second, only using data from *G. rossii* host plants, we tested whether N, *D. cespitosa* removal, or their interaction had an effect on *G. rossii* RAB diversity (N + removal + N x removal).

Phylogenetic diversity takes into account relatedness, or shared branch lengths, between species within a category. The other alpha diversity measures do not, so results can differ depending on how coarsely OTUs are grouped. For example, it is possible *D. cespitosa* RAB could represent more orders but fewer species relative to *G. rossii*-RAB. In the results, for simplicity, we focus only on phylogenetic diversity and Phylum-level richness, evenness and diversity. Analyses at all other taxonomic levels are presented in Supplemental Information.

RESULTS

Host effects on RAB Communities. Initially, sequencing resulted in 3,419 (s.d. = 1,693) sequences/sample in *G. rossii*, and 2,472 (s.d. = 464) sequences/sample in *D. cespitosa.* Chloroplasts made up a larger percentage of sequences in *G. rossii* (2,678 sequences/sample) than in *D. cespitosa* (101 sequences/sample). Chloroplast removal resulted in 741 (s.d. = 435) sequences/sample in *G. rossii* and 2,371 (s.d. = 465) sequences/sample in *D. cespitosa*.

Under ambient conditions, RAB communities were host specific, with only 175 out of a total of 1,051 (16.65%) OTUs shared by both hosts after rarefaction. Although the dominant phyla were similar in both hosts, *D. cespitosa* RAB communities had lower evenness than those of *G. rossii* (S.T1). For instance, 74% of *D. cespitosa* RAB belong to the phylum Proteobacteria, while Bacterioidetes comprised 9% of sequences, and Actinobacteria 6%. *G. rossii*-RAB was dominated by Proteobacteria as well, but these only comprised 45% of sequences, followed by Actinobacteria at 18%, and Bacteroidetes and Acidobacteria, both comprising 12% of sequences (Fig. 1).

Hosts significantly differed from each other in relative abundances of six phyla. *Deschampsia cespitosa* had higher relative abundance of Proteobacteria (n = 23, $F_{1,13} = 21.309$, $P < 0.001$) and sequences that retrieved no blast hit from Greengenes ($F_{1,13} = 5.47$, $P = 0.036$), and *G. rossii* had higher relative abundances of Acido and Actinobacteria, Chloroflexi, and Armatimonadetes ($F_{1,13} = 26.086$, $P < 0.001$; $F_{3,19} = 10.770$, $P = 0.006$; $F_{1,13} = 11.06$, $P = 0.006$; $F_{1,13} = 5.20$, $P = 0.040$) (Fig. 1).

Distance-based redundancy analysis (dbRDA) indicates compositional differences between *D. cespitosa*- and *G. rossii*-RAB communities (n = 24, $P_{MonteCarlo} = 0.001$, Fig. 2). Phylogenetic diversity was marginally significantly lower in *D. cespitosa* compared to *G. rossii* RAB communities in control conditions (n = 12, $F_{1,10} = 4.72$, $P = 0.055$; Fig. 3; Table 2). Phylum evenness was also significantly lower in *D. cespitosa* RAB communities, as was phylum and class diversity, and phylum, class and order richness (Table 2, ST.1).

Effects of Nitrogen. Plant host effect was maintained at high N (Fig. 1, Table 1), with host explaining 30% of the variance in RAB regardless of nitrogen environment. Nitrogen also significantly affected RAB composition, explaining an additional 12% of the variation in composition (Fig. 1, Table 1). Nitrogen affected *G. rossii* marginally significantly more than *D. cespitosa*, and also caused RAB communities of the two hosts to diverge (n = 24, host × N interaction, $P_{MonteCarlo}$=0.093, Fig. 2, Table 1), contrary to our predictions.

Nitrogen effects on individual RAB taxa were strongly dependent on host. More RAB OTUs were responsive to N in *G. rossii* compared to *D. cespitosa* (S.T3). The order Chromatiales from *G. rossii* significantly decreased in abundance with N, decreasing from 19% to 3% of sequences. This decline was driven almost entirely by the family Sinobacteraceae, which also declined in *D. cespitosa*, but was much more dominant in *G. rossii*. At the phylum level, Acidobacteria in *G. rossii* decreased with N from 13% to 5%. Nitrogen significantly reduced Planctomycetes in *D. cespitosa*, but this was a rare phylum, and made up <1% of sequences under ambient conditions.

Many OTUs could not be classified to species, and the ecological functions of those that were assigned specific taxonomies are poorly understood. Putative N fixers (*Bradyrhizobium spp*., *Burkholdariales spp.*) declined with N, and putative denitrifiers (*Rhodanobacter lindaniclasticus*) increased with N in both hosts (S.T3). Twenty-two OTUs were affected significantly differently by N in the two hosts prior to FDR correction (significant host x N effect, n = 23, S.T4).

Phylogenetic diversity declined with N in *D. cespitosa* RAB communities but was maintained in *G. rossii* RAB communities (significant host x N effect, n = 23, $F_{1,19}$ = 5.25, P=0.03, Fig. 3, Table 2), even though *G. rossii* RAB community composition changed in response to N (Fig. 2). This appears to be driven by a reduction in richness (ST.1).

Effects of Neighborhood (*D. cespitosa* removal). While host and N affected RAB community composition, *G. rossii*-RAB communities were unaffected by removal of *D. cespitosa* (Fig. 2, Table 1). In contrast, N caused *G. rossii* RAB phylogenetic diversity to significantly decline in the absence of *D. cespitosa* (significant N x removal effect, n = 27, $F_{1,23}$ = 7.32, P=0.01, Fig. 3, Table 2), and absence of *D. cespitosa* affected the N response of several *G. rossii* RAB taxa (significant N x removal effect, n = 27, S.T5).

DISCUSSION

Host influences abiotic assembly mechanisms. Host identity is the primary structuring force for these RAB communities, regardless of the N environment. The effect of increased N availability was over 50% weaker than host identity in determining RAB composition. Host effect may be driven by root exudate biochemistry (Walker *et al.,* 2003), which is known to be very different between these two host plants, with more recalcitrant substrates exuded by *G. rossii*, including allelopathic chemicals (Meier *et al.,* 2008). It has been suggested this difference in root exudates is responsible for *G. rossii*'s larger microbial biomass (Bowman *et al.* 2004), and we suggest it also affects RAB community composition.

Contrary to our hypothesis, *G. rossii* RAB communities shifted more in response to N relative to *D. cespitosa* RAB communities, though this effect was marginal. Perhaps *D. cespitosa* RAB are inherently more nitrophilic, and therefore experience little turnover with N enrichment, or *D. cespitosa* is better able to regulate its RAB communities. Research suggests plants may actively control their microbial communities through immune responses (Kogel *et al.,* 2006) and chemical attractants/repellants (Walker *et al.,* 2003). The larger shift in *G. rossii* RAB community composition could represent an inability to control infection by foreign RAB under N enrichment (Johnson *et al.,* 1997). Indeed, Farrer *et al.* (2013) found G. rossii allocated more C to soil bacteria and was C-limitated in N addition plots. These data contrast with RAF responses to N from the same sites; *D. cespitosa* root fungi were more sensitive to N relative to *G. rossii* root fungi (Dean *et al.* 2014). So is a stable or a flexible microbial community beneficial under N enrichment? It probably depends on the taxa that are affected, and their relationship with the host.

Examining biotic (host and neighbor) and N effects on individual taxa revealed that indeed both factors contribute to RAB community composition. Host not only affected the relative abundance of taxa, but also how those taxa responded to N. Many of the responsive *G. rossii* OTUs belonged to the Sphingobacteriales order. Members of this order have been found in a great diversity of environments, but little is known about their ecological functions. Members of the Sinobacteraceae family were very abundant in *G. rossii* roots, and nearly disappeared with N in both hosts, but little is known about this taxonomic group either. Several less abundant taxa were affected differently by N depending on which plant species was host. Our

ability to assess the effects of RAB community shifts on host fitness is limited by the lack of ecological information available for most taxa.

The effect of N on alpha diversity was dependent upon the host: RAB phylogenetic diversity decreased with N in *D. cespitosa* but not *G. rossii* roots. *Deschapmsia cespitosa* shed taxa at high N. Symbionts are not strongly needed at high N and may instead drain carbon or other resources (Johnson *et al.,* 2008), perhaps explaining reduced richness here. In contrast, *G. rossii* RAB communities shifted in composition with no overall change in phylogenetic diversity, due to replacement of original taxa with new taxa. Interestingly, *G. rossii* RAF diversity was found to increase while *D. cespitosa* RAF diversity did not change with N at these sites (Dean *et al.* 2014). These patterns illustrate differences in microbe-plant interactions between hosts, which may contribute to how plant communities respond to a changing environment.

Neighbor influences abiotic assembly mechanisms. We found neighborhood, measured here as the effect of presence/absence of the principle competitor, *D. cespitosa,* on *G. rossii* RAB*,* to play a negligible role in community assembly. In contrast, neighbor did play a role in structuring fungal communities (Dean *et al.,* 2014). This may be because fungi can establish networks that extend from one plant to another (Simard *et al.,* 2012), and so more directly influence a neighbor's fungal symbionts.

Interestingly, *G. rossii* RAB phylogenetic diversity declined at high N in plots where *D. cespitosa* had been removed, though it did not decline with N when *D. cespitosa* was present. This decline mostly constituted the loss of rare RAB taxa, as it

was not reflected in compositional changes in ordination analyses. These data indicate that *G. rossii* and *D. cespitosa* RAB influence each other. Several *G. rossii* taxa were affected by N differently depending on *D. cespitosa*'s presence, further evidencing the effect of plant neighbor on RAB. Plants can affect the rhizosphere biota of other plants by altering soil chemistry and nutrient availability (Bais *et al.,* 2006; Meier *et al.,* 2009; Meinhardt and Gehring, 2012), through plant-produced allelopathic, antibiotic, or symbiont attracting molecules, or through microbially-produced quorum sensing molecules (Bais *et al.,* 2004; Bais *et al.,* 2006). Neighborhood effects have been shown for root-associated fungal communities (Meinhardt and Gehring, 2012; Dean *et al.,* 2014), but as far as we know, ours is the first evidence of plant neighbor effects on RAB.

Individual taxa and host response to N. The dominant taxonomic group can have an important functional role within an ecological community (Smith and Knapp, 2003). The dominant root-associated fungal order was Helotiales, a group that includes important ericoid mycorrhizal and dark septate fungi. Helotiales were more dominant in *G. rossii*, and declined with N in *G. rossii* but not in *D. cespitosa,* perhaps indicating a loss of mutualists is responsible for *G. rossii* dieback with N (Dean *et al.,* 2014). In contrast, the dominant RAB taxonomic group, Proteobacteria, did not respond to N in either host. Over a third of this phylum was composed of *Pseudomonas* in *D. cespitosa*, which was also unaffected by N. The sensitivity of the dominant fungal taxonomic group and lack of sensitivity of the dominant bacterial taxonomic group to N may indicate fungal dynamics play a more important role in differing host response to N. The ecological functions of most RAB taxonomic groups

affected by N are not well known. However, host-specific effects of N on RAB may indicate that RAB influence host-specific responses to N enrichment.

Summary. We found that RAB communities are structured by both biotic and abiotic assembly mechanisms. Host had a more profound effect than N on RAB assembly. Furthermore, the host with the less diverse RAF community (*G. rossii*) had the more diverse RAB community, and RAB diversity and community composition responded to N enrichment inversely from RAF at the same site (Dean *et al.,* 2014). Plant neighborhood did not affect RAB assembly even though neighborhood at this site has been found to affect RAF (Dean *et al.,* 2014). The presence or absence of *D. cespitosa* in experimental plots did, however, influence how *G. rossii* RAB diversity and individual *G. rossii* RAB taxa responded to N, indicating that hosts and their microbial communities interact, and affect neighboring RAB responses to nutrient enrichment, which has not been found before to our knowledge (but has been shown before for RAF, Dean *et al.,* 2014). Considering root microbes can have a profound effect on host health, vegetation community response to N enrichment may be more complex than previously thought due to complex plant-microbe interactions.

Data Accessibility: DNA sequences, data tables, and R code are available on Dryad (doi:10.5061/dryad.7535k). SFF, FASTA and QUAL files can be accessed through SRA by the study accession SRP049180.

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Figures and Tables

Figure 1: Phylum profiles of *D. cespitosa* and *G. rossii* RAB under each treatment. Proteobacteria is broken down into different classes, shown in gray scale.

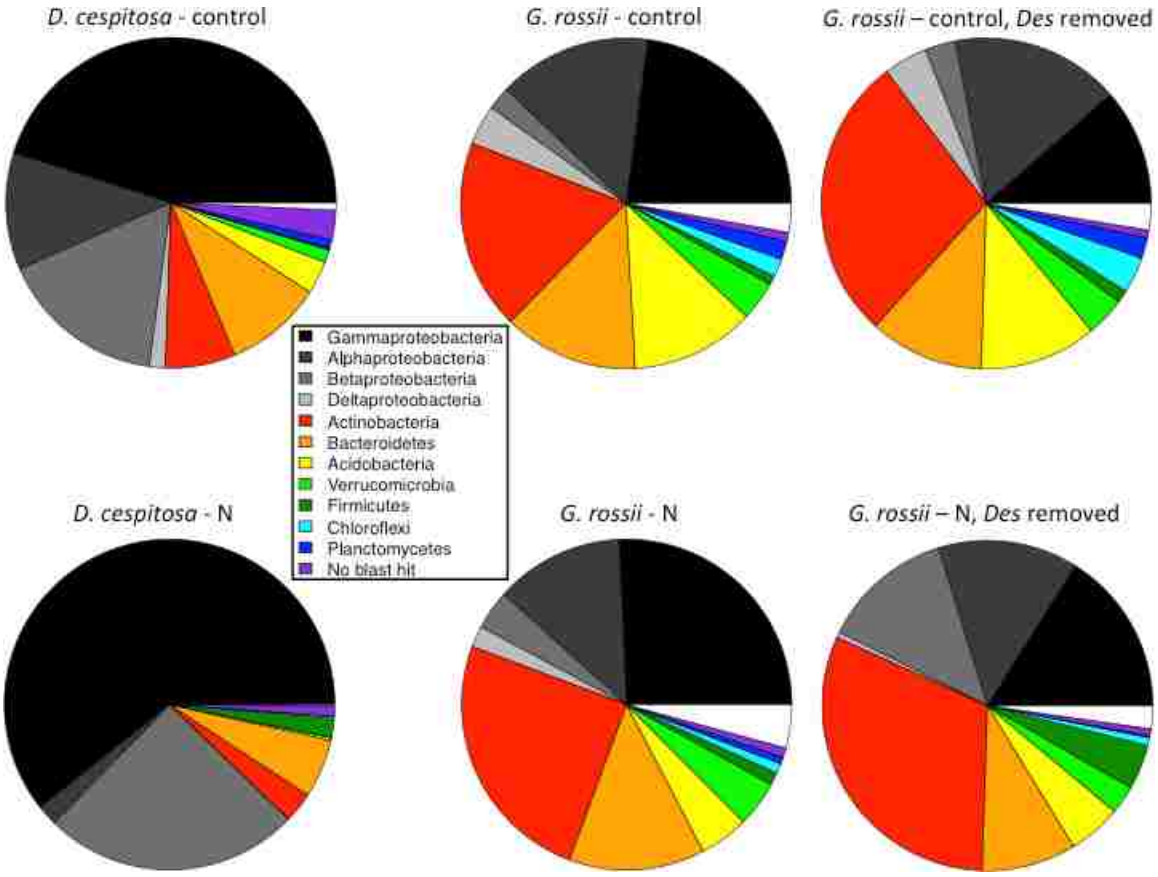

Figure 2: Distance-based redundancy analysis showing the effect of host, N, and competitor removal on RAB community assembly. For significance of treatments see Table I. Abbreviations are as follows: *Des = Deschampsia* host plant; *Geum = Geum* host plant; CC= no removal, no N; CN = no removal, N; DC = *Deschampsia* removed, no N; DN = *Deschampsia* removed, N.

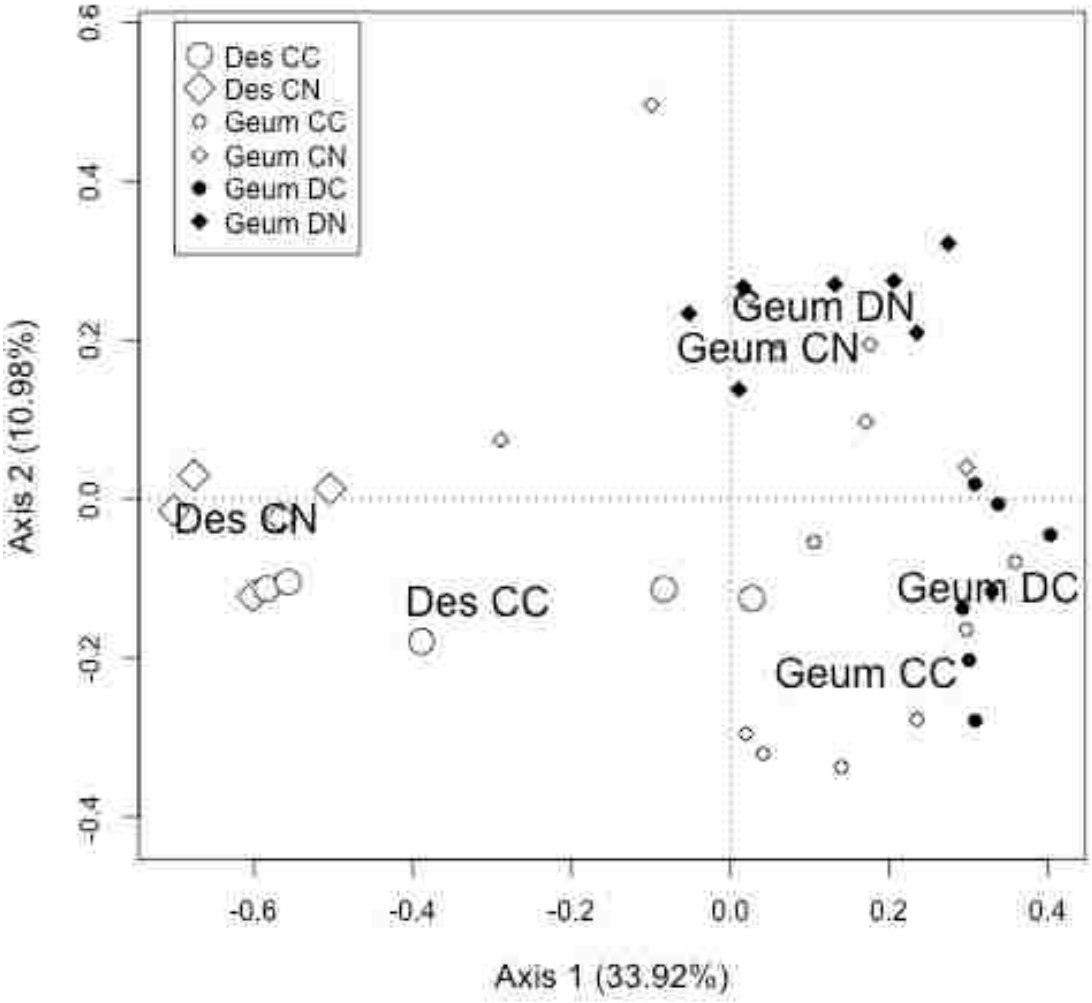

Figure 3: Phylogenetic diversity of *D. cespitosa* and *G. rossii* in control and N plots. *Geum rossii* samples are divided between those from plots where *D. cespitosa* was removed and those where *D. cespitosa* was present. We performed a Tukey post hoc test on a model including all the data to test pairwise differences among the six host x treatment combinations. Bars sharing a letter are not significantly different ($P < 0.05$).

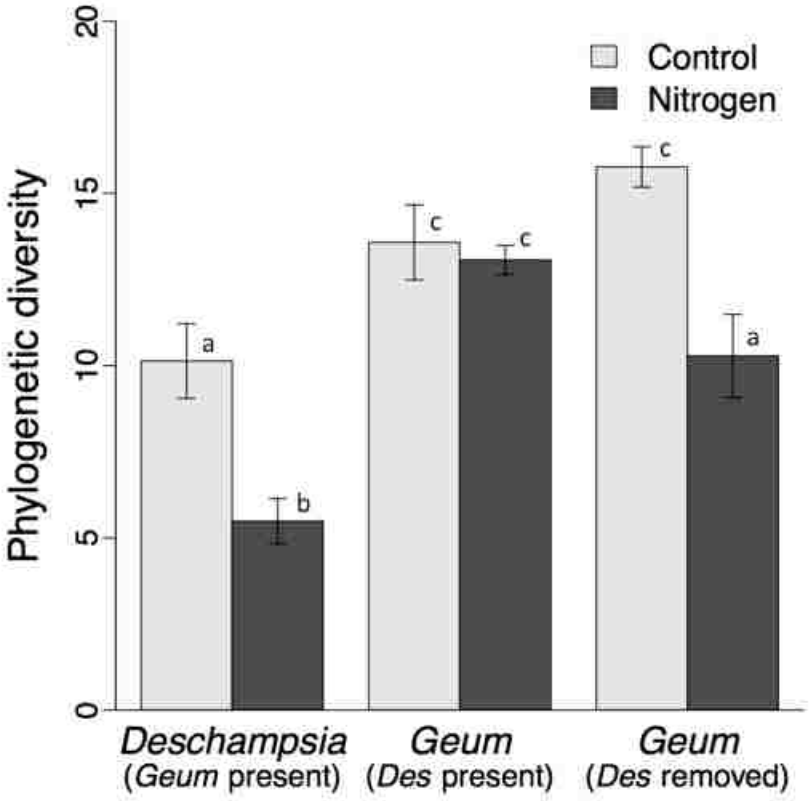

Fig. S1: Alpha rarefaction curves. One hundred rarefactions for each sample at each sequencing depth.

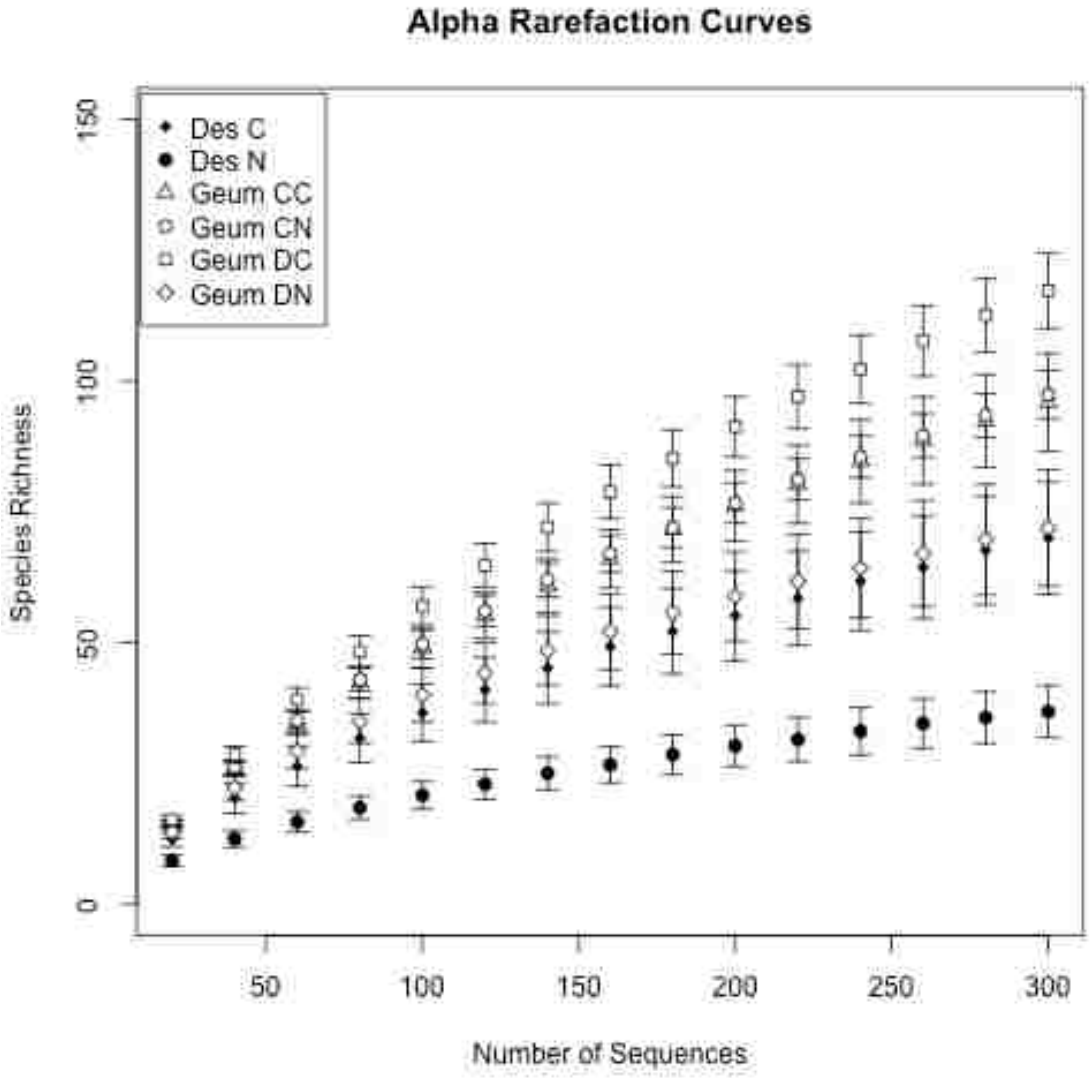

Table 1: The effect of host, N fertilization, and competitor removal on RAB community composition (phylogenetic distance), using permutation tests. Due to the unbalanced design, two models were tested. The first model uses only the data from *Deschampsia* (n = 10) and *Geum* (n = 14) hosts in control and N plots and tests the effect of host and N on community composition. The second uses all data from *Geum* hosts (n = 28) and tests the effect of N and *Deschampsia* removal. Marginal and significant effects are italicized, *** $P<0.001$, ** $P<0.01$, * $P<0.05$, † $P<0.1$.

Model	Explanatory variable	% Variance explained	*P*
Host + N + Host x N	Host	30.0	*<0.001****
	N	12.1	*<0.001****
	Host x N	4.5	*0.093*†
N + removal + N x removal	N	21.2	*<0.001****
	Removal	2.7	0.438
	Removal x N	2.8	0.432

Table 2: The effect of host, N fertilization, and competitor removal on RAB phylogenetic diversity and phylum diversity, evenness, and richness, tested using linear mixed effects models. Due to the unbalanced design, two models were tested. The first model uses only the data from *Deschampsia* (n = 10) and *Geum* (n = 13) hosts in control and N plots and tests the effect of host and N on diversity. The second uses all data from *Geum* hosts (n = 27) and tests the effect of N and *Deschampsia* removal. Marginal and significant effects are italicized, *** $P<0.001$, ** $P<0.01$, * $P<0.05$, † $P<0.1$.

Taxonomic level	Host (Ambient only)	Host + N + Host × N			N + removal +N × removal		
	Host	Host	N	Host × N	N	Removal	N × Removal
PD	*0.055*†	*<0.001***	*0.010**	*0.033**	*0.003***	0.751	*0.012**
Phylum diversity	*0.005***	*<0.001***	0.146	0.263	*0.040**	0.730	0.259
Phylum evenness	*0.010**	*0.004***	0.751	0.405	0.912	0.378	0.398
Phylum richness	*0.008***	*<0.001***	*0.004***	*0.003***	*0.006***	0.873	*0.004***

S.T1: Effect of N on alpha diversity measures of RAB in *G. rossii* and *D. cespitosa* at each taxonomic level. Diversity measure means for each host x N combination as well as P-values are displayed. Samples from *D. cespitosa* removal plots are excluded from this analysis (n = 23). P.D. = Phylogenetic diversity. DesC = *D. cespitosa* control, DesN = *D. cespitosa* N addition, GeumC = *G. rossii* control, GeumN = *G. rossii* N addition. Marginal and significant effects are italicized, ** *P*<0.01, * *P*<0.05, † *P*<0.1.

	Means				**Model: host + N + host*N**			**Model: host (control plots)**
Species	**DesC**	**DesN**	**GeumC**	**GeumN**	**P(Host)**	**P(N)**	**P(interaction)**	**P(host)**
P.D.	10.13	5.48	13.58	13.07	*<0.01***	*0.01**	*0.03**	*0.05**
Simpson's	0.89	0.75	0.96	0.95	*<0.01***	*0.06*†	*0.09*†	*0.09*†
Evenness	0.22	0.14	0.29	0.27	*0.01**	0.20	0.42	0.18
Richness	70.36	37.36	96.63	98.00	*<0.01***	*0.07*†	*0.05**	0.10
Genus								
Simpson's	0.83	0.69	0.92	0.94	*<0.01***	0.15	*0.06*†	0.14
Evenness	0.21	0.13	0.26	0.31	*0.01**	0.63	0.10	0.42
Richness	46.70	28.44	53.60	60.03	*<0.01***	0.19	*0.01**	0.37
Family								
Simpson's	0.82	0.67	0.91	0.93	*<0.01***	0.10	*0.06*†	0.11
Evenness	0.24	0.16	0.28	0.32	*0.02**	0.55	0.17	0.52
Richness	38.10	21.99	46.73	48.40	*<0.01***	*0.04**	*0.01**	0.15
Order								
Simpson's	0.80	0.65	0.88	0.88	*<0.01***	*0.06*†	*0.08*†	0.10
Evenness	0.27	0.22	0.29	0.28	0.22	0.42	0.63	0.63
Richness	24.26	14.15	30.67	29.79	*<0.01***	*<0.01***	*0.01**	*0.02**
Class								
Simpson's	0.67	0.47	0.85	0.81	*<0.01***	*0.09*†	0.22	*0.05**
Evenness	0.27	0.23	0.33	0.30	0.11	0.39	0.99	0.27
Richness	15.46	9.10	20.39	19.18	*<0.01***	*<0.01***	*0.03**	*0.01**
Phylum								
Simpson's	0.39	0.22	0.72	0.70	*<0.01***	0.15	0.26	*0.01**
Evenness	0.21	0.24	0.31	0.30	*<0.01***	0.75	0.41	*0.01**
Richness	9.37	5.87	11.72	11.79	*<0.01***	*<0.01***	*<0.01***	*0.01**

S.T2: Effect of treatment on alpha diversity of RAB in *G. rossii* at each taxonomic level. Diversity measure means for each treatment combination, and P-values, are displayed. *D. cespitosa* is excluded from this analysis (n = 27). P.D. = Phylogenetic diversity. CC = control, CN = N addition, DC = *D. cespitosa* removal, DN = *D. cespitosa* removal + N addition. Marginal and significant effects are italicized, ** *P*<0.01, * *P*<0.05, † *P*<0.1.

	Means				**Model: N + removal + N*removal**		
Species	**CC**	**CN**	**DC**	**DN**	**P(N)**	**P(removal)**	**P(interaction)**
P.D.	13.58	13.07	15.77	10.28	*<0.01***	0.75	*0.01**
Simpson's	0.96	0.95	0.96	0.93	0.19	0.47	0.40
Evenness	0.29	0.27	0.29	0.28	0.66	0.88	0.92
Richness	96.63	98.00	118.21	72.46	*0.02**	0.82	*0.01**
Genus							
Simpson's	0.92	0.94	0.94	0.91	0.84	1.00	0.19
Evenness	0.26	0.31	0.31	0.30	0.49	0.49	0.35
Richness	53.60	60.03	65.40	49.21	0.34	0.92	*0.03**
Family							
Simpson's	0.91	0.93	0.94	0.91	0.64	0.91	0.25
Evenness	0.28	0.32	0.33	0.33	0.65	0.32	0.49
Richness	46.73	48.40	54.06	41.61	0.15	0.94	*0.07*†
Order							
Simpson's	0.88	0.88	0.89	0.84	*0.04**	0.30	*0.09*†
Evenness	0.29	0.28	0.28	0.27	0.61	0.62	0.97
Richness	30.67	29.79	34.87	25.16	*0.01**	0.91	*0.03**
Class							
Simpson's	0.85	0.81	0.85	0.80	*0.01**	0.77	0.53
Evenness	0.33	0.30	0.29	0.34	0.74	0.84	*0.06*†
Richness	20.39	19.18	23.56	15.95	*<0.01***	0.98	*0.01**
Phylum							
Simpson's	0.72	0.70	0.76	0.68	*0.04**	0.73	0.26
Evenness	0.31	0.30	0.31	0.33	0.91	0.38	0.40
Richness	11.72	11.79	13.36	9.97	*0.01**	0.87	*<0.01***

S.T3: *G. rossii* (n = 13) and *D. cespitosa* (n = 10) RAB taxa significantly correlated with N using type III ANOVA. N effect is tested on each host separately, and *D. cespitosa* removal plots are excluded. Uncorrected P-values, direction of N effect and relative abundance of taxa are listed. Taxa significantly correlated with N after FDR correction are indicated by an asterisk.

G. rossii	Taxon	Effect Direction	P	Relative Abundance (C)	Relative Abundance (N)
Phylum	Acidobacteria	-	0.008	12.71	5.09
Class	Betaproteobacteria	+	0.012	2.37	3.96
	Solibacteres	-	0.013	3.96	0.23
	Unknown	-	0.014	7.88	3.26
Order	Burkholderiales	+	0.025	2.10	3.69
	Chromatiales*	-	0.001	19.40	2.91
	Rhizobiales	-	0.016	10.07	5.26
	Solibacterales	-	0.013	4.00	0.23
	Unknown Acidobacteria	-	0.014	7.95	3.28
	Xanthomonadales	+	0.004	2.16	13.70
Family	Bradyrhizobiaceae	-	0.008	7.95	3.16
	Rhodospirillaceae	-	0.013	1.46	0.22
	Sinobacteraceae	-	0.001	19.25	2.79
	Solibacteraceae	-	0.012	3.97	0.22
	Sphingobacteriaceae	+	0.041	3.11	7.62
	Unknown Acidobacteria	-	0.022	7.07	2.92
	unknwon Myxococcales	-	0.025	1.61	0.33
	Xanthomonadaceae	+	0.004	1.76	13.67
Genus	Bradyrhizobium	-	0.009	6.95	2.39
	Dokdonella	+	0.043	0.00	1.03
	Polaromonas	-	0.004	0.44	0.17
	Rhodanobacter	+	0.004	0.15	10.34
	Unknown Acidobacteria	-	0.023	7.20	3.02
	Unknown Candidatus Solibacter	-	0.012	4.04	0.23
	Unknown Frankiaceae	+	0.029	0.25	0.76
	Unknown Microbacteriaceae	+	0.049	0.25	2.47
	Unknown Myxococcales	-	0.025	1.64	0.34
	Unknown Rhodospirillaceae	-	0.034	1.24	0.23
	Unknown Sinobacteraceae	-	0.001	19.42	2.87
	Unknown Sphingobacteriaceae	+	0.033	2.78	7.61
	Unknown Sphingomonadaceae	+	0.015	0.00	0.76
Species	*Acidobacteria spp.*	-	0.015	2.23	0.13
	Acidobacteriaceae spp	+	0.034	0.13	1.08
	Bradyrhizobium spp.	-	0.008	8.19	2.89
	Candidatus Solibacter spp.	-	0.048	1.30	0.00
	Caulobacteraceae spp.	+	0.035	0.06	0.87
	Dokdonella spp.	+	0.045	0.00	1.24
	Microbacteriaceae spp.	+	0.044	0.13	2.94
	Polaromonas spp.	-	0.018	0.48	0.21
	Rhodanobacter lindaniclasticus	+	0.006	0.05	10.30
	Sinobacteraceae spp.	-	0.001	10.63	1.16
	Sinobacteraceae spp.	-	0.001	9.58	1.01
	Sphingobacteriaceae spp.	+	0.021	0.18	0.54
	Sphingobacteriaceae spp.	+	0.037	0.00	0.34
	Sphingobacteriaceae spp.	+	0.035	1.91	6.30
	Sphingobacteriales	-	0.008	6.28	0.89
	Sphingobacteriales	-	0.025	1.93	0.21
	Sphingomonadaceae spp.	+	0.013	0.00	0.88
D.	Taxon	Effect	P	Relative	Relative

cespitosa		Direction		Abundance (C)	Abundance (N)
Phylum	Planctomycetes	-	0.008	0.87	0.00
Class	Alphaproteobacteria	-	0.025	11.63	2.08
Order	Chromatiales	-	0.039	3.01	0.33
	Rhizobiales	-	0.013	4.55	0.73
	Xanthomonadales	+	0.003	3.23	9.86
Family	Rhizobiaceae	-	0.041	2.26	0.00
	Sinobacteraceae	-	0.040	3.08	0.13
	Unknown Burkholderiales	-	0.019	5.77	0.74
	Xanthomonadaceae	+	0.004	3.36	9.88
Genus	Rhizobium	-	0.042	2.34	0.00
	Unknown Burkholderiales	-	0.013	5.46	0.62
	Unknown Sinobacteraceae	-	0.041	3.19	0.13
Species	*Rhodanobacter lindaniclasticus*	+	0.039	1.00	7.19
	Unknown Burkholderiales	-	0.017	6.32	0.65
	Unknown Sinobacteraceae	-	0.046	2.02	0.14

S.T4: RAB that are significantly correlated with host and N addition (n = 23). The direction of N effect on each taxon, uncorrected P-values, and average relative abundance are listed. DesC = *D. cespitosa* control, DesN = *D. cespitosa* N addition, GeumC = *G. rossii* control, GeumN = *G. rossii* N addition. Species showing significant interactions between host and N are emboldened, P-values that were significant after FDR correction are indicated by an asterisk.

Taxa	Species name	N Effect	P (N)	P (host)	P (N x host)	DesC	DesN	GeumC	GeumN
Acidobacteria									
Unknown	*unknown*	0	0.340	0.005	0.110	0.37	0.00	2.16	0.12
	unknown	0	1.000	0.009	0.051	0.00	0.00	1.00	0.00
	unknown	0	1.000	0.014	0.070	0.00	0.00	0.30	0.00
Solibacteres Solibacterales Solibacteraceae	*Candidatus Solibacter spp.*	0	1.000	0.024	0.099	0.00	0.00	0.33	0.00
	Candidatus Solibacter spp.	-/0	0.005	0.003	0.026	0.30	0.00	0.00	0.00
	Candidatus Solibacter spp.	0	1.000	0.032	0.141	0.00	0.00	1.13	0.07
	Candidatus Solibacter spp.	0	0.716	0.010	0.061	0.14	0.00	1.26	0.00
Actinomycetales unknown	*unknown*	0	0.926	0.043	0.736	0.00	0.00	0.66	0.49
	unknown	0	0.977	0.004	0.251	0.00	0.00	3.39	1.82
	unknown	0	0.969	0.005	0.895	0.07	0.00	5.73	5.98
Actinobacteria Actinomycetales Actinosynnemataceae	*Kutzneria spp.*	-	0.015	0.003	0.055	0.91	0.14	0.00	0.00
Actinobacteria Actinomycetales Microbacteriaceae	***unknown***	-/+	0.846	0.363	0.030	0.90	0.75	0.12	2.77
Actinobacteria Actinomycetales Pseudoncardiaceae	***Amycolatopsis spp.***	-/+	0.251	0.218	0.044	0.45	0.00	0.00	0.64
Bacteroidetes									
Sphingobacteria Sphingobacteriales unknown	*unknown*	0	0.120	0.007	0.247	2.14	0.14	6.07	0.85
	unknown	-	0.689	0.001*	0.030	0.22	0.00	1.85	0.20
Sphingobacteria Sphingobacteriales Sphingobacteriaceae	***unknown***	-/+	0.120	0.399	0.042	0.32	0.00	0.17	0.52
	unknown	-/+	0.093	0.161	0.006	4.06	1.11	1.82	6.00
Planctomycetes									
Phycisphaerae unknown	*unknown*	0	1.000	0.050	0.156	0.00	0.00	0.24	0.00
Proteobacteria									
Alphaproteobacteria unknown	***unknown***	-/+	0.479	0.246	0.042	0.55	0.22	0.06	0.97
Alphaproteobacteria Caulobacterales Caulobacteraceae	*unknown*	-	0.043	0.094	0.232	0.46	0.00	0.12	0.00
	unknown	-/+	0.742	0.913	0.019	0.08	0.00	0.06	0.84
	Asticcacaulis biprosthecium	-/+	0.080	0.062	0.046	0.36	0.00	0.00	0.20
	Phenylobacterium spp.	0	0.803	0.033	0.654	0.00	0.00	0.30	0.26
Alphaproteobacteria Rhizobiales Bradyrhizobiaceae	*unknown*	0	0.444	0.041	0.482	0.00	0.00	0.71	0.66
	Bradyrhizobium spp.	-	0.290	<0.001*	0.020	1.57	0.35	7.87	2.72
Alphaproteobacteria Rhizobiales Hyphomicrobiaceae	***Devosia spp.***	-/+	0.344	0.501	0.040	0.16	0.00	0.06	0.39
Alphaproteobacteria Rhizobiales Rhizobiaceae	***Rhizobium spp.***	-	<0.001*	0.001*	0.005	2.40	0.00	0.23	0.12
Alphaproteobacteria Sphingomonadales Sphingomonadaceae	***unknown***	-/0	1.000	1.000	0.007	0.00	0.00	0.00	0.84
	Sphingomonas spp.	+	0.030	0.947	0.949	0.00	0.21	0.00	0.20
	Sphingomonas asaccharolytica	-/+	0.015	0.020	0.028	2.87	0.70	0.87	1.35
	Sphingomonas wittichii	-/0	0.008	0.005	0.036	0.30	0.00	0.00	0.00

Betaproteobacteria Burkholderiales unknown	***unknown***	-/+	<0.001*	<0.001*	0.001	5.45	0.64	0.89	1.16
Betaproteobacteria Burkholderiales Alcaligenaceae	*unknown*	+	0.005	0.894	0.056	0.00	0.41	0.00	0.07
Betaproteobacteria Burkholderiales Burkholderiaceae	*Burkholderia sordidicola*	0	0.137	0.013	0.178	3.76	1.60	0.11	0.53
Betaproteobacteria Burkholderiales Comamonadaceae	*Polaromonas spp.*	0	0.948	0.016	0.236	0.08	0.07	0.46	0.20
Betaproteobacteria Burkholderiales Oxalobacteraceae	***unknown***	-/+	0.056	0.004	0.048	0.81	0.28	0.00	0.20
	unknown	0	0.411	0.039	0.512	1.76	2.52	0.00	0.00
	Janthinobacterium lividum	+/0	0.017	0.897	0.059	0.84	19.13	0.00	0.00
	Massilia timonae	0	0.912	0.027	0.447	0.97	0.93	0.00	0.42
Deltaproteobacteria Myxococcales unknown	*unknown*	0	0.651	0.039	0.170	0.15	0.00	0.79	0.07
Deltaproteobacteria Myxococcales Haliangiaceae	*unknown*	0	0.910	0.017	0.154	0.08	0.00	1.32	0.26
	unknown	-/+	0.012	0.008	0.016	0.22	0.00	0.00	0.06
	unknown	-/0	0.008	0.005	0.035	0.52	0.00	0.00	0.00
Gammaproteobacteria Chromatiales Sinobacteraceae	*unknown*	0	0.901	0.043	0.292	0.07	0.00	1.21	0.34
	unknown	-	0.551	<0.001*	0.006	0.91	0.00	10.24	1.10
	unknown	-	0.234	<0.001*	<0.001*	1.70	0.14	9.23	0.97
Gammaproteobacteria Pseudomonadales Pseudomonadaceae	*Pseudomonas spp.*	-/+	0.166	0.037	0.069	11.33	4.07	0.68	6.47
	Pseudomonas spp.	+	0.285	0.020	0.465	19.38	28.15	0.00	0.88
	Pseudomonas spp.	+	0.012	0.478	0.066	1.00	5.32	0.00	0.37
Gammaproteobacteria Xanthomonadales Xanthomonadaceae	*Luteibacter rhizovicinus*	-/+	0.329	0.135	0.046	1.75	0.55	0.00	2.29
	Rhodanobacter lindaniclasticus	+	0.016	0.705	0.257	0.85	7.05	0.05	9.79
Verrucomicrobia									
Opitutae Opitutales Opitutaceae	*Opitutus spp.*	0	0.911	0.031	0.236	0.00	0.00	0.23	0.07
No blast hit	*unknown*	0	0.101	0.011	0.143	1.99	0.75	0.00	0.13

S.T5: *G. rossii* RAB taxa that are significantly correlated with treatment (n = 27), uncorrected P-values, and average relative abundance in each treatment are listed. N = Nitrogen, rem = *D. cespitosa* removal, CC = control, CN = N addition, DC = *D. cespitosa* removal, DN = *D. cespitosa* removal + N addition. Species showing significant interactions between N and *D. cespitosa* removal are emboldened, P-values that were significant after FDR correction are indicated with an asterisk.

Taxa		P (N)	P (rem)	P (N x rem)	CC	CN	DC	DN
Acidobacteria								
unknown	*Unknown*	0.002	0.007	0.075	2.05	0.12	0.62	0.05
	Unknown	0.007	0.084	0.114	0.95	0.00	0.40	0.16
	Unknown	1.000	0.009	0.059	0.00	0.00	0.17	0.00
	Unknown	0.005	0.004	0.032	0.29	0.00	0.00	0.00
	Unknown	0.041	0.055	0.214	0.61	0.00	0.11	0.00
Acidobacteria Acidobacteriales								
Acidobacteriaceae	*Unknown*	1.000	0.009	0.058	0.00	0.00	0.17	0.00
	Unknown	0.011	0.833	0.857	0.11	0.97	0.05	0.83
Solibacteres Solibacterales								
Solibacteraceae	*Candidatus Solibacter spp.*	0.653	0.034	0.058	0.11	0.06	0.34	0.00
	Candidatus Solibacter spp.	0.042	0.317	0.487	0.31	0.00	0.17	0.00
	Candidatus Solibacter spp.	1.000	0.009	0.057	0.00	0.00	0.52	0.00
	Candidatus Solibacter spp.	0.021	0.022	0.122	1.06	0.06	0.11	0.00
	Candidatus Solibacter spp.	0.002	0.002	0.025	1.19	0.00	0.11	0.00
Actinobacteria								
Actinobacteria Actinomycetales								
Microbacteriaceae	*Unknown*	0.043	0.542	0.362	0.12	2.70	0.79	4.77
Actinobacteria Solirubrobacterales								
unknown	***unknown***	0.045	0.293	0.048	0.00	0.48	0.23	0.05
	unknown	0.055	0.243	0.011	0.17	0.51	0.34	0.05
	unknown	0.428	0.002	0.008	0.05	0.27	1.02	0.12
Armatimonadetes								
incertae sedis CH21	*Unknown*	0.033	0.014	0.053	0.41	0.00	0.05	0.13
Bacteroidetes								
Sphingobacteria Sphingobacteriales								
unknown	*Unknown*	0.001	0.165	0.545	5.74	0.83	4.03	0.27
	Unknown	0.003	0.013	0.111	1.75	0.19	0.59	0.06
Sphingobacteria Sphingobacteriales								
incertae sedis	*Flavisolibacter spp.*	0.912	0.029	0.118	0.05	0.00	1.13	0.00
Sphingobacteria Sphingobacteriales								
Sphingobacteriaceae	*Unknown*	0.011	0.770	0.606	1.69	5.84	2.11	5.16
Chloroflexi								
Bljii12 B07_WMSP1 FFCH4570	*Unknown*	0.465	0.015	0.078	0.17	0.00	0.74	0.00
Ktedonobacteria	*Unknown*	0.687	0.032	0.112	0.06	0.13	0.40	0.11
Gemmatimonadetes								
Gemmatimonadetes								
Gemmatimonadales unknown	*Unknown*	1.000	0.017	0.086	0.00	0.00	0.22	0.00
Gemmatimonadetes								
Gemmatimonadales								
Gemmatimonadaceae	***Gemmatimonas spp.***	0.004	1.000	0.028	0.00	0.33	0.00	0.00
Planctomycetes								
Phycisphaerae unknown	*Unknown*	0.021	0.016	0.083	0.22	0.00	0.00	0.00
Proteobacteria								
Alphaproteobacteria	*Unknown*	0.014	0.580	0.365	0.06	0.92	0.23	0.69
Alphaproteobacteria Caulobacterales								
Caulobacteraceae	*Unknown*	0.651	0.018	0.118	0.11	0.00	0.73	0.06
	Unknown	0.005	0.317	0.165	0.05	0.80	0.28	0.56
	Phenylobacterium spp.	0.535	0.016	0.034	0.00	0.06	0.23	0.00
Alphaproteobacteria Rhizobiales								
Bradyrhizobiaceae	*Bradyrhizobium spp.*	0.008	0.581	0.973	7.45	2.63	6.58	1.83

Alphaproteobacteria Rhizobiales Hyphomicrobiaceae	*Rhodoplanes spp.*	0.251	0.048	0.135	0.27	0.00	0.68	0.00
Alphaproteobacteria Sphingomonadales unknown	*unknown*	0.016	1.000	0.153	0.00	0.27	0.00	0.06
Alphaproteobacteria Sphingomonadales Sphingomonadaceae	*unknown*	0.004	1.000	0.431	0.00	0.82	0.00	0.55
	Sphingomonas spp.	0.003	1.000	0.022	0.00	0.20	0.00	0.00
Betaproteobacteria Burkholderiales Oxalobacteraceae	*Massilia timonae*	0.038	1.000	0.262	0.00	0.41	0.00	0.12
Deltaproteobacteria Myxococcales unknown	*Unknown*	0.014	0.126	0.337	0.74	0.06	0.35	0.00
Deltaproteobacteria Myxococcales Haliangiaceae	*Unknown*	0.018	0.280	0.705	1.23	0.25	0.81	0.00
	Unknown	0.544	0.040	0.151	0.16	0.00	0.76	0.00
Gammaproteobacteria Chromatiales Sinobacteraceae	***Unknown***	<0.001*	0.000	0.021	9.68	1.08	3.03	0.00
	Unknown	<0.001*	0.008	0.105	8.76	0.94	6.06	0.44
Gammaproteobacteria Pseudomonadales Pseudomonadaceae	*Pseudomonas spp.*	0.015	1.000	0.113	0.00	0.84	0.00	0.12
Gammaproteobacteria Xanthomonadales Xanthomonadaceae	*Unknown*	0.047	0.104	0.145	0.27	0.00	0.06	0.05
	Unknown	0.044	0.489	0.543	0.67	0.00	0.46	0.05
	Dokdonella spp.	0.005	0.614	0.101	0.00	1.12	0.17	0.45
	Luteibacter rhizovicinus	0.008	1.000	0.118	0.00	2.21	0.00	0.53
	Rhodanobacter	0.033	0.919	0.909	0.11	1.39	0.06	1.24
	Rhodanobacter lindaniclasticus	<0.001*	0.975	0.033	0.05	9.48	0.00	4.17
Verrucomicrobia Opitutae Opitutales Opitutaceae	*Unknown*	0.003	0.775	0.140	0.21	2.14	0.06	0.77
No blast hit	*Unknown*	0.300	0.004	0.282	0.00	0.13	0.39	0.33

S.T6: pH measurements for control (C) and N addition (N) plots. Average and results of a t-test are provided at the bottom of the table.

Plot	C	N
2	5.1	5.1
3	5.2	5.2
4	5.4	4.6
5	5.5	5.35
average	5.3	5.0625
t-test P	0.25	

CHAPTER 4

Root Associated Fungal Community Response to Drought-Associated Changes in Vegetation Community

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Abstract: Recent droughts in southwestern North America have led to large-scale mortality of piñon (*Pinus edulis*) in piñon-juniper woodlands. Piñon mortality alters soil moisture, nutrient and carbon availability, which could affect the root-associated fungal (RAF) communities, and therefore the fitness, of the remaining plants. We collected fine root samples at piñon-juniper woodland and a juniper

savannah site in central New Mexico. Roots were collected from piñon and juniper (*Juniperus monosperma*) trees whose nearest neighbors were live piñon, live juniper or dead piñon. RAF communities were analyzed by 454 pyrosequencing of the universal fungal ITS region. The most common taxa were Hypocreales, and Chaetothyriales. Over 10% of the ITS sequences could not be assigned taxonomy at the phylum level. Two of the unclassified OTUs significantly differed between savanna and woodland, had few like sequences in GenBank, and formed new fungal clades with other unclassified RAF from arid plants, highlighting how little study has been done on the RAF of arid ecosystems. Neither plant host nor neighbor effected RAF community composition. However, there was a significant difference between RAF communities from woodland vs. savanna, indicating abiotic factors such as temperature and aridity may be more important in structuring these RAF communities than biotic factors such as plant host or neighbor identity. Ectomycorrhizal fungi (EM) were present in juniper as well as piñon in the woodland site, in contrast with previous research, but did not occur in juniper savanna, suggesting that piñon can share it's EM with juniper. RAF richness was lower in hosts that were neighbored by the opposite host. This may indicate competitive exclusion between fungi from different hosts. Characterizing these communities and their responses to environment and neighbor effects is a step toward understanding of the effects of drought on a biome that spans 19 million ha of the American southwest.

Key Words: Arid ecosystems, fungal ITS, juniper savanna, next generation sequencing, piñon dieback, piñon juniper woodlands, root associated fungi

INTRODUCTION

Climate change is expected to cause many areas of the world, such as southwestern North America, to become hotter and more arid, with increased incidences of severe drought (Leung *et al.* 2004, IPCC 2007, Christensen *et al.* 2007, Seager *et al.* 2007, Weiss *et al.* 2009). Drought can alter the relative abundance of plant species. For example, in piñon-juniper woodlands, which cover 19 million hectares of the southwestern United States (Gottfried *et al.* 1995), piñon (*Pinus edulis*) severely died back during droughts from 1994 to 2004 while juniper (*Juniperus monosperma*) largely survived (Breshears *et al.* 2005, Mueller *et al.* 2005, Breshears *et al.* 2009), causing a major restructuring of the plant community.

Root associated fungi (RAF) can affect host fitness through mutualistic or parasitic interactions (Rodriguez *et al.* 2008, Badri *et al.* 2009, Porras-Alfaro and Bayman 2011). Some RAF in extreme environments aid with environmental stress tolerance such as drought (Rodriguez *et al.* 2008, Porras-Alfaro and Bayman 2011). Thus, gain or loss of individual RAF could alleviate or amplify the effects of drought, and feedback into restructuring of the plant community. Changes in the vegetation community have been shown to alter the composition of RAF associated with remaining plant species (Meinhart *et al.* 2012, Bogar *et al.* 2013, Dean *et al.* 2014). Previous studies in piñon-juniper woodlands suggest competition between the two co-dominant plants affects both fine root growth and associated RAF (Haskins and Gehring 2004, Haskins and Gehring 2005), but these RAF have yet to be described using DNA sequencing, which has the potential to provide more detailed information on RAF community composition, and on phylogenetic information for

poorly classified fungi. In this study we use next generation sequencing to explore the effect of piñon and juniper on each other's RAF, as well as the effect of piñon death on the RAF of surviving junipers.

We collected fine root samples from piñon and juniper trees whose nearest neighbors were live piñon, live juniper, or dead piñon. For comparison, we also collected roots from junipers neighboring junipers from a nearby juniper savanna (where, to our knowledge, RAF have yet to be described). Juniper savannas are warmer, drier, and lack piñon trees, and may represent the ecosystem state to which piñon-juniper woodland may transition should these extreme drought events continue (Swaty *et al.* 2004). DNA from the fungal ITS region was sequenced using 454 pyrosequencing. We hypothesized that plant neighbor identity would impact fungal community structure, and dead piñons would introduce saprobes and pathogens to their neighbors. We thought this because the roots of these two host plants grow in very close proximity and even in contact with each other, which may encourage sharing of microbes. Finally, we expected plant host to have the strongest influence on RAF community structure, followed by environment (woodland vs. savanna), and lastly neighbor identity.

METHODS

Field Site.— The two sampling sites were within the fetch of eddyflux covariance towers, part of the Ameriflux network , for which extensive above and belowground monitoring data are available. Climate data and site information can be found both at FLUXNET (piñon-juniper woodland: http://fluxnet.ornl.gov/site/2713, and juniper savanna: http://fluxnet.ornl.gov/site/2712) and AmeriFlux

(http://cdiac.esd.ornl.gov/programs/ameriflux/data_system/aamer.html where piñon-juniper woodland is titled "Heritage Wood" and juniper savanna is titled "Tablelands"). Briefly, the piñon-juniper woodland site is located south of Mountainair, NM (34.45 N, -106.21 W), at 2100m in elevation. The area has a mean annual temperature 14.8 C, mean annual precipitation of 418mm, with a canopy co-dominated by piñon and juniper. The juniper savanna site (34.43 N, -105.86 W) is 1926m in elevation, has mean annual temperature 15.2 C, mean annual precipitation 361mm. The only woody vegetation is juniper.

At the piñon-juniper site, the mature piñon trees (~16 000) were girdled in a 4 ha area in Sept. 2009 (Eitel *et al.* 2011). These trees were dead by the following April. From this site, on 26 and 27 July 2012, we collected root samples from live piñon trees (LP) that neighbored other live piñons (LP-LP) or live junipers (LP-LJ). We also collected roots from live juniper trees (LJ) that neighbor other live junipers (LJ-LJ), live piñons (LJ-LP), or dead piñons (LJ-DP). At the juniper savanna site we only collected roots from LJ-LJ pairs, because piñon are not present in this biome. Because all mature piñons were girdled within the fetch of the eddyflux tower, and because juniper-neighbor dynamics were the focus of this study (since juniper is the remaining co-dominant post-drought), we did not collect from live piñons neighboring dead piñons.

Tree pairs were selected randomly. Because we collected roots growing in three different directions from the main trunk, we targeted trees that neighbored at least two of the neighbor species of interest. For example, LJ-LP samples were collected from juniper that had at least two live piñon neighbors. Because most of

the mature piñons had been girdled within the fetch of the eddyflux tower, pairs including live piñon were selected just outside the fetch of the tower, and trees neighboring dead piñons were selected from just within the fetch of the tower.

Fine root tips were collected from each host tree in three directions from the trunk towards the relevant neighbors. Identity of the roots was determined visually in the field, as the root morphology of the two plants is distinct. Two 2-3 cm root tips were collected from each direction, washed with DI and alcohol, stored in a ziplock bag with other root tips from the same tree (each tree represents one sample), and placed in a cooler filled with dry ice until return to the University of New Mexico, where roots were stored at −80 C until DNA extraction (no more than two weeks).

Laboratory Preparation.— All root tissue from each sample was lysed by grinding in liquid nitrogen using a mortar and pestle. DNA was extracted from a small sample of ground tissue using Qiagen DNEasy plant minikit, according to manufacturer instructions. Conifer trees are high in phenolics (Chang *et al.* 1993, Franceschi *et al.* 2005), which can inhibit polymerase reactions. Diluting the extraction can overcome the inhibitory effect of phenolics. We performed PCR on all samples to be sure the samples amplified successfully. One µL DNA extract was added to 24µL PCR mix, which included 1µL of both the fungal ITS 1F-4 primers (White *et al.* 1990). The thermocycles were as follows: initial denaturation at 95 C for 5min, then 30 cycles of 94 C for 30s, 53 C for 30s, and 72 C for 45s, followed by a final extension at 72 C for 7min. Samples that did not amplify were diluted until they could amplify. All

samples successfully amplified at either 1:1, 1:10 or 1:100 dilution with milliQ filtered water.

The most concentrated DNA extracts or dilutions that successfully amplified were sent to MRDNA in Shallowater, TX for 454 pyrosequencing of the fungal ITS region, using the same primers and thermocycles described above, but using HotStartTaq Plus Master Mix Kit (Qiagen, Valencia CA). Due to low sequence retrieval of some samples, all samples were amplified and sequenced several times. All amplicon products were mixed, with equal concentrations from each sample, and purified using Agencourt Ampure beads (Agencourt Bioscience Corporation, MA, USA). Purified amplicon products were sequenced using Roche 454 FLX titanium instruments and reagents, following manufacturer's guidelines.

Sequence Processing.— Sequences have been deposited in NCBI SRA under the study accession SRP046474. Sequences were denoised, quality filtered and checked for chimeras using the default titanium settings in AMPLICONNOISE (v1.25) for MACQIIME (v1.6.0, Caporaso *et al.* 2010). All sequences were truncated to 400bp at the 3' end, retaining the highest quality region, and sequences shorter than 200bp or with a quality score <25 were discarded. One sample had retrieved only one sequence and so was discarded.

Sequences with 97% similarity to each other were clustered into operational taxonomic units (OTUs) using UCLUST as implemented in QIIME v1.7.0. Representative sequences (the most frequent sequence) for each OTU were BLASTed against the non-redundant dynamic UNITE+INSDC fungal ITS reference database (version 6, 04/07/2014 release, http://unite.ut.ee/repository.php) to

assign taxonomies. To the reference database we added plant ITS sequences from the Fungal Metagenomics Project's curated ITS database (University of Alaska, Fairbanks), as well as one ITS sequence from GenBank representing each of the host plants (*J. monosperma* and *P. edulis*). Sequences identified as having plant origin were discarded from the dataset.

Statistical Analyses.— Due to the uneven experimental design, we used four different models to assess the effect of host, neighbor and environment on RAF alpha diversity, community composition, and individual taxa. To assess the effect of environment, because piñons do not grow in juniper savanna, we compared only same neighbor juniper pairs across the juniper savanna and piñon-juniper woodland sites (savanna LJ-LJ vs. woodland LJ-LJ). Piñon and juniper roots grew in close proximity, and even in contact, with each other, which we worried would increase risk of mixing roots of the two hosts when collecting from opposite neighbor pairs. So we assessed the effect of host identity on RAF by comparing only hosts from same neighbor pairs within piñon-juniper woodland (woodland: LJ-LJ vs. LP-LP). To assess the effect of neighbor we looked at the interaction between host and neighbor within piñon-juniper woodland (host + neighbor + host × neighbor), excluding trees with dead piñon neighbors (woodland: LJ-LJ, LJ-LP, LP-LP and LP-LJ). To assess the effect of dead piñon neighbors on juniper RAF, we compared junipers from each neighbor pair within piñon-juniper woodlands (woodland: LJ-LJ vs. LJ-LP vs. LJ-DP). To examine which taxonomic levels were most affected by host, neighbor and environment, we collapsed OTUs into genera, families, orders, classes and phyla, and performed all of the following analyses at each taxonomic level.

We analyzed the effect of host, neighbor and environment on community composition by conducting an ANOVA like permutation test on Bray-Curtis distances in R (adonis, vegan package, Oksanen et al. 2011). Distance matrices were built from unrarefied OTU tables to retain the most information from each sample. We visualized distances using NMDS.

Alpha diversity metrics were calculated in QIIME. Variability of sequencing depth across samples was high (range: 37 to 20,175 sequences). The most shallowly sequenced sample (37 sequences) was removed, and to avoid the biasing effects of sequencing depth, each sample was sub-sampled 100 times to 202 sequences (the depth of the second most shallowly sequenced sample). Simpson's diversity index, Simpson's evenness, and taxonomic richness were calculated for each sub-sample, and averaged within a sample. Thus, the alpha diversity measures reported for each sample are calculated from 100 rarefactions. The effect of host, neighbor and environment on alpha diversity was assessed by type III ANOVA performed in R (package nlme, Pinheiro *et al.* 2011; R Development Core Team 2011).

To see which taxonomic groups were most affected by host, neighbor and environment, we conducted type III ANOVA on each individual taxonomic group, from OTU through phylum level, in R (package nlme, Pinheiro *et al.* 2011). Due to the large number of comparisons we conducted a false discovery rate (FDR) correction (Yoav & Yosef 1995).

RESULTS

Sequence Summaries.— After quality filtering we obtained a total of 131 616 ITS sequences. These averaged to 3 988.4 sequences per sample (s.d. = 4 540.3). Overall,

11.1% of sequences hit to unknown fungi, and 73.2% to fungi within Ascomycota. Two of the unknown OTUs were more closely examined, as will be discussed below, and were found to be Ascomycota. These accounted for 72% of the unknown sequences (8% of total sequences). The next most abundant phylum was Basidiomycota (12.2%), then Glomeromycota (3.2%). The most abundant orders were Hypocreales (18.8%), Chaetothyreales (12.0%), unidentified fungi (11.1%), Pleosporales (6.5%), Helotiales (6.3%), unidentified orders within Sordariomycetes (6.1%), and Pezizales (5.8%). At the genus level, unidentified fungi are the largest group, followed by unidentified Chaetothyriales (10.0%), *Ilyonectria* (7.4%), unidentified Sordariales (6.1%), unidentified Helotiales (5.5%), and *Fusarium* (5.1%). Some of the dominant groups differed across host plants and environments (FIG. 1). Significant differences are discussed below.

Community Composition.— Effects of host or neighbor on RAF community composition were insignificant. However, the effect of environment on juniper RAF was significant at all taxonomic levels except phylum (species level, $P < 0.01$, $F = 4.15$, FIG. 2, SUPPLEMENTARY TABLE I).

Alpha Diversity.— Juniper RAF richness, Simpson's evenness, and Simpson's diversity were significantly higher in piñon-juniper woodlands relative to juniper savanna. This was true at all taxonomic levels except phylum, where evenness was higher in woodland (due to the dominance of Ascomycota in juniper savanna), and class, where there was no significant evenness effect (TABLE I).

Host had mostly marginal effects on alpha diversity measures, except that juniper RAF were more diverse at the genus and family level, and less even at the

phylum level (seemingly due to dominance of Ascomycota) relative to piñon RAF. However, when both hosts were considered together juniper RAF were marginally or significantly less even at most taxonomic levels, while diversity and richness were unaffected. There were only marginal effects of neighbor, and only on Simpson's diversity. However, richness was significantly affected by the interaction between host and neighbor, in that richness was always lower when the host was neighbored by the other co-dominant (LP-LJ and LJ-LP pairs) relative to same neighbor pairs at nearly all taxonomic levels (TABLE I). When piñon hosts were excluded and the effects of all three neighbor species on juniper RAF were examined, the effect of neighbor on RAF richness was preserved. Junipers neighboring junipers were found to have the highest richness, and junipers neighboring live piñons the lowest richness. This effect was significant or marginal at all taxonomic levels except phylum (TABLE I).

Individual Taxa.—

Environment. More taxa differed between environments (woodland juniper vs. savanna juniper) than between hosts or neighbors (TABLE I). After FDR correction, the only fungal group significantly affected by any of the independent factors (host, neighbor or environment) were fungi that could not be assigned to phylum ($F_{1,9} = 10.71$, $P_{FDR} = 0.038$), which were more abundant in piñon-juniper woodland relative to juniper savanna. Pezizomycetes and Glomeromycota were marginally more abundant in woodland relative to savanna after FDR correction ($F_{1,9} = 7.66$, $P_{FDR} = 0.098$; $F_{1,9} = 5.36$, $P_{FDR} = 0.091$ respectively). The small number of replicates afforded in this study, and thus the low power, could be in part

responsible for lack of significance after FDR correction, so we will discuss significant correlations with the independent factors according to raw *P*-values, as these occasionally represent interesting trends.

The taxa that differed with the largest effect size were fungi within Eurotiomycetes, Sordariomycetes, and unclassified fungi. The trend in unclassified fungi was driven by a single OTU, OTU386, which was more abundant in woodland. We blasted OTU386 against GenBank to find close relatives and conducted a phylogenetic analysis, focusing on voucher and published sequences. Few like sequences were available in GenBank, and all similar sequences belonged to unclassified, uncultured RAF from arid plants (Khidir *et al.* 2010, Maciá-Vicente *et al.* 2012). OTU386 formed a well-supported clade with these arid unclassified root fungi, which represented a new putative clade at the order level within the class Eurotiomycetes (FIG. 3).

The class Eurotiomycetes was also more abundant in piñon-juniper woodland, mostly due to a single unclassified Chaetothyriales species. This order includes melanized fungi with several extremophile lineages (Sterflinger *et al.* 1999, Ruibal 2004). Sordariomycetes were more abundant in juniper savanna, mostly due to an OTU closely related to *Ilyonectria macrodidyma*, a root rot. Indeed, juniper RAF differed between environments primarily by putative pathogens: *Coniochaeta prunicola* (more abundant in savanna, a pathogen found on damaged leaves and twigs, the genus is known for it's woody host pathogens; Damm *et al.* 2010; Ivanová and Bernadovičová 2012; Ivanová and Bernadovičová 2013), *Pyrenochaeta lycopersici* (more abundant in savanna, a root rot), and *Readeriella sp.* (more

abundant in woodlands, often opportunistic pathogens, particularly of Eucalyptus; Barber *et al.* 2003; Andjic *et al.* 2010). A *Helotiales sp.* and another unclassified fungus (OTU60) were also more abundant in woodlands. The unclassified fungus OTU60 had very low sequence similarity with other sequences deposited in GenBank, no more than 94%, but it formed a well supported clade with other unclassified root fungi from arid and xeric environments (particularly with those found by Porras-Alfaro *et al.* 2014 in gypsophilic plants), and with Monosporascus (Ascomycota), which can be pathogenic (de Souza Bezerra *et al.* 2013), but is also an abundant taxon in roots of arid plants (Porras-Alfaro *et al.* 2008, 2014) (FIG. 4).

Host. Fungi that could not be classified to a phylum were more abundant in piñon than in juniper roots (within the woodland site), though individual unclassified OTUs did not significantly differ between hosts (TABLE II). In fact, no individual OTUs at the 97% similarity cutoff significantly differed across hosts. When collapsed to genera, Clonostachys was found in juniper but not in piñon. This genus includes many species used as biocontrol agents, parasites of other fungi and insects (Martijn ten Hoopen *et al.* 2010).

Host-Neighbor Interactions. Clonostachys rosea was affected by the interaction between host and neighbor but the effect is likely spurious, as the effect size is small and it is associated with juniper neighbors, but not with juniper itself (TABLE II). Fungi that could not be identified to a phylum were more abundant in piñon with a fairly large effect size. Unclassified Sordariomycetes are more abundant in roots of opposite host-neighbor pairs. An OTU belonging to the genus

Chalara, which includes plant pathogens, was found only in same neighbor pairs, and particularly in juniper.

Juniper Neighbors. The order Helotiales was more abundant in juniper neighboring dead piñon, but no individual Helotiales species were significantly affected by neighbor in juniper roots. An OTU classified as a *Readeriella sp*. was very abundant in juniper same neighbor pairs but rare when neighbored by live or dead piñon. The OTU classified as *I. macrodidyma*, a root rot, was associated with dead piñon neighbors with a fairly large effect size (TABLE II).

Ectomychorrhizal Fungi.— We found a few putative ectomycorrhizal fungi (EM) in common with previous studies in piñon-juniper woodland (Allen *et al.* 2010, Gehring *et al.* 1998, Treseder *et al.* 2004, Haskins and Gehring 2004 and 2005): several OTUs classified as *Cenococcum sp., Geopora*, *Inocybe*, *Tricholoma*, and *Rhizopogon sp.* (TABLE III). Additionally, several of our OTUs hit to other EM genera listed in the UNITE EM lineages list (http://unite.ut.ee/EcM_lineages.php). These included *Tarzetta*, *Humaria*, *Tricharina*, *Tuber*, *Amphinema*, *Clavulina*, *Sebacina* and *Tomentella sp*. None of the OTUs that hit to EM species significantly differed between hosts, neighbors or environment, but are notably absent from the savannah site where piñon does not occur (TABLE III).

DISCUSSION

Drought has been shown to have rapid impacts on the boundaries of piñon-juniper woodlands (Allen and Breshears 1998). Piñon and juniper expansion seem to be controlled by a complex combination of climatic factors, cattle grazing and fire suppression (Blackburn and Tueller 1970). The role of climate change still plays an

unclear role, as juniper expansion is traditionally attributed to wet periods, but is currently observed to occur with drought (Belsky 1996, Van Auken 2000). Complex interactions between the changing climatic conditions and vegetation community may explain the observed vegetation shifts in response to drought.

In this study we used next generation sequencing (NGS) to examine how RAF communities of two co-dominant plants in piñon-juniper woodland respond to vegetation shifts associated with global-change type drought, independent of actual drought conditions. We also compared juniper RAF from piñon-juniper woodlands to juniper RAF from juniper savanna, an environment that resembles conditions projected for piñon-juniper woodlands should extreme drought events become more frequent as climate change progresses.

Very few studies have examined RAF in piñon-juniper woodlands. Those that have, have examined RAF visually using microscopy (Swaty *et al.* 2004, Haskins and Gehring 2004 and 2005), sporocarps (Allen *et al.* 2010), and micro-video cameras (Treseder *et al.* 2005, Allen *et al.* 2010), or molecularly by RFLP analysis specifically of EM tips (e.g. Gehring *et al.* 1998, Treseder *et al.* 2004, Swaty *et al.* 2004, Haskins and Gehring 2004 and 2005). Aside from one study that used Sanger Sequencing to identify a small subset of RFLP types (Haskins and Gehring 2004), we are not aware of any studies prior to this that used DNA sequencing to describe total RAF in piñon-juniper woodlands. This lack of study is reflected in the high number of unidentified fungi uncovered by this dataset, many more than were uncovered by Haskins and Gehring (2004) due to the detailed community description provided by NGS techniques.

Environment Effect on RAF.—Of the three independent factors measured (host, neighbor and environment) environment had the strongest effect on RAF. It was the only factor that had any affect on community composition, it impacted RAF richness, evenness and diversity (all were higher in piñon-juniper woodland), and it affected many more fungal taxa relative to the biotic factors (plant host and neighbor).

OTU 629, classified as *I. macrodydima* (a root rot), was extremely abundant in juniper from savanna, where it made up >25% of sequences. Because of it's high abundance in apparently healthy trees, if this OTU is truly *I. macrodydima* it seems it engages in an unusual relationship with juniper here. Several other fungi related to putative pathogens were associated with one of the two environments: *Readeriella sp*., *Pyrenochaeta lycopersici, Coniochaeta prunicola,* and an unclassified fungi that was most closely related to a *Monosporascus sp*. It is likely that so many OTUs were identified as putative pathogens in this study because of the economic importance of plant pathogens and the resulting bias in research and reference databases towards pathogenic fungi.

Fungi unassigned to a phylum were more abundant in woodland. The two unclassified OTUs we looked at more thoroughly have few similar sequences in GenBank, and form clades with other unclassified root fungi from arid plants, indicating these systems may harbor new fungal groups that are specific to arid environments (Khidir *et al.* 2010, Maciá-Vicente *et al.* 2012, Porras-Alfaro *et al.* 2014).

Host Effect on RAF.— The RAF community composition of the two host plants did not significantly differ, and host effect on alpha diversity measures was minimal.

This is unusual, as host identity has repeatedly been shown to have a strong influence on RAF community composition (e.g. Morris *et al.* 2007, Tedersoo *et al.* 2012, Bogar and Kennedy 2013, Dean *et al.* 2014). The roots of these two plant species grow in extremely close proximity, and even in contact with each other. Perhaps this allows them to share RAF in a way that few competing plant neighbors do, or is responsible for detecting fungi on roots that they are not in fact colonizing. Other studies in arid ecosystems of New Mexico show plants share similar RAF communities (Porras Alfaro *et al.* 2008 and 2014, Khidir *et al.* 2010), consistant with lack of host effect within woodland.

Few taxa varied between hosts either. There were nearly three times as many fungi that could not be classified to a phylum in piñon relative to juniper, though no individual unidentifiable OTUs significantly differed between hosts. Future studies with larger sample sizes could probably identify OTUs that differ between hosts, which would help focus culture and inoculation experiments designed to identify their function.

Neighbor Effect on RAF.— When both hosts were examined together, neighbor had no effect on RAF community composition or diversity, and affected only one rare OTU prior to FDR correction. As mentioned earlier, the roots of the two plant hosts grow inter-entwined. If roots in opposite neighbor pairings were even slightly mixed, it could have confounded our analysis of neighbor effects on RAF, especially if neighbor effects are weak, as suggested by other studies (Meinhart and Gehring 2013, Bogar and Kennedy 2013, Dean *et al.* 2014). Alternatively, hosts may share

fungal communities, as has been found by Porras-Alfaro *et al.* (2008 and 2014) and Khidir *et al.* (2010).

In contrast, Haskins and Gehring (2005) found piñon EM colonization was significantly lower when growing with juniper in comparison to growing without juniper. The comparisons, however, were made between ecosystems in which juniper was or was not present, and so may more closely parallel our comparisons between savanna and woodland rather than our comparisons between tree pairs that did or did not include juniper within woodland. Haskins and Gehring (2004) found that piñon EM community composition was significantly different in piñons that had been cut off from juniper roots by trenching in comparison with control piñons. That we found no neighbor effects may indicate that a continuous soil environment allows RAF to colonize same neighbor and opposite neighbor pairs equally in piñon-juniper woodland, suggesting neutral processes, such as barriers to dispersion, are more important to structuring RAF in these ecosystems relative to biotic factors.

There was a significant interaction, however, between neighbor and host on RAF richness. In both hosts, richness was higher in same neighbor pairs and lower in opposite neighbor pairs. Dead piñon neighbors also had a negative effect on juniper RAF richness. Because community composition and individual taxa were unaffected, the effect of host and neighbor on richness is likely driven by rare species, the effect size too small to detect in this study. This may indicate competitive interactions occur between root microbiomes of different hosts, resulting in exclusion of less dominant fungal taxa. This might occur if, for example,

taxa colonizing the different hosts are functionally redundant, drawing from the same resource pool.

Ilyonectria macrodidyma was associated with dead piñon neighbors with a fairly large effect size, seeminly supporting our hypothesis that dead piñon can infect live neighbors with pathogens. However, *I. macrodidyma* was also extremely abundant in juniper savanna (>25% of sequences) where no piñon grow at all. These trees did not appear to be ill, so the relationship between juniper and *I. macrodidyma* in these systems is unclear.

Mycorrhizal Fungi.— Previous microscopy work suggests piñon hosts EM while juniper is associated with arbuscular mycorrhizal fungi (AMF) (Haskins and Gehring 2004, though Haskins and Gehring 2005 found AMF in piñon seedlings that did not produce coils or arbuscules, and Reinsvold and Reeves 1986 photographed EM on roots of an uprooted *J. osteosperma*). In piñon-juniper woodlands, we did not find host differences in either EM or AMF taxa. However, EM taxa were nearly absent from juniper savanna where piñon are also absent, even though the effect of environment was non-significant on individual EM OTUs.

Ectomycorrhizae and AMF were both found in same neighbor pairs of both hosts, reducing the likelihood that the lack of differences between hosts is due to mixing. Because pines in general are EM, previous studies have focused on EM root tips from piñon, which may have exaggerated the differences between hosts. We targeted the mycobiome of any living root tip. Additionally, though we sampled after a rain event (11mm on July 19th, preceded by other precipitation events July 2nd-7th and 9th-10th), 2012 was a particularly dry year (151.9mm accoding to the AmeriFlux

network, vs. the 418mm/y average). Swaty *et al.* (2004) shows EM senesce during drought. The dry conditions may have reduced EM formation in piñon relative to what is typical in other years. Sampling over a time series would be useful to identifying environmental effects on EM and on RAF community composition in general.

Glomeromycota (essentially entirely AMF) were found in significantly lower abundance in savanna relative to woodland, which is counter-intuitive, as one might expect this phylum to be more abundant in an environment where it's host is more dominant, and some research suggests AMF are more dominant in drier conditions (Gehring *et al.* 2006). We suggest abiotic differences between woodland and savanna drive this difference in abundance.

Biotic vs. Abiotic Assembly Mechanisms.— Different plant species have been shown to harbor different RAF communities (e.g. Morris *et al.* 2007, Dean *et al.* 2014), probably due to host specific immune responses (Kogel *et al.* 2006) and differences in carbon root exudates (Broekling *et al.* 2008). The identity of neighboring vegetation has also been shown to impact a host plant's root microbial community composition (Meinhardt and Gehring 2012, Bogar and Kennedy 2013, Dean *et al.* 2014). The abiotic environment, such as soil nutrients (Edgarton-Warburton *et al.* 2007), moisture (Shi *et al.* 2002), and structure (Pankhurst *et al.* 2002, Jansa *et al.* 2003) can impact RAF community composition as well, though biotic factors such as host identity generally seem more important (Tedersoo *et al* 2012).

We found no effect of biotic assembly mechanisms on RAF community composition, and minimal effect on other RAF measures in these hosts, while

environment had a strong effect on all RAF measures assessed here. The two environments, savanna and woodland, differ in moisture, temperature, and vegetation. The presence or absence of the co-dominant plant, piñon, had no effect on juniper RAF community composition, suggesting abiotic differences likely drive the differences in RAF communities across the two environments. Gehring *et al.* (1998) found that piñon EM community composition significantly differed in two piñon-juniper sites that differed by soil moisture, nutrients, and type. The piñon trees in the more nutrient and moisture poor site were found to contain more Ascomycota relative to the wetter, more nutrient rich site, just as we found more Ascomycota in drier juniper savanna, further suggesting abiotic factors drive RAF community differences in these arid systems.

Little research exists on what causes biotic or abiotic factors to be more or less important in different systems. It may be the environmental conditions in these systems are so stressful, that abiotic selective pressures overwhelm biotic selection processes that usually control host-fungal compatibility.

The fungal loop hypothesis (Collins *et al.* 2008) suggests that fungal networks may be essential in arid systems to transport nutrients and water across arid landscapes. The same fungi may colonize more than one plant, or both plants and biotic soil crusts, transporting water and nutrients between them (Smith and Read 2008). Perhaps few fungal species can survive the intense abiotic stresses of arid systems, and both plants and fungi benefit when fungi colonize more than one plant even when different plant species are colonized by the same fungus, allowing hyphal networks greater reach across the landscape.

Summary.— A large proportion of sequences retrieved from piñon juniper woodland belonged to unidentified fungi, indicating how little work has been done to describe the root-associated fungal communities in this arid system. One previous study that sequenced a subset of dominant RFLPs (Haskins and Gehring 2004) found a comparable percentage of unclassifiable fungi (8.4%), but because they found only nine unique RFLP types, this amounted to only 2 unclassifiable fungi, highlighting the insights provided by NGS technologies.

Two of these unidentified OTUs which we looked at more closely matched few sequences in GenBank, and seemed to belong to poorly resolved or new fungal clades with other unclassified root fungi from arid plants. Putative pathogens were found in extremely high abundance in what appear to be healthy plants. Extensive work, particularly isolation and inoculation experiments, is warranted to identify and describe the RAF present in these ecosystems. Research on some well known fungi would be useful as well, as the nature of their relationships with their hosts might be unusual in these systems.

We found that typical biotic RAF assembly mechanisms (host and neighbor identity), had little affect on RAF of these hosts. One interesting biotic effect was what appears to be competitive exclusion of rare RAF when the two different hosts grow in close proximity with each other. In contrast, environment had a pronounced effect on RAF of juniper roots, indicating that abiotic factors may be more important in structuring RAF communities in these arid systems than biotic factors. Perhaps the environmental selective pressures in the arid southwest, particularly during a drought year, are so strong that they overwhelm the selective pressures imposed by

the host plant on these fungal communities. Description and classification of these fungal communities may be critical to understanding response of these ecosystems to abiotic change.

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Figures and Tables

FIG. 1: The relative abundance of top 10 fungal orders found in each host-neighbor-environment combination. White wedges represent relative abundance of fungi not within the top 10.

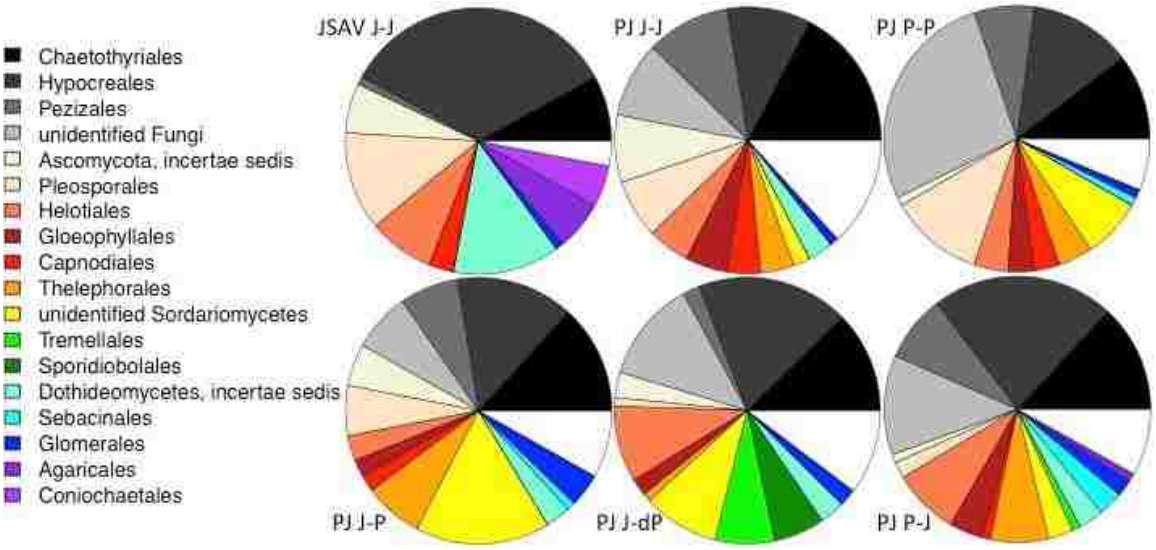

FIG. 2: An NMDS ordination of species level Bray-Curtis distances between samples. Large points represent juniper savanna samples, smaller points represent piñon-juniper woodland. Closed symbols represent juniper hosts, open symbols represent piñon hosts. Circles represent same neighbor pairs, triangles represent live opposite neighbor pairs, and stars indicate LJ-DP pairs. Ellipsoids are drawn around the standard deviation of samples from their centroid.

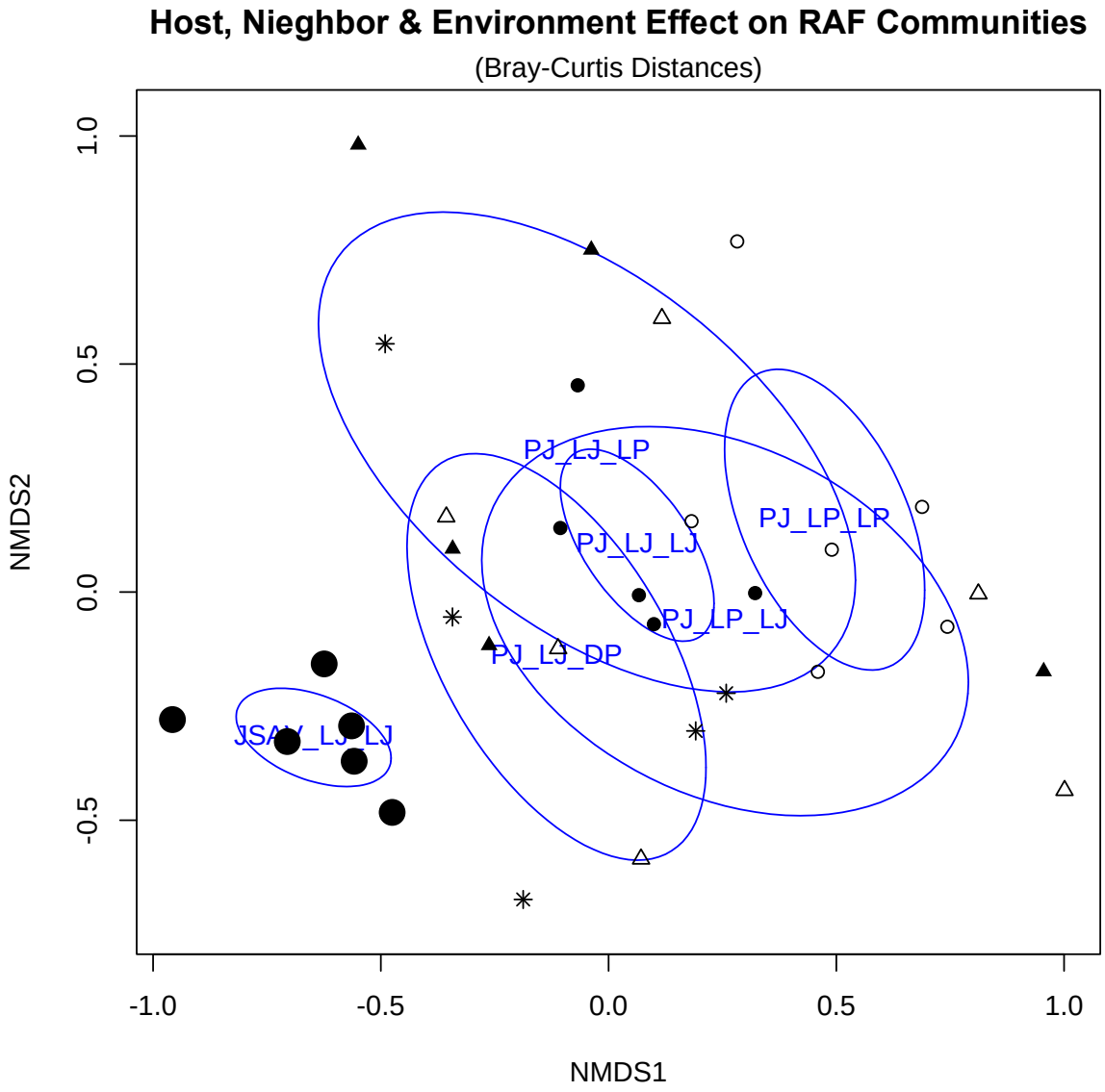

FIG. 3: Maximum likelihood tree of OTU386, closest GenBank blast hits, and relatives of closest hits. We aligned sequences using CLUSTALW and built a maximum likelihood tree using default settings in MEGA (v.6.06). Branches are labeled with bootstrap values from 1000 replications. Black circles= query sequence and direct blast hits, dark grey circles= Eurotiales, light grey circles = Coryneliales, black triangle = Chaetothyriales, dark grey triangle = Verrucariales, light grey triangle = Pyrenulales, white triangle = Onygenales, diamond = outgroup (Sordariomycetes). Alignment access: http://purl.org/phylo/treebase/phylows/study/TB2:S16316

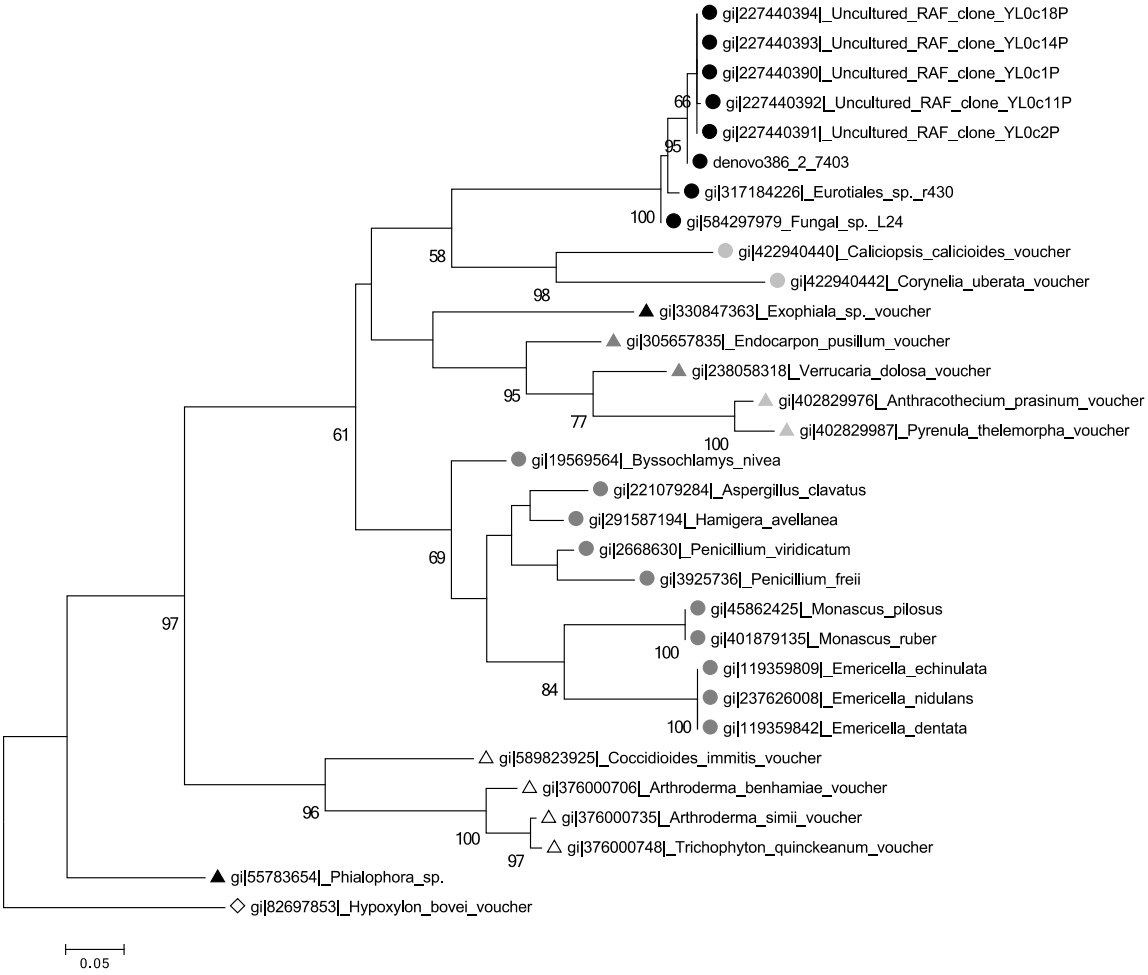

FIG. 4: Phylogenetic tree of OTU60, closest GenBank blast hits, and relatives of closest hits. We aligned sequences using CLUSTALW and built a maximum likelihood tree using default settings in MEGA (v.6.06). Branches are labeled with bootstrap values from 1000 replications. Black circles = query sequence and direct blast hits, dark grey circle = Monosporascus, light grey circles = Xylariaceae (Xylariales), grey diamond = Bolinieaceae (Boliniales), dark grey triangles = Lasiosphaeriaceae complex, black triangles = Chaetomiaceae (Sordariales), light grey triangles = Sordariaceae (Sordariales), white diamonds = outgroups (Eurotiomycetes and Dothidiomycetes). Though Monosporascus are currently placed within Sordariales, this tree places them in a clade with Xylariales, which is in agreement with other molecular studies on Monosporascus (Collado *et al.* 2002). Alignment access: http://purl.org/phylo/treebase/phylows/study/TB2:S16370

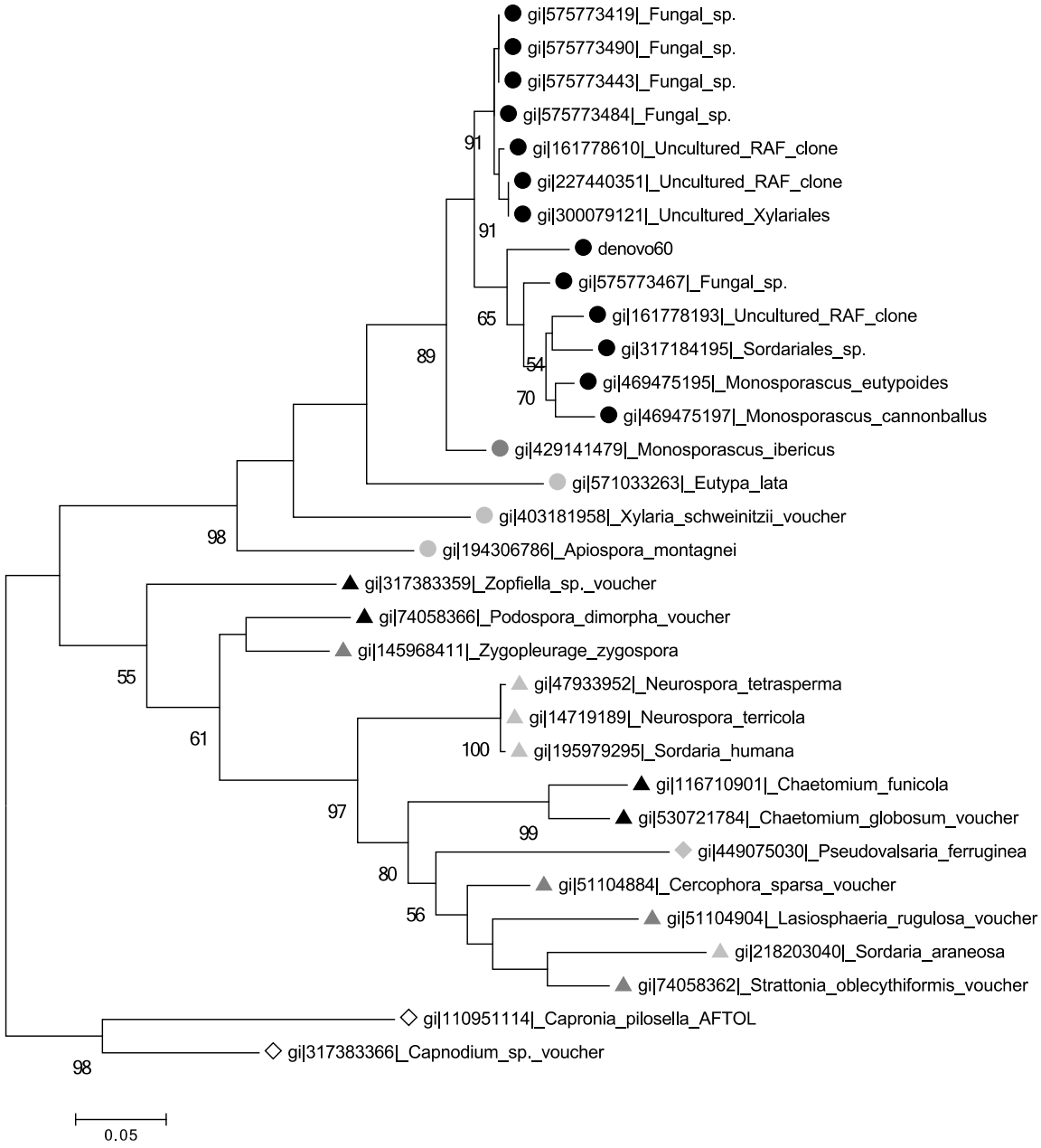

TABLE I: Means and environment, host and neighbor effects on RAF alpha diversity measures. H1 indicates piñon and juniper comparison from same neighbor pairs. N1 indicates comparison of the three neighbors (LJ, LP, and DP) on woodland juniper RAF. Env. Indicates comparisons of junipers from same neighbor pairs across savanna and woodland. H2, N2, and their interaction are the result of comparisons from within woodland alone, excluding DP neighbors. Sav. = savanna, wl = woodland. Significant and marginal effects are italicized, and an asterisk indicates a significant effect.

	Means						**Significance**					
Species	sav. LJ-LJ	wl. LJ-LJ	wl. LJ-LP	wl. LJ-DP	wl. LP-LP	wl. LP-LJ	Env.	H1	N1	H2	N2	H2 x N2
Simpson's	0.81	0.93	0.85	0.86	0.86	0.80	*0.01**	0.12	*0.04**	0.23	0.89	0.17
Evenness	0.30	0.45	0.41	0.35	0.35	0.35	*<0.01**	*0.09*	0.30	0.10	0.67	0.61
Richness	20.54	31.77	19.56	22.41	25.71	21.81	*0.04**	0.18	*0.02**	0.51	0.16	*0.01**
Genus												
Simpson's	0.81	0.91	0.84	0.83	0.83	0.78	*0.01**	*0.05**	*0.02**	0.14	0.81	0.19
Evenness	0.33	0.46	0.45	0.36	0.36	0.38	*<0.01**	0.11	0.15	*0.08*	0.79	0.98
Richness	17.86	25.30	15.57	18.02	19.63	17.01	*0.05**	*0.08*	*0.01**	0.30	*0.09*	*0.01**
Family												
Simpson's	0.77	0.90	0.84	0.83	0.83	0.78	*<0.01**	*0.05**	*0.03**	0.15	0.84	0.22
Evenness	0.31	0.47	0.49	0.37	0.37	0.39	*<0.01**	*0.09*	0.14	*0.05**	0.97	0.70
Richness	15.84	23.11	14.23	17.33	18.20	16.24	*0.03*	*0.07*	*0.01**	0.42	*0.06*	*0.01**
Order												
Simpson's	0.72	0.88	0.83	0.80	0.81	0.77	*<0.01**	*0.08*	*0.02**	0.16	0.92	0.25
Evenness	0.34	0.49	0.54	0.38	0.41	0.42	*0.01*	0.10	*0.05**	*0.03**	0.70	0.54
Richness	11.96	17.86	11.59	13.50	14.70	13.09	*0.02*	*0.09*	*<0.01**	0.50	*0.07*	*<0.01**
Class												
Simpson's	0.61	0.82	0.76	0.74	0.77	0.73	*0.02*	0.18	0.10	0.36	0.80	0.27
Evenness	0.45	0.56	0.58	0.44	0.49	0.47	0.24	0.26	0.17	0.12	0.67	0.97
Richness	7.06	10.37	7.99	9.22	9.51	9.68	*0.03*	0.30	*0.04**	0.54	*0.07*	0.11
Phylum												
Simpson's	0.11	0.39	0.36	0.41	0.50	0.44	*0.02*	0.10	0.88	0.11	0.80	0.44
Evenness	0.54	0.42	0.50	0.49	0.56	0.57	0.36	*0.05**	0.43	*0.05**	0.47	0.40
Richness	2.72	4.09	3.33	3.78	3.73	3.42	*0.04*	0.33	0.11	0.59	0.39	*0.05**

TABLE II: RAF taxa significantly correlated with specific hosts, neighbors, and/or environments are listed. Due to the uneven experimental design, several different models were used to test effects of the independent factors. Model descriptions are listed above the statistics outputs. Mean relative abundances of each taxa are listed only for the site-neighbor-environment combinations tested in each model. In the model that tests host × neighbor interactions, the factor that exerted a significant influence on each taxon are indicated in parentheses (Nghbr = neighbor, Intrctn = host × neighbor interaction). Taxa that were significantly or marginally affected after FDR correction are emboldened. Sav. = juniper savanna, wl. = pinon-juniper woodland, LJ = live juniper, LP = live pinon, DP = dead pinon. The first plant species in each pair is the host plant, the second is the neighbor.

		Environment: sav. LJ-LJ vs wl. LJ-LJ		**Means**					
level	**Taxonomy assignment**	**F**	***P***	**sav. LJ-LJ**	**wl.LJ-LJ**	**wl.LJ-LP**	**wl.LJ-DP**	**wl.LP-LP**	**wl.LP-LJ**
phylum	**Glomeromycota**	5.36	0.05	0.83	2.89				
	Unidentified	10.71	0.01	0	9.13				
class	Eurotiomycetes	5.36	0.05	9.24	20.51				
	Pezizomycetes	7.66	0.02	1.08	9.76				
	Sordariomycetes	5.14	0.05	39.55	15.73				
	Unidentified fungi	10.59	0.01	0.00	9.36				
order	Dothideomycetes incertae	5.76	0.04	13.72	2.89				
	Chaetothyriales	6.33	0.03	8.08	18.66				
	Pezizales	7.4	0.02	1.12	10.53				
	Coniochaetales	11.87	0.01	4.7	0				
	Hypocreales	5.57	0.04	35	11.46				
	Unidentified fungi	12.75	0.01	0	10.3				
family	Dothideomycetes incertae	6.6	0.03	14.76	3.07				
	Coniochaetaceae	7.83	0.02	5.5	0				
	Hypocreales incertae sedis	8.74	0.02	29.98	3.58				
	Mycenaceae	6.09	0.04	0	0.47				
	Thelephoraceae	5.17	0.05	0	5.14				
	Unidentified fungi	10.43	0.01	0	11.19				
genus	Unidentified Pyronemataceae	5.45	0.04	0	5.95				
	Coniochaeta	9.1	0.01	5.75	0				
	Ilyonectria	11.77	0.01	24.09	4.35				
	Mycena	5.62	0.04	0	0.58				
	Unidentified Thelephoraceae	6.1	0.04	0	5.93				

	Unidentified fungi	9.23	0.01	0	14.13				
species	*Chaetothyriales sp*	5.56	0.04	5.47	13.64				
	uncultured RAF 60	5.59	0.04	0	2.36				
	Coniochaeta prunicola	7.62	0.02	5.67	0				
	Readeriella sp	10.87	0.01	2.31	9.98				
	Pyrenochaeta lycopersici	5.47	0.04	1.12	0				
	uncultured RAF 386	6.33	0.03	0	14.36				
	Ilyonectria macrodidyma	7.33	0.02	25.73	6.31				
	uncultured Helotiales	6.61	0.03	0	1.82				
		Host: LJ-LJ vs LP-LP		**Means**					
level	**Taxonomy assignment**	**F**	***P***	**sav. LJ-LJ**	**wl.LJ-LJ**	**wl.LJ-LP**	**wl.LJ-DP**	**wl.LP-LP**	**wl.LP-LJ**
phylum	Unidentified fungi	6.06	0.04		9.13			26.4	
class	Ascomycota incertae sedis	8.99	0.02		7.63			1.24	
	Unidentified fungi	6.07	0.04		9.21			26.47	
order	Ascomycota incertae sedis	7.63	0.02		8.66			1.26	
	Unidentified Sordariomycetes	5.89	0.04		2.17			7.98	
	Unidentified fungi	6.46	0.03		9.84			27.23	
family	Ascomycota incertae sedis	9.23	0.01		9.77			1.29	
	Bionectriaceae	9.8	0.01		0.89			0	
	Unidentified fungi	6.14	0.04		11.42			29.13	
genus	Clonostachys	9.49	0.01		1.01			0	
		Host x Neighbor: LJ-LJ, LJ-LP, LP-LP, LP-LJ		**Means**					
level	**Taxonomy assignment**	**F**	***P***	**sav. LJ-LJ**	**wl.LJ-LJ**	**wl.LJ-LP**	**wl.LJ-DP**	**wl.LP-LP**	**wl.LP-LJ**
phylum	Unidentified fungi	4.64(H)	0.04(H)		9.11	6.53		26.4	11.88
class	Ascomycota incertae sedis	7.78(H)	0.01(H)		7.56	5.15		1.24	1.24
	Unidentified fungi	4.64(H)	0.04(H)		9.13	6.53		26.43	11.88
order	Ascomycota incertae sedis	7.98(H)	0.01(H)		8.38	5.15		1.26	1.25
	Unidentified Sordariomycetes	7.97(N)	0.01(N)		2.16	16.27		7.92	3.08
	Unidentified fungi	4.77(H)	0.04(H)		9.71	6.57		27.12	12.02
family	Ascomycota incertae sedis	8.97(H)	0.01(H)		9.19	5.28		1.28	1.25
	Unidentified Sordariomycetes	7.62(N)	0.01(N)		2.69	16.41		8.85	3.08
	Unidentified fungi	4.56(H)	0.05(H)		10.97	6.85		28.53	12.03
genus	Chalara	4.43(HxN)	0.05(HxN)		5.15	0		0.34	0
	Unidentified Sordariomycetes	7.86(N)	0.01(N)		2.76	17.11		9.52	3.08
species	Clonostachys rosea f.	4.6(N)	0.05(N)		1.48	0		0	1.16
		Juniper neighbors: LJ-LJ vs LJ-LP vs LJ-DP		**Means**					
level	**Taxonomy assignment**	**F**	***P***	**sav. LJ-LJ**	**wl.LJ-LJ**	**wl.LJ-LP**	**wl.LJ-DP**	**wl.LP-LP**	**wl.LP-LJ**

class	Leotiomycetes	4.9	0.03	5.55	3.27	10.78
order	Helotiales	5.03	0.03	5.57	3.34	11.26
family	Pyronemataceae	4.4	0.04	6.71	0.71	0.48
	Mycenaceae	4.3	0.04	0.5	0	0
genus	Mycena	4.52	0.03	0.55	0	0
species	*Readeriella sp.*	5.47	0.02	8.29	2.38	0.43
	Ilyonectria macrodidyma	4.65	0.03	5.44	4.18	14.14

TABLE III: Ectomycorrhizal (EM) taxa found among RAF in piñon and juniper roots from piñon-juniper woodland and juniper savanna, and indication whether the genus was found in PJ woodland in the past (we looked to Gehring et al. 1998, 2004 and 2005, Treseder et al. 2004, and Allen et al. 2010). OTUs were determined to be EM if they hit to a genus that is listed in the UNITE EM list. JS = juniper savanna, PJ = piñon-juniper woodland, J = juniper, P = piñon, dP = dead piñon.

	Relative Abundance (%)						
Ectomycorrhizal Taxa	**JS: J-J**	**PJ: J-J**	**PJ: J-P**	**PJ: J-dP**	**PJ: P-P**	**PJ: P-J**	**Found in PJ before?**
Ascomycota; Dothideomycetes; Hysteriales; Gloniaceae; Cenococcum; *Cenococcum sp.*	-	-	-	0.099	-	-	N
Ascomycota; Pezizomycetes; Pezizales; Pyronemataceae; Geopora; *Geopora sp.*	-	2.277	0.297	-	3.630	4.043	Y
Ascomycota; Pezizomycetes; Pezizales; Pyronemataceae; Tarzetta; *Tarzetta sp.*	-	0.198	-	-	-	-	N
Ascomycota; Pezizomycetes; Pezizales; Pyronemataceae; unidentified; uncultured *Humaria*	-	0.990	0.297	-	1.155	0.165	N
Ascomycota; Pezizomycetes; Pezizales; Pyronemataceae; unidentified; uncultured *Tricharina*	-	-	-	-	-	0.083	N
Ascomycota; Pezizomycetes; Pezizales; Tuberaceae; unidentified; uncultured *Tuber*	-	1.188	6.238	0.594	0.825	3.713	N
Basidiomycota; Agaricomycetes; Agaricales; Cortinariaceae; unidentified; uncultured *Inocybe*	-	-	0.396	-	-	0.083	Y
Basidiomycota; Agaricomycetes; Agaricales; Tricholomataceae; Tricholoma; *Tricholoma sp.*	-	-	-	0.495	0.083	-	Y
Basidiomycota; Agaricomycetes; Atheliales; Atheliaceae; Amphinema; *Amphinema sp.*	-	-	-	-	0.083	-	N
Basidiomycota; Agaricomycetes; Boletales; Rhizopogonaceae; Rhizopogon; *Rhizopogon sp.*	-	-	-	-	0.165	-	Y
Basidiomycota; Agaricomycetes; Cantharellales; Clavulinaceae; unidentified; uncultured *Clavulina*	0.083	0.099	0.297	-	-	-	N
Basidiomycota; Agaricomycetes; Sebacinales; Sebacinaceae; Sebacina; *Sebacina incrustans*	-	-	0.198	0.495	1.238	3.300	N
Basidiomycota; Agaricomycetes; Thelephorales; Thelephoraceae; Tomentella; *Tomentella sp.*	-	0.099	0.990	-	0.578	0.495	N

SUPPLEMENTARY TABLE I: Environment, host and neighbor effects on RAF community composition. These are the results of ANOVA like permutation tests conducted in R on Bray-Curtis distances (F represents the pseudo-F statistic). The environment statistics are the result of comparing junipers from same neighbor pairs only across savanna and woodland. $Host_1$ tests compared piñon and juniper from same neighbor pairs. $Neighbor_1$ tests compared the effect of the three neighbor identities (LJ, LP, and DP) on woodland juniper RAF only. The statistics listed for $host_2$, $neighbor_2$, and their interaction are the result of comparisons from within woodland alone, and excluding DP neighbors (to balance the statistical analysis). Asterisks indicate a significant effect.

Taxonomic Level		Environment	$Host_1$	$Neighbor_1$	$Host_2$	$Neighbor_2$	Interaction
		sav.LJ-LJ, wl.LJ-LJ	LJ-LJ, LP-LP	LJ-LJ, LJ-LP, LJ-DP	LJ-LJ, LJ-LP, LP-LP, LP-LJ		
Species	F	4.15	1.38	1.09	1.19	0.77	0.98
	P	**<0.01***	**0.15**	**0.33**	**0.26**	**0.71**	**0.46**
Genus	F	3.81	1.39	0.92	0.73	1.11	0.68
	P	**<0.01***	**0.18**	**0.61**	**0.76**	**0.34**	**0.81**
Family	F	3.63	1.63	1.00	0.89	1.11	0.63
	P	**<0.01***	**0.11**	**0.45**	**0.55**	**0.30**	**0.87**
Order	F	3.60	1.21	0.95	0.78	0.87	0.57
	P	**0.01***	**0.28**	**0.53**	**0.67**	**0.57**	**0.87**
Class	F	2.71	0.78	0.99	0.52	0.70	0.79
	P	**0.04***	**0.60**	**0.44**	**0.80**	**0.65**	**0.56**
Phylum	F	1.60	0.33	0.41	1.44	0.44	0.42
	P	**0.31**	**0.70**	**0.81**	**0.24**	**0.68**	**0.72**

CHAPTER 5

Conclusion

Cross-Microbial Group Comparisons, Fungi vs. Bacteria--

Moist meadow alpine tundra at Niwot is co-dominated by *D. cespitosa* and *G. rossii*. These two plant species show contrasting responses to N enrichment; *D. cespitosa* numbers increase while *G. rossii* numbers decline. We looked at root-associated fungal and bacterial community responses to N in each of these hosts. The microbial communities of the two co-dominant plant hosts responded very differently to N. Because of the essential role root associated microbes play in host fitness, this suggests root microbial response to N may play a role in contrasting responses of these hosts to N.

Interestingly, the fungal and bacterial communities within each host had nearly inverse responses to N and to host identity. *Geum rossii* fungal community composition was less sensitive to N enrichment relative to *D. cespitosa* fungal community composition. Conversely, *G. rossii* bacterial community composition was more sensitive to N enrichment relative to *D. cespitosa* bacterial community composition. Fungal diversity was lower in *G. rossii* because *G. rossii* was strongly dominated by a single fungal order, Helotiales. In contrast, bacteria diversity was lower in *D. cespitosa*, because *D. cespitosa* was strongly dominated by a single bacterial genus, *Pseudomonas*. Nitrogen caused an increase in *G. rossii* fungal diversity but not in bacterial diversity. Nitrogen caused a decrease in *D. cespitosa* bacterial diversity but not fungal diversity. Fungal vs. bacterial response to the

abiotic environment have not before been compared using DNA sequencing, to our knowledge. Studies that compare fungal and bacterial biomass response to N find the two microbial groups respond differently (Frey *et al.* 2004; Lauber *et al.* 2008; van der Heijden *et al.* 2008). In the case of biomass it could be assumed that when fungi are more successful at acquiring the limiting resources, bacteria are outcompeted and vice versa. Why other aspects of bacteria and fungal communities should behave inversely to each other in other ways is not known. More high-throughput environmental sequencing studies comparing these two microbial groups are needed to determine whether these trends are universal or a quirk particular to Niwot moist meadow alpine tundra.

Perhaps the most important difference between microbial response to N in the two hosts is the response of the dominant microorganisms. Helotiales, the fungal group that dominates *G. rossii* root fungal communities, was extremely sensitive to N, declining from 83% to 60% of fungal sequences. In contrast, *Pseudomonas*, the bacterial genus that dominated *D. cespitosa* root bacterial communities, did not respond significantly to N. The dominant taxonomic group often plays an important functional role in an ecological community, in part due to its sheer abundance (Smith and Knapp 2003). It is therefore probably important that a taxonomic group that makes up 83% of fungal sequences in our nitrophobic host experiences a massive decline in abundance with N, especially since this taxonomic group has been shown in several studies to include many important mutualists for hosts in cold-stressed habitats (Newsham *et al.* 2009; Upson *et al.* 2009; Newsham 2011). Perhaps this indicates loss of fungal mutualists with N enrichment is responsible for

G. rossii decline. No known pathogens increased with N, and while this could be a result of incomplete databases, it could also indicate that mutualists may sometimes be more important than pathogens to host fitness, and may play an underappreciated role in host survival, particularly in extreme environments. Future studies inoculating the host plants with fungal and bacterial isolates are necessary to determine the relationships between these two plant species and the most dominant and N-sensitive microbial species.

Cross-Ecosystem Comparison, Alpine Tundra vs. Arid Woodlands--

In both alpine tundra at Niwot and piñon-juniper woodland in New Mexico we examined the impact of plant neighborhood on root associated microbial communities. At Niwot we looked at root-associated microbial response to *D. cespitosa* removal in *G. rossii* roots, to compare the effects of N enrichment and co-dominant competition in the plant species that suffers under N enrichment. In piñon-juniper woodland we collected roots from piñon and juniper trees that were growing amongst piñon, juniper, or dead piñon, to examine the effect of plant neighbor as well as the effects of piñon mortality on root associated fungal communities. We found evidence that root microbiomes are affected by neighboring plants, which has been shown in other studies (e.g. Meinhardt and Gehring 2012). Our Niwot data also indicate that neighboring plants mediate how a root microbial community responds to abiotic change. Such findings are unprecedented, and suggest that plant-microbe-environment feedbacks are complex, and that the impacts of environmental changes on plant-microbe relationships will be difficult to

predict, as microbe-plant relationships may continue to change as the surrounding vegetation changes.

Fungal species richness was over four times higher at Niwot than in piñon-juniper woodlands, but also more heavily dominated by a single fungal taxa, making them less even. The effect of evenness on a community's stability in the face of perturbation has been debated. Some researchers suggest that if the dominant taxonomic group has an important functional role in a community, extreme dominance, or community unevenness, can create instability because the community structure relies heavily on that one taxon (Ives and Carpenter 2007; Hillebrand *et al.* 2008; Wittebolle *et al.* 2009). This hypothesis has not been tested in the field with microbial communities. It would be interesting to quantify microbial response to environmental perturbations in more and less even microbial communities to elucidate the role of alpha diversity measures such as evenness and taxonomic richness on microbial community stability in the face of environmental change.

Perhaps the most important differences between the alpine and arid sites were the controls on microbial community assembly. Host identity had a powerful effect on both fungal and bacterial community assembly at Niwot, but was surprisingly unimportant to fungal community assembly in piñon-juniper woodlands. Neighbor also had a stronger effect on alpine tundra microbes relative to piñon-juniper woodland microbes. So biotic factors were important to microbial community assembly in alpine tundra, but not at all important to fungal community assembly in piñon-juniper woodland. This is an unusual finding, as host plant

identity is generally among the most important factors structuring root microbial community assembly (Tedersoo *et al.* 2012). We also sequenced juniper root fungi from a slightly warmer and drier New Mexican ecosystem, juniper savanna, to explore the effect of environment on root microbial assembly. Effect of environment on juniper root associated root fungi was significant. Because plant community did not seem to have an effect or root fungi, these differences are probably driven by the abiotic differences between the two environments. This suggests abiotic factors may be more important than biotic factors to structuring root fungal communities in the arid southwest.

Why biotic or abiotic factors are more or less important to microbial community assembly in different systems has yet to be explored. Extreme environmental conditions may exert such strong selective pressures upon microbial communities that they overwhelm the selective pressures imposed by the host, driving different hosts towards similar microbial communities. Only by describing the microbial communities of more plants in more environments can we begin to understand the interactions between abiotic and biotic assembly mechanisms on microbial communities. Understanding how root microbial communities are assembled will provide insight into how global change affects microbe-plant relationships. With many ecosystems around the world experiencing rapid anthropogenically induced changes, research into these relationships is critical to our understanding of our impact on natural systems.

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