THE INFLUENCE OF PLANT-SOIL INTERACTIONS ON PLANT AND SOIL MICROBIAL RESPONSES TO NITROGEN DEPOSITION

By

Teal Steinkraus Potter

B.A., The University of Montana, 2009

A thesis submitted to the

Faculty of the Graduate School of the

University of Colorado in partial fulfillment

of the requirement for the degree of

Doctor of Philosophy

Department of Ecology and Evolutionary Biology

2018

This thesis entitled:
The Influence of Plant-Soil Interactions
on Plant and Soil Microbial Responses to Nitrogen Deposition
written by Teal Steinkraus Potter
has been approved for the Department of Ecology and Evolutionary Biology

____
Dr. William D. Bowman, Chair

____
Dr. Noah Fierer

____
Dr. Katharine N. Suding

____
Dr. Nichole N. Barger

____
Dr. Eve-Lyn Hinckley

Date_________________

A final copy of this thesis has been examined by the signatories,
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Potter, Teal Steinkraus (Ph.D., Ecology and Evolutionary Biology)
The Influence of Plant-Soil Interactions on Plant and Soil Microbial Responses to Nitrogen Deposition
Thesis directed by Professor William D. Bowman

How plants interact with the soil around their roots is critical to how plant species function. Given the enormous ecological complexity of soils, it remains a challenge to understand the unique associations of plants and soils across plant species. Such knowledge is needed, however, to predict plant responses to changes in their environments. This work addresses questions on the theme of understanding how plant-soil interactions differ across among plant species and impact plant responses to environmental changes. More specifically, I examined how soil chemistry, nitrogen (N) availability, microbial communities, and abiotic conditions influence both plant responses to environmental change as well as plant species effects on soil microbial communities.

In three chapters I address 1) why dominant alpine sedge species shift in abundance as a result of long-term simulated N deposition, 2) a set of potential changes in environmental conditions that would promote invasion of alpine ecosystems by a non-native grass (*Bromus tectorum*), and 3) how variation among plant species in the same genus influences plant-soil microbial associations and plant and microbial responses to elevated N availability. Pot experiments using local plant species and their native soils in Colorado's Front Range were used to isolate the effects of N and other factors on plant-soil associations. I found that 1) alpine sedges, *Kobresia myosuroides* and *Carex rupestris,* are not changing in abundance due to N induced shifts in bacterial composition or exposure to soluble aluminum. However, ectomycorrhizal fungi may play a role in shifts in sedge abundance with N addition. Alpine invasion 2) by *Bromus tectorum* would likely be inhibited by alpine soil, while the beneficial effects of increasing growing season temperatures and N deposition could promote growth of lower elevation populations. Finally, 3) congeneric *Poa* species have unique effects on soil fungal community composition but not bacteria composition, and N addition enhanced the unique effects of plant species for both fungi and bacteria. Together, these results demonstrate how soils contribute to variation in

plant species' responses to environmental change and plant species' effects on soil microbial communities.

ACKNOWLEDGEMENTS

Many people contributed their expertise to this dissertation. Their contributions are deeply appreciated. I offer a special thank you to Bill Bowman, my PhD advisor, for his dependable and thoughtful mentoring. I thank my committee members, Noah Fierer, Katie Suding, Nichole Barger, and Eve Hinckley for their invaluable mentoring and feedback. Amber Churchill, Christine Avena, Aidan Beers, Toby Hammer, Megan Blanchard, and Chiara Forrester were also instrumental to my successes in graduate school. I would also like to acknowledge current and past members of the Ecology and Evolutionary Biology department, who may not be named individually here, who's contributions to making EBIO a supportive and functional community I value greatly. The following people and funding sources made specific contributions to the research conducted within chapters.

Chapter II. I thank Max Owens for his collaboration on this project and the data he contributed from his honors thesis. I thank Sam Simkin and Jared Stewart for their suggestions while designing the experiment and writing the manuscript. Mariska Hamstra, Jessica Henley, and Thomas Lemieux helped with data collection, and Jon Leff assisted with microbial data analyses. This study was funded by the Niwot Ridge LTER Program, the Indian Peaks Wilderness Alliance and University of Colorado grants including the John W. Marr Fund and the Ecology and Evolutionary Biology Graduate Research Fund.

Chapter III. I thank Sophie Thilenius, Andrew Xicto, Tyler Justice, and Shelby Kaminski for assistance with the greenhouse harvest, as well as Ben Murphy, Megan Budnik, Amber Churchill, and Robert Andrus for help with project development and editing. This study was funded by the Undergraduate Research Opportunities Program at the University of Colorado and the John Marr Memorial Fellowship.

Chapter IV. I thank Brian Anacker for collaborating on part of this project. I thank greenhouse staff Tom Lemieux, and Janice Harvey, and herbarium staff Tim Hogan and Dina Clark for their assistance with the project setup. Jessica Henley, Holly Archer, and Matt Gebert assisted with lab work and Jon

Leff, Toby Hammer, and Josh Grinath assisted with data analyses. Black Dog LED generously donated a LED panel for the duration of the greenhouse experiments. This study was funded by the Beverly Sears Fund, the John Marr Memorial Fund, Evolutionary Biology Graduate Research Fund, and the Undergraduate Research Opportunities Program at the University of Colorado.

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CHAPTER I

Introduction

The soil environment around a terrestrial plant's roots is a dynamic zone where soil chemistry and activities of microorganisms impact plant health and reproduction (Wardle et al. 2004; Philippot et al. 2013). Through multiple types of interactions, microbes and plants mediate each other's access to resources (Philippot *et al.* 2013). Soil bacteria and fungi have important effects on plants in their roles as decomposers, with different taxa specializing on degrading different types of organic matter and inorganic molecules (Wickings et al. 2012). A microbially-mediated increase in a limiting resource, for example, can promote plant growth and fitness (Bever *et al.* 2010). Soil microbes that compete with plants for resources, can also negatively impact plants (Bever *et al.* 2010). Since plants are influenced by activities in root-associated microbial communities, understanding the drivers of variation in plant-soil interactions is necessary for improving predictions of how plants respond to environmental changes.

Plant species differ in how they interact with soil microbes and how they respond to changes in soil conditions, such as increases nitrogen (N) availability (Bardgett et al. 1999; Veresoglou and Rillig 2014). Differences in plant species' effects on soil processes and soil microbes, through chemical inputs via litter and root exudates, also likely contribute to plant species' differences in their responses to changes in soil conditions (Innes, Hobbs & Bardgett 2004; Classen *et al.* 2015). Thus, understanding how plant species' differences in their below-ground ecology is needed to improve predictions of plant species' responses to environmental changes. Closely related plant species share morphological and physiological traits that can potentially reveal similarities in how plants interact with other organisms, including mutualists and pathogens (Fitzpatrick *et al.* 2016; Emmett *et al.* 2017). Ecologists use plant evolutionary history (Kembel and Cahill 2011; Münzbergová and Šurinová 2015; Emmett et al. 2017; Saar 2017), aboveground plant traits, and more recently, belowground plant traits to describe ecological

variation among plant species (Orwin et al. 2010; Gould et al. 2016; Faucon et al. 2017; Freschet et al. 2017). However, given the enormous heterogeneity in the physical properties of soils and consequently the enormous diversity of biological life in soils, it remains difficult to generalize plant-soil interactions across plant species or predict plant species' responses to environmental change.

Plant species' responses to resource addition can manifest in changes in plant community structure and even nutrient cycling in ecosystems (Bever et al. 2010; Suding et al. 2013). For example, increases in N deposition have led to losses of native plant diversity in many regions around the world (Bobbink *et al.* 2010). Additionally, growing evidence suggests that plant-microbial associations can create feedback loops which may alter plant species composition and rates of nutrient cycling and ecosystem function (Weidenhamer & Callaway 2010; Classen *et al.* 2015). Understanding how plant species affect soils around their roots and how plant-microbe associations impact plant responses to changes in soil conditions is therefore essential for predicting changes in plant communities and ultimately, ecosystem function.

Uncovering the effects of resource availability on plants and microbes is complicated by the fact that plants and microorganisms require many of the same resources to survive, reproduce and maintain populations over time. Organisms capitalize on each other's adaptations to acquire resources, through competition, abstraction, or exchange of resources between plants and microbes in the rooting zone (Nguyen 2003; Jones et al. 2004; Kiers et al. 2011; Kuzyakov and Xu 2013). For example, many bacteria are effective decomposers of organic matter, making inorganic and organic N and other nutrients available to plants (Wardle et al. 2004; Wickings et al. 2012). Plants release labile carbon (C) compounds from their roots, which can be an important source of C for microbes (Nguyen 2003; Bais et al. 2006). The exchange of C and N among plants and microbes in the rhizosphere is well described, although the variation in plant controls on these processes among plant species is still poorly understood (Berg & Smalla 2009; Bever *et al.* 2010). Plants, bacteria and fungi can experience N limitation in soils which

stimulates plant-microbial interactions to acquire N (Kaye & Hart 1997; Bell *et al.* 2015).

Much research has described the effects of N addition on plants (Vitousek and Howarth 1991), as well effects of N addition on soil chemistry (Guo *et al.* 2011; Lieb, Darrouzet-Nardi & Bowman 2011) and soil microbial communities (Ramirez et al. 2010; Fierer et al. 2012). However, these studies generally target plant, soil chemistry or microbial community responses separately without considering their interactions. Given that N is necessary for both plant and microbial growth and N availability also can be manipulated in soils to determine how change in resource availability impacts plant-soil interactions in meaningful ways, I chose to investigate the role of N in plant-soil interactions as a theme in this dissertation research.

In this thesis, I present research that seeks to inform how N availability, abiotic factors and microbial communities operate separately and interactively to understand plant-soil interactions and plant responses to N deposition. My research employs experimental manipulations to investigate theses linkages. In chapter 2 of this thesis, I investigated potential belowground factors that were hypothesized to influence alpine sedge species' responses to long-term N deposition. *Kobresia myosuroides* has decreased significantly in cover while *Carex rupestris* has increased significantly with almost 20 years of N additions in a long term N addition study on Niwot Ridge (Bowman *et al.* 2006). In this study, I tested the potential roles of soil bacterial communities, mycorrhizal associations and aluminum toxicity in explaining differences in plant species' responses to simulated N deposition.

In chapter 3, I explored whether N deposition and/or a warming climate could improve a nitrophilic invasive species' ability to invade an alpine ecosystem where native species tend to not increase growth in response to N additions (Bowman et al. 2006). I manipulated growing temperatures, freezing event temperatures, and soil types to determine whether current or future alpine conditions would allow for cheatgrass (*Bromus tectorum*) to establish and reproduce in the absence of dispersal limitation.

Chapter 4 was developed to identify consistencies in plant-soil interactions across plant species. While some plant communities do exhibit more ecological similarity among closely related plant species (Burns and Strauss 2011; Anacker and Strauss 2016), it remains to be determined how similar (or closely related) plants can be and still detect unique effects of plant species on soil microbial composition (Innes *et al.* 2004; Emmett *et al.* 2017). Thus, in chapter 4 I used seven grass species in the genus *Poa* to determine whether there are differences among congeneric plant species on rhizosphere composition of bacteria and fungi. I found that fungal composition differed among *Poa* species but bacteria communities were only influenced by N addition. I then explored whether phylogenetic relatedness or traits were useful predictors of variation in microbial community composition, and how elevated N deposition influenced plant species' effects on soil microbial communities.

This thesis describes the interactive effects and responses of plants and soils and emphasizes the importance of manipulating soil conditions to determine factors that determine the outcomes of plant and soil responses to N deposition. More specifically, this research demonstrates that experimental manipulations that incorporate soil and microbial parameters are necessary to develop hypotheses for how plant species differ in their responses to N addition (chapters 2 and 4) and differ in their effects on soil microbial communities (chapter 4). The presented data and findings offer directions for further research. For example, chapter 3 shows that cheatgrass is inhibited by alpine soil despite this species being known for being quite plastic in its ability to thrive on many soil types. The utility of this finding could be increased if it were further investigated in the context of soil microbes, soil chemistry or the interactive effects of both as contributors of limited cheatgrass growth. In addition, a rich microbial dataset is associated with chapter 4; in which fungi and bacteria taxa are shown to preferentially associate with some plant hosts and not others, and ambient N is compared to high N conditions, thus providing clues to examine the functional roles of these taxa. This thesis moves the field of plant-soil interactions one step closer to predicting variation within plant species (chapters 2 and 3) and among

plant species (chapter 4) towards predicting plant responses to environmental change, and N deposition in particular.

CHAPTER II

Do plant-microbe interactions and aluminum tolerance influence alpine sedge species' responses to nitrogen deposition?

Abstract

A common response of plant communities to increased nitrogen (N) deposition is a shift in species' abundances. Multiple factors have been proposed to explain the changes in abundance, notably competition and soil acidification. We hypothesized that a plant species that decreased in abundance with elevated N would have lower ectomycorrhizal fungi, altered root-associated bacteria communities, and/or greater susceptibility to Al toxicity than a species that increased in abundance with increasing N deposition. We examined changes in plant-microbe associations and Al toxicity in two dominant species from an alpine dry meadow community subjected to long-term low-level N addition. *Carex rupestris* has increased in cover over time with N addition and *Kobresia myosuroides* has decreased. We conducted field sampling of soil microbes from treatment plots and tested whether field levels of Al have toxic effects on sedge species in a greenhouse study. Declines in ectomycorrhizal infection of *Cenococcum geophilum* occurred on *Kobresia* with increasing N treatment. In contrast, neither Al level nor changes in bacteria community composition corresponded with the change in cover of sedge species. Decreased ectomycorrhizal infection may have contributed to the decrease in abundance of *Kobresia*. This study contributes to an understanding of the types of plant-soil interactions that may influence how plant species respond to N deposition and rejects Al toxicity and changes in bacteria composition as factors that likely play a role in changes in sedge abundance.

Introduction

Atmospheric nitrogen (N) deposition from anthropogenic sources has led to eutrophication and loss of diversity, followed by acidification in many aquatic and terrestrial ecosystems worldwide (Galloway *et al.* 2004). While changes in plant diversity is one of the first and most commonly reported

indicators associated with increasing N deposition in terrestrial plant communities (Bobbink, Hornung & Roelofs 1998; Bowman *et al.* 2006; Simkin *et al.* 2016), changes in diversity are variable in magnitude and even direction, with decreases in species richness and evenness commonly reported in experimental N studies (Stevens *et al.* 2004; Clark *et al.* 2008, 2013; de Schrijver *et al.* 2011). Several possible mechanisms have been proposed to explain how N deposition elicits changes in plant community diversity, including competition (Bobbink *et al.* 1998, 2010; Brooker 2006; Hautier, Niklaus & Hector 2009; Dickson & Foster 2011), changes in plant-microbial interactions (Johnson *et al.* 2008; Suding *et al.* 2008), and soil acidification (Houdijk *et al.* 1993; Roem, Klees & Berendse 2002; Van Den Berg *et al.* 2005; Stevens *et al.* 2010). Altered competitive interactions among plant species is a primary hypothesis explaining N induced shifts in plant diversity, but there is little experimental evidence to demonstrate how it operates. One hypothesis suggests that greater productivity leads to decreased light availability in the community understory, which drives a decrease in diversity (Hautier *et al.* 2009). Alternatively, altered competition among plant species may be induced by indirect effects of N on soil chemistry or soil biota. Some evidence suggests that acid tolerant species are favored when high N deposition conditions lead to soil acidification (Stevens *et al.* 2010). Less attention has been given to changes in soil microbial composition (Lilleskov *et al.* 2002; Farrer *et al.* 2013; Dean *et al.* 2014; Allen *et al.* 2016) and aluminum (Al) toxicity in plants as it relates to soil acidity (Houdijk *et al.* 1993; De Graaf *et al.* 1997) as contributors to changes in species abundances. These soil components are directly influenced by N concentrations and are known to influence plant species differently, and thus could play important roles in changes in species abundances due to N deposition.

Plant health and fitness can be influenced by pathogenic and/or mutualistic soil microorganisms that interact with plant roots (van der Heijden, Bardgett & van Straalen 2008). Species-specific changes in plant fitness over time can lead to changes in plant species diversity. Since microbial community composition and function are tightly linked with nutrient availability (Fierer *et al.* 2012; Ramirez, Craine

& Fierer 2012), elevated levels of N deposition can lead to changes in the interactions between plants and microorganisms. Individual plants can be directly affected by changes in the abundance of microbial mutualists and pathogens (van der Putten, van Dijk & Peters 1993; Mills & Bever 1998), or indirectly via microbial alteration of the supply of plant resources (Hodge *et al.* 2000b; Schimel & Bennett 2004; Suding *et al.* 2008). Additionally, these processes can involve feedback loops that influence the plant species responses to N deposition (Sigüenza, Corkidi & Allen 2006; Bezemer *et al.* 2006; Kardol *et al.* 2007; Kulmatiski *et al.* 2008a). Thus, microbial community composition is believed to be an important but poorly understood influence on changes in plant responses to N deposition (van der Heijden *et al.* 2008; Mitchell *et al.* 2010; Farrer *et al.* 2013).

Nitrogen deposition can also impact plants by altering soil chemistry. Elevated N increases the leaching of nutrient base cations (calcium, magnesium, potassium), eventually leading to enhanced weathering and mobility of acidic cations—namely Al in acidic soils (van Breemen, Mulder & Driscoll 1983; Bowman *et al.* 2008; Stevens, Dise & Gowing 2009). Increased Al mobility is detrimental because Al is toxic to plants and soil microorganisms (Thompson & Medve 1984; Delhaize & Ryan 1995; Chen *et al.* 2013). Differences in plant species' tolerances to Al could therefore contribute to the different responses to N deposition among plant species. The links between N availability, soil pH, and Al availability are well established, (Bowman *et al.* 2008; Guo *et al.* 2011; Lieb *et al.* 2011; Greaver *et al.* 2012), but Al toxicity is rarely considered in plant community ecology (as opposed to agriculture), and few studies have tested Al toxicity as a contributing factor to the decline of native plant species as a result of N deposition (De Graaf *et al.* 1997).

Long-term N addition experiments that simulate elevated N deposition in alpine plant communities have resulted in changes in diversity, a consequence of increases and decreases in different species' abundances (Bowman *et al.* 2006; Suding *et al.* 2008). In dry meadow communities of the southern Rocky Mountains, cover of *Carex rupestris* (hereafter referred to as *Carex*) tripled with

increases in N deposition. Conversely, cover of co-dominant *Kobresia myosuroides'* (hereafter referred to as *Kobresia*) decreased by half (Bowman et al. 2006, in prep). These responses are noteworthy because these two sedge species are the dominant plant species in this community, and are among the few species that have exhibited significant change in cover since the initiation of this experiment in 1997. Changes in abundance of dominant species drives changes in species diversity in this alpine community (Bowman *et al.* 2006). Additionally, a study assessing acid buffering capacity of soil from the plots reported a significant decrease in pH (from 5.4 to 4.6) and increase in extractable Al^{3+} with increasing N level(from 12 to 34 mg kg^{-1} dry mass of soil) (Lieb *et al.* 2011). Aluminum toxicity in plants usually occurs when soil pH drops below pH 5.5 (Vitorello, Capaldi & Stefanuto 2005), and soil pH is below pH 5.0 in our long-term high N treatment plots (Lieb *et al.* 2011). This finding suggested that Al toxicity may contribute to the decline in *Kobresia*'s abundance.

This alpine plant community is a good model system to investigate the poorly understood mechanisms of N deposition, since species shifts occur in the absence of changes in light limitation (leaf area index is < 1), plant productivity, or changes in functional groups (Bowman et al. 2006). Thus, in this system we can essentially control for these better understood processes (Gough *et al.* 2000; Hautier *et al.* 2009) and explore potential contributions of soil microorganisms and Al toxicity to sedge responses to long-term N addition. Furthermore, current uncertainty about the primary mechanisms of plant community responses to N deposition is perhaps due to the complexity of multiple mechanisms operating together (Clark *et al.* 2007; Simkin *et al.* 2016). This implies that investigations that test multiple potential mechanisms are necessary to advance the current understanding N deposition's effects on plant communities within and across ecosystems.

Our goal for the research reported here was to examine pathways by which changing conditions below ground may drive changes in abundances of *Carex* and *Kobresia* with long-term N addition. We tested the influence of sedge species' differential sensitivity to Al toxicity as well as changes in microbial

community structure, which included assessment of both mycorrhizal colonization and soil bacteria community composition for each sedge species. For plant-microbe associations, we predicted that soil microbial community composition (both bacteria in the rhizosphere and mycorrhizal fungi) would correspond to changes in plant cover in response to N deposition. We hypothesized that mycorrhizal infection levels would differ between sedges species and show divergent responses to N addition. A decrease in mycorrhizal infection on *Kobresia* or an increase on *Carex* could suggest a shift to a new limiting resource other than N could be involved in sedge species' change in cover. We hypothesized that bacteria taxa on *Kobresia*'s roots that increase in relative abundance with N addition would be candidates to test for pathogenic effects that could explain *Kobresia*'s decline. We also hypothesized that *Kobresia* and *Carex* differ in their tolerance of Al and predicted that *Kobresia's* growth would be more inhibited by high Al availability than *Carex.*

Materials and methods

We evaluated the factors influencing changes in *Carex rupestris* ssp. *drummondiana* (Dewey) and *Kobresia myosuroides* (Villars) abundance due to elevated N deposition using plants and soils from an ongoing experiment initiated in 1997 (Bowman *et al.* 2006; Lieb *et al.* 2011). *Carex* and *Kobresia* are both rhizomatous sedge species and are co-dominant species in the long-term N addition plots.

The experiment simulated a range of N deposition rates in a species-rich dry meadow alpine community on Niwot Ridge, Colorado for 17 year before these data were collected. Five replicate 1 m x 1.5 m plots receive N fertilizer at rates of 0, 20, 40, or 60 kg N ha^{-1} yr^{-1}, applied as NH_4NO_3 in aqueous solution, within five blocks to help account for microsite variation. Ambient N deposition (wet + dry) at this site is approximately 6 kg N ha^{-1} yr^{-1} (Sievering et al. 2001). Soils are cryumbrepts with granitic parent material, contain 28.8±1.5% organic matter, and C:N is 16.9±0.2 (Seastedt, 2001).

Microbial sampling

To test whether the long-term N manipulation has altered soil microbial structure associated with *Carex* and *Kobresia's* roots, in July of 2013 four ramets of each sedge species were harvested per plot. Ramets were removed with root-associated soil attached and transported from the field on ice before subsampling for fungi and bacteria. In the lab, a rhizospere soil sample was taken from each plant by massaging soil from roots after loose soil had been removed from the root system. These samples were frozen at -20°C before extracting DNA.

Mycorrhizal colonization of roots

Kobresia is known to associate with *Cenococcum geophilum,* a species of ectomycorrhizal fungi that has experimentally been shown to supply N in the form of the amino acid glycine to *Kobresia* roots (Lipson *et al.* 1999). We believe that this is the first study to rigorously examine *Carex rupestris* for mycorrhizal associations.

To assess whether mycorrhizal infection differed across N levels in both species, five or more root fragments per plant were used to quantify the proportion of root tips harboring fungal hyphae per plant (n = 13-16 per treatment within species). Individual coarse roots were traced back to the base of each plant, clipped into 3cm fragments, rinsed with water and surveyed for ectomycorrhizal fungi using a 10x magnification dissecting scope. Root fragments were also cleared with 10% KOH and stained with 0.05% Trypan-Blue stain to search for endomycorrhizal fungi (Giovannetti & Mosse 1979). Plot averages of the number of infected root tips (hyphae mantel present) out of 50 root tips for each species were used in analyses.

Rhizosphere bacteria

Root-associated soil was collected from the field-harvested sedges by shaking and gently massaging soil off roots. Following established protocols (Leff *et al.* 2015; Prober *et al.* 2015), soil samples were processed for 16S rRNA gene sequencing to characterize microbial community composition across N treatments. DNA was extracted from 0.25g of soil from 40 samples stratified across blocks and N treatments using the Mo Bio PowerSoil DNA Isolation Kit (Mo Bio Laboratories, Inc., Carlsbad, CA, USA). The V4 region of the 16S rRNA gene was PCR-amplified using barcoded primers. Dilutions (1:100) were conducted to improve amplification across samples and the resulting PCR products from duplicate reactions were normalized for DNA concentration (Quant-iT PicoGreen® dsDNA Assay Kit, Life Technologies). Normalized samples were pooled for PCR clean-up (Mo Bio Labs UltraClean® PCR Cleanup kit), and cleaned PCR product was sequenced on an Illumina MiSeq at the University of Colorado. Raw sequences were demultiplexed and processed using the UPARSE pipeline (Edgar 2013), applying quality-filtering criteria used in previous studies (McDonald *et al.* 2012; Leff *et al.* 2015). To account for differences in sequencing depth, samples were rarefied to 10,000 sequences each (n = 14 for *Kobresia* and 19 for *Carex*).

Aluminum toxicity experiment

In order to test the effects of soluble Al on *Carex* and *Kobresia* growth, an Al addition experiment was conducted under greenhouse conditions. Sedges were harvested from the field site in June of 2014 within 20 meters of the field plots. The proximity to the plots was intended to acquire sedges that had experienced similar environmental conditions to those in the plots, but would not have been subjected to experimental N application and subsequent changes in soluble Al^{3+} (Lieb et al. 2011). Sedges were brought to the alpine room at the University of Colorado Greenhouse, where roots were separated from chunks of soil. One ramet per plot was planted in 164 ml conical pots filled to within a centimeter of the top of each pot with homogenized alpine soil from the field site. The sedges were

grown under uniform conditions (mean temperature was 15 °C, and soil moisture was maintained just below saturation) for four weeks prior to the initiation of the Al manipulation, to minimize variation in field effects on the plant responses to the experimental Al treatments. To assess Al effects on aboveground growth, shoots were clipped to 2.5 cm length prior to initiating Al additions. Plants were watered with 20 ml of tap water every other day throughout the experiment.

Al additions were initiated after the four-week acclimation period. Al was added as aluminum chloride hexahydrate ($AlCl_3H_{12}O_6$) at levels of 0, 10, 50, and 100 µmol Al^{3+} L^{-1} in 20 ml applications. Al treatments were applied every four days (20 ml of a tap water = Al solution). The range of Al additions in the greenhouse experiment spanned soluble Al concentrations in the field plots collected using lysimeters. Twelve replicates per Al treatment level were maintained for each sedge species. The experiment ended after 12 weeks, corresponding to the short alpine growing season. Three soil samples per treatment were analyzed for pore water Al concentrations using microlysimeters. Samples were analyzed on an ARL Inductively Coupled Plasma Emission Spectrophotometer (Laboratory for Environmental and Geological Studies, University of Colorado).

At the end of the experiment, we quantified variation in sedge growth by measuring root dry mass and shoot dry mass. Biomass allocation was measured using root to shoot ratios. Shoot length was also calculated as the length of the longest leaf blade at the end of the experiment minus the 2.5 cm present at the start of the treatments. A subset of plants across treatment levels were also visually inspected throughout the experiment to observe potential tissue damage associated with Al toxicity. Soil pH was measured in all pots at the end of the experiment using a Beckman 340 pH probe. Soil samples were shaken for 30 minutes in a 2:1 water to soil slurry, and tubes were shaken again before measuring pH.

Statistical analyses

Plot level means (n = 5) were used in analyses for bacteria communities and ectomycorrhizal infections. All statistical analyses were performed using R statistical software (R Core Team 2016). To determine the relationship between N level and percent ectomycorrhizal colonization on sedge roots, a linear mixed model was fit to these data using the LME4 package (Bates *et al.* 2015), where N level was the fixed effect and block was included as a random effect. The mctoolsr package for microbial community analysis was used with the bacteria data to aid in formatting and graphing multivariate data structures (Leff 2016). Differences in bacteria community composition between species and across N treatments in field plots were assessed with a permutation multivariate ANOVA (PERMANOVA) test using the “Adonis” function in the vegan package (Oksanen *et al.* 2016). This test uses Bray-Curtis dissimilarity matrices to test for differences in bacteria composition across treatments. Specifically, the relative abundances of OTUs within genera and families were compared among samples. Additionally, Pearson’s moment correlation tests were used to determine whether any common families (>5% of sequences within samples) exhibited a significant correlation in relative abundance of sequences with increasing N level. This was done to determine if any bacteria taxa may be candidate pathogens. Families that exhibit increasing abundance of sequences with N level would also correlate with a decrease in Kobresia cover and could be further examined for potential pathogenic effects. Common taxa were used in these analyses because rare taxa (at low relative abundances near plant roots) may be less likely to impart strong effects on plants, and by reducing the number of correlations tests performed we lowered the probability of making a type I statistical error. For the Al experiment, general linear models were used to test for a relationship between Al level and plant mass for each sedge species. Initial pre-Al treatment plant height were included as covariates in separate linear mixed effects models (Paine *et al.* 2012).

Results

Both *Kobresia* and *Carex* were infected with *Cenococcum geophilum*, a cosmopolitan species of ectomycorrhizal fungi distinguished by black mantel hyphae on root tips (Lobuglio 1999). Dark-septate endophytic fungi in roots occurred in < 2% of samples, and therefore were not included in analyses. Colonization of *C. geophilum* on *Kobresia* decreased with increasing N level in the long-term N addition plots, with a 30% decrease between control plots and the highest N level ($p = 0.10$, $R^2 = 0.14$, $n = 5$ (4 plants averaged per plot per species; Figure 2.1).

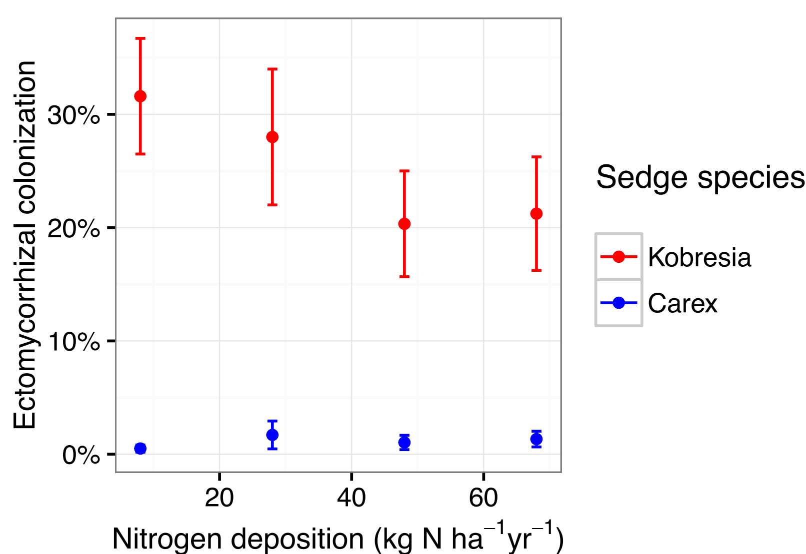

Figure 2.1. Colonization of ectomycorrhizal fungi (percent of root tips per plant) on *Carex rupestris* and *Kobresia myosuroides*' root tips for each N level. Means and standard error of the means are shown. N level is represented as the treatment levels (0, 20, 40, 60 kg N ha^{-1} yr^{-1}) added to the ambient N addition level of 6 kg N ha^{-1} yr^{-1} (n=5 plots per treatment level).

Kobresia harbored *C. geophilum* on *0-86%* of root tips surveyed, with a median colonization of 23% infected root tips per plant (out of 50 tips surveyed per plant). *Carex* had much lower infection levels than *Kobresia*, and showed no relationship between colonization and N level ($p = 0.60$, $R^2 = 0.02$, $n = 5$). Across all N levels, 9% of *Carex* plants harbored *C. geophilum* at > 2 root tips infected per plant. Infected *Carex* plants had less than 10% infected root tips per plant for all but one individual, which had 26% infected root tips.

Bacteria communities adhering to sedge roots showed no significant change in community composition across N levels ($p = 0.12$, $R^2 = 0.09$, $n = 2$), but did differ between the two sedge species ($p < 0.01$, $R^2 = 0.06$, $n = 2$) and among blocks ($p < 0.01$, $R^2 = 0.16$, $n = 5$) although species, block and the significant interaction between these two variables ($p = 0.04$, $R^2 = 0.21$) explained little of the overall variance in bacteria community composition among treatments. *Actinobacteria* and *proteobacteria* were the dominant phyla in similar proportions for both *Carex* and *Kobresia* (Figure 2.2).

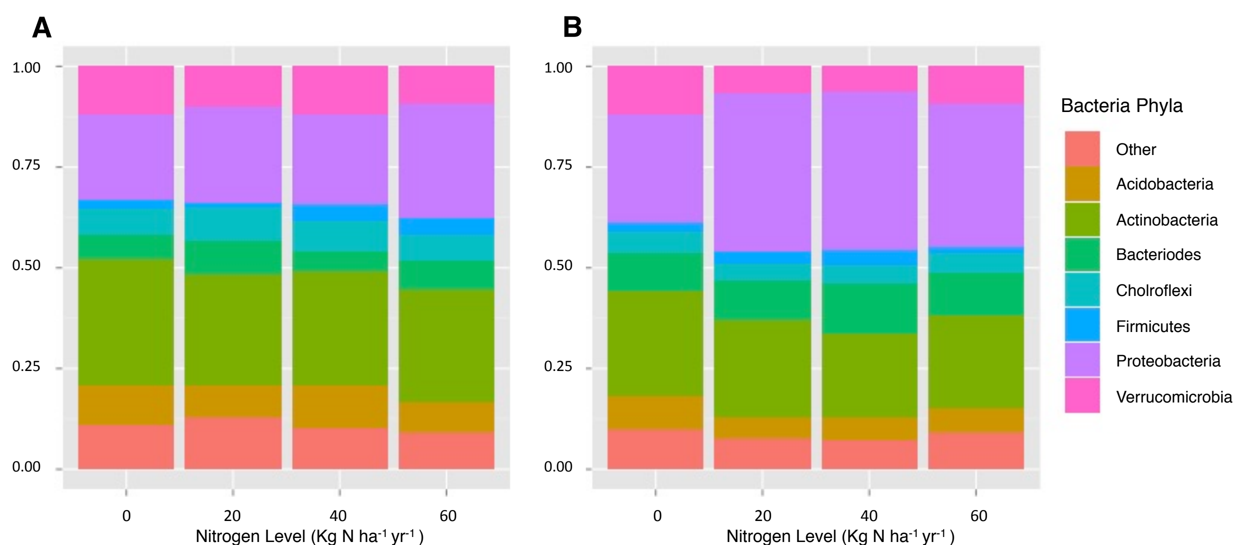

Figure 2.2. The relative proportions of bacteria OTUs grouped by phylum from soil adhering to *Carex rupestris'* roots (A) and *Kobresia myosuroides'* roots (B). Sedge species roots' harbored similar proportions of the same dominant bacteria phyla, which are common phyla in alpine soils.

Bacteria OTU frequencies grouped by higher taxonomic levels (family and genus) did not reveal any shifts in composition to indicate an N treatment effect. Correlation tests on the 67 common families with N treatment did not result in any families that varied significantly with N level ($n = 67$, $p > 0.05$). Correlation tests for the 120 common genera also did not reveal any taxa that varied significantly in relative abundance with N treatment ($n = 120$, $p > 0.05$).

The Al treatments had no negative effects on sedge growth for either species (Figure 2.3). Root dry mass (*Kobresia*: $p = 0.39$, $R^2 = 0.015$, *Carex*: $p = 0.28$, $R^2 = 0.026$), shoot dry mass (*Kobresia*: $p = 0.69$,

$R^2 = 0.003$, *Carex*: $p = 0.59$, $R^2 = 0.01$), root to shoot ratio (*Kobresia*: $p = 0.95$, $R^2 < 0.001$, *Carex*: $p = 0.5$, $R^2 = 0.01$), and shoot growth with pre-treatment shoot length included as a random effect (*Kobresia*: $p < 0.01$, $R^2 = 0.04$, *Carex*: $p < 0.01$, $R^2 = 0.02$) did not vary significantly for either species.

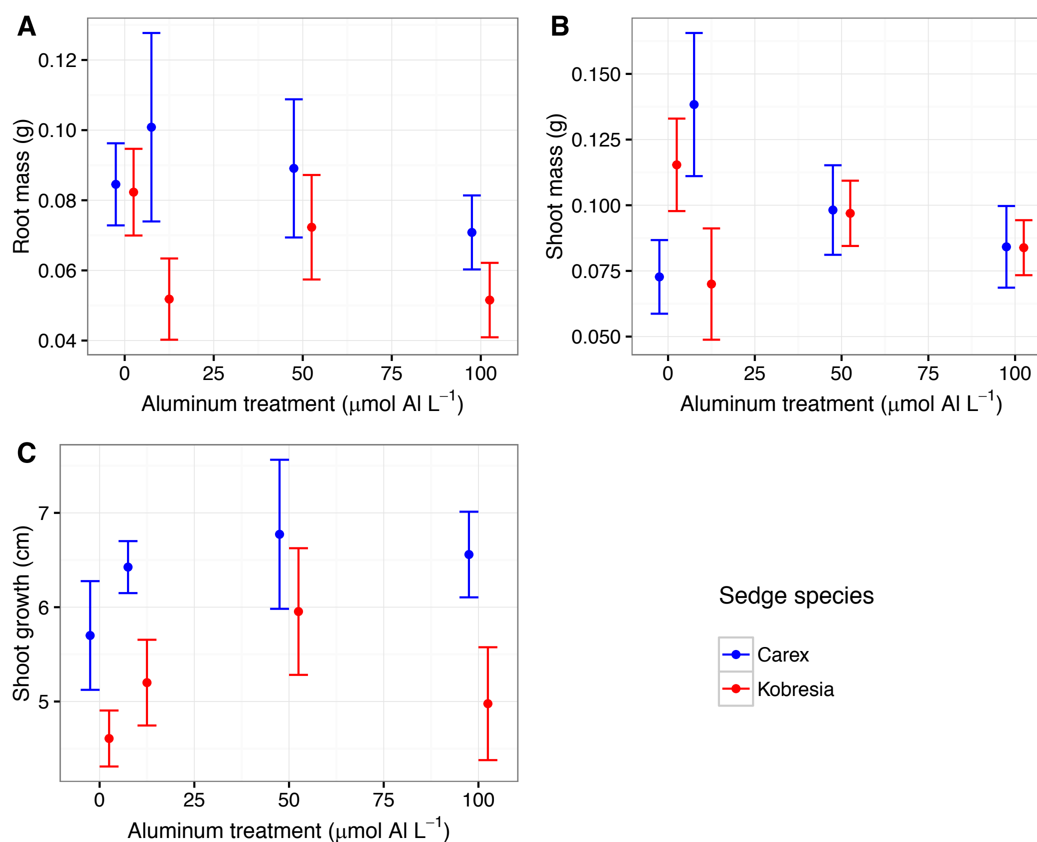

Figure 2.3. Root dry mass (A), shoot dry mass (B) and shoot growth (change in shoot length of the longest blade per plant) (C) for *Kobresia myosuroides* and *Carex rupestris* plants subjected to three levels of Al with a control treatment (tap water). Data are means and standard error of the means (n=5 plots per treatment level).

At the end of the experiment, soil pH was significantly lower in the high Al treatments than in the control treatment ($P = 0.01$, $R^2 = 0.07$, $n = 96$). The mean pH in the control pots was 5.88 ± 0.05 (s.e.m.) and 5.67 ± 0.09 in the highest Al addition treatment. Soil solution concentration of Al ranged from a mean of 28 ppb in control pots compared to a mean of 608 ppb in the 100 μmol Al^{3+} L^{-1} pots, effectively spanning measurements from the field plots as intended. The mean soil solution concentration of Al in

field control plots was 394 ppb, and the soil pH was 4.97 ± 0.07. The mean Al concentration from the high N plots was 246 ppb and the pH was 4.73 ± 0.12.

Discussion

The goal of this study was to explore belowground processes that could contribute to plant species' responses to elevated N deposition. We hypothesized that soil microbes and/or Al toxicity contribute to the observed increase in cover of *Carex rupestris* and decrease in cover of *Kobresia myosuroides* following 17 years of N addition in an alpine dry meadow community. There was a relationship between sedge species' cover and ectomycorrhizal fungi colonization, where root tip infection decreased with increasing N for *Kobresia.* We did not find evidence to support a relationship between root-associated bacteria and N addition. Al toxicity also does not appear to be a mechanism affecting the sedge species differently; neither species was affected by Al addition. These results suggest that the ectomycorrhizal status of the sedge species may be involved in the observed decrease in *Kobresia* cover associated with simulated N deposition, while bacterial community change and Al tolerance do not contribute to the species' responses.

The observed decrease in ectomycorrhizal colonization on *Kobresia* with increasing N is consistent with the hypothesis that mycorrhizal host plants reduce their carbon investment in the mutualism when N is abundant (Wallenda & Kottke 1998; Treseder 2004; Suding *et al.* 2005; Shantz, Lemoine & Burkepile 2015). The observed decrease in *Kobresia*'s cover with increasing N could be explained by the carbon costs of associating with mycorrhizal fungi when the fungi are no longer needed for N acquisition. An alternative hypothesis for *Kobresia*'s decline in cover may be related to a change in its limiting resource(s), from N to phosphorus (P) or water availability which would be expected limiting resources in this system after N (Bowman *et al.* 1993; Fan, Neff & Wieder 2016). A change from 30% decrease in infection may or may not be a meaningful change in resource acquisition for *Kobresia*, but

we considered that it could be meaningful given the large decrease in hyphae surface area and access to resources with each plant-mycorrhizal connection lost. Experimental work is needed to identify conditions where a shift in ectomycorrhizal colonization represents a shift in ectomycorrhizal function, since plant-mycorrhizal relationships and colonization are often responsive to changes in resource availability and most of this research on this topic has been conducted on arbuscular mycorrhizal fungi (Johnson 2010; Lekberg & Helgason 2018; Treseder *et al.* 2018). Recent research that characterizes plant-mycorrhizal relationships with N addition (Lilleskov, Hobbie & Horton 2011; Treseder *et al.* 2018) provides additional plant-mycorrhizal species pairs to investigate functional relationships.

Additionally, *Kobresia* and *Carex*'s cover is negatively correlated in the high N level field plots and the relationship increases in magnitude over time (Bowman, unpublished data). Plant-plant competition for a resource other than N is one possible explanation for this pattern. We did not directly test competition between *Carex* and *Kobresia* because *Kobresia*'s response to experimental manipulation requires several years to detect (Bowman, unpublished data), however changes in competition may very well be both a consequence of changes in resource availability as well as a reason for the observed changes in cover of the sedge species.

Our hypothesis that changes in root-associated bacteria are linked to sedge species' responses to long-term N addition was not supported, since bacterial community composition did not differ across N levels. Since there was no significant difference in common bacteria taxa (genera or families) across N levels, it is unlikely that specific bacteria taxa had enhanced pathogenic effects with elevated N that could contribute to *Kobresia*'s decline in cover. This null result differs from field fertilization experiments that have demonstrated shifts in abundance of bacteria taxa with added N (Allison & Martiny 2008; Nemergut *et al.* 2008; Ramirez *et al.* 2012; Coolon *et al.* 2013), but is useful in eliminating a potential mechanism for *Kobresia*'s decline in abundance. More recently, a few studies have reported no change in bacterial community composition in N addition experiments (Leff *et al.* 2015; Jing *et al.* 2016; McHugh

et al. 2017), suggesting that responsiveness of soil bacteria to long-term changes in N addition may be more specific to local site conditions and species composition than previously thought. However, differences in experimental designs among studies likely contribute to differences in the magnitude of the treatment effects. First, experimental manipulations differ greatly in the dosage and duration of N fertilization (ranging from 10-800 kg N ha^{-1} yr^{-1})(Ramirez *et al.* 2010), and N addition does not have a consistent effects on bulk soil bacteria biomass (Treseder 2008) or composition (Leff *et al.* 2015) across ecosystems. Second, the response variables that target microbial function also differ among studies. Here, taxonomic composition of root-associated bacteria was chosen to reveal whether plant-bacteria interactions are altered in response to N deposition. The majority of N studies that describe microbial community responses to abiotic changes measure microbial biomass or respiration (reviewed by Treseder 2008; Wei *et al.* 2013). However, changes in microbial biomass and respiration cannot reveal whether changes to microbial composition occur. Thus, examining changes in the relative abundance of taxa is potentially a useful approach to identify taxa that may affect plants. In this study rhizosphere bacteria composition did not differ across N levels. Since the relative abundances of taxonomic groups did not change, we can conclude that there is likely no change in bacteria function due to long-term N addition.

Contrary to our expectations, Al addition had no effect on aboveground or belowground growth for either sedge species. To our knowledge, this is one of the first studies to test for Al toxicity as a consequence of N deposition in a native plant community (De Graaf *et al.* 1997). We considered that soils with high organic matter content are buffered from the effects of soluble Al, because most Al is chemically bound to organic matter in basic to slightly acidic soils (Berggren & Mulder 1995). Niwot Ridge alpine soils are relatively high in organic matter, but long-term N addition has resulted in elevated soluble Al^{3+} in treatment plots (Lieb et al. 2011). Additionally, Al concentrations in pore water in the Al addition pots were 22 times higher on average in the highest Al treatment compared to controls. Thus,

organic matter in the pots did not immobilize all added Al and make it inaccessible for plant uptake.Since initial root growth responses to elevated soluble Al are very rapid (within minutes to hours) the greenhouse experiment provided ample time to observe sedge responses (Barceló & Poschenrieder 2002). The lack of response of plants to Al addition in this experiment, which effectively spanned the range of Al concentration in field plots, suggests that these alpine plants may be resistant to modest changes in pH and Al availability that can occur with elevated N deposition. It should be noted though that N can accumulate in dry meadow soils over time, and the threshold concentrations for plant responses to Al and pH may decrease over time as NO_3^- and H^+ accumulate due to increased N cycling (Stevens *et al.* 2009; Humbert *et al.* 2016).

We tested two potential mechanisms to explain how atmospheric N deposition might alter plant species cover and thereby plant diversity through changes in species evenness. In an alpine dry meadow plant community, long-term N addition did not impact soil bacteria communities associated with the dominant plant species, nor did Al toxicity explain differential responses of these sedge species. A generalist ectomycorrhizal fungus may play a role in plant-plant competition, although the mechanism is not clear. We hypothesize that lower abundance of *C. geophilum* would lessen the ability of *Kobresia* to access P and/or water, the likely limiting resources. Further research is needed to determine the nature of competition among plant species in the alpine and other ecosystems affected by N deposition to determine how competition among plant species as well as function of mycorrhizal fungi shifts with increasing N deposition. While we have no clear evidence for precise mechanisms, we contribute to this field by rejecting the potential roles of Al toxicity and bacterial pathogens in mediating changes *Kobresia*'s and *Carex*'s cover. Understanding the mechanisms by which N deposition drives species-specific responses is important, especially in communities like alpine dry meadow communities where changes in cover of these dominant species impacts plant diversity and composition. Studies like this

one are needed to address the potential for multiple factors to be involved in plant responses to elevated N deposition in order to determine which are most common across different ecosystems.

CHAPTER III

Testing invasion filters for the alpine: the roles of climate, nitrogen deposition and soil

Abstract

Alpine areas will likely experience an increase in non-native plant species invasions as a result of increased human activity and environmental changes that will lower the environmental constraints for their establishment and spread. To understand and prepare for high elevation plant invasions, it is necessary to evaluate the changes in environmental factors that make alpine regions susceptible to potential invaders. The alpine of the Rocky Mountains has very few occurrences of non-native species to date, but anthropogenic environmental changes may facilitate invasion. We tested whether climate change would promote cheatgrass (*Bromus tectorum*) invasion in the Rocky Mountain alpine, through increases in alpine growing season temperature and plants' tolerance to spring sub-freezing temperatures. We also tested whether nitrogen (N) deposition and alpine soils may modify *B. tectorum* responses to climate change. Our findings suggest that alpine soils strongly inhibited growth of *B. tectorum* regardless of temperature or simulated N deposition. However, higher minimum growing temperatures and increased N addition did enhance *B. tectorum* growth for plants grown in upper montane soils. These results indicate that alpine invasion by *B. tectorum* is unlikely in the near future given low growth and reproduction in alpine soils even under warmer growing seasons and increased N availability, but these changes may promote population growth of *B. tectorum* within montane regions, and further adaptation of *B. tectorum* to more alpine-like environments could eventually lead to alpine invasions.

Introduction

Non-native plant species have altered ecological conditions in most terrestrial ecosystems (Lonsdale 1999). Efforts to restore communities to native states are expensive and rarely achieve

desired long-term outcomes (Reid *et al.* 2009; Suding 2011). As such, the enormous challenges associated with restoration suggest preventing non-native (or introduced) species from becoming invasive (able to spread away from sites of introduction) should be prioritized (Leung *et al.* 2002; Lodge *et al.* 2006; Mcdougall *et al.* 2011). Effective prevention of invasive species establishment requires a better understanding of the environmental factors that make ecosystems vulnerable to invasion.

Alpine ecosystems are some of the least invaded environments due both to low seed dispersal and environmental constraints such as low temperatures (Lonsdale 1999; Alexander *et al.* 2016). However concurrent increases in nitrogen (N) deposition and climate change have the potential to facilitate invasions in high mountain ecosystems (Dukes & Mooney 1999; Pauchard & Alaback 2004; Concilio, Loik & Belnap 2013; Petitpierre *et al.* 2015; Lembrechts *et al.* 2016). Invasive species are now established and spreading along alpine roadsides in the Andes (Cavieres *et al.* 2005), the Northern Calcareous Alps (Dullinger, Dirnböck & Grabherr 2003), and the Australian Alps (Johnston & Pickering 2001), demonstrating that the alpine is not immune to invasive species establishment (reviewed by Alexander et al. 2016).

To successfully establish in a new habitat such as the alpine, non-native species must overcome multiple barriers (i.e. ecological filters). Seed availability is the initial filter (i.e. dispersal limitation), which is followed by multiple site-level filters including abiotic conditions and biotic interactions that affect the success of plants (Grubb 1977, (Theoharides & Dukes 2007). Increasing human activity in alpine regions such as road building and recreation is likely relaxing the dispersal filter, and contributing to the presence of non-native species in mountain ecosystems (Marini *et al.* 2009). At the same time, site-level filters are also changing in alpine ecosystems. A long-standing hypothesis posits that alpine ecosystems are too cold for invasive species to establish (Pauchard et al. 2009). However, anthropogenic climate change is increasing growing season temperatures and growing season length (Clow 2010), which may benefit non-native species that are adapted to lower a elevation climate (Dukes and Mooney

1999; Hellmann et al. 2008; Walther et al. 2009).

Resource availability may also play an important role in determining establishment and spread of non-native species that are able to overcome dispersal and environmental constraints (Davis *et al.* 2000; Gross, Mittelbach & Reynolds 2005; Flores-Moreno *et al.* 2016; Liu & van Kleunen 2017). Invasives often grow best in nutrient rich soils, and compete well for limiting nutrients relative to native species (Davis et al. 2000; Levine et al. 2003; Funk and Vitousek 2007; Rao and Allen 2010). Resource availability likely differs between invaded upper montane soils and uninvaded alpine soils given differences in plant species composition, microbial community composition, soil texture, organic matter chemistry, and availability of mineral nutrients. Invaded soils also differ in resource availability compared to uninvaded soils due to negative or positive feedbacks caused by changes in resource use and rates of nutrient cycling by the non-native plant species (Suding *et al.* 2013). In the alpine, cold temperatures and short growing season limit rates of nutrient cycling (Fisk, Brooks & Schmidt 2001). Some alpine soils would likely be suitable for non-native species' growth, especially under the recent climate scenario of earlier spring snowmelt and longer growing seasons (Clow 2010). Another environmental change that can increase resource availability in the alpine is N deposition. While native alpine plant species grow slowly, and exhibit conservative changes in N use with increasing N availability (Bowman and Bilbrough 2001), the opposite is generally true for non-native species in their invaded ranges (Vasquez, Sheley & Svejcar 2008; Milberg *et al.* 2010). It is also probable that shifts in multiple site-level factors (i.e. increased N availability + increased growing season temperatures) may increase the likelihood of invasions. This idea has been examined in the context of community assembly with some evidence to support it (Myers & Harms 2009; Pinto, Pearson & Maron 2014), but only recently has it been applied in invasion biology (Maron *et al.* 2014; Eskelinen, Kaarlejarvi & Olofsson 2017; Lembrechts 2017).

To experimentally test how climate change (growing season temperature and subfreezing temperatures), soil type and N deposition affect a potential alpine invader's growth and reproduction

we conducted growth chamber experiments with cheatgrass (*Bromus tectorum). Bromus tectorum* is a problematic invader throughout the Western United States that occurs in the montane and subalpine in some areas of the Rocky Mountain (Mack 1981; Chapin *et al.* 2000). We hypothesized that cold growing season temperatures and spring freezing temperatures currently limit *B. tectorum* establishment but warming will enhance growth and reproduction. We also hypothesized that increased N availability promotes invasive species establishment when temperature is not limiting. Finally, we hypothesized that alpine soils will be suitable for *B. tectorum* growth especially under warmer temperatures and enhanced N availability. We expected that *B. tectorum* growth and reproduction would differ between invaded and uninvaded soils and used an uninvaded montane soil treatment in addition to the alpine soil treatment to be able to distinguish between abiotic differences and biotic feedbacks on *B. tectorum* performance.

Methods

Study species

Bromus tectorum is a winter annual grass species that was introduced to the western United States in the late nineteenth century. Populations were established throughout the Intermountain West by 1930 (Mack 1981). In its native range, which includes most of Europe, the northern edge of Africa and western Asia, it is found at upper montane elevations below 3,000 m (Upadhyaya, Turkington & McIlvride 1986; Novak & Mack 2001). There is no evidence of *B. tectorum* occurring above treeline in the Rocky Mountains at this time. Germination studies have shown that *B. tectorum* can germinate and grow well in 5°C nighttime & 10°C daytime temperatures (Aguirre & Johnson 1991; Meyer *et al.* 1997), and root growth persists below this range and ceases around 3°C (Harris 1967). *Bromus tectorum* is also adapted to a relatively wide range of physical soil properties (i.e. soil texture)(Norton *et al.* 2004; Reisner *et al.* 2013).

Growing Season experiment

To explore the effects of growing season temperature and N availability on *B. tectorum* growth we conducted pot experiments in temperature and light-controlled growth chambers. We compared *B. tectorum* germination and growth in current and expected future alpine growing season temperatures for Niwot Ridge, a long-term alpine study site. The 4°C temperature increase we used for our future temperature scenario is consistent with estimates for Colorado's mountain regions, which are predicted to experience an increase in temperatures between 3-5 °C between 2035 and 2064 (Lukas *et al.* 2014). Additionally, we tested whether simulated increases in N deposition would enhance the growth of *B. tectorum* and whether increased growth depended on temperature. For this experiment we planted *B. tectorum* seeds collected from a montane population (elev 1780m, 40.1262N, -105.3078W) in alpine soil collected from Niwot Ridge, CO (elev 3466m, lat 40.052486N, long-105.582467W).

Temperatures for the control growing season temperature treatment were set to 12°C daytime and 8°C nighttime to simulate average July growing season temperature in the alpine (elevation 3739m) on Niwot Ridge (Greenland & Losleben 2001). We germinated *B. tectorum* seeds on filter paper in petri dishes in both temperature treatments to determine whether temperature influenced germination success. Seedlings were transplanted into 164mL 3.8 x 21cm conical pots filled with mixed and sieved (2mm) alpine soil. The warm treatment was set to 16°C daytime and 12°C nighttime temperatures. Both temperature treatments were applied using a growth chamber and received 14 hours of daylight at 400 mmol photons m^{-2} s^{-2}.

Half of the pots started receiving the N addition treatment 40 days after transplanting, resulting in a total of 40 pots with 10 replicates for each temperature and N level combination. The N addition treatment was 20 kg N ha^{-1} yr^{-1}, approximately double the current rate on Niwot Ridge, and was applied as $NH_4^+NO_3^-$ dissolved in tap water at a concentration of 1mmol N L^{-1} applied at 30 ml increments. The

control treatment received the same volume of tap water without N added. We allowed plants to grow for 78 days total to simulate the short alpine growing season, and then we measured shoot length (longest leaf), dry shoot mass, and root mass. None of the plants in either treatment produced flowers in this experiment.

Freeze recovery experiment

The goal of the freezing experiment was to determine whether frost events influence *B. tectorum* establishment and reproduction, and whether soil type and N addition influence *B. tectorum* responses to simulated frost events. Seeds from a montane population of *Bromus tectorum* (elevation 2632m, lat 40.0024°N, long -105.5013W) were collected in August of 2015. The alpine soils used in this experiment were from the same source as the soils used for the growing season experiment. The two montane soil types were collected from within a population of *B. tectorum* [elevation 2611m, location (40.0024˚N, 105.5013˚W)] and just outside of that population (elevation 2611m, 40.0481N, 105.4665W). We assume that differences in growth and reproduction between invaded montane soils collected from within the *B. tectorum* population and non-conditioned uninvaded soils can be attributed to effects of *B. tectorum* on soil such as microbial community composition and carbon chemistry. These two soil types will be referred to as ‘conditioned’ and ‘non-conditioned’ hereafter. Each soil type was mixed and sieved to 2mm separately to homogenize and remove rocks and coarse organic material and placed in conical pots in September 2015. Seeds were germinated in November and seedlings were planted in the prepared pots in December.

Seedlings were grown in 10°C daytime and 5°C nighttime temperatures for 50 days before subjected to one of four subfreezing treatments with 14 hours per day with lights on. For the freezing treatments, plants were randomly assigned to a subfreezing temperature, -8°C, -6°C, -4°C or control (5°C nighttime temperature). These temperatures were based on a minimum June temperature of -6°C at a

high alpine weather station at 3739 m elevation on Niwot Ridge averaged across 1994-2014 (median = -6.5°C) (Losleben 1994). The longest leaf was recorded for each plant, and blemishes were noted before exposure to the sub-freezing temperatures. Freezing occurred in an incubator and one freezing event occurred for three hours during between 3:00 am and 6:00 am to simulate a realistic time period where when minimum temperatures would occur. Mortality and freezing damage was recorded and photographed the day after each freezing event.

Plants were allowed to recover and grow in growth chambers for another 54±3 days to determine whether soil type or N addition affected recovery from freezing. At the end of the experiment plant height was measured and plants were harvested to measure dry shoot mass and root mass. Additionally, reproduction potential was estimated as the number of fully emerged spikelets per plant. This estimate has been used previously (Griffith & Loik 2010; Concilio *et al.* 2013), and was used for this experiment because, while many plants matured to this flowering stage, none produced fruit over this time interval.

Data analyses

Two-way ANOVAs were conducted to determine important treatment combinations and interactions among treatments for both the growing season experiment (using N treatment and growing season temperatures as categorical variables) and freeze recovery experiments (using N treatment, freezing temperature and soil type). While total plant mass was the response variable for ANOVAs for both experiments, an additional test was conducted using reproductive spikelet production as the response variable for analyzing the freeze recovery data. Distributions of residuals were reasonably normal and sample sizes within treatments were sufficiently large for ANOVAs. All statistical analyses were performed using R statistical software (R version 3.2.4).

Results

Growing Season experiment

Under the control temperature treatment, 46% of seeds germinated, while 88% of seeds germinated in the warm treatment. At the end of the experiment, plant dry mass was significantly greater under the warmer growing season temperature compared to the current temperature treatment (2-way ANOVA, $F_{1,36}$ = 12.6, p = 0.001). Plants in both temperature treatments were small at the end of the experiment (mean height = 4.7 cm, mean total mass = 0.015 g; Figure 3.1) relative to individuals grown in the field from the populations where the seeds were collected (>20cm).

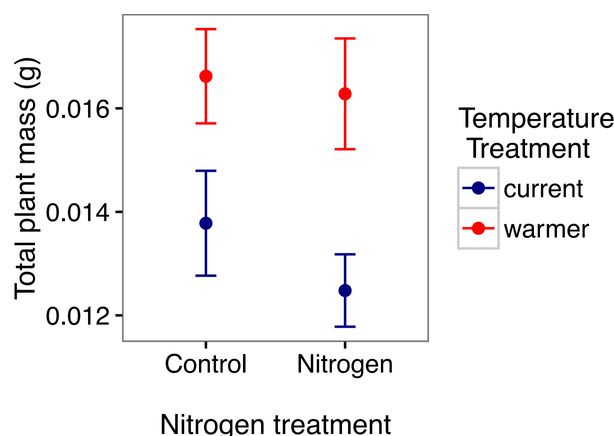

Figure 3.1. Means and standard errors of dry total plant mass from the growing season experiment. The warmer growing season treatment (12°C daytime, 8°C nighttime) resulted in significantly larger plants by mass than the control temperature (16°C daytime, 12°C night time)(2-way ANOVA, F1,36 = 12.6, p = 0.001), and N addition had no effect on mass.

As noted above, none of the plants produced flowers in either control or warmer growing season treatments. Finally, the N addition treatment did not influence plant growth in either temperature treatment (2-way ANOVA, $F_{1,36}$ = 0.8, p = 0.386).

Freeze recovery experiment

Sub-freezing temperatures significantly affected *B. tectorum* total dry mass per plant (2-way ANOVA, $F_{3,325}$ = 018.3, p < 0.001; Figure 3.2).

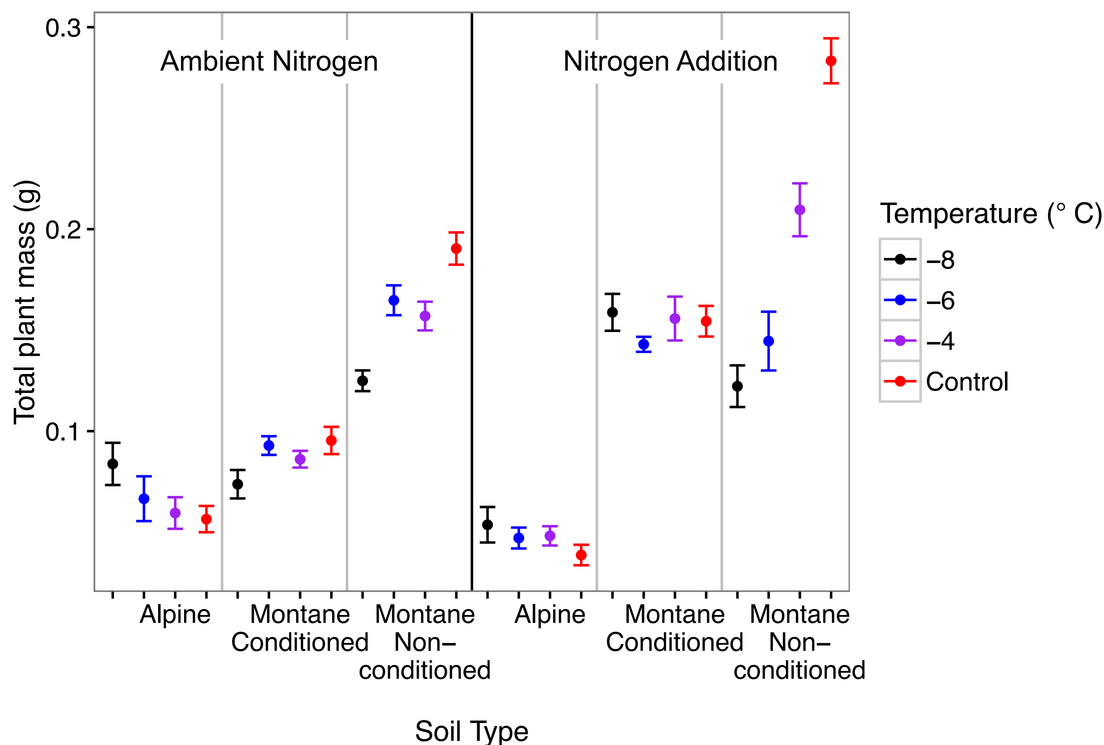

Figure 3.2. tectorum total dry mass from the freezing temperature experiment. Each treatment combination of nitrogen, soil type and freezing temperature is depicted as means and standard errors.

However, the effect of freezing temperature differed depending on which soil type plants were grown in ($F_{6,325}$ = 24.7, p < 0.001). Across all treatments, there was a significant effect of soil type on plant mass ($F_{2,325}$ = 407.3, p < 0.001), with significant interactions between soil type and N level ($F_{2,325}$ = 52.7, p < 0.001), and all three predictor variables together (2-way ANOVA, $F_{6,325}$ = 6.9, p < 0.001). Total plant mass was 38% lower in alpine soil compared to upper montane soil (Figure 3.2). Since the effect of temperature differed depending on the soil type plants were grown in, and a main interest was how plants fared between treatments within alpine soil, we conducted ANOVAs within soil type in addition to interpreting results from the full model. Within the alpine soil treatment, nitrogen addition decreased total plant mass (2-way ANOVA, $F_{1,106}$ = 13.1, p < 0.001), and the warmer temperatures resulted in marginally significant lower biomass than colder subfreezing temperatures (2-way ANOVA, $F_{3,106}$ = 2.5, p = 0.061). There was not a significant interaction between N treatment and subfreezing temperature. For

plants grown in the non-conditioned soil, there was a significant decrease in plant mass after exposure to lower sub-freezing temperatures (2-way ANOVA, $F_{3,109} = 45.5$, $p < 0.001$). Nitrogen addition also influenced total mass in the non-conditioned soil type (2-way ANOVA, $F_{1,109} = 23.1$, $p < 0.001$), and the magnitude of the subfreezing temperature effect on growth was greater within the N addition treatment (2-way ANOVA, $F_{3,109} = 13.6$, $p < 0.001$). For plants grown in the conditioned soil from the upper montane *B. tectorum* population, N addition again significantly enhanced total mass (2-way ANOVA, $F_{1,110} = 166.2$, $p < 0.001$), but exposure to subfreezing temperatures did not impact mass accumulation during the recovery period (2-way ANOVA, $F_{3,110} = 0.6$, $p = 0.63$).

Nitrogen addition increased total plant mass (2-way ANOVA, $F_{1,325} = 49.6$, $p < 0.001$), and there was a significant interaction between N treatment and soil type (2-way ANOVA, $F_{2,325} = 52.7$, $p < 0.001$). Under ambient N conditions, mass was significantly greater in the soil from the non-conditioned population soil type (1-way ANOVA, $F_{2,168} = 136.3$, $p < 0.001$).

The different treatments did not influence potential reproduction in the same way as total plant mass. Subfreezing temperatures did not influence the number of spikelets produced per plant (2-way ANOVA, $F_{3,325} = 1.5$, $p = 0.213$; figure 3.3).

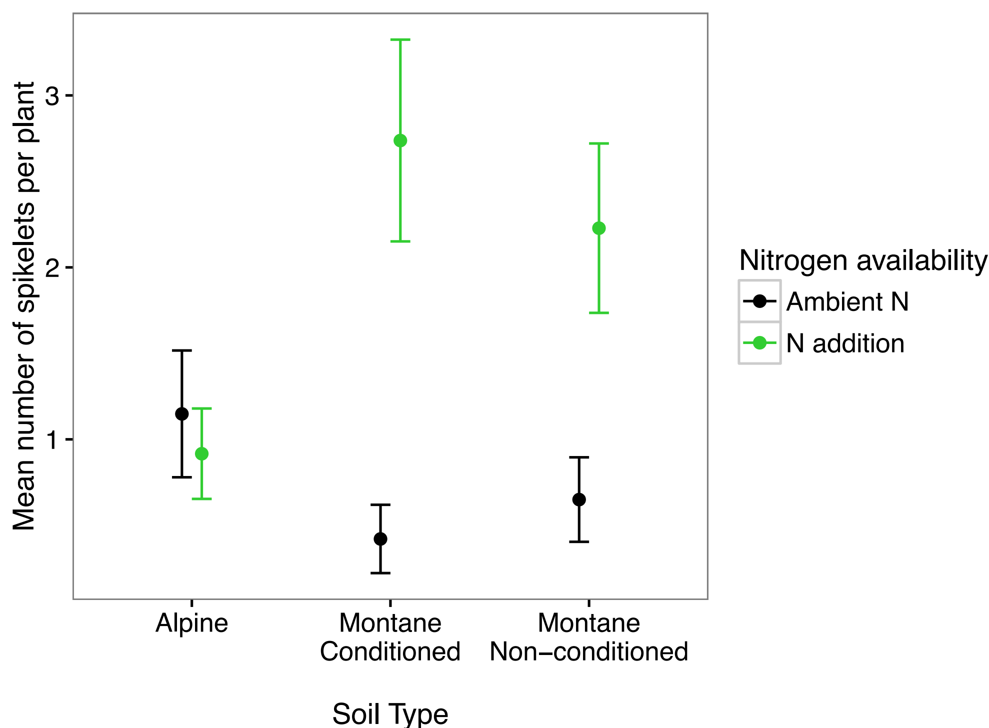

Figure 3.3. Effects of nitrogen addition and soil type on mean spikelet production per plant. The different freezing temperature treatments were pooled since there was no effect of freezing temperature on spikelet production. Nitrogen addition significantly increased spikelet production (2-way ANOVA, $F_{1,325} = 49.6$, $p < 0.001$), and the effect of N depended on soil type (2-way ANOVA, $F_{2,325} = 5.7$, $p < 0.003$). Plants with zero spikelets are included.

Spikelet production was, however, significantly greater in the N addition treatment relative to ambient N (2-way ANOVA, $F_{1,325} = 15.2$, $p < 0.001$), and there was a significant interaction between soil type and N with no effect of N in plants growing in the alpine soil (2-way ANOVA, $F_{2,325} = 5.7$, $p < 0.003$) despite soil types not having unique effects on spikelet production (2-way ANOVA, $F_{2,325} = 1.3$, $p = 0.273$).

Discussion

The goal of this study was to evaluate the vulnerability of alpine ecosystems to invasion by a non-native grass species under anthropogenic environmental change. We tested the effects of climate change and N deposition on *B. tectorum* growth and reproduction in alpine and montane soils. We hypothesized that the cold temperatures associated with alpine growing seasons is a key filter

preventing alpine invasions. Contrary to our expectations, we found that while low growing season temperature and exposure to subfreezing temperatures did conditionally influence growth and reproduction of *B. tectorum,* alpine soil was the most important filter we examined that inhibits *B. tectorum* invasion into the alpine. In montane soils, where *B. tectorum* is currently established, N and temperature did enhance *B. tectorum* growth, but overall the effects of N and temperature were small compared to the effects of the different soil types.

Alpine soils inhibit growth and reproduction

Evidence from both experiments indicate that alpine soils may inhibit *B. tectorum* invasion. In the freezing experiment, we found that total biomass of plants grown in montane soils was more than 2.5 times greater than plants grown in alpine soil. Nitrogen addition and freezing treatments had no effect on this difference. In the growing season experiment, where all plants were grown in alpine soil, total plant height and biomass was low (plants were <7cm tall) and the plants did not produce flowers. *B. tectorum* is generally thought to be well adapted to a wide range of soil conditions (Bradford & Lauenroth 2006a; Blank 2008), and yet our results suggest that the transition between upper montane and alpine soil may be an effective invasion barrier. The alpine soil used in this study was within the range of soil texture and soil pH that *B. tectorum* grows in its invaded range (Bradford & Lauenroth 2006a; Miller *et al.* 2006; Concilio *et al.* 2013), however we note that the vast majority of studies reporting soil characteristics in *B. tectorum* populations were conducted in low elevation, arid ecosystems of the Great Basin. In comparison, the alpine soil used in our study is lower in pH and higher in organic matter than cool desert soils, and this may have contributed to poorer growth of *B. tectorum* in this study.

Given that *B. tectorum* is well adapted to a wide range of soil types (Norton *et al.* 2004; Reisner *et al.* 2013), we hypothesize that the soil microbial community may also inhibit *B. tectorum* growth.

Most research on *B. tectorum's* interactions with soil microogranisms has focused on the effects of *B. tectorum* on already invaded soils (Belnap & Phillips 2001; Hawkes *et al.* 2006; Schaeffer *et al.* 2012; Concilio, Vargas & Cheng 2015). To our knowledge, no studies have specifically tested whether microbial communities in uninvaded soils influence establishment or whether change in microbial communities after establishment leads to feedbacks that would affect population growth of subsequent generations of *B tectorum*. Increased growth in the montane non-conditioned population soil type compared to the conditioned montane soil type may also be driven by microbes, and supports the enemy release hypothesis (Keane & Crawley 2002). Further investigations should determine whether alpine soil inhibits growth of invasives across different alpine soils in the Rocky Mountains and determine whether soil microbes or other attributes of alpine soil inhibit *B. tectorum* growth.

Interacting effects of nitrogen, soil, and temperature

Contrary to our hypothesis, N did not enhance cheatgrass' growth in alpine soils. Thus, it is unlikely that N deposition would improve establishment and/or spread of *B. tectorum* into the alpine. In the growing season experiment, plants did not respond to N addition, even in the warmer than average growing season treatment. The amount of N added (20 kg N ha^{-1} yr^{-1}) is within the range of forecasted rates near urban and agricultural centers in the Western United States for the middle of the 21st century (Dentener *et al.* 2006). We consider this strong evidence that N deposition will not facilitate *B. tectorum* establishment in the alpine, even if rates of N deposition increase in the Rocky Mountains. This alpine-specific finding differs from lower elevations (low elevation biomes) in *B. tectorum'* invaded range wherein *B. tectorum* often responds positively to high N availability, alters N cycling and changes competitive outcomes in invaded communities (Sperry, Belnap & Evans 2006; He, Yu & Sun 2011; Concilio & Loik 2013). For example, Uresk (1979) showed that soil temperature constrained growth rates

of *B. tectorum* below 11°C, and that above this temperature, N availability influenced growth (Uresk, Cline & Rickard 1979).

Although N addition had no effect on *B. tectorum* grown in alpine soils, N addition did have a significant positive effect on total biomass accumulation and flower production for plants grown in the two montane soil treatments. For plants grown in the non-conditioned soil treatment, N enhanced growth significantly for the control treatment and plants that recovered from the -4°C subfreezing event but did not affect growth in plants that experienced -6°C and -8°C subfreezing events. This suggests that increased N deposition could promote persistence and spread of current populations *within* montane ecosystems. Larger, persistent populations in the montane are cause for concern, because this will provide more opportunities for adaptation to alpine conditions over time (Haider *et al.* 2010).

We originally hypothesized that cold alpine temperatures limit establishment of non-native species in the alpine, as has been proposed previously (Pauchard *et al.* 2009). We found that the variation in growing season and extreme minimum temperatures had different effects on *B. tectorum* growth and reproduction respectively. This implies that there are multiple ways in which temperature could act as an invasion filter (Haider, Alexander & Kueffer 2011). First, no *B. tectorum* plants reached reproductive maturity under current or warmer growing season temperatures, which may be due to the inhibiting effect of the alpine soil and less to do with growing season temperature. Second, none of the sub-freezing temperature treatments resulted in *B. tectorum* mortality, and 25% of plants produced flowers, suggesting that spring freezing events will not prevent *B. tectorum* from establishing in the alpine. In the context of climate warming, a shift in plant phenology with early spring snow melt would increase plants' exposure to subfreezing temperatures (Synder & de Melo-Abreu 2005).

Conclusion

Based on the evidence from this study, the Rocky Mountain alpine does not show immediate risk of alpine invasion by *B. tectorum*. However, the spread of *B. tectorum* within the montane is concerning, and our results show that both increased N availability and fewer freezing events and/or warmer freezing temperatures will aid in growth and reproduction within montane populations. Haider (2010 & 2011) demonstrated that upslope range expansion of non-native plant species relies on genetic changes for species to become adapted to high elevation conditions (Haider *et al.* 2010, 2012), indicating that adaptation within high elevation *B. tectorum* populations may be necessary for alpine invasions to occur.

Low temperatures and resource availability have been hypothesized as potentially important invasion filters in high elevation systems (Pauchard *et al.* 2009), but to our knowledge, this is the first time they have been experimentally tested together. Our results demonstrate that alpine soil could be an effective invasion barrier for *B. tectorum* in some alpine areas. Contrary to our expectations, warmer growing season temperatures, warmer minimum temperatures and increased N availability did not lower constraints on growth and reproduction when plants are grown in alpine soil.

CHAPTER IV

Closely related grass species differ in their effects on soil microbial communities with and without nitrogen addition

Abstract

Both soil conditions and plant species have considerable influence on the composition of soil microbial communities near plant roots. However, the drivers of variation in plant species effects on soil microbial communities are poorly understood. We examined how grasses in the genus *Poa* influence soil microbial community composition and examined whether the variation in differences in microbial composition was related to plant phylogenetic relationships, plant traits and variables associated with plant species' environmental niches. We also explored whether plant-microbe associations differ between ambient nutrient conditions and elevated N addition to address whether plant-microbial associations are important for understanding plant and soil responses to changes in resource availability, such as anthropogenic nitrogen deposition. We conducted a greenhouse experiment with 7 *Poa* species and their native soils and found that *Poa* species had unique effects on fungal community composition, but not bacterial community composition. None of the hypothesized traits explained the variation in plant species' effects on fungal community composition. *Poa* species' effects on microbial communities were accentuated within N addition treatments. These results suggest that plant species' associations with both bacteria and fungi differ across closely related plant species, and that these associations may be important even when N is abundant in soils.

Introduction

Together, plant species identity and the composition of soil microbes near a plant's roots creates unique interactions that influence both plant function and microbial communities. A wealth of research has demonstrated the importance of plant-microbe associations for many plant species, but

generalizing plant-microbe associations across plant species remains an enormous challenge (Bever et al. 2010; Bardgett 2017). It is also poorly understood how plant-microbial community interactions are affected by environmental changes such as elevated nitrogen availability (Classen et al. 2015). Understanding differences in plant species effects on soils is necessary for improving predictions about plant species' coexistence and how plant species and soils respond to anthropogenic environmental changes (Reynolds et al. 2003; van der Heijden et al. 2008; Bever et al. 2010). Species-specific effects on nutrient cycling influences the composition of plant communities and ecosystem function (van der Heijden et al. 2008; Grigulis et al. 2013).

Plants are both affected by microbial activities and can affect rhizosphere microbial communities in several ways (Chapman et al. 2006; Hartmann et al. 2008). Plant roots secrete a wide variety of compounds that foster or inhibit colonization by microbial groups, interfere with microbial signaling and alter the acidity in the rhizosphere to favor nutrient uptake by roots (Nguyen 2003; Bais et al. 2006). Different plant species may have unique effects on soil and soil microbes, as a result of exudation of different amounts and combinations of chemicals and litter production (Hamilton William E and Frank 2001; Meier and Bowman 2008; Philippot et al. 2013).

Aboveground plant traits and evolutionary history often explain variation in aboveground ecological interactions (Gilbert and Parker 2010; Burns and Strauss 2011). The link between phylogenetic relatedness and similarity of plant traits that influence plant processes belowground is an area of active investigation (Veresoglou and Rillig 2014; Mehrabi and Tuck 2015; Münzbergová and Šurinová 2015; Leff et al. 2018). Studies that have compared plant species' effects on soil microbes often use very distantly related, dissimilar plant species, with a single species typically representing a family or order (Bardgett et al. 1999; Smalla et al. 2001; Innes et al. 2004; Garcia et al. 2005). Relatively few studies report phylogenetic and trait-based signals for distantly related species for aspects of plant species' belowground ecology compared to above ground ecology (Burns and Strauss 2011; Anacker et

al. 2014; Cantarel et al. 2014; Moreau et al. 2015). Comparisons using multiple genotypes of single crop species also detect phylogenetic signal, but differences in plants' effects on soils among plant genotypes are not consistently found across studies (Wagner et al. 2016; Emmett et al. 2017; Leff et al. 2017). There is evidence of phylogenetic and trait-based influences in plant-soil feedbacks (Brandt et al. 2009; Anacker et al. 2014), which further sets up the expectation that closely related and/or morphologically similar plants should have more similar effects on soil microbial communities when appropriate variation in plant traits and relatedness is assessed. While there may be few good predictors for plant species' effects on soils using plant species aboveground ecological interactions, the appropriate scale of variation among plant species is still not clear (Kembel and Cahill 2011).

In order to detect plant trait-based and/or plant phylogenetic influences on microbial community structure, we first investigated whether congeneric grasses differed in their effects on microbial community composition. We hypothesized that congeneric plant species have unique, non-random effects on rhizosphere microbial composition. To our knowledge, this level of taxonomic relatedness has not been examined, despite its intermediate level of trait conservation compared to previous studies (Bardgett et al. 1999; Innes et al. 2004; Berg and Smalla 2009; Wagner et al. 2016; Emmett et al. 2017; Leff et al. 2017). Phylogenetic signal most commonly is lost above family level despite considerable variation across studied clades (Anacker and Strauss 2016). Second, we hypothesized that differences in soil microbial community composition across plant species can be explained by plant relatedness. We also tested alternative and non-mutually exclusive hypotheses for factors that contribute to plant differences in their effects on the rhizosphere. We expected that variation in soil microbial communities among plant species would be strongly associated with plant traits that relate to nutrient use (Moreau et al. 2015) such as root and shoot mass and plant height. Finally, attributes of plant species' habitat preferences may be correlated with plant effects on microbial community composition. Presumably, rhizosphere microbial community assembly depends partly on

abiotic attributes that compose the niches of the plant species they associate with (Berg and Smalla 2009). We tested whether mean annual temperature, elevation range, or soil pH for each plant species geographical range explains differences in microbial community composition.

Characterizing plant-soil associations across plant species is important for understanding how plant species may respond to environmental change (Classen et al. 2015). In certain contexts, plant effects on soils can initiate feedback loops: when plants alter their rhizosphere microbial community and nutrient cycling in a way that promotes or inhibits the growth of subsequent generations of plants in a population To identify microbial taxa that have important roles in the function of different plant species, it is first necessary to identify how microbial taxa differ in their relative abundance across plant species and soil conditions to develop hypotheses about the function of individual taxa (Bever et al. 2010; Classen et al. 2015).

Increases in anthropogenic N deposition has affected plants and soils in both natural and managed ecosystems (Galloway et al. 2008; Bobbink et al. 2010; Simkin et al. 2016; Payne et al. 2017). The influence of plants on microbial species composition and abundance will additionally have an important impact on ecosystem responses to environmental change. N deposition is an environmental change that directly impacts both plant and microbial communities (Bardgett et al. 1999; Suding et al. 2008), and these impacts have been extensively described for plants (Tilman and Wedin 1991; Vitousek and Howarth 1991; Stevens et al. 2004) and soil microbes (Treseder 2004; Ramirez et al. 2010; Geisseler and Scow 2014) in separate studies. Although research has compared microbial community biomass, respiration, and composition among plant species in N addition studies (Groffman et al. 1996; Bardgett et al. 1999; Kennedy et al. 2004; Grman and Robinson 2013; Farrer and Suding 2016) the interactive effects have not been sufficiently addressed by separating the direct effects of N on microbes from the effects of plants on microbes.

We characterized plant-microbe associations with and without N among species in the genus *Poa* and along with no-plant control soils with and without N to gain insight into plant species control on soil microbial communities (Pennings et al. 2005). Plant species would have unique effects on microbial community composition if different types and amounts of exudates create microbial niches. Microbial competition with plants for N can be high even in fertile soils (Kaye and Hart 1997; Bell et al. 2015), which suggests plants may use microbial processed N and maintain close associations with these microbes in high N conditions. Thus, we hypothesized that N addition enhances the distinct effects of plant species on microbial community composition. On the other hand, an increase in N availability may have a larger direct effect on microbial community composition than the effect of plant species. To address whether plant responses to N addition explain variation in microbial communities among *Poa* species, we tested for a relationship between plant growth and change in microbial community composition between N addition and ambient N treatments. We hypothesized that there is a positive relationship between the responsiveness of plant species to N addition and the magnitude of change in the rhizosphere communities to N addition would occur if plant species consistently allocated more carbon (C) belowground with elevated N availability.

Methods

To assess whether closely related grasses have unique effects on rhizosphere microbial composition, seven grass species in the genus *Poa* were used in a greenhouse experiment with live soil. The species are *Poa alpina*, *Poa arctica*, *Poa compressa*, *Poa glauca*, *Poa nemoralis*, *Poa Pratensis*, and *Poa reflexa*. This set of plant species was chosen for a variety of reasons including local geographical convenience, the existence of a fully resolved phylogeny (Gillespie et al. 2007), low rates of hybridization for closely related species, and variation in phylogenetic distance among the seven species, none of which are sister taxa. Additionally, species that share similar plant traits and habitat attributes such as

the three alpine species (*Poa alpina*, *Poa arctica*, and *Poa glauca)* are distantly related within the group, providing an opportunity to determine whether phylogentic signal is a better or worse predictor of microbial composition than plant traits or environmental niche. Two species are non-native to this region, therefore possessing similar traits associated with successful invasion necessary in the Colorado Front Range (*Poa Pratensis*, *Poa compressa*) where seeds and soils were collected. All seven selected *Poa* are perennial and rhizomatous.

A population of each *Poa* species was identified using both flora for the region (Weber and Wittmann 2012; Ackerfield 2015) during the summer of 2014. All populations used in the study were located in Boulder County and spanned an elevational gradient from low montane (elev 1764 m, lat 40.124012˚ N, long -105.30666˚ W) to alpine (elev 3466 m, lat 40.052486˚ N, long-105.582467˚ W). Seeds were collected from approximately 20 inflorescences distributed across the population of each species. Nine liters of combined bulk and rhizosphere soil was collected from each *Poa* species population in September.

The soils from the seven *Poa* populations were mixed separately for each soil type, sieved (2mm), and then an equal volume of soil from each site was combined and thoroughly mixed to create a uniform soil type. This uniform soil environment therefore allowed soil microbial communities associated with each species population in each pot, creating a similar starting composition. An initial uniform microbial community allowed for statistical differentiation of the effect of plant species on this similar initial microbial composition. Extra soil from each of the populations was also reserved for a comparison with the mixed soil. Conical pots (164 ml) were filled with the mixed soil to within 2 cm of the top of the pots.

Seeds from each species were surface sterilized with 10% bleach and seeds were planted in half of the prepared pots. The number of seeds planted per pot (<10) was determined by a germination trial in petri dishes and plants were thinned to a single seedling per pot. In a few instances where no plants

germinated in a pot, a seedling from another pot was surface sterilized in 7% bleach for 10 minutes, rinsed with DI water and transplanted.

Pots were then thinned to one individual seedling per pot, and tools were sterilized with bleached before thinning a new pot to minimize contamination of microbes between pots. Four treatments were then established at this time, where the mixed soil was used for all treatments. These treatments were plant + N, plant, soil + N, and soil only. These treatments allow for distinguishing plant effects, N effects and plant + N effects on soil microbes. Ten replicates per treatment per plant species totaled to 160 pots.

A 595 watt LED panel was installed above the racks to supplement low levels of natural light without increasing solar radiation (360 umol/photons/m^2 below light to 215 umol/photons/m^2 on edge of racks on a cloudy afternoon). Racks were randomly rotated under the LED panel every week to minimize the effects of light intensity variation. Lights were on for 13 hours a day, which was increased to 14 hours a day half way through the experiment to better mimic natural photoperiod of the growing season. Greenhouse temperatures ranged from 10-21°C, which is well within the range all *Poa* species experience in the field during their growing season.

Four weeks after germination, half of the planted pots and half of the soil-only pots began receiving N treatments twice each week as a solution of 1mmol NH_4NO_3 for a total of 11 N applications. The young plants were watered with 20 ml of tap water (with or without N), which was poured onto each pot's soil twice per week to maintain constant soil moisture throughout the experiment. This application rate is equivalent to 70 kg N ha^{-1}. Tap water contained <0.05 mg N L^{-1} in the form of nitrate. After 17 weeks the plants were harvested. Bulk soil was collected for soil pH measurements and plant height (length of longest leaf) was measured. Soil was shaken from roots and the remaining soil adhering to roots was then shaken and gently massaged from roots onto a clean sheet of weigh paper. This rhizosphere soil was then transferred to a sterile tube and kept on ice until samples could be moved

to a -20°C freezer at the end of each harvest day. Roots were then washed, and roots and shoots were separated and placed in labeled envelopes for measuring dry weight. One representative leaf per plant was attached to a white piece of printer paper using clear tape, which was scanned the same day and used for leaf area measurement using the software program Image J. Leaves were then removed from paper, dried and added to individual's dry shoot mass. Soil pH was measured using a 2:1 soil:tap water slurry which was shaken for 30 minutes and measured using a Beckman 340 pH probe.

DNA from soil samples was extracted using MoBio Laboratories PowerSoil DNA isolation kit (Mo Bio Laboratories, Inc.), according the manufacturer's instructions. To ensure that a representative sample of microbial taxa from each plant's rhizosphere was used for DNA extraction, an additional step was taken in which .25 g from each soil sample was combined with 0.4 ml ultra pure water in a sterile microcentrifuge tube and shaken before subsampling. From the slurry, 0.25 g were transferred to a well in 96 well power bead plates to begin the normal DNA extraction protocol.

To obtain microbial composition for each pot, the V4 region of the16S ribosomal RNA gene and the ribosomal internal spacer ITS1 were amplified using unique barcodes for each sample for multiplexing with 16S and ITS primers to amplify bacterial and fungal DNA respectively. PCR product was pooled after performing duplicate reactions and cleaned using a SequalPrep Normalization kit (Thermo Fischer Scientific Inc.). The cleaned and pooled amplicon was then sequenced on an IlluminaMiSeq running 2 x 250 bp sequences. Sequences were demultiplexed using a custom R script (https://github.com/leffj/helper-code-for-uparse), and quality filtering and phylotype clustering were conducted using the UPARSE pipeline (Edgar 2013). Raw sequences were then mapped to phylotypes (97% similarity) after removing singletons and clustering. Reads were mapped to the Greengenes database to obtain taxonomy classifications for OTUs using the RDP classifier. Datasets were then checked for contamination, and samples were rarefied to 3997 sequences per sample for 16S and 1000 sequences per sample for ITS to account for differences in sequencing depth.

To estimate pair-wise phylogenetic distances among the seven *Poa* species, two *Poa* phylogenies were used to determine typology and estimate phylogenetic distances for all seven species (Gillespie and Soreng 2005; Gillespie et al. 2007). We created a molecular phylogeny for the 7 plants species based on four genes: ITS, rbcL, matK, and trnH-psbA. Sequences were downloaded from GenBank (accessed Jan. 18, 2017; Table A4.1) and aligned using MUSCLE.

Phylogenetic reconstruction based on the aligned sequence data was performed with maximum likelihood (ML) using RAxML-HPC2 v.7.2.6. To guide the ML search, we created a topological constraint tree based on a recent Angiosperm Phylogeny Working Group tree (R20100428) using Phylomatic. The ML search was conducted using a GTRCAT model and 100 bootstrap replicates. Branch length calibration was conducted in BEAST using the RAxML tree to constrain the topology and calibration points for the root node of 50 Ma and an arbitrary standard deviation of 1.0 Ma with a normal distribution.

We ran an MCMC chain for 10 million generations, sampling every 1000 generations. We checked convergence of the posterior distribution, using TRACER v1.5. From the combined BEAST posterior distribution of 10,000 trees, we removed the first 2,000 trees as burnin and combined the remaining 8,000 trees into a single maximum credibility tree using TreeAnnotator v1.8. The newick-formatted phylogeny follows:

(Poa_alpina:49.94841223,((Poa_compressa:14.6524015,(Poa_glauca:5.437345329,Poa_nemoralis:5.437345329):9.215056172):17.46971806,(Poa_reflexa:11.73752126,(Poa_pratensis:5.136634192,Poa_arctica:5.136634192):6.600887064):20.3845983):17.82629268);

In addition to estimating phylogenetic distances among *Poa* species to predict differences in microbial community structure among plant species, we also identified a set of traits to test alternative hypotheses since traits that may be important for explaining plant effects on microbial communities would not necessarily be phylogenetically conserved. We measured plant height (longest leaf), shoot mass, and root mass of plants at the end of the experiment. These are traits that we predicted would

represent plant species' effects on soil microbial communities as they are relevant for plant nutrient use and allocation. Our intent was not to perform an exhaustive analysis of potential traits to find the best surrogate for exudate composition that drives variation in microbial communities among plant species. Instead, our goal was to test a few alternative hypotheses to phylogenetic relatedness, and test traits that are easy to measure and would therefore have practical applications for future research if they correlate with plant species' effects on microbial community composition. Accordingly, we chose two attributes that represent environmental variation among the *Poa* species geographical ranges to determine if plant effects on soil microbes may be influence by their adaptations to particular environments. We obtained geographical and elevation ranges for each plant species from the GBIF database and used this information to estimate species' means for soil pH from the ISRIC Soil Data Hub and mean annual temperature from PRISM.

Data Analyses

Statistical analyses were performed in R (R Core Team 2016). The package 'mctoolsr' (http://leffj.github.io/mctoolsr/) was used to aid in formatting of multivariate datasets for analysis preparation.

We used PerMANOVAs (adonis function in R-vegan) to determine the amount of variation in bacterial and fungal community composition explained by plant species. Microbial communities associated with no-plant controls were included at first to determine if there was a general effect of plants on soil communities regardless of plant species. Then no-plant controls were removed, and the analysis was run again to determine whether microbial communities differed among *Poa* species. Differences in microbial communities were calculated by comparing the relative abundance of all OTUs per treatment. PerMANOVA was also used to test how N addition altered plant effects on rhizosphere microbial communities by determining differences in microbial communities among plant species,

between N treatments and the potential interaction between plant species and N addition. For significant perMANOVAS, a follow-up test of independent contrasts was performed to determine which treatments differed significantly from one another (https://www.researchgate.net/post /How_can_I_do_PerMANOVA _pairwise_contrasts_in_R). A T-test was also performed on each fungal and bacterial family that contained ≥ 10 sequence copies (individual organisms) within replicate pots for each *Poa* species, and compared rhizosphere composition to no-plant controls. This analysis was included to provide a more detailed view on how *Poa* species influenced the absolute abundances of sequence copies within families instead of the whole community together. Bonferroni corrections were made using a 0.0014 alpha level to avoid issues of Type 1 and Type 2 error.

Linear regressions were used to test for monotonic relationships between plant phylogeny and plant traits to determine whether evolutionary histories determined similarities in the traits we measured for *Poa* species. Linear regressions were then used to test for a relationship between plant phylogeny and microbial community structure, plant traits and microbial community structure and finally environmental predictors and microbial community structure to determine which predictors if any explain observed variation in microbial community structure. To obtain mean differences in microbial community composition between *Poa* species pairs a dissimilarity matrix containing the relative abundance of bacterial OTUs across soil samples was collapsed into a three-column matrix where a distance (between 0 and 1) was estimated for every combination of samples in the experiment. Distances were then calculated between every combination of samples for which a comparison could be made between two plant species. This method allowed us to retain replicates for each *Poa* species and distances to be calculated for perMANOVAs. The same procedure was used prepare data for a linear regression test to determine whether plant species' growth responses to add N impact the degree of microbial community change. The difference between microbial communities with and without N addition was calculated within each *Poa* species and among species.

Since pH differed among soil samples, and therefore pH may have contributed to microbial community composition among samples, we ran a variance partitioning model with N treatment and soil pH. We used the varpart function in the vegan package to attribute variation in microbial community composition to either N or pH or both while including plant species information (Mitchell et al. 2012). Using this method, explanatory variables were run as partial regressions with community dissimilarity matrices derived from bacterial and fungal OTU tables.

Results

We first tested whether the plant traits we measured were correlated with phylogenetic relationships among *Poa* species. There was no relationship between phylogenetic distance and trait similarity for root mass (slope = -0.39, P = 0.63 R^2 = 0.01), shoot mass (slope = -1.03, P = 0.18, R^2 = 0.09), or SLA (slope = -0.79, P = 0.45, R^2 = 0.03), but was a good predictor for plant height (slope = 7.22, $P < 0.01$, R^2 = 0.36).

Plant effects on microbial composition

There was a significant effect of plants on bacterial and fungal community structure when no-plant controls were included in perMANOVAs (bacteria: p = 0.01, R^2 = 0.10, n = 8; fungi: $p < 0.01$, R^2 = 0.13, n = 8; Figure 4.1 A & B). Additionally, *Poa* species had unique effects on fungal community composition when no-plant controls were removed from the analysis ($p < 0.01$, R^2 = 0.11, n = 7), which was done to determine if differences in microbial communities among plant species existed.

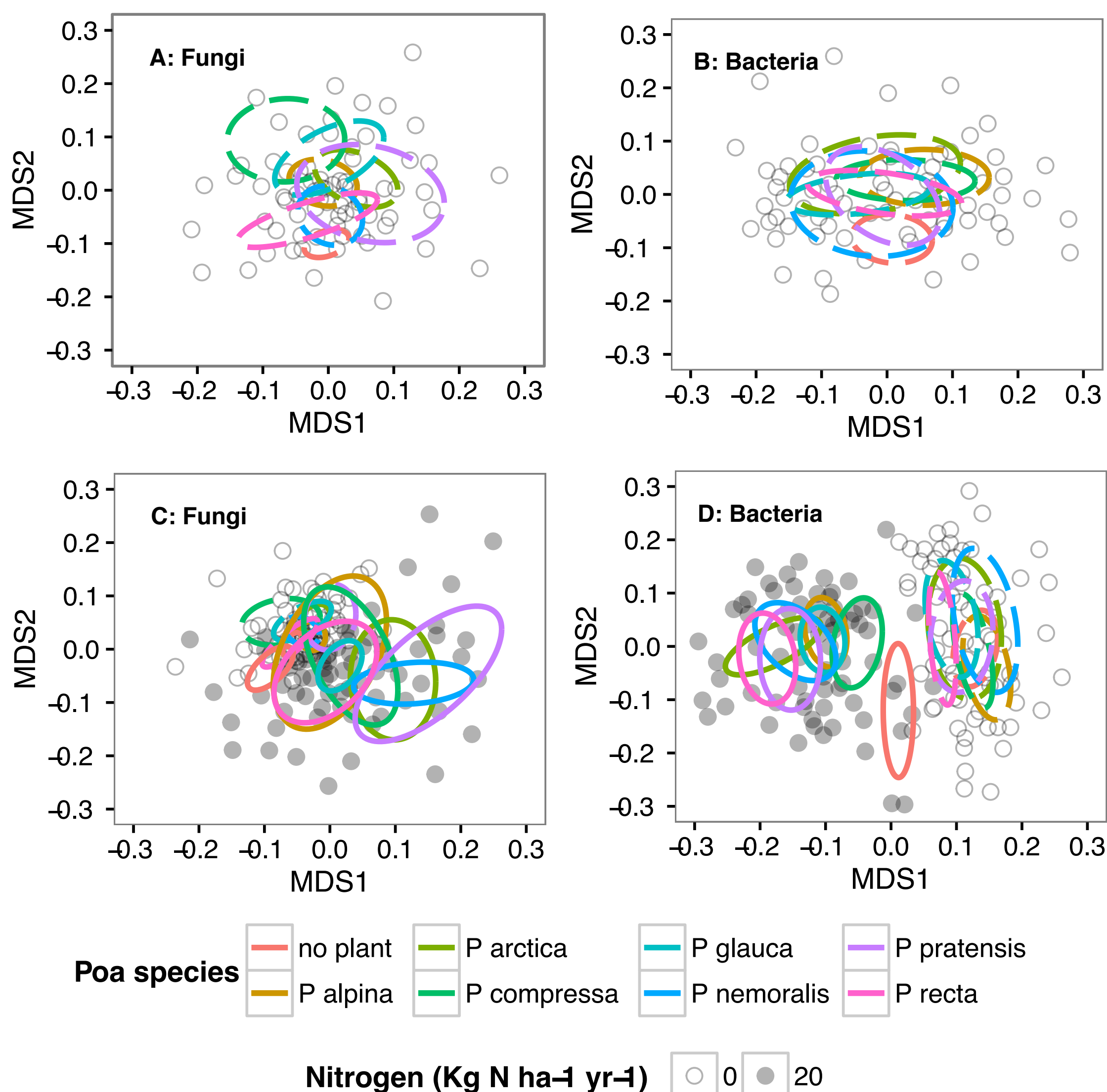

Figure 4.1. microbial community differences associated with rhizosphere samples and no-plant control samples. Orbitals are 95% confidence limits for each *Poa* species. Panel A) is fungal composition and B) is bacteria composition among plant species in non-fertilized soils, while is C) fungal communities and d) bacteria communities from ambient N (open circles) and N addition treatments (filled circles). Orbitals with dashed, colored lines indicate microbial communities associated with *Poa* species in the ambient N treatment, whereas solid, colored lines indicate microbial communities among *Poa* species in the N addition treatment. Multiple microbial communities differ significantly among *Poa* species in panels A, C, and D (perMANOVAs, $p < 0.05$).

Pair-wise contrasts among *Poa* species pairs revealed that rhizosphere bacterial community associated with each *Poa* species differed significantly from at least two other *Poa* species' rhizosphere communities except for *Poa glauca,* which overlapped strongly with all species combinations. Bacterial

communities did not differ among *Poa* species once no-plant controls were removed (p = 0.10, R^2 = 0.10, n = 7), and thus only fungal data were used to determine whether hypothesized predictors could explain differences in bacteria community structure across plant species.

Moderate changes in 8% of tested fungal families (118-129 fungal families had >10 sequences among pot samples, depending on *Poa* species; Table A4.2) occurred with at least one *Poa* species (relative to no-plant control communities), and likely contributed to the change in overall community composition (alpha < 0.0014). On the other hand, 22% of tested bacteria families (200-224 bacterial families were tested per plant species as these families had 10 or more sequences represented by a family across samples) were significantly different between one or more *Poa* species and no plant controls (Table A4.3) but overall community composition was not significantly altered by plant species, suggesting that a lack of change in many depauperate bacterial families was responsible for the lack of change in overall bacteria community composition.

None of the predictor variables were good predictors of plant species' similarity in bacteria community composition. Fungal community composition for plant species pairs was tested against *Poa* phylogenetic distance (slope = -0.02, P = 0.34 R^2 = 0.05). Follow up tests to determine if phylogenetic distance explained variation in the number of sequences within each common fungal family revealed only 3 significant tests out of 171 families. Only 2 out of 292 bacterial families showed evidence of phylogenetic signal (Table A4.4). Phylogenetic signal was also not found for any of the plant measurements (p < 0.05) except for plant height (slope = 7.21, p < 0.01, R^2 = 0.36). The plant growth and traits tested against fungal community differences included plant height (slope = 0.02, P < 0.01, R^2 = 0.36), root mass (slope = 0.03, P = 0.44, R^2 = 0.03), shoot mass (slope = -0.05, P = 0.13, R^2 = 0.012), and SLA (slope = -0.04, P = 0.14, R^2 = 0.11). Additionally, the two environmental niche variables, soil pH means associated with each *Poa* species native range (slope < -0.01, P = 0.51, R^2 = 0.02) and mean

annual temperature for each *Poa* species (slope < -0.01, $P = 0.93$, $R^2 < 0.01$) were not predictive of fungal community composition.

Nitrogen and plant effects on microbial composition

When N-treated samples were included in the analysis to determine whether N addition disrupted or enhanced plant species' unique effects on rhizosphere communities, we found that both N and plant species had unique effects on bacteria communities, and the effect of plant species was dependent on N level (plant species: $p < 0.01$, $R^2 = 0.05$, n = 8; N: $p < 0.01$, $R^2 = 0.04$, n = 2; plant species*N: $p < 0.01$, $R^2 = 0.05$, n = 8; Figure 4.1 D). Nitrogen addition and *Poa* species had similarly significant in their effects of fungal communities (plant species: $p < 0.01$, $R^2 = 0.06$, n = 8; N: $p < 0.01$, $R^2 = 0.04$, n = 2; plant species*N: $p < 0.01$, $R^2 = 0.05$, n = 8; Figure 4.1C). Differences in bacterial and fungal community composition remained significantly different across plant species and the two N treatments after no-plant controls were removed from the analysis (bacteria: plant species: $p < 0.01$, $R^2 = 0.09$, n = 7; N: $p < 0.01$, $R^2 = 0.05$, n = 2; plant species*N: $p < 0.01$, $R^2 = 0.05$, n = 7; fungi: plant species: $p < 0.01$, $R^2 = 0.05$, n = 7; N: $p < 0.01$, $R^2 = 0.04$, n = 2; plant species*N: $p < 0.01$, $R^2 = 0.05$, n = 7). Differences in community composition between N addition and ambient N treatments can be attributed to increases in taxa that showed preference for either ambient N or elevated N conditions (Tables A4.5 & A4.6).

Soil pH diverged over time among the plant species, with the mean pH of 5.9 in ambient N treated pots and a mean of 5.4 in N treated soils at the end of the experiment. Variance partitioning revealed that 5% of the explained variance in microbial community structure could be ascribed to N while only 1% of the variation could be attributed to variation in pH. Both N and pH together explained an additional 6% of the variation in microbial community composition together.

No relationship was found between the magnitude of plant growth responses to N (difference in average biomass for species pairs) and the magnitude in microbial community turnover between

ambient N and N addition treatments (difference in community composition for pairs of *Poa* species). The following are results for linear regression tests for differences in bacteria community between N and ambient N treatments vs. plant growth responses (plant height: slope < 0.01, p = 0.81, R^2 < 0.01, n = 49; root mass: slope = 0.02, p = 0.51, R^2 = 0.01, n = 49; shoot mass: slope = 0.01, p = 0.54, R^2 = 0.01, n = 49) and tests for change in fungal composition with plant growth responses (plant height: slope < 0.01, p = 0.74, R^2 < 0.01, n = 49; root mass: slope = 0.02, p = 0.38, R^2 = 0.02, n = 49; shoot mass: slope = -0.02, p = 0.45, R^2 = 0.01, n = 49).

Discussion

Our objective in this study was to investigate the effects of plants and N on bacterial and fungal community development in the rhizospheres of closely related grasses. It was hypothesized that congeneric plants would have unique effects on soil microbial communities which could be explained by phylogenetic relatedness. We found that congeneric *Poa* species did have unique effects on rhizosphere fungal communities. Additionally, there was a significant effect of the presence of plants on microbial community composition compared to no-plant controls. None of the hypothesized plant traits nor phylogenetic relationships among *Poa* species were good predictors of fungal community composition across *Poa* species. To address how environmental change would impact plant effects on soils, we hypothesized that species-specific plant responses to N deposition would enhance plant species' unique influence on microbial communities. Our data supported this hypothesis within both fungal and bacterial communities, demonstrating that N deposition may alter plant-microbe associations differently among similar plant species.

Differences in fungal community composition among *Poa* species were associated with four combinations of *Poa* species pairs which harbored significantly different fungal rhizosphere communities. Additionally, *Poa* species had unique effects on 10% of common fungal families (>10

sequences across samples). The specificity of plant species-fungal relationships provides support for our first hypothesis that congeneric plant species have unique, non-random effects on microbial communities. On the other hand, *Poa* species did not cause bacterial communities to develop differently under ambient N conditions despite the fact that 20% of common bacteria families showed significant change between *Poa* species and no-plant control communities. Overall bacterial community change among *Poa* species was perhaps not detected due to a lack of change in abundances in the many depauperate bacterial families. While the influence of closely related plant species on rhizosphere fungal communities has rarely been investigated, and a greater emphasis has been placed on examining the specificity of plant-mycorrhizal relationships (Johnson et al. 1992). While evidence from mycorrhizal work indicates that mycorrhizal colonization of plant roots is non-random (Johnson et al. 1992; Davison et al. 2011; Maherali and Klironomos 2012), the mycelia that compose individual organisms extend far beyond a single rhizosphere and share resources among multiple plant species (Fellbaum et al. 2014) which complicates understanding the predictability of general plant-fungal relationships among plant species (Hodge et al. 2000). We did not see differences in relative abundance of *Glomermycota* (division including arbuscular mycorrhizal fungi) among *Poa* species in this experiment, indicating that differences in the *Poa* species relationships with mycorrhizal fungi did not contribute to differences in fungal composition among *Poa* species.

We examined the influence of plant phylogenetic relationships, the factors that characterize the environmental niche of *Poa* species, and several plant traits, and attributes which we hypothesized may explain variation in *Poa* species' impacts on fungal communities. None of these factors were found to be good predictors of fungal community composition. We offer three potential reasons for the lack of relationships. First, we hypothesized that comparisons of plant species in the same genus would represent an appropriate degree of variation on the continuum of plant relatedness to detect a phylogenetic signal because it is an intermediate level of similarity on the continuum of relatedness

examined in previous studies (Bardgett et al. 1999; Zancarini et al. 2012; Bouffaud et al. 2014; Barberán et al. 2015). However, including members of sister genera in *Poaceae* may be necessary to detect phylogenetic-based pattern in rhizosphere microbial community composition. Including both confamilial and congeneric plants and replicating a range of species relatedness across different target plant species does reveal that phylogeny can predict plant-soil feedbacks in particular (Anacker et al. 2014). It is also possible that *Poa* species did not possess sufficient variation in factors such as the chemistry of root exudates to detect phylogenetic signal in plant effects on soils, while other genera may have sufficient variation to detect this signal. Second, it is possible that evolutionary relationships among plant species do play a role in rhizosphere microbial community assembly, but only for certain microbial taxa. We addressed this alternative hypothesis and found that there was a significant relationship between the phylogenetic distance among *Poa* species and total abundance of sequences within a microbial family for only 3 out of 171 fungal families tested and 2 out of 292 bacterial families tested. This finding indicates that for some genera, plant phylogenetic relationships may not be useful predictors of plant effects on soil microbes. Third, basic plant traits and attributes that represent a species' unique environmental constraints such as soil pH and mean annual temperature may not correlate with the factors that determine differences in microbial communities (Cantarel et al. 2014; Moreau et al. 2015). While these traits generally relate to plant resource use, they do not appear to explain differences in plant species' allocation of resources to exudate diversity or quantity.

Nitrogen addition was used in this study to determine whether N deposition would enhance the unique effects of *Poa* species on rhizosphere microbes or disrupt the unique effects of *Poa* species, making microbial communities less distinguishable among *Poa* species. We found that N addition enhanced species-specific plant effects on both fungal and bacterial community composition, as hypothesized. Moderation of plant effects on bacteria community composition by N addition has previously been demonstrated with distantly related plant species (Frank and Groffman 2009; Moreau

et al. 2015). In our study, *Poa* species' unique effects on bacterial community composition is particularly noteworthy, since differences in bacteria communities were not detectable within the ambient N treatment. This finding also suggests that even when N is abundant, plant-microbe interactions may be important for plant or microbial nutrient acquisition as opposed to plants and microbes operating more independently (Kaye and Hart 1997; Bell et al. 2015). We also found that N addition affected bacterial and fungal community composition directly which has been described in many studies (Frederick and Klein 1994; Groffman et al. 1996; Innes et al. 2004; Farrer et al. 2013). These findings are consistent the previous research and provide improved resolution on how rhizosphere microbial community composition changes with and without N for a set of closely related plant species (Table A4.5 & A4.6).

To address whether plant species' responses to N addition influenced the magnitude of change in microbial community composition in response to N, we evaluated whether variation in the *Poa* species' growth responses to simulated N deposition explained variation in the microbial responses. Plant growth was hypothesized to indicate belowground plant effects on soil microbes activities because plant growth responses to N can vary among species (Wardle et al. 2004; Rinnan et al. 2007) and plants alter allocation of resources to growth and exudates when N availably changes (Bowsher et al. 2017). A divergence in microbial community composition among plant species would potentially occur through changes in the amount of carbon inputs belowground. We did not find a relationship to support this hypothesis, despite the fact that *Poa* species differed in their responsiveness to N addition. This may suggest that instead of increased aboveground plant growth correlating with more C exudation, plant species' allocation of C towards growth results in less root exudation or no change from ambient N conditions. While examined changes in microbial composition, others have found a positive relationship between increases in plant biomass and greater microbial biomass (Rinnan 2007) or in some cases no relationship (Bardgett et al. 1999), indicating that plant growth is not likely a consistent predictor of microbial community attributes. A review found that increases in N availability has varied effects on

rhizodeposition, even after accounting for variation in C pools and units across studies (Bowsher et al. 2017).

This research contributes to the field of plant-soil interactions by demonstrating congeneric plant species can have a species-specific influence on fungal community composition. This species-specific effect was accentuated by simulated N deposition. Variation in plant relatedness, plant traits, and niches did not explain variation in soil microbial communities. The findings help us understand that plant influence on microbial communities can be species dependent and influenced by environmental change.

CHAPTER V

Conclusions

My goal for this thesis was to advance the field of plant-soil interactions by conducting experiments that inform how plants and soils respond to N deposition and other environmental factors that impact plants and soils. In chapter 2, I examined plant-soil interactions to determine why alpine sedge species respond differently to long-term simulated N deposition. In chapter 3, I tested environmental filters including temperature and soil type that would allow N deposition to promote invasive species' range expansion. In chapter 4, I examined how N availability influences soil microbial communities among closely related plant species. These chapters address how plant-soil interactions are altered by N availability and other environmental factors to answer three sets of questions predicated on the local ecology of graminoid species in the Colorado Front Range.

In chapter 2, I tested three non-mutually exclusive hypotheses to investigate the underlying reasons why two dominant plant species in dry meadow communities of the Front Range alpine exhibit opposite responses to simulated N deposition. *Kobresia myosuroides* has decreased in abundance and *Carex rupestris* has increased significantly with 20 years of N treatments in a long-term N addition experiment at Niwot Ridge, CO (Bowman et al. 2006; Bowman et al. 2012). I hypothesized that bacterial communities, mycorrhizal associations and aluminum toxicity may explain differences in plant species' responses to simulated N deposition. None of these factors were good predictors of sedge species responses to N addition. We did, however, observe a decline in colonization of a generalist ectomycorrhizal species, *Cenococcum geophilum*, on *K. myosuroides'* roots. This mycorrhizal association may be associated with C costs that would harm *K. myosuroides'* ability to acquire limiting resources via reduced colonization or simply allow *C. rupestris* to be a stronger competitor as a non-mycorrhizal species. This study suggests that plant-bacteria associations and Al toxicity are not likely important. Instead, plant-plant competition may better explain differences in plant species' responses to long-term

N deposition.

Among alpine plant species, *K. myosuroides* and *C. rupestris* are somewhat unusual because they do change in abundance in response to increased N availability. Most alpine species show little change in biomass or cover with increasing N availability. Some alpine species do perform luxury uptake of N (a mechanism describing storage of N in tissues for future use), and this uptake of N is generally not observed as a change in biomass (Bowman 1994; Lipson et al. 1996). It is also likely that alpine species lack the ability to substantially increase N uptake or use additional N towards biomass accumulation given physiological constraints (Monson et al. 2001). In chapter 3, I considered whether an annual, invasive species with an affinity for N rich soils, may benefit from N deposition by increasing N availability in alpine soils, hypothesizing that elevated N availability may promote invasion in the alpine if dispersal and temperature constraints do not limit establishment. Two growth chamber experiments revealed that cheatgrass' (*Bromus tectorum*) growth and reproduction was determined by multiple factors. In montane soil, *B. tectorum* grew best in warmer, nutrient rich conditions. Alpine soil, on the other hand, inhibited *B. tectorum* growth regardless of other treatment conditions. This finding contrasts *B. tectorum's* putative indifference to soil type in the Western United States, which leads me to hypothesize that the soil microbial community suppresses *B. tectorum* growth in alpine soils. *B. tectorum* is not known to have meaningful associations with soil microbes, as observational and feedback experiments show inconsistent results (Bradford and Lauenroth 2006; Sperry et al. 2006; Rowe and Brown 2008; Blank et al. 2013; Concilio et al. 2015). However, a comparison of *B. tectorum* growth in conditioned and non-conditioned montane soils in this experiment showed that *B. tectorum* growth was inhibited by *B. tectorum*-conditioned soil, providing some evidence for negative plant-microbe interactions.

While experimental manipulations are useful for comparing belowground associations of plant species with abiotic and biotic variables (chapter 2), as well as characterizing species-specific

environmental constraints (chapter 3), these findings remain difficult to generalize to other plant species. Using functional traits and phylogenetic relationships is a popular approach to explain variation in ecological interactions across different plant species (Burns and Strauss 2011; Cadotte et al. 2013; Münzbergová and Šurinová 2015). However, such approaches have yet to determine how similar plant species must be to resolve systematic differences in plant species' effects on soils (Wagner et al. 2016; Leff et al. 2017). This is demonstrated by the lack of consistency in findings from studies seeking to predict plant effects on soil attributes, both when they compare closely related plant species or when they compare dissimilar, distantly related species (Barberán et al. 2015; Anacker and Strauss 2016; Wagner et al. 2016; Emmett et al. 2017; Leff et al. 2018). Intermediate levels of plant similarity had not yet been explored (i.e. within a genus). The level of plant trait variation represented by plant genera may capture the necessary degree of plant variation to both distinguish plant species effects on rhizosphere communities and potentially use plan traits or phylogenetic relationships to explain this variation. Thus, in chapter 4 I tested whether 7 congeneric grasses in the genus *Poa* have unique effects on rhizosphere microbial communities, and whether those potential differences could be explained by the phylogenetic relationships among *Poa* species, morphological traits, or attributes representing potentially important aspects of each plant species' environmental niche. Fungal communities were significantly different between some *Poa* species, but bacteria communities did not differ. Our results suggest that closely related plant species differ in exudate composition that relates to fungal niches, but congeneric plant species may not always produce the necessary diversity of root exudates to develop unique bacteria communities in the rhizosphere.

Additionally, to address the need for understanding how plant-microbe associations respond to N deposition or changes in resource availability in general, I investigated whether plant effects on rhizosphere microbial compositions are altered by N addition. I hypothesized that N addition would enhance the unique effects of plant species on microbial communities. I also hypothesized that

differences in microbial communities may be explained by differential growth responses of each plant species to N. Microbial community responses to N addition did depend on plant species for both fungal and bacteria communities. However, plant species that grew more in response to N additions were not associated with the microbial communities that changed the most in response to N addition. This indicates that increased plant growth does not equate to an increase in plant resources which determines the quantity or diversity of exudates released into the rhizosphere. However, the fact that plants exhibited species-specific microbial associations suggests that microbial communities may have a meaningful role in plant responses to changes in resource addition. For example, plant-soil feedbacks may differ among plant species due to associations with different microbial assemblages that potentially differ in functions they perform in plant species' rhizsopheres (Classen et al. 2015).

Together, the research that composes this thesis contributes to knowledge of how N availability impacts multiple aspects of plant ecology. I found that the effects of N are limited when environmental constraints such as temperature and soil attributes restrict plants' N use for growth and reproduction. In the context of natural systems, such inhibition of N use may serve as a filter for invasive species establishment in the alpine (chapter 3). On the other hand, N can enhance plant species' effects on soil microbes, which has implications for plant species responses to environmental change (chapter 4). All three chapters highlight new nuanced roles of soil and soil microbes that drive important variation in the ecology of plant species, which fits with current understanding that many factors contribute to plant-soil interactions and plant and microbial responses to resource addition (Bever et al. 2010).

The lack of clear mechanisms associated with outcomes of plant-soil interactions from this work and in the field of plant-soil interactions in general highlights the complexity of plant-soil interactions. I demonstrate that despite this complexity, controlled manipulations are generally sufficient for isolating the abiotic conditions and biotic controls associated with plant growth and abundance, which may help guide further research to identify the underlying mechanisms driving variation in plant-soil and plant-

microbe associations. Detecting this variation in plant-soil associations is necessary for continued efforts in building a mechanistic framework for understanding plant-soil interactions. This thesis contributes to the field of plant-soil interactions by addressing how plant-soil interactions differ with elevated N availability in different environmental contexts and across plant species, towards the goal of understanding the role of these interactions in predicting plant and soil microbial responses to anthropogenic N deposition.

REFERENCES

Ackerfield, J. (2015) *Flora of Colorado* (ed B Lipscomb). BRIT Press.

Ackerly, D. (2009) Conservatism and diversification of plant functional traits: Evolutionary rates versus phylogenetic signal. *Proceedings of the National Academy of Sciences of the United States of America*, **106 Suppl**, 19699–706.

Aguirre, L. & Johnson, D. a. (1991) Influence of temperature and cheatgrass competition on seedling development of two bunchgrasses. *Journal of Range Management*, **44**, 347–354.

Alexander, J.M., Lembrechts, J.J., Cavieres, L.A., Daehler, C., Haider, S., Kueffer, C., Liu, G., McDougal, K., Milbau, A., Pauchard, A., Rew, L. & Seipel, T. (2016) Plant invasions into mountains and alpine ecosystems : current status and future challenges. *Alpine Botany*, **126**, 89–103.

Allen, E.B., Egerton-Warburton, L.M., Hilbig, B.E. & Valliere, J.M. (2016) Interactions of arbuscular mycorrhizal fungi, critical loads of nitrogen deposition, and shifts from native to invasive species in a southern California shrubland. *Botany*, **94**, 425–433.

Allison, S.D. & Martiny, J.B.H. (2008) Colloquium paper: resistance, resilience, and redundancy in microbial communities. *Proceedings of the National Academy of Sciences*, **105**, 11512–11519.

Anacker, B.L., Klironomos, J.N., Maherali, H., Reinhart, K.O. & Strauss, S.Y. (2014) Phylogenetic conservatism in plant-soil feedback and its implications for plant abundance. *Ecology Letters*, **17**, 1613–1621.

Anacker, B.L. & Strauss, S.Y. (2016) Ecological similarity is related to phylogenetic distance between species in a cross-niche field transplant experiment. *Ecology*, **97**, 1807–1818.

Bais, H.P., Weir, T.L., Perry, L.G., Gilroy, S. & Vivanco, J.M. (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. *Annual review of plant biology*, **57**, 233–266.

Barberán, A., McGuire, K.L., Wolf, J. a., Jones, F.A., Wright, S.J., Turner, B.L., Essene, A., Hubbell, S.P.,

Faircloth, B.C. & Fierer, N. (2015) Relating belowground microbial composition to the taxonomic, phylogenetic, and functional trait distributions of trees in a tropical forest. *Ecology Letters*, n/a-n/a.

Barceló, J. & Poschenrieder, C. (2002) Fast root growth responses, root exudates, and internal detoxification as clues to the mechanisms of aluminium toxicity and resistance: A review. *Environmental and Experimental Botany*, **48**, 75–92.

Bardgett, R.D., Mawdsley, J.L., Edwards, S., Hobbs, P.J., Rodwell, J.S. & Davies, W.J. (1999) Plant species and nitrogen effects on soil biological properties of temperate upland grasslands. *Functional Ecology*, **13**, 650–660.

Bates, D., Maechler, M., Bolker, B. & Walker, S. (2015) Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, **67**, 1–48.

Bell, C.W., Asao, S., Calderon, F., Wolk, B. & Wallenstein, M.D. (2015) Plant nitrogen uptake drives rhizosphere bacterial community assembly during plant growth. *Soil Biology and Biochemistry*, **85**, 170–182.

Belnap, J. & Phillips, S.L. (2001) Soil biota in an ungrazed grassland: Response to annual grass (Bromus tectorum) invasion. *Ecological Applications*, **11**, 1261–1275.

Berg, G. & Smalla, K. (2009) Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *FEMS microbiology ecology*, **68**, 1–13.

Van Den Berg, L.J.L., Dorland, E., Vergeer, P., Hart, M.A.C., Bobbink, R. & Roelofs, J.G.M. (2005) Decline of acid-sensitive plant species in heathland can be attributed to ammonium toxicity in combination with low pH. *New Phytologist*, **166**, 551–564.

Berggren, D. & Mulder, J. (1995) The role of organic matter in controlling aluminum solubility in acidic mineral soil horizons. *Geochimica et Cosmochimica Acta*, **59**, 4167–4180.

Bever, J.D., Dickie, I.A., Facelli, E., Facelli, J.M., Klironomos, J., Moora, M., Rillig, M.C., Stock, W.D., Tibbett, M. & Zobel, M. (2010) Rooting theories of plant community ecology in microbial

interactions. *Trends in Ecology and Evolution*, **25**, 468–478.

Bezemer, T.M., Lawson, C.S., Hedlund, K., Edwards, A.R., Brook, A.J., Igual, J.M., Mortimer, S.R. & Van Der Putten, W.H. (2006) Plant species and functional group effects on abiotic and microbial soil properties and plant-soil feedback responses in two grasslands. *Journal of Ecology*, **94**, 893–904.

Blank, R.R. (2008) Biogeochemistry of Plant Invasion: A Case Study with Downy Brome (Bromus tectorum). *Invasive Plant Science and Management*, **1**, 226–238.

Blank, R.R., Morgan, T., Clements, C.D. & Mackey, B.E. (2013) Bromus tectorum L. Invasion. *Soil Science*, **178**, 1.

Bobbink, R., Hicks, K., Galloway, J., Spranger, T., Alkemade, R., Ashmore, M., Bustamante, M., Cinderby, S., Davidson, E., Dentener, F., Emmett, B., Erisman, J.-W., Fenn, M., Gilliam, F., Nordin, a, Pardo, L. & De Vries, W. (2010) Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. *Ecological applications : a publication of the Ecological Society of America*, **20**, 30–59.

Bobbink, R., Hornung, M. & Roelofs, J.G.M. (1998) The effects of air-borne nitrogen pollutants on species diversity in natural and semi-natural European vegetation. *Journal of Ecology*, **86**, 717–738.

Bouffaud, M.L., Poirier, M.A., Muller, D. & Moenne-Loccoz, Y. (2014) Root microbiome relates to plant host evolution in maize and other Poaceae. *Environmental Microbiology*, **16**, 2804–2814.

Bowman, W.D. (1994) Accumulation and use of nitrogen and phosphorus following fertilization in two alpine tundra communities. *Oikos*, **70**, 261–270.

Bowman, W.D., Cleveland, C.C., Halada, Ĺ., Hreško, J. & Baron, J.S. (2008) Negative impact of nitrogen deposition on soil buffering capacity. *Nature Geoscience*, **1**, 767–770.

Bowman, W.D., Gartner, J.R., Holland, K. & Wiedermann, M. (2006) Nitrogen critical loads for alpine vegetation and terrestrial ecosystem response: are we there yet? *Ecological applications : a publication of the Ecological Society of America*, **16**, 1183–93.

Bowman, W.D., Murgel, J., Blett, T. & Porter, E. (2012) Nitrogen critical loads for alpine vegetation and soils in Rocky Mountain National Park. *Journal of environmental management*, **103**, 165–71.

Bowman, W., Theodose, T., Schardt, J. & Conant, R. (1993) Constraints of Nutrient Availability on Primary Production in Two Alpine Tundra Communities. *Ecology*, **74**, 2085–2097.

Bradford, J.B. & Lauenroth, W.K. (2006a) Controls over invasion of Bromus tectorum: The importance of climate, soil, disturbance, and seed availability. *Journal of Vegetation Science*, **17**, 693–704.

Bradford, J.B. & Lauenroth, W.K. (2006b) Controls over invasion of Bromus tectorum: The importance of climate, soil, disturbance, and seed availability. *Journal of Vegetation Science*, **17**, 693–704.

van Breemen, N., Mulder, J. & Driscoll, C.T. (1983) Acidification and alkalinization of soils. *Plant and Soil*, **75**, 283–308.

Brooker, R.W. (2006) Plant-plant interactions and environmental change. *New Phytologist*, **171**, 271–284.

Burns, J.H. & Strauss, S.Y. (2011) More closely related species are more ecologically similar in an experimental test. *Proceedings of the National Academy of Sciences*, **108**, 3302–5307.

Cadotte, M., Albert, C.H. & Walker, S.C. (2013) The ecology of differences: assessing community assembly with trait and evolutionary distances. *Ecology letters*, 1–11.

Cantarel, A. a. M., Pommier, T., Desclos-Theveniau, M., Diquélou, S., Dumont, M., Grassein, F., Kastl, E.-M., Grigulis, K., Laîné, P., Lavorel, S., Lemauviel-Lavenant, S., Personeni, E., Schloter, M. & Poly, F. (2014) Using plant traits to explain plant-microbe relationships involved in nitrogen acquisition. *Ecology*, **96**, 140822142010004.

Cassman, N.A., Leite, M.F., Pan, Y., de Hollander, M., van Veen, J.A. & Kuramae, E.E. (2016) Plant and soil fungal but not soil bacterial communities are linked in long-term fertilized grassland. *Sci Rep*, **6**, 23680.

Cavieres, L.A., Quiroz, C.L., Molina-montenegro, M.A., Munoza, A.A. & Pauchard, A. (2005) Nurse effect

of the native cushion plant Azorella monantha on the invasive non-native Taraxacum officinale in the high-Andes of central Chile. *Perspectives in Plant Ecology, Evolution and Systematics*, **7**, 217–226.

Chapin, F.S., Zavaleta, E.S., Eviner, V.T., Naylor, R.L., Vitousek, P.M., Reynolds, H.L., Hooper, D.U., Lavorel, S., Sala, O.E., Hobbie, S.E., Mack, M.C. & Díaz, S. (2000) Consequences of changing biodiversity. *Nature*, **405**, 234–42.

Chapman, S.K., Langley, J.A., Hart, S.C. & Koch, G.W. (2006) Plants actively control nitrogen cycling: uncorking the microbial bottleneck. *The New phytologist*, **169**, 27–34.

Chen, D., Lan, Z., Bai, X., Grace, J.B. & Bai, Y. (2013) Evidence that acidification-induced declines in plant diversity and productivity are mediated by changes in below-ground communities and soil properties in a semi-arid steppe. *Journal of Ecology*, **101**, 1322–1334.

Clark, C.M., Cleland, E.E., Collins, S.L., Fargione, J.E., Gough, L., Gross, K.L., Pennings, S.C., Suding, K.N. & Grace, J.B. (2007) Environmental and plant community determinants of species loss following nitrogen enrichment. *Ecology Letters*, **10**, 596–607.

Clark, C.M., Morefield, P.E., Gilliam, F.S. & Pardo, L.H. (2008) Estimated losses of plant biodiversity in the United States from historical N deposition (1985-2010). *Ecology*, **94**, 1441–1448.

Clark, C.M., Morefield, P.E., Gilliam, F.S. & Pardo, L.H. (2013) Estimated losses of plant biodiversity in the Unitied States from historical N deposition (1985-2010). *Ecology*, **94**, 1441–1448.

Classen, A.T., Sundqvist, M.K., Henning, J.A., Newman, G.S., Moore, J.A.M., Cregger, M.A., Moorhead, L.C. & Patterson, C.M. (2015) Direct and indirect effects of climate change on soil microbial and soil microbial-plant interactions: What lies ahead? *Ecosphere*, **6**, 1–21.

Clow, D.W. (2010) Changes in the timing of snowmelt and streamflow in Colorado: A response to recent warming. *Journal of Climate*, **23**, 2293–2306.

Concilio, A.L. & Loik, M.E. (2013) Elevated nitrogen effects on Bromus tectorum dominance and native

plant diversity in an arid montane ecosystem. *Applied Vegetation Science*, **16**, 598–609.

Concilio, A.L., Loik, M.E. & Belnap, J. (2013) Global change effects on Bromus tectorum L. (Poaceae) at its high-elevation range margin. *Global Change Biology*, **19**, 161–172.

Concilio, A., Vargas, T. & Cheng, W. (2015) Rhizosphere-mediated effects of the invasive grass Bromus tectorum L. and native Elymus elymoides on nitrogen cycling in Great Basin Desert soils. *Plant and Soil*, **393**, 245–257.

Coolon, J.D., Jones, K.L., Todd, T.C., Blair, J.M. & Herman, M.A. (2013) Long-Term Nitrogen Amendment Alters the Diversity and Assemblage of Soil Bacterial Communities in Tallgrass Prairie. *PLoS ONE*, **8**.

Davis, M. a, Grime, J.P., Thompson, K., Davis, A. & Philip, J. (2000) in plant communities: Fluctuating resources a general of invasibility theory. *Journal of Ecology*, **88**, 528–534.

Davison, J., Öpik, M., Daniell, T.J., Moora, M. & Zobel, M. (2011) Arbuscular mycorrhizal fungal communities in plant roots are not random assemblages. *FEMS Microbiology Ecology*, **78**, 103–115.

Dean, S.L., Farrer, E.C., Taylor, D.L., Porras-Alfaro, A., Suding, K.N. & Sinsabaugh, R.L. (2014) Nitrogen deposition alters plant-fungal relationships: linking belowground dynamics to aboveground vegetation change. *Molecular ecology*, **23**, 1364–78.

Delhaize, E. & Ryan, P.R. (1995) Aluminum Toxicity and Tolerance in Plants. *Plant physiology*, **107**, 315–321.

Dentener, F., Drevet, J., Lamarque, J.F., Bey, I., Eickhout, B., Fiore, a. M., Hauglustaine, D., Horowitz, L.W., Krol, M., Kulshrestha, U.C., Lawrence, M., Galy-Lacaux, C., Rast, S., Shindell, D., Stevenson, D., Van Noije, T., Atherton, C., Bell, N., Bergman, D., Butler, T., Cofala, J., Collins, B., Doherty, R., Ellingsen, K., Galloway, J., Gauss, M., Montanaro, V., Müller, J.F., Pitari, G., Rodriguez, J., Sanderson, M., Solmon, F., Strahan, S., Schultz, M., Sudo, K., Szopa, S. & Wild, O. (2006) Nitrogen and sulfur deposition on regional and global scales: A multimodel evaluation. *Global Biogeochemical Cycles*, **20**.

De Deyn, G.B. & Van der Putten, W.H. (2005) Linking aboveground and belowground diversity. *Trends in ecology & evolution*, **20**, 625–33.

Dickson, T.L. & Foster, B.L. (2011) Fertilization decreases plant biodiversity even when light is not limiting. *Ecology Letters*, **14**, 380–388.

Dukes, J.S. & Mooney, H.A. (1999) Does global change increase the success of biological invaders ? *Trends in Ecology and Evolution*, **14**, 135–139.

Dullinger, S., Dirnböck, T. & Grabherr, G. (2003) Patterns of Shrub Invasion into High Mountain Grasslands of the Northern Calcareous Alps , Austria. *Arctic, Antarctic, and Alpine Research*, **35**, 434–441.

Edgar, R.C. (2013) UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nature methods*, **10**, 996–8.

Emmett, B.D., Youngblut, N.D., Buckley, D.H. & Drinkwater, L.E. (2017) Plant phylogeny and life history shape rhizosphere bacterial microbiome of summer annuals in an agricultural field. *Frontiers in Microbiology*, **8**, 1–16.

Eskelinen, A., Harrison, S. & Tuomi, M. (2012) Plant traits mediate consumer and nutrient control on plant community productivity and diversity. *Ecology*, **93**, 2705–18.

Eskelinen, A., Kaarlejarvi, E. & Olofsson, J. (2017) Herbivory and nutrient limitation protect warming tundra from lowland species ’ invasion and diversity loss. *Global Change Biology*, 245–255.

Fan, Z., Neff, J.C. & Wieder, W.R. (2016) Model-based analysis of environmental controls over ecosystem primary production in an alpine tundra dry meadow. *Biogeochemistry*, **128**, 35–49.

Farrer, E.C., Herman, D.J., Franzova, E., Pham, T. & Suding, K.N. (2013) Nitrogen deposition, plant carbon allocation, and soil microbes: Changing interactions due to enrichment. *American Journal of Botany*, **100**, 1458–1470.

Farrer, E.C. & Suding, K.N. (2016) Teasing apart plant community responses to N enrichment: the roles

of resource limitation, competition and soil microbes. *Ecology Letters*, **19**, 1–10.

Fellbaum, C.R., Mensah, J.A., Cloos, A.J., Strahan, G.E., Pfeffer, P.E., Kiers, E.T. & Bücking, H. (2014) Fungal nutrient allocation in common mycorrhizal networks is regulated by the carbon source strength of individual host plants. *New Phytologist*, **203**, 646–656.

Fierer, N., Lauber, C.L., Ramirez, K.S., Zaneveld, J., Bradford, M.A. & Knight, R. (2012) Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. *The ISME Journal*, **6**, 1007–17.

Fisk, M.C., Brooks, P.D. & Schmidt, S.K. (2001) Nitrogen Cycling. *Structure and function of an alpine ecosystem* (eds W.D. Bowman), & T.R. Seastedt), pp. 237–252. Oxford University Press.

Fitzpatrick, C.R., Gehant, L., Kotanen, P.M. & Johnson, M.T.J. (2016) Phylogenetic relatedness, phenotypic similarity, and plant-soil feedbacks. *Journal of Ecology*.

Flores-Moreno, H., Reich, P.B., Lind, E.M., Sullivan, L.L., Seabloom, E.W., Yahdjian, L., MacDougall, A.S., Reichmann, L.G., Alberti, J., Báez, S., Bakker, J.D., Cadotte, M.W., Caldeira, M.C., Chaneton, E.J., D'Antonio, C.M., Fay, P.A., Firn, J., Hagenah, N., Harpole, W.S., Iribarne, O., Kirkman, K.P., Knops, J.M.H., La Pierre, K.J., Laungani, R., Leakey, A.D.B., McCulley, R.L., Moore, J.L., Pascual, J. & Borer, E.T. (2016) Climate modifies response of non-native and native species richness to nutrient enrichment. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **371**, 20150273.

Frank, D. & Groffman, P. (2009) Plant rhizospheric N processes: what we don't know and why we should care. *Ecology*, **90**, 1512–1519.

Frederick, B.A. & Klein, D.A. (1994) Nitrogen effects on rhizosphere processes of range grasses from different successional seres. , 241–250.

Galloway, J.N., Dentener, F.J., Capone, D.G., Boyer, E.W., Howarth, R.W., Seitzinger, S.P., Asner, G.P., Cleveland, C.C., Green, P. a, Holland, E. a, Karl, D.M., Michaels, a F., Porter, J.H., Townsend, a R. & Vörösmarty, C.J. (2004) *Nitrogen Cycles: Past, Present, and Future*.

Galloway, J.N., Townsend, A.R., Erisman, J.W., Bekunda, M., Cai, Z., Freney, J.R., Martinelli, L. a, Seitzinger, S.P. & Sutton, M. a. (2008) Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. *Science (New York, N.Y.)*, **320**, 889–892.

Garcia, C., Roldan, A. & Hernandez, T. (2005) Ability of different plant species to promote microbiological processes in semiarid soil. *Geoderma*, **124**, 193–202.

Geisseler, D. & Scow, K.M. (2014) Long-term effects of mineral fertilizers on soil microorganisms - A review. *Soil Biology and Biochemistry*, **75**, 54–63.

Gilbert, G.S. & Parker, I.M. (2010) ORIGINAL ARTICLE: Rapid evolution in a plant-pathogen interaction and the consequences for introduced host species. *Evolutionary Applications*, **3**, 144–156.

Gillespie, L.J., Archambault, A. & Soreng, R.J. (2007) Phylogeny of Poa (Poaceae) based on trnT-trnF sequence data: major clades and basal relationships. *Aliso: A Journal of Systematic and Evolutionary Botany*, **23**, 420–434.

Gillespie, L. & Soreng, R. (2005) A phylogenetic analysis of the bluegrass genus Poa based on cpDNA restriction site data. *Systematic Botany*, **30**, 84–105.

Giovannetti, M. & Mosse, B. (1979) An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytologist*, **84**, 489–500.

Gough, L., Osenberg, C.W., Gross, K.L. & Collins, S.L. (2000) Fertilization effects on species density and primary productivity in herbaceous plant communities. *Oikos*, **89**, 428–439.

De Graaf, M.C.C., Bobbink, R., Verbeek, P.J.M. & Roelofs, J.G.M. (1997) Aluminium toxicity and tolerance in three heathland species. *Water Air and Soil Pollution*, **98**, 229–239.

Gransee, A. & Wittenmayer, L. (2000) Qualitative and quantitative analysis of water-soluble root exudates in relation to plant species and development. *Journal of Plant Nutrition and Soil Science*, **163**, 381–385.

Greaver, T.L., Sullivan, T.J., Herrick, J.D., Barber, M.C., Baron, J.S., Cosby, B.J., Deerhake, M.E., Dennis,

R.L., Dubois, J.J.B., Goodale, C.L., Herlihy, A.T., Lawrence, G.B., Liu, L., Lynch, J.A. & Novak, K.J. (2012) Ecological effects of nitrogen and sulfur air pollution in the US: What do we know? *Frontiers in Ecology and the Environment*, **10**, 365–372.

Greenland, D. & Losleben, M. (2001) Climate. *Structure and function of an alpine ecosystem* pp. 15–31.

Griffith, A.B. & Loik, M.E. (2010) Effects of climate and snow depth on Bromus tectorum population dynamics at high elevation. *Oecologia*, **164**, 821–832.

Grigulis, K., Lavorel, S., Krainer, U., Legay, N., Baxendale, C., Dumont, M., Kastl, E., Arnoldi, C., Bardgett, R.D., Poly, F., Pommier, T., Schloter, M., Tappeiner, U., Bahn, M. & Clément, J.-C. (2013) Relative contributions of plant traits and soil microbial properties to mountain grassland ecosystem services (ed M Hutchings). *Journal of Ecology*, **101**, 47–57.

Grman, E. & Robinson, T.M. (2013) Resource availability and imbalance affect plant – mycorrhizal interactions : a field test of three hypotheses. *Ecology*, **94**, 62–71.

Groffman, P.M., Eagan, P., Sullivan, W.M. & Lemunyon, J.L. (1996) Grass species and soil type effects on microbial biomass and activity. *Plant and soil*, **183**, 61–67.

Gross, K.L., Mittelbach, G.G. & Reynolds, H.L. (2005) Grassland invasibility and diversity: Responses to nutrients, seed input, and disturbance. *Ecology*, **86**, 476–486.

Guo, J.H., Liu, X., Zhang, Y., Shen, J., Han, W., Zhang, W., Christie, P., Goulding, K., Vitousek, P. & Zhang, F. (2011) Significant acidification in major chinese croplands. *Science*, **327**, 1008–1010.

Haider, S., Alexander, J., Dietz, H., Trepl, L., Edwards, P.J. & Kueffer, C. (2010) The role of bioclimatic origin, residence time and habitat context in shaping non-native plant distributions along an altitudinal gradient. *Biological Invasions*, **12**, 4003–4018.

Haider, S., Alexander, J.M. & Kueffer, C. (2011) Elevational distribution limits of non-native species: combining observational and experimental evidence. *Plant Ecology & Diversity*, **4**, 363–371.

Haider, S., Kueffer, C., Edwards, P.J. & Alexander, J.M. (2012) Genetically based differentiation in growth

of multiple non-native plant species along a steep environmental gradient. *Oecologia*, **170**, 89–99.

Hamilton William E & Frank, D.A. (2001) Can Plants Stimulate Soil Microbes and Their Own Nutrient Supply? Evidence From a Grazing Tolerant Grass. *Ecology*, **82**, 2397–2402.

Harris, G.A. (1967) Some Competitive Relationships between Agropyron spicatum and Bromus tectorum. *Ecological Monographs*, **37**, 89–111.

Hartmann, A., Schmid, M., Tuinen, D. Van & Berg, G. (2008) Plant-driven selection of microbes. *Plant and Soil*, **321**, 235–257.

Hautier, Y., Niklaus, P. a. & Hector, A. (2009) Competition for light causes plant biodiversity loss after eutrophication. *Science*, **324**, 636–638.

Hawkes, C. V., Belnap, J., D'Antonio, C. & Firestone, M.K. (2006) Arbuscular mycorrhizal assemblages in native plant roots change in the presence of invasive exotic grasses. *Plant and Soil*, **281**, 369–380.

He, W.M., Yu, G.L. & Sun, Z.K. (2011) Nitrogen deposition enhances Bromus tectorum invasion: Biogeographic differences in growth and competitive ability between China and North America. *Ecography*, **34**, 1059–1066.

van der Heijden, M.G. a, Bardgett, R.D. & van Straalen, N.M. (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology letters*, **11**, 296–310.

Hodge, A., Robinson, D. & Fitter, A. (2000a) Are microorganisms more effective than plants at competing for nitrogen? *Trends in Plant Science*, **5**, 304–308.

Hodge, A., Stewart, J., Robinson, D., Griffiths, B.S. & Fitter, A.H. (2000b) Competition between roots and soil micro-organisms for nutrients from nitrogen-rich patches of varying complexity. *Journal of Ecology*, **88**, 150–164.

Houdijk, A.L.F.M., Verbeek, P.J.M., Van Dijk, H.F.G. & Roelofs, J.G.M. (1993) Distribution and decline of endangered herbaceous heathland species in relation to the chemical composition of the soil. *Plant and Soil*, **148**, 137–143.

Humbert, J.Y., Dwyer, J.M., Andrey, A. & Arlettaz, R. (2016) Impacts of nitrogen addition on plant biodiversity in mountain grasslands depend on dose, application duration and climate: A systematic review. *Global Change Biology*, **22**, 110–120.

Innes, L., Hobbs, P.J. & Bardgett, R.D. (2004) The impacts of individual plant species on rhizosphere microbial communities in soils of different fertility. *Biology and Fertility of Soils*, **40**, 7–13.

Jing, X., Yang, X., Ren, F., Zhou, H., Zhu, B. & He, J.S. (2016) Neutral effect of nitrogen addition and negative effect of phosphorus addition on topsoil extracellular enzymatic activities in an alpine grassland ecosystem. *Applied Soil Ecology*, **107**, 205–213.

Johnson, N.C. (2010) Resource stoichiometry elucidates the structure and function of arbuscular mycorrhizas across scales. *New Phytologist*, **185**, 631–647.

Johnson, N.C., Rowland, D.L., Corkidi, L. & Allen, E.B. (2008) Plant Winners and Losers During Grassland N-Eutrophication Differ in Biomass Allocation and Mycorrhizas. *Ecology*, **89**, 2868–2878.

Johnson, N.C., Tilman, D. & Wedin, D. (1992) Plant and Soil Controls on Mycorrhizal Fungal Communities. *Ecology*, **73**, 2034–2042.

Johnston, F.M. & Pickering, C.M. (2001) Alien Plants in the Australian Alps. *Mountain Research and Development*, **21**, 284–291.

Kardol, P., Cornips, N.J., van Kempen, M.M., Bakx-Schotman, T.J. & Van der Putten, W.H. (2007) Microbe-Mediated Plant – Soil Feedback Causes Historical Contingency Effects in Plant Community Assembly. *Ecological Monographs*, **77**, 147–162.

Kaye, J.P. & Hart, S.C. (1997) Competition for nitrogen between plants and soil microorganisms. *Trends in Ecology and Evolution*, **12**, 139–143.

Keane, R.M. & Crawley, M.J. (2002) Exotic plant invasions and the enemy release hypothesis. , **17**, 164–170.

Kembel, S.W. & Cahill, J.F. (2011) Independent evolution of leaf and root traits within and among

temperate grassland plant communities. *PLoS ONE*, **6**, 12–15.

Kennedy, N., Brodie, E., Connolly, J. & Clipson, N. (2004) Impact of lime, nitrogen and plant species on bacterial community structure in grassland microcosms. *Environmental Microbiology*, **6**, 1070–1080.

Kulmatiski, A., Beard, K.H., Stevens, J.R. & Cobbold, S.M. (2008a) Plant-soil feedbacks: a meta-analytical review. *Ecology letters*, **11**, 980–92.

Kulmatiski, A., Beard, K.H., Stevens, J.R. & Cobbold, S.M. (2008b) Plant-soil feedbacks: a meta-analytical review. *Ecology letters*, **11**, 980–92.

Leff, J.W. (2016) mctoolsr: Microbial Community Data Analysis Tools.

Leff, J.W., Bardgett, R.D., Wilkinson, A., Jackson, B.G., Pritchard, W.J., de Long, J.R., Oakley, S., Mason, K.E., Ostle, N.J., Johnson, D., Baggs, E.M. & Fierer, N. (2018) Predicting the structure of soil communities from plant community taxonomy, phylogeny, and traits. *ISME Journal*, 1–12.

Leff, J.W., Jones, S.E., Prober, S.M., Barberán, A., Borer, E.T., Firn, J.L., Harpole, W.S., Hobbie, S.E., Hofmockel, K.S., Knops, J.M.H., McCulley, R.L., La Pierre, K., Risch, A.C., Seabloom, E.W., Schütz, M., Steenbock, C., Stevens, C.J. & Fierer, N. (2015) Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. *Proceedings of the National Academy of Sciences*, 201508382.

Leff, J.W., Lynch, R.C., Kane, N.C. & Fierer, N. (2017) Plant domestication and the assembly of bacterial and fungal communities associated with strains of the common sunflower, Helianthus annuus. *New Phytologist*, **214**.

Lekberg, Y. & Helgason, T. (2018) In situ mycorrhizal function - knowledge gaps and future directions. *New Phytologist*.

Lembrechts, J. et al. (2017) Microclimate variability in alpine ecosystems as stepping stones for non-native plant establishment above their current elevational limit. *Ecography*.

Lembrechts, J.J., Pauchard, A., Lenoir, J., Nuñez, M.A., Geron, C., Ven, A., Bravo-Monasterio, P., Teneb, E., Nijs, I. & Milbau, A. (2016) Disturbance is the key to plant invasions in cold environments. *Proceedings of the National Academy of Sciences*, **113**, 201608980.

Leung, B., Lodge, D.M., Finnoff, D., Shogren, J.F., Lewis, M.A. & Lamberti, G. (2002) An ounce of prevention or a pound of cure: bioeconomic risk analysis of invasive species. *Proceedings of the Royal Society B: Biological Sciences*, **269**, 2407–2413.

Lieb, A.M., Darrouzet-Nardi, A. & Bowman, W.D. (2011) Nitrogen deposition decreases acid buffering capacity of alpine soils in the southern Rocky Mountains. *Geoderma*, **164**, 220–224.

Lilleskov, E., Fahey, T., Horton, T. & Lovett, G. (2002) Belowground ectomycorrhizal fungal community change over a nitrogen deposition gradient in Alaska. *Ecology*, **83**, 104–115.

Lilleskov, E. a., Hobbie, E. a. & Horton, T.R. (2011) Conservation of ectomycorrhizal fungi: Exploring the linkages between functional and taxonomic responses to anthropogenic N deposition. *Fungal Ecology*, **4**, 174–183.

Lipson, D.A., Bowman, W.D. & Monson, R.K. (1996) Luxury Uptake and Storage of Nitrogen in the Rhizomatous Alpine Herb , Bistorta Bistortoides LUXURY UPTAKE AND STORAGE OF NITROGEN IN THE. , **77**, 1277–1285.

Lipson, D., Schadt, C., Schmidt, S.K. & Monson, R.K. (1999) Ectomycorrhizal transfer of amino acid-nitrogen to the alpine sedge Kobresia myosuroides. *New Phytologist*, **142**, 163–167.

Liu, Y. & van Kleunen, M. (2017) Responses of common and rare aliens and natives to nutrient availability and fluctuations. *Journal of Ecology*, **105**, 1111–1122.

Lobuglio, K.F. (1999) Cenococcum. *Ectomycorrhizal Fungi Key Genera in Profile* (eds J.W.. Cairney), & S.M. Chambers), pp. 287–309.

Lodge, D.M., Williams, S., MacIsaac, H.J., Hayes, K.R., Leung, B., Reichard, S., Mack, R.N., Moyle, P.B., Smith, M., Andow, D.A., Carlton, J.T. & McMichael, A. (2006) Biological invasions:

Recommendations for U.S. policy and management. *Ecological Applications*, **16**, 2035–2054.

Lonsdale, W. (1999) Global patterns of plant invasion and the concept of invasibility. *Ecology*, **80**, 1522–1536.

Losleben, M. (1994) D-1 (3743 m) climate station: CR21X data.

Lukas, J., Barsugli, J., Doesken, N., Imtiaz, R. & Wolter, K. (2014) *Climate Change in Colorado: A Synthesis to Support Water Resources Management and Adaptation*.

Mack, R.N. (1981) Invasion of Bromus tectorum L. into Western North America: An ecological chronicle. *Agro-Ecosystems*, **7**, 145–165.

Maherali, H. & Klironomos, J.N. (2012) Phylogenetic and trait-based assembly of arbuscular mycorrhizal fungal communities. *PLoS ONE*, **7**.

Marini, L., Gaston, K.J., Prosser, F. & Hulme, P.E. (2009) Contrasting response of native and alien plant species richness to environmental energy and human impact along alpine. , 652–661.

Maron, J., Auge, H., Pearson, D., Korell, L., Hensen, I., Suding, K. & Stein, C. (2014) Staged invasions across disparate grasslands: effects of seed provenance, consumers and disturbance on productivity and species richness. *Ecology letters*, **17**, 499–507.

McDonald, D., Price, M.N., Goodrich, J., Nawrocki, E.P., DeSantis, T.Z., Probst, A., Andersen, G.L., Knight, R. & Hugenholtz, P. (2012) An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *The ISME journal*, **6**, 610–8.

Mcdougall, K.L., Khuroo, A.A., Loope, L.L., Parks, C.G., Pauchard, A., Reshi, Z.A., Rushworth, I. & Kueffer, C. (2011) Plant Invasions in Mountains: Global Lessons for Better Management. *Source: Mountain Research and Development*, **31**, 380–387.

McHugh, T.A., Morrissey, E.M., Mueller, R.C., Gallegos-Graves, L.V., Kuske, C.R. & Reed, S.C. (2017) Bacterial, fungal, and plant communities exhibit no biomass or compositional response to two years of simulated nitrogen deposition in a semiarid grassland. *Environmental Microbiology*, 3–12.

Mehrabi, Z. & Tuck, S.L. (2015) Relatedness is a poor predictor of negative plant-soil feedbacks. *New Phytologist*, **205**, 1071–1075.

Meier, C.L. & Bowman, W.D. (2008) Links between plant litter chemistry, species diversity, and below-ground ecosystem function. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 19780–5.

Meyer, S.E., Allen, P.S., Beckstead, J. & Seed, J. (1997) Seed germination regulation in Bromus tectorum (Poaceae) and its ecological significance. *Oikos*, **78**, 475–485.

Milberg, P., Lamont, B.B., Pérez-fernández, M.A., Ecology, S.P., Lamont, B. & Mar, A.P. (2010) Survival and Growth of Native and Exotic Composites in Response to a Nutrient Gradient Stable URL : http://www.jstor.org/stable/20050846 Survival and growth of native and exotic composites nutrient gradient in response to a. , **145**, 125–132.

Miller, M.E., Belnap, J., Beatty, S.W. & Webb, B.L. (2006) Performance of Bromus tectorum L. in relation to soil properties, water additions, and chemical amendments in calcareous soils of southeastern Utah, USA. *Plant and Soil*, **288**, 19–29.

Mills, K.E. & Bever, J.D. (1998) Maintenance of diversity within plant communities: soil pathogens as agents of negative feedback. *Ecology*, **79**, 1595–1601.

Mitchell, R.J., Hester, A.J., Campbell, C.D., Chapman, S.J., Cameron, C.M., Hewison, R.L. & Potts, J.M. (2010) Is vegetation composition or soil chemistry the best predictor of the soil microbial community? *Plant and Soil*, **333**, 417–430.

Mitchell, R.J., Hester, A.J., Campbell, C.D., Chapman, S.J., Cameron, C.M., Hewison, R.L. & Potts, J.M. (2012) Explaining the variation in the soil microbial community: Do vegetation composition and soil chemistry explain the same or different parts of the microbial variation? *Plant and Soil*, **351**, 355–362.

Monson, R.K., Mullen, R.B. & Bowman, W.D. (2001) Plant nutrient relations. *Structure and function of an*

alpine ecosystem pp. 198–221.

Moreau, D., Pivato, B., Bru, D., Busset, H., Deau, F., Faivre, C., Matejicek, A., Strbik, F., Philippot, L. & Mougel, C. (2015) Plant traits related to nitrogen uptake influence plant- microbe competition. *Ecology*, **96**, 2300–2310.

Münzbergová, Z. & Šurinová, M. (2015) The importance of species phylogenetic relationships and species traits for the intensity of plant-soil feedback. *Ecosphere*, **6**, art234.

Myers, J.A. & Harms, K.E. (2009) Seed arrival, ecological filters, and plant species richness: A meta-analysis. *Ecology Letters*, **12**, 1250–1260.

Nemergut, D.R., Townsend, A.R., Sattin, S.R., Freeman, K.R., Fierer, N., Neff, J.C., Bowman, W.D., Schadt, C.W., Weintraub, M.N. & Schmidt, S.K. (2008) The effects of chronic nitrogen fertilization on alpine tundra soil microbial communities: implications for carbon and nitrogen cycling. *Environmental microbiology*, **10**, 3093–105.

Nguyen, C. (2003) Rhizodeposition of organic C by plants: mechanisms and controls. *Agronomie*, **23**, 375–396.

Norton, J.B., Monaco, T.A., Norton, J.M., Johnson, D.A. & Jones, T.A. (2004) Soil morphology and organic matter dynamics under cheatgrass and sagebrush-steppe plant communities. *Journal of Arid Environments*, **57**, 445–466.

Novak, S.J. & Mack, R.N. (2001) Tracing Plant Introduction and Spread: Genetic Evidence from Bromus tectorum (Cheatgrass). *BioScience*, **51**, 114–122.

Oksanen, J., Blanchet, F. Guillaume Friendly, M., Roeland, K., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Gavin L. Simpson, Peter Solymos, M. Henry H. Stevens, E.S. & Wagner, and H. (2016) vegan: Community Ecology Package. R package version 2.4-1.

Orwin, K.H., Buckland, S.M., Johnson, D., Turner, B.L., Smart, S., Oakley, S. & Bardgett, R.D. (2010) Linkages of plant traits to soil properties and the functioning of temperate grassland. *Journal of*

Ecology, **98**, 1074–1083.

Paine, C.E.T., Marthews, T.R., Vogt, D.R., Purves, D., Rees, M., Hector, A. & Turnbull, L.A. (2012) How to fit nonlinear plant growth models and calculate growth rates: An update for ecologists. *Methods in Ecology and Evolution*, **3**, 245–256.

Pauchard, a & Alaback, B.P. (2004) Influences of evaluation, land use and landscape context on patterns of alien plant invasions along roadsides in protected areas of south -central chile. *Conservation Biology*, **18**, 238–248.

Pauchard, A., Kueffer, C., Dietz, H., Daehler, C.C., Alexander, J., Edwards, P.J., Arévalo, J.R., Cavieres, L. a, Guisan, A., Haider, S., Jakobs, G., McDougall, K., Millar, C.I., Naylor, B.J., Parks, C.G., Rew, L.J. & Seipel, T. (2009) Ain't no mountain high enough: plant invasions reaching new elevations. *Frontiers in Ecology and the Environment*, **7**, 479–486.

Payne, R.J., Dise, N.B., Field, C.D., Dore, A.J., Caporn, S.J. & Stevens, C.J. (2017) Nitrogen deposition and plant biodiversity: past, present, and future. *Frontiers in Ecology and the Environment*.

Pennings, S.C., Clark, C.M., Cleland, E.E., Collins, S.L., Gough, L., Gross, K.L., Milchunas, D.G. & Suding, K.N. (2005) Do individual plant species show predictable responses to nitrogen addition across multiple experiments? *Oikos*, **110**, 547–555.

Petitpierre, B., MacDougall, K., Seipel, T., Broennimann, O., Guisan, A. & Kueffer, C. (2015) Will climate change increase the risk of plant invasions into mountains? *Ecological Applications*, **26**, 150709023716008.

Philippot, L., Raaijmakers, J.M., Lemanceau, P. & Van Der Putten, W.H. (2013) Going back to the roots: The microbial ecology of the rhizosphere. *Nature Reviews Microbiology*, **11**, 789–799.

Pinto, S.M., Pearson, D.E. & Maron, J.L. (2014) Seed dispersal is more limiting to native grassland diversity than competition or seed predation. *Journal of Ecology*, n/a-n/a.

Porazinska, D., Bardgett, R., Blaauw, M., Hunt, H., Parsons, A., Seastedt, T. & Wall, D. (2003)

Relationships at the aboveground-belowground interface: plants, soil biota, and soil processes. *Ecological Monographs*, **73**, 377–395.

Prober, S.M., Leff, J.W., Bates, S.T., Borer, E.T., Firn, J., Harpole, W.S., Lind, E.M., Seabloom, E.W., Adler, P.B., Bakker, J.D., Cleland, E.E., Decrappeo, N.M., Delorenze, E., Hagenah, N., Hautier, Y., Hofmockel, K.S., Kirkman, K.P., Knops, J.M.H., La Pierre, K.J., Macdougall, A.S., Mcculley, R.L., Mitchell, C.E., Risch, A.C., Schuetz, M., Stevens, C.J., Williams, R.J. & Fierer, N. (2015) Plant diversity predicts beta but not alpha diversity of soil microbes across grasslands worldwide. *Ecology Letters*, **18**, 85–95.

van der Putten, W.H., van Dijk, C. & Peters, B.A.. (1993) Plant-specific soil-borne diseases contribute to succession in foredune vegetation. *Nature*, **362**, 53–56.

R Core Team. (2016) R: A language and environment for statistical computing.

Ramirez, K.S., Craine, J.M. & Fierer, N. (2012) Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. *Global Change Biology*, **18**, 1918–1927.

Ramirez, K.S., Lauber, C.L., Knight, R., Bradford, M. a & Fierer, N. (2010) Consistent effects of nitrogen fertilization on soil bacterial communities in contrasting systems. *Ecology*, **91**, 3463-70; discussion 3503–14.

Redford, A.J., Bowers, R.M., Knight, R., Linhart, Y. & Fierer, N. (2010) The ecology of the phyllosphere: Geographic and phylogenetic variability in the distribution of bacteria on tree leaves. *Environmental Microbiology*, **12**, 2885–2893.

Reid, A.M., Morin, L., Downey, P.O., French, K. & Virtue, J.G. (2009) Does invasive plant management aid the restoration of natural ecosystems? *Biological Conservation*, **142**, 2342–2349.

Reisner, M.D., Grace, J.B., Pyke, D.A. & Doescher, P.S. (2013) Conditions favouring Bromus tectorum dominance of endangered sagebrush steppe ecosystems. *Journal of Applied Ecology*, **50**, 1039–1049.

Reynolds, H.L., Packer, A., Bever, J.D. & Clay, K. (2003) Grassroots ecology: Plant-microbe-soil interactions as drivers of plant community structure and dynamics. *Ecology*, **84**, 2281–2291.

Rinnan, R., Michelsen, A., Bååth, E. & Jonasson, S. (2007) Fifteen years of climate change manipulations alter soil microbial communities in a subarctic heath ecosystem. *Global Change Biology*, **13**, 28–39.

Roem, W.J., Klees, H. & Berendse, F. (2002) Effects of nutrient addition and acidification on plant species diversity and seed germination in heathland. *Journal of Applied Ecology*, **39**, 937–948.

Rowe, H. & Brown, C.S. (2008) Native Plant Growth and Seedling Establishment in Soils Influenced by Bromus tectorum. *Rangeland Ecology & Management*, **61**, 630–639.

Schaeffer, S.M., Ziegler, S.E., Belnap, J. & Evans, R.D. (2012) Effects of Bromus tectorum invasion on microbial carbon and nitrogen cycling in two adjacent undisturbed arid grassland communities. *Biogeochemistry*, **111**, 427–441.

Schimel, J. & Bennett, J. (2004) Nitrogen mineralization: challenges of a changing paradigm. *Ecology*, **85**, 591–602.

de Schrijver, A., de Frenne, P., Ampoorter, E., van Nevel, L., Demey, A., Wuyts, K. & Verheyen, K. (2011) Cumulative nitrogen input drives species loss in terrestrial ecosystems. *Global Ecology and Biogeography*, **20**, 803–816.

Sigüenza, C., Corkidi, L. & Allen, E.B. (2006) Feedbacks of soil inoculum of mycorrhizal fungi altered by N deposition on the growth of a native shrub and an invasive annual grass. *Plant and Soil*, **286**, 153–165.

Simkin, S.M., Allen, E.B., Bowman, W.D., Clark, C.M., Belnap, J., Brooks, M.L., Cade, B.S., Collins, S.L., Geiser, L.H., Gilliam, F.S., Jovan, S.E., Pardo, L.H., Schulz, B.K., Stevens, C.J., Suding, K.N., Throop, H.L. & Waller, D.M. (2016) Conditional vulnerability of plant diversity to atmospheric nitrogen deposition across the USA. *Proceedings of the National Academy of Sciences of the United States of America*, **113**, In press.

Smalla, K., Wieland, G., Buchner, a, Zock, a, Parzy, J., Roskot, N., Heuer, H. & Berg, G. (2001) Bulk and Rhizosphere Soil Bacterial Communities Studied by Denaturing Gradient Gel Electrophoresis : Plant-Dependent Enrichment and Seasonal Shifts Revealed Bulk and Rhizosphere Soil Bacterial Communities Studied by Denaturing Gradient Gel Electrophoresis. *Applied and environmental microbiology*, **67**, 4742–4751.

Sperry, L.J., Belnap, J. & Evans, R.D. (2006) Bromus tectorum invasion alters nitrogen dynamics in an undisturbed arid grassland ecosystem. *Ecology*, **87**, 603–615.

Stevens, C.J., Dise, N.B. & Gowing, D.J. (2009) Regional trends in soil acidification and exchangeable metal concentrations in relation to acid deposition rates. *Environmental Pollution*, **157**, 313–319.

Stevens, C.J., Dise, N.B., Mountford, J.O. & Gowing, D.J. (2004) Impact of nitrogen deposition on the species richness of grasslands. *Science (New York, N.Y.)*, **303**, 1876–9.

Stevens, C.J., Thompson, K., Grime, J.P., Long, C.J. & Gowing, D.J.G. (2010) Contribution of acidification and eutrophication to declines in species richness of calcifuge grasslands along a gradient of atmospheric nitrogen deposition. *Functional Ecology*, **24**, 478–484.

Suding, K.N. (2011) Toward an Era of Restoration in Ecology: Successes, Failures, and Opportunities Ahead. *Annual Review of Ecology, Evolution, and Systematics*, **42**, 465–487.

Suding, K., Ashton, I., Bechtold, H., Bowman, W., Mobley, M. & Winkleman, R. (2008) Plant and microbe contribution to community resilience in a directionally changing environment. *Ecological Monographs*, **78**, 313–329.

Suding, K.N., Stanley Harpole, W., Fukami, T., Kulmatiski, A., MacDougall, A.S., Stein, C. & van der Putten, W.H. (2013) Consequences of plant-soil feedbacks in invasion (ed M Hutchings). *Journal of Ecology*, **101**, 298–308.

Synder, R.L. & de Melo-Abreu, J.P. (2005) *Frost Protection: Fundamentals, Practice and Economics*. Food and Agriculture Organization of the United Nations, Rome.

Theoharides, K. a & Dukes, J.S. (2007) Plant invasion across space and time: factors affecting nonindigenous species success during four stage of invasion. *New Phytologist*, **176**, 256–273.

Thompson, G.W. & Medve, R.J. (1984) Effects of aluminum and manganese on the growth of ectomycorrhizal fungi. *Applied and environmental microbiology*, **48**, 556–560.

Tilman, D. & Wedin, D. (1991) Dynamics of nitrogen competition between successional grasses. *Ecology*, **72**, 1038–1049.

Treseder, K.K. (2004) A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO2 in field studies. *New Phytologist*, **164**, 347–355.

Treseder, K.K. (2008) Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. *Ecology letters*, **11**, 1111–20.

Treseder, K.K., Allen, E.B., Egerton-Warburton, L.M., Hart, M.M., Klironomos, J.N., Maherali, H. & Tedersoo, L. (2018) Arbuscular mycorrhizal fungi as mediators of ecosystem responses to nitrogen deposition: A trait-based predictive framework. *Journal of Ecology*, **106**, 480–489.

Upadhyaya, M.K., Turkington, R. & McIlvride, D. (1986) The biology of Canadian weeds. 75. Bromus tectorum L. *Canadian Jornal of Plant Sciences*, **66**, 689–709.

Uresk, D., Cline, J. & Rickard, W. (1979) Growth rates of a cheatgrass community and some associated factors. *Journal of Range Management*, **32**, 168–170.

Vasquez, E., Sheley, R. & Svejcar, T. (2008) Nitrogen Enhances the Competitive Ability of Cheatgrass (Bromus tectorum) Relative to Native Grasses. *Invasive Plant Science and Management*, **1**, 287–295.

Veresoglou, S.D. & Rillig, M.C. (2014) Do closely related plants host similar arbuscular mycorrhizal fungal communities? A meta-analysis. *Plant and Soil*, **377**, 395–406.

Vitorello, V.A., Capaldi, F.R. & Stefanuto, V.A. (2005) Recent advances in aluminum toxicity and resistance in higher plants. *Brazilian Journal of Plant Physiology*, **17**, 129–143.

Vitousek, P.M., Aber, J.D., Howarth, R.W., Likens, G.E., Matson, P.A., Schindler, D.W., Schlesinger, W.H. & Tilman, D.G. (1997) Human alteration of the global nitrogen cycle: sources and consequences. *Ecological Applications*, **7**, 737–750.

Vitousek, P.M. & Howarth, R.W. (1991) Nitrogen Limitation on Land and in the Sea : How Can It Occur ? *Biogeochemistry*, **13**, 87–115.

Wagner, M.R., Lundberg, D.S., Del Rio, T.G., Tringe, S.G., Dangl, J.L. & Mitchell-Olds, T. (2016) Host genotype and age shape the leaf and root microbiomes of a wild perennial plant. *Nature Communications*, **7**, 1–15.

Wallenstein, M.D., McNulty, S., Fernandez, I.J., Boggs, J. & Schlesinger, W.H. (2006) Nitrogen fertilization decreases forest soil fungal and bacterial biomass in three long-term experiments. *Forest Ecology and Management*, **222**, 459–468.

Wardle, D. a, Bardgett, R.D., Klironomos, J.N., Setälä, H., van der Putten, W.H. & Wall, D.H. (2004) Ecological linkages between aboveground and belowground biota. *Science (New York, N.Y.)*, **304**, 1629–33.

Weber, W.A. & Wittmann, R.C. (2012) *Colorado Flora: Eastern Slope, Fourth Edition, A Field Guide to the Vascular Plants*. University Press of Colorado.

Wei, C., Yu, Q., Bai, E., Lü, X., Li, Q., Xia, J., Kardol, P., Liang, W., Wang, Z. & Han, X. (2013) Nitrogen deposition weakens plant-microbe interactions in grassland ecosystems. *Global change biology*, **19**, 3688–3697.

Weidenhamer, J.D. & Callaway, R.M. (2010) Direct and indirect effects of invasive plants on soil chemistry and ecosystem function. *Journal of chemical ecology*, **36**, 59–69.

Zancarini, A., Mougel, C., Voisin, A.-S., Prudent, M., Salon, C. & Munier-Jolain, N. (2012) Soil Nitrogen Availability and Plant Genotype Modify the Nutrition Strategies of M. truncatula and the Associated Rhizosphere Microbial Communities. *PLoS ONE*, **7**, e47096.

APPENDIX

Table A4.1. Species by gene matrix with genes used to calculate phylogenetic distance among *Poa* species.

taxon	ets	its	matk	rbcl	trnT-trnL
Poa alpina	297375143	297375339	459929648	459994671	86169391
Poa arctica	297375147	297375343	371533758	459994755	86169414
Poa compressa	685211931	209887699	744393645	685212046	86169408
Poa glauca	297375180	209887701	459929766	459994799	86169409
Poa nemoralis	297375211	297375385	607345128	607344860	297375296
Poa pratensis	297375225	297375398	459929844	459994878	86169416
Poa reflexa	NA	297375399	NA	NA	297375306

Table A4.2. Fungal families affected by Poa species compared to no-plant controls.

Fungal families that differed significantly (alpha = 0.007) in relative abundance between individual *Poa* species' rhizospheres and no-plant control pots. Change relative to control indicates whether the absolute abundance of organisms within a family decreased (-) significantly or increased (+) significantly in the rhizosphere compared to no-plant control communities. Means are the mean abundance of sequence copies within a family for a Poa rhizosphere or no-plant control pot. Taxonomy classification, including placeholder names and unidentified taxa, are labeled according to assignments given by the Greengenes RDP classifier.

Fungal family								
Phylum	**Class**	**Order**	**Family**	**Poa species**	**p**	**Change relative to control**	**Control mean**	**Poa mean**
Ascomycota	Leotiomycetes	Helotiales	Vibrisseaceae	arctica	0.004	-	117	75
Ascomycota	Leotiomycetes	Helotiales	Vibrisseaceae	glauca	0.001	-	117	71
Ascomycota	unclassified	unclassified	unclassified	glauca	0.008	+	132	184
Basidiomycota	Agaricomycetes	Sebacinales	unidentified	alpina	0.01	+	2	10
Basidiomycota	Agaricomycetes	Sebacinales	unidentified	compressa	<0.001	+	2	28
Basidiomycota	Agaricomycetes	Sebacinales	unidentified	pratensis	0.006	+	2	16
Basidiomycota	Agaricomycetes	unclassified	unclassified	alpina	0.003	+	7	30
Basidiomycota	Agaricomycetes	unclassified	unclassified	arctica	0.001	+	7	71
Basidiomycota	Tremellomycetes	Filobasidiales	Piskurozymaceae	glauca	0.005	-	138	101
unclassified	unclassified	unclassified	unclassified	alpina	0.004	+	491	652
unclassified	unclassified	unclassified	unclassified	arctica	0.001	+	491	768
unclassified	unclassified	unclassified	unclassified	compressa	0.004	+	491	741
unclassified	unclassified	unclassified	unclassified	glauca	0.002	+	491	736
unclassified	unclassified	unclassified	unclassified	nemoralis	0.014	+	491	630
Ascomycota	Leotiomycetes	Helotiales	Vibrisseaceae	arctica	0.004	-	117	75
Ascomycota	Leotiomycetes	Helotiales	Vibrisseaceae	glauca	0.001	-	117	71
Ascomycota	unclassified	unclassified	unclassified	glauca	0.008	+	132	184
Basidiomycota	Agaricomycetes	Sebacinales	unidentified	alpina	0.01	+	2	10
Basidiomycota	Agaricomycetes	Sebacinales	unidentified	compressa	<0.001	+	2	28
Basidiomycota	Agaricomycetes	Sebacinales	unidentified	pratensis	0.006	+	2	16

Basidiomycota	Agaricomycetes	unclassified	unclassified	alpina	0.003	+	7	30
Basidiomycota	Agaricomycetes	unclassified	unclassified	arctica	0.001	+	7	71

Table A4.3. Bacterial families affected by *Poa* species compared to no-plant controls.

Bacterial families that differed significantly (alpha = 0.007) in relative abundance between individual *Poa* species' rhizospheres and no-plant control pots. Change relative to control indicates whether the absolute abundance of organisms within a family decreased (-) significantly or increased (+) significantly in the rhizosphere compared to no-plant control communities. Means are the mean abundance of sequence copies within a family for a Poa rhizosphere or no-plant control pot. Taxonomy classification, including placeholder names and unidentified taxa, are labeled according to assignments given by the Greengenes RDP classifier.

Bacterial family								
Phylum	**Class**	**Order**	**Family**	**Poa species**	**p**	**Change relative to control**	**Control mean**	**Poa mean**
Acidobacteria	Acidobacteriia	Acidobacteriales	Acidobacteriaceae	glauca	0.003	+	34	51
Acidobacteria	Sva0725	Sva0725	unidentified	arctica	0.004	+	6	11
Acidobacteria	Sva0725	Sva0725	unidentified	compressa	0.002	+	6	12
Acidobacteria	Sva0725	Sva0725	unidentified	nemoralis	0.013	+	6	11
Actinobacteria	Acidimicrobiia	Acidimicrobiales	unidentified	arctica	0.005	+	11	15
Actinobacteria	Acidimicrobiia	Acidimicrobiales	unidentified	pratensis	0.002	+	11	17
Actinobacteria	Acidimicrobiia	Acidimicrobiales	Iamiaceae	nemoralis	0.008	+	0	1
Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	compressa	0.013	+	3	6
Actinobacteria	Actinobacteria	Actinomycetales	Sporichthyaceae	compressa	0.008	+	0	1
Actinobacteria	MB-A2-108	0319-7L14	unidentified	alpina	0.011	-	6	4
Actinobacteria	Rubrobacteria	Rubrobacterales	Rubrobacteraceae	alpina	0.004	+	5	10
Actinobacteria	Rubrobacteria	Rubrobacterales	Rubrobacteraceae	compressa	0.012	+	5	10
Actinobacteria	Thermoleophilia	Solirubrobacterales	unclassified	alpina	0.008	+	0	1
Actinobacteria	Thermoleophilia	Solirubrobacterales	unclassified	compressa	0.012	+	0	1
Bacteroidetes	[Saprospirae]	[Saprospirales]	unidentified	alpina	0.002	-	5	2
Bacteroidetes	[Saprospirae]	[Saprospirales]	unidentified	arctica	0.002	-	5	1
Bacteroidetes	[Saprospirae]	[Saprospirales]	unidentified	nemoralis	0.009	-	5	2
Bacteroidetes	[Saprospirae]	[Saprospirales]	unidentified	pratensis	0.009	-	5	2

Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	compressa	0.005	+	146	179
Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	glauca	0.001	+	146	185
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	unidentified	alpina	0.003	-	37	29
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	unidentified	pratensis	0.005	-	37	29
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	unidentified	reflexa	0.004	-	37	25
Chlamydiae	Chlamydiia	Chlamydiales	Rhabdochlamydiaceae	compressa	0.004	-	9	3
Chlamydiae	Chlamydiia	Chlamydiales	Rhabdochlamydiaceae	pratensis	0.004	-	9	3
Chlorobi	unidentified	unidentified	unidentified	alpina	0.003	-	4	2
Chlorobi	unidentified	unidentified	unidentified	glauca	0.003	-	4	2
Chlorobi	SJA-28	unidentified	unidentified	nemoralis	0.012	+	2	4
Chloroflexi	Anaerolineae	A31	S47	alpina	0.006	+	3	6
Chloroflexi	Anaerolineae	A31	S47	nemoralis	0.001	+	3	6
Chloroflexi	Anaerolineae	CFB-26	unidentified	compressa	0.006	+	0	1
Chloroflexi	Anaerolineae	H39	unidentified	glauca	0.001	-	7	4
Chloroflexi	Chloroflexi	[Roseiflexales]	[Kouleothrixaceae]	arctica	0.004	+	2	4
Chloroflexi	Chloroflexi	[Roseiflexales]	[Kouleothrixaceae]	compressa	0.008	+	2	5
Chloroflexi	Chloroflexi	[Roseiflexales]	[Kouleothrixaceae]	glauca	0.001	+	2	5
Chloroflexi	Chloroflexi	[Roseiflexales]	[Kouleothrixaceae]	nemoralis	0.004	+	2	4
Chloroflexi	Chloroflexi	[Roseiflexales]	[Kouleothrixaceae]	pratensis	0.006	+	2	4
Cyanobacteria	4C0d-2	MLE1-12	unidentified	arctica	0.008	-	11	5
Cyanobacteria	4C0d-2	MLE1-12	unidentified	compressa	0.012	-	11	6
Cyanobacteria	4C0d-2	MLE1-12	unidentified	glauca	0.013	-	11	6
Cyanobacteria	4C0d-2	MLE1-12	unidentified	pratensis	0.003	-	11	5
Cyanobacteria	Oscillatoriophycideae	Oscillatoriales	Phormidiaceae	nemoralis	0.001	+	0	2
Elusimicrobia	Elusimicrobia	IIb	unidentified	compressa	0.011	-	9	4
Elusimicrobia	Elusimicrobia	IIb	unidentified	glauca	0.012	-	9	4
FCPU426	unidentified	unidentified	unidentified	alpina	0.002	-	7	2
FCPU426	unidentified	unidentified	unidentified	arctica	0.001	-	7	1

FCPU426	unidentified	unidentified	unidentified	compressa	0.001	-	7	1
FCPU426	unidentified	unidentified	unidentified	glauca	0.002	-	7	1
FCPU426	unidentified	unidentified	unidentified	pratensis	0.002	-	7	1
FCPU426	unidentified	unidentified	unidentified	reflexa	0.004	-	7	2
Fibrobacteres	Fibrobacteria	258ds10	unidentified	arctica	0.002	+	6	9
Fibrobacteres	Fibrobacteria	258ds10	unidentified	nemoralis	0.002	+	6	14
Fibrobacteres	Fibrobacteria	258ds10	unidentified	pratensis	0.006	+	6	14
Firmicutes	Bacilli	Bacillales	Paenibacillaceae	alpina	0.004	+	2	4
Gemmatimonadetes	Gemm-1	unidentified	unidentified	arctica	0.006	-	15	10
Gemmatimonadetes	Gemm-1	unidentified	unidentified	glauca	0.006	-	15	10
Gemmatimonadetes	Gemm-1	unidentified	unidentified	nemoralis	0.011	-	15	10
Gemmatimonadetes	Gemmatimonadetes	unidentified	unidentified	glauca	0.006	-	15	9
Gemmatimonadetes	Gemmatimonadetes	unidentified	unidentified	nemoralis	0.013	-	15	10
Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Ellin5301	reflexa	0.009	-	20	13
OP3	koll11	unidentified	unidentified	alpina	0.013	-	6	3
OP3	koll11	unidentified	unidentified	arctica	0.01	-	6	2
OP3	koll11	unidentified	unidentified	compressa	0.004	-	6	2
OP3	koll11	unidentified	unidentified	glauca	0.01	-	6	2
OP3	koll11	unidentified	unidentified	nemoralis	0.005	-	6	2
Planctomycetes	Planctomycetia	Gemmatales	Gemmataceae	alpina	0.014	+	61	76
Planctomycetes	vadinHA49	DH61	unidentified	alpina	0	-	14	4
Planctomycetes	vadinHA49	DH61	unidentified	arctica	0	-	14	5
Planctomycetes	vadinHA49	DH61	unidentified	compressa	0	-	14	5
Planctomycetes	vadinHA49	DH61	unidentified	glauca	0	-	14	6
Planctomycetes	vadinHA49	DH61	unidentified	nemoralis	0	-	14	7
Planctomycetes	vadinHA49	DH61	unidentified	pratensis	0	-	14	4
Planctomycetes	vadinHA49	DH61	unidentified	reflexa	0.006	-	14	8
Proteobacteria	Alphaproteobacteria	BD7-3	unidentified	alpina	0.002	-	16	6

Proteobacteria	Alphaproteobacteria	BD7-3	unidentified	arctica	0.014	-	16	9
Proteobacteria	Alphaproteobacteria	BD7-3	unidentified	compressa	0.004	-	16	7
Proteobacteria	Alphaproteobacteria	BD7-3	unidentified	glauca	0.006	-	16	8
Proteobacteria	Alphaproteobacteria	Ellin329	unidentified	arctica	0.002	-	80	66
Proteobacteria	Alphaproteobacteria	Ellin329	unidentified	nemoralis	0	-	80	60
Proteobacteria	Alphaproteobacteria	Ellin329	unidentified	pratensis	0.005	-	80	68
Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	arctica	0.007	+	3	8
Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	glauca	0.007	+	3	7
Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	nemoralis	0.002	+	3	6
Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	pratensis	0	+	3	8
Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	reflexa	0.002	+	3	6
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	pratensis	0.004	+	1	5
Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	compressa	0.003	+	1	2
Proteobacteria	Alphaproteobacteria	unclassified	unclassified	alpina	0.007	-	5	2
Proteobacteria	Alphaproteobacteria	unclassified	unclassified	compressa	0.01	-	5	2
Proteobacteria	Alphaproteobacteria	unclassified	unclassified	reflexa	0.002	-	5	2
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	arctica	0.004	+	20	29
Proteobacteria	Deltaproteobacteria	Myxococcales	unidentified	arctica	0.002	-	135	106
Proteobacteria	Deltaproteobacteria	Myxococcales	unidentified	compressa	0	-	135	92
Proteobacteria	Deltaproteobacteria	Myxococcales	unidentified	glauca	0	-	135	101
Proteobacteria	Deltaproteobacteria	Myxococcales	Haliangiaceae	arctica	0	-	44	27
Proteobacteria	Deltaproteobacteria	Myxococcales	Haliangiaceae	glauca	0	-	44	27
Proteobacteria	Deltaproteobacteria	Myxococcales	Haliangiaceae	pratensis	0.013	-	44	33
Proteobacteria	Deltaproteobacteria	Myxococcales	Haliangiaceae	reflexa	0.002	-	44	29
Proteobacteria	Deltaproteobacteria	Myxococcales	Polyangiaceae	alpina	0.002	+	9	16
Proteobacteria	Deltaproteobacteria	Myxococcales	Polyangiaceae	arctica	0.001	+	9	15
Proteobacteria	Deltaproteobacteria	Myxococcales	Polyangiaceae	compressa	0.009	+	9	16
Proteobacteria	Deltaproteobacteria	Myxococcales	Polyangiaceae	glauca	0.005	+	9	15

Proteobacteria	Deltaproteobacteria	Myxococcales	Polyangiaceae	nemoralis	0	+	9	17
Proteobacteria	Deltaproteobacteria	unclassified	unclassified	glauca	0.004	-	4	2
Proteobacteria	Deltaproteobacteria	unclassified	unclassified	nemoralis	0.007	-	4	2
Proteobacteria	Deltaproteobacteria	unclassified	unclassified	pratensis	0.012	-	4	2
Tenericutes	Mollicutes	Anaeroplasmatales	Anaeroplasmataceae	alpina	0	+	1	13
Tenericutes	Mollicutes	Anaeroplasmatales	Anaeroplasmataceae	arctica	0	+	1	11
Tenericutes	Mollicutes	Anaeroplasmatales	Anaeroplasmataceae	compressa	0	+	1	19
Tenericutes	Mollicutes	Anaeroplasmatales	Anaeroplasmataceae	glauca	0.001	+	1	12
Tenericutes	Mollicutes	Anaeroplasmatales	Anaeroplasmataceae	nemoralis	0.002	+	1	6
Tenericutes	Mollicutes	Anaeroplasmatales	Anaeroplasmataceae	pratensis	0	+	1	10
Tenericutes	Mollicutes	Anaeroplasmatales	Anaeroplasmataceae	reflexa	0.01	+	1	6
TM6	SJA-4	unidentified	unidentified	pratensis	0.013	+	7	13
Verrucomicrobia	[Pedosphaerae]	[Pedosphaerales]	unidentified	alpina	0.004	-	32	22
Verrucomicrobia	[Pedosphaerae]	[Pedosphaerales]	unidentified	arctica	0.007	-	32	23
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	arctica	0.002	+	2	6
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	reflexa	0.003	+	2	5

Table A4.4. Fungal and bacterial families with a significant relationship with phylogenetic distance among Poa species.

Families that showed a significant relationship in linear regressions with plant phylogenetic distance (alpha = 0.00058 for fungi; alpha = 0.00034 for bacteria). Families that contain > 20 sequence copies within a family within all replicate pots per *Poa* species were included in this analysis. Taxonomy classification, including placeholder names and unidentified taxa, are labeled according to assignments given by the Greengenes RDP classifier.

Kingdom	**Phylum**	**Class**	**Order**	**Family**	**p**	**R^2**	**Coefficient**	**Standard error**
Fungi	Ascomycota	Emycetes	incertae sedis	incertae sedis	0.0001	0.99	1303.71	77.96
Fungi	Basidiootta	Agarmycetes	Agaricales	Typhulaceae	<0.0001	0.99	12989.26	575
Fungi	Basidiootta	Tremeymycetes	Cysilobasidiales	Cystofilobasidiaceae	0.0005	0.96	6861.13	667.84
Bacteria	Bacteroidetes	Flbacteriia	Flavobacteriales	Cryomorphaceae	0.0003	0.97	311.1	27.35
Bacteria	Chloroflexi	Analineae	CFB.26	unidentified	0.0002	0.97	166.96	13.42

Table A4.5. Fungal families that differed between ambient N and N addition treatments.
Fungal families that differed significantly ($P < 0.007$) in relative abundance between ambient N no-plant control pots and N addition no-plant control pots. Change relative to control indicates whether the absolute abundance of organisms within a family decreased (-) significantly or increased (+) significantly in the rhizosphere compared to no-plant control communities. Means are the mean abundance of sequence copies within a family for a *Poa* ambient N or N addition pots. Taxonomy classification, including placeholder names and unidentified taxa, are labeled according to assignments given by the Greengenes RDP classifier.

Phylum	**Class**	**Order**	**Family**	**Poa species**	**p**	**Change relative to control**	**Ambient N mean**	**N addition mean**
Ascomycota	Dothideomycetes	Capnodiales	Antennulariellaceae	reflexa	0.01	-	35	13
Ascomycota	Dothideomycetes	incertae sedis	incertae sedis	compressa	0.014	-	6	2
Ascomycota	Dothideomycetes	unclassified	unclassified	pratensis	0.004	-	5	2
Ascomycota	Eurotiomycetes	Chaetothyriales	unidentified	glauca	0.006	+	8	26
Ascomycota	Leotiomycetes	Helotiales	Hyaloscyphaceae	arctica	0.011	+	43	80
Ascomycota	Leotiomycetes	Leotiales	Leotiaceae	pratensis	0.014	-	13	7
Ascomycota	Sordariomycetes	Hypocreales	Clavicipitaceae	nemoralis	0.007	+	6	12
Basidiomycota	Agaricomycetes	Agaricales	Amanitaceae	glauca	0.01	+	8	14
Basidiomycota	Agaricomycetes	Agaricales	Clavariaceae	nemoralis	0.006	-	56	35
Basidiomycota	Agaricomycetes	Agaricales	Cortinariaceae	pratensis	0.002	+	0	2
Basidiomycota	Agaricomycetes	unclassified	unclassified	arctica	0.004	-	72	23
Basidiomycota	Agaricomycetes	unclassified	unclassified	nemoralis	0.007	+	16	42
Chytridiomycota	Chytridiomycetes	Chytridiales	Chytridiaceae	arctica	0.004	+	0	6
Zygomycota	Mortierellomycotina incertae sedis	Mortierellales	Mortierellaceae	nemoralis	0.002	-	1159	850
Zygomycota	Mortierellomycotina incertae sedis	Mortierellales	Mortierellaceae	pratensis	<0.001	-	1005	712

Table A4.6. Bacterial families that differed between ambient N and N addition treatments.
Bacterial families that differed significantly (alpha = 0.007) in relative abundance between ambient N no-plant control pots and N addition no-plant control pots. Change relative to control indicates whether the absolute abundance of organisms within a family decreased (-) significantly or increased (+) significantly in the rhizosphere compared to no-plant control communities. Means are the mean abundance of sequence copies within a family for a *Poa* ambient N or N addition pots. Taxonomy classification, including placeholder names and unidentified taxa, are labeled according to assignments given by the Greengenes RDP classifier.

Phylum	**Class**	**Order**	**Family**	**Poa species**	**p**	**Change relative to control**	**Ambient N mean**	**N addition mean**
Acidobacteria	[Chloracidobacteria]	11-24	unidentified	alpina	0.013	-	5	2
Acidobacteria	Acidobacteria-6	CCU21	unidentified	alpina	<0.001	-	7	2
Acidobacteria	Acidobacteria-6	CCU21	unidentified	arctica	0.003	-	7	2
Acidobacteria	Acidobacteria-6	CCU21	unidentified	compressa	0.002	-	6	2
Acidobacteria	Acidobacteria-6	CCU21	unidentified	glauca	<0.001	-	5	1
Acidobacteria	Acidobacteria-6	CCU21	unidentified	nemoralis	<0.001	-	9	2
Acidobacteria	Acidobacteria-6	CCU21	unidentified	pratensis	0.001	-	4	1
Acidobacteria	Acidobacteria-6	iii1-15	unidentified	alpina	<0.001	-	93	58
Acidobacteria	Acidobacteria-6	iii1-15	unidentified	arctica	0.002	-	95	64
Acidobacteria	Acidobacteria-6	iii1-15	unidentified	glauca	0.001	-	91	63
Acidobacteria	Acidobacteria-6	iii1-15	unidentified	nemoralis	<0.001	-	100	58
Acidobacteria	Acidobacteria-6	iii1-15	unidentified	pratensis	<0.001	-	92	62
Acidobacteria	Acidobacteria-6	iii1-15	unidentified	reflexa	0.009	-	92	56
Acidobacteria	Acidobacteria-6	iii1-15	RB40	glauca	0.007	-	10	5
Acidobacteria	Acidobacteria-6	iii1-15	RB40	nemoralis	<0.001	-	10	5
Acidobacteria	Acidobacteriia	Acidobacteriales	Acidobacteriaceae	alpina	0.001	+	42	77
Acidobacteria	Acidobacteriia	Acidobacteriales	Acidobacteriaceae	arctica	0.002	+	47	72
Acidobacteria	Acidobacteriia	Acidobacteriales	Acidobacteriaceae	pratensis	0.006	+	40	68
Acidobacteria	Acidobacteriia	Acidobacteriales	Acidobacteriaceae	reflexa	0.004	+	44	76
Acidobacteria	iii1-8	DS-18	unidentified	glauca	0.003	-	28	17

Acidobacteria	Solibacteres	Solibacterales	unidentified	arctica	0.003	-	66	50
Acidobacteria	Solibacteres	Solibacterales	unidentified	glauca	0.001	-	75	51
Acidobacteria	Solibacteres	Solibacterales	unidentified	reflexa	0.006	-	74	49
Actinobacteria	Acidimicrobiia	Acidimicrobiales	unidentified	compressa	0.001	+	16	27
Actinobacteria	Acidimicrobiia	Acidimicrobiales	unidentified	glauca	0.014	+	13	22
Actinobacteria	Acidimicrobiia	Acidimicrobiales	unidentified	nemoralis	0.004	+	13	22
Actinobacteria	Acidimicrobiia	Acidimicrobiales	unidentified	pratensis	<0.001	+	15	28
Actinobacteria	Acidimicrobiia	Acidimicrobiales	EB1017	glauca	0.001	+	7	12
Actinobacteria	Actinobacteria	Actinomycetales	Frankiaceae	alpina	0.012	+	10	17
Actinobacteria	Actinobacteria	Actinomycetales	Frankiaceae	arctica	0.002	+	8	16
Actinobacteria	Actinobacteria	Actinomycetales	Frankiaceae	compressa	0.005	+	9	16
Actinobacteria	Actinobacteria	Actinomycetales	Frankiaceae	glauca	0.001	+	8	18
Actinobacteria	Actinobacteria	Actinomycetales	Frankiaceae	nemoralis	<0.001	+	10	20
Actinobacteria	Actinobacteria	Actinomycetales	Frankiaceae	pratensis	0.002	+	10	18
Actinobacteria	Actinobacteria	Actinomycetales	Intrasporangiaceae	alpina	<0.001	+	2	7
Actinobacteria	Actinobacteria	Actinomycetales	Intrasporangiaceae	arctica	0.01	+	2	7
Actinobacteria	Actinobacteria	Actinomycetales	Intrasporangiaceae	nemoralis	<0.001	+	2	8
Actinobacteria	Actinobacteria	Actinomycetales	Intrasporangiaceae	pratensis	0.001	+	2	7
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	alpina	0.007	+	1	4
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	arctica	<0.001	+	2	10
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	compressa	0.012	+	1	4
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	pratensis	0.001	+	2	9
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	reflexa	<0.001	+	1	9
Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	alpina	0.006	+	5	12
Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	nemoralis	0.004	+	4	12
Actinobacteria	Actinobacteria	Actinomycetales	Nocardioidaceae	reflexa	0.011	+	4	10
Actinobacteria	Actinobacteria	Actinomycetales	Sporichthyaceae	arctica	0.001	+	1	7
Actinobacteria	Actinobacteria	Actinomycetales	Sporichthyaceae	compressa	0.002	+	1	6

Actinobacteria	Actinobacteria	Actinomycetales	Sporichthyaceae	nemoralis	<0.001	+	1	6
Actinobacteria	Actinobacteria	Actinomycetales	Sporichthyaceae	pratensis	<0.001	+	1	5
Actinobacteria	Actinobacteria	Actinomycetales	Sporichthyaceae	reflexa	0.008	+	1	5
Actinobacteria	Actinobacteria	Actinomycetales	Streptomycetaceae	alpina	0.012	+	4	8
Actinobacteria	Actinobacteria	Actinomycetales	Streptomycetaceae	arctica	<0.001	+	3	6
Actinobacteria	Actinobacteria	Actinomycetales	Streptomycetaceae	glauca	<0.001	+	2	6
Actinobacteria	Actinobacteria	Actinomycetales	Streptomycetaceae	nemoralis	0.008	+	2	6
Actinobacteria	Actinobacteria	Actinomycetales	Streptomycetaceae	pratensis	0.003	+	3	8
Actinobacteria	Actinobacteria	Actinomycetales	unclassified	arctica	0.002	-	5	2
Actinobacteria	Thermoleophilia	Gaiellales	Gaiellaceae	glauca	0.008	+	36	52
Actinobacteria	Thermoleophilia	Solirubrobacterales	unidentified	glauca	<0.001	+	25	47
Actinobacteria	Thermoleophilia	Solirubrobacterales	unidentified	nemoralis	0.002	+	26	44
Actinobacteria	Thermoleophilia	Solirubrobacterales	Conexibacteraceae	glauca	0.006	+	12	20
Actinobacteria	Thermoleophilia	Solirubrobacterales	Patulibacteraceae	arctica	0.001	+	4	11
Actinobacteria	Thermoleophilia	Solirubrobacterales	Patulibacteraceae	nemoralis	0.011	+	3	6
Actinobacteria	Thermoleophilia	Solirubrobacterales	Patulibacteraceae	pratensis	0.007	+	6	11
AD3	ABS-6	unidentified	unidentified	glauca	0.008	+	17	22
Armatimonadetes	Armatimonadia	FW68	unidentified	alpina	0.007	+	2	8
Armatimonadetes	Armatimonadia	FW68	unidentified	arctica	0.001	+	2	7
Armatimonadetes	Armatimonadia	FW68	unidentified	compressa	0.004	+	1	4
Armatimonadetes	Armatimonadia	FW68	unidentified	glauca	0.001	+	1	4
Armatimonadetes	Armatimonadia	FW68	unidentified	nemoralis	0.011	+	1	6
Armatimonadetes	Armatimonadia	FW68	unidentified	pratensis	<0.001	+	2	9
Armatimonadetes	Armatimonadia	FW68	unidentified	reflexa	<0.001	+	1	13
Armatimonadetes	Chthonomonadetes	SJA-22	unidentified	glauca	0.007	-	2	1
Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	arctica	0.007	-	176	147
Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	glauca	0.005	-	180	152
Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	arctica	0.005	-	17	9

Bacteroidetes	Sphingobacteriia	Sphingobacteriales	unidentified	alpina	<0.001	-	28	15
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	unidentified	arctica	<0.001	-	31	15
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	unidentified	compressa	0.002	-	30	20
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	unidentified	glauca	0.011	-	30	17
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	unidentified	nemoralis	0.003	-	30	17
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	unidentified	pratensis	<0.001	-	29	11
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	unidentified	reflexa	0.009	-	22	14
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	alpina	0.001	+	14	71
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	arctica	<0.001	+	15	115
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	compressa	0.001	+	12	71
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	glauca	<0.001	+	14	65
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	nemoralis	<0.001	+	18	87
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	pratensis	<0.001	+	16	103
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	reflexa	<0.001	+	17	107
Bacteroidetes	unclassified	unclassified	unclassified	glauca	0.012	+	2	4
Bacteroidetes	unclassified	unclassified	unclassified	nemoralis	0.007	+	1	4
Bacteroidetes	unclassified	unclassified	unclassified	reflexa	<0.001	+	1	4
Chlamydiae	Chlamydiia	Chlamydiales	Rhabdochlamydiaceae	pratensis	0.001	-	3	1
Chlorobi	SJA-28	unidentified	unidentified	arctica	0.001	-	4	1
Chloroflexi	Anaerolineae	SBR1031	A4b	pratensis	0.007	-	22	13
Chloroflexi	Anaerolineae	SBR1031	A4b	reflexa	0.003	-	19	8
Chloroflexi	Anaerolineae	SBR1031	oc28	arctica	0.009	-	18	9
Chloroflexi	Anaerolineae	SBR1031	oc28	nemoralis	0.007	-	17	10
Chloroflexi	Anaerolineae	SBR1031	oc28	pratensis	<0.001	-	20	10
Chloroflexi	TK10	AKYG885	Dolo_23	glauca	0.014	+	2	5
Chloroflexi	TK10	B07_WMSP1	unidentified	pratensis	0.001	+	0	2
Chloroflexi	TK10	B07_WMSP1	FFCH4570	pratensis	<0.001	-	19	11
Cyanobacteria	unidentified	unidentified	unidentified	reflexa	0.005	+	2	8

Cyanobacteria	4C0d-2	MLE1-12	unidentified	alpina	0.001	-	8	3
Cyanobacteria	4C0d-2	MLE1-12	unidentified	compressa	0.001	-	6	2
Cyanobacteria	4C0d-2	MLE1-12	unidentified	pratensis	0.002	-	4	2
Cyanobacteria	4C0d-2	MLE1-12	unidentified	reflexa	0.001	-	7	1
Cyanobacteria	4C0d-2	SM1D11	unidentified	compressa	0.008	-	7	3
Cyanobacteria	4C0d-2	SM1D11	unidentified	reflexa	0.007	+	5	27
Elusimicrobia	Elusimicrobia	FAC88	unidentified	arctica	<0.001	-	14	6
Elusimicrobia	Elusimicrobia	IIb	unidentified	alpina	0.014	-	5	3
Elusimicrobia	Elusimicrobia	IIb	unidentified	pratensis	0.001	-	6	2
FBP	unidentified	unidentified	unidentified	alpina	0.009	+	3	11
FBP	unidentified	unidentified	unidentified	arctica	<0.001	+	4	14
FBP	unidentified	unidentified	unidentified	glauca	0.008	+	3	6
FBP	unidentified	unidentified	unidentified	pratensis	0.01	+	3	11
Fibrobacteres	Fibrobacteria	258ds10	unidentified	glauca	0.002	+	8	21
Fibrobacteres	Fibrobacteria	258ds10	unidentified	nemoralis	0.01	+	11	26
Firmicutes	Bacilli	Bacillales	unclassified	reflexa	0.012	+	8	14
Gemmatimonadetes	Gemmatimonadetes	Ellin5290	unidentified	nemoralis	0.002	-	17	10
Gemmatimonadetes	Gemmatimonadetes	Ellin5290	unidentified	pratensis	0.006	-	15	9
Gemmatimonadetes	Gemmatimonadetes	Ellin5290	unidentified	reflexa	0.004	-	16	8
OD1	ZB2	unidentified	unidentified	compressa	0.006	-	4	1
OD1	ZB2	unidentified	unidentified	pratensis	0.002	-	3	1
Planctomycetes	OM190	agg27	unidentified	pratensis	0.001	-	4	1
Planctomycetes	Phycisphaerae	CPla-3	unidentified	alpina	0.01	+	3	5
Planctomycetes	Phycisphaerae	Phycisphaerales	unidentified	glauca	0.003	-	4	2
Planctomycetes	Planctomycetia	Gemmatales	Gemmataceae	alpina	0.007	-	74	56
Planctomycetes	Planctomycetia	Gemmatales	Gemmataceae	nemoralis	0.013	-	69	51
Planctomycetes	Planctomycetia	Planctomycetales	Planctomycetaceae	glauca	0.006	+	14	22
Planctomycetes	vadinHA49	DH61	unidentified	compressa	0.011	-	4	2

Planctomycetes	vadinHA49	DH61	unidentified	nemoralis	0.002	-	8	2
Planctomycetes	vadinHA49	DH61	unidentified	reflexa	0.008	-	7	2
Planctomycetes	vadinHA49	p04_C01	unidentified	arctica	0.001	-	4	2
Planctomycetes	vadinHA49	p04_C01	unidentified	nemoralis	0.01	-	4	2
Planctomycetes	vadinHA49	p04_C01	unidentified	pratensis	0.011	-	4	2
Proteobacteria	Alphaproteobacteria	unidentified	unidentified	compressa	0.004	-	18	10
Proteobacteria	Alphaproteobacteria	unidentified	unidentified	pratensis	<0.001	-	18	9
Proteobacteria	Alphaproteobacteria	unidentified	unidentified	reflexa	0.012	-	18	12
Proteobacteria	Alphaproteobacteria	BD7-3	unidentified	pratensis	<0.001	-	10	2
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	alpina	<0.001	+	16	29
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	arctica	<0.001	+	15	45
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	compressa	0.004	+	14	27
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	glauca	<0.001	+	15	34
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	nemoralis	0.001	+	17	35
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	pratensis	<0.001	+	14	44
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	reflexa	<0.001	+	18	42
Proteobacteria	Alphaproteobacteria	Ellin329	unidentified	alpina	0.003	+	69	90
Proteobacteria	Alphaproteobacteria	Ellin329	unidentified	arctica	<0.001	+	60	90
Proteobacteria	Alphaproteobacteria	Ellin329	unidentified	compressa	0.001	+	66	85
Proteobacteria	Alphaproteobacteria	Ellin329	unidentified	glauca	0.013	+	74	90
Proteobacteria	Alphaproteobacteria	Ellin329	unidentified	nemoralis	<0.001	+	63	97
Proteobacteria	Alphaproteobacteria	Ellin329	unidentified	pratensis	0.01	+	72	91
Proteobacteria	Alphaproteobacteria	Ellin329	unidentified	reflexa	0.007	+	72	90
Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	alpina	0.002	+	3	8
Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	compressa	0.009	+	5	10
Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	nemoralis	<0.001	+	5	13
Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	reflexa	0.01	+	5	10
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	arctica	0.004	+	3	12

Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	glauca	0.011	+	2	6
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	nemoralis	<0.001	+	3	6
Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	reflexa	0.008	+	3	7
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Hyphomonadaceae	compressa	0.008	-	14	8
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Hyphomonadaceae	pratensis	0.007	-	12	7
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Hyphomonadaceae	reflexa	0.008	-	11	5
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	alpina	<0.001	-	43	28
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	reflexa	0.001	-	64	48
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Erythrobacteraceae	compressa	0.013	+	0	2
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	alpina	0.001	+	31	61
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	arctica	0.002	+	37	75
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	compressa	<0.001	+	27	61
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	glauca	0.001	+	29	51
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	nemoralis	0.012	+	33	52
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	pratensis	<0.001	+	31	68
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	reflexa	0.002	+	34	60
Proteobacteria	Betaproteobacteria	A21b	EB1003	arctica	0.012	-	57	40
Proteobacteria	Betaproteobacteria	A21b	EB1003	reflexa	0.008	-	51	37
Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	alpina	<0.001	+	5	15
Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	nemoralis	0.008	+	8	15
Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	reflexa	0.001	+	6	15
Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	arctica	0.004	+	23	44
Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	pratensis	0.005	+	19	38
Proteobacteria	Betaproteobacteria	Ellin6067	unidentified	arctica	<0.001	-	30	15
Proteobacteria	Betaproteobacteria	Ellin6067	unidentified	compressa	0.009	-	34	25
Proteobacteria	Betaproteobacteria	Ellin6067	unidentified	glauca	0.001	-	29	18
Proteobacteria	Betaproteobacteria	Ellin6067	unidentified	nemoralis	<0.001	-	33	19
Proteobacteria	Betaproteobacteria	Ellin6067	unidentified	pratensis	<0.001	-	27	14

Proteobacteria	Betaproteobacteria	Ellin6067	unidentified	reflexa	0.004	-	27	16
Proteobacteria	Betaproteobacteria	MND1	unidentified	alpina	0.001	-	7	2
Proteobacteria	Betaproteobacteria	MND1	unidentified	arctica	0.001	-	7	3
Proteobacteria	Betaproteobacteria	MND1	unidentified	compressa	0.009	-	7	4
Proteobacteria	Betaproteobacteria	MND1	unidentified	pratensis	<0.001	-	6	1
Proteobacteria	Betaproteobacteria	unclassified	unclassified	alpina	<0.001	-	8	2
Proteobacteria	Betaproteobacteria	unclassified	unclassified	arctica	<0.001	-	7	1
Proteobacteria	Betaproteobacteria	unclassified	unclassified	pratensis	0.009	-	5	2
Proteobacteria	Deltaproteobacteria	unidentified	unidentified	alpina	0.006	-	4	2
Proteobacteria	Deltaproteobacteria	unidentified	unidentified	arctica	0.002	-	4	2
Proteobacteria	Deltaproteobacteria	unidentified	unidentified	glauca	0.004	-	4	2
Proteobacteria	Deltaproteobacteria	unidentified	unidentified	reflexa	<0.001	-	4	2
Proteobacteria	Deltaproteobacteria	[Entotheonellales]	[Entotheonellaceae]	compressa	0.006	-	4	2
Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bdellovibrionaceae	arctica	0.002	-	6	3
Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bdellovibrionaceae	reflexa	0.005	-	5	3
Proteobacteria	Deltaproteobacteria	FAC87	unidentified	reflexa	0.001	-	3	1
Proteobacteria	Deltaproteobacteria	MIZ46	unidentified	alpina	0.014	-	10	6
Proteobacteria	Deltaproteobacteria	MIZ46	unidentified	arctica	<0.001	-	11	3
Proteobacteria	Deltaproteobacteria	MIZ46	unidentified	pratensis	0.001	-	9	4
Proteobacteria	Deltaproteobacteria	Myxococcales	unidentified	alpina	<0.001	-	114	63
Proteobacteria	Deltaproteobacteria	Myxococcales	unidentified	arctica	<0.001	-	108	54
Proteobacteria	Deltaproteobacteria	Myxococcales	unidentified	glauca	0.001	-	100	68
Proteobacteria	Deltaproteobacteria	Myxococcales	unidentified	nemoralis	<0.001	-	114	64
Proteobacteria	Deltaproteobacteria	Myxococcales	unidentified	pratensis	<0.001	-	120	52
Proteobacteria	Deltaproteobacteria	Myxococcales	unidentified	reflexa	<0.001	-	108	56
Proteobacteria	Deltaproteobacteria	Myxococcales	Haliangiaceae	alpina	0.006	-	36	22
Proteobacteria	Deltaproteobacteria	Myxococcales	Haliangiaceae	glauca	0.004	-	30	20
Proteobacteria	Deltaproteobacteria	Myxococcales	Haliangiaceae	nemoralis	0.007	-	40	21

Proteobacteria	Deltaproteobacteria	Myxococcales	Haliangiaceae	pratensis	<0.001	-	35	16
Proteobacteria	Deltaproteobacteria	Myxococcales	Haliangiaceae	reflexa	<0.001	-	33	17
Proteobacteria	Deltaproteobacteria	Myxococcales	Myxococcaceae	alpina	0.012	-	10	5
Proteobacteria	Deltaproteobacteria	Myxococcales	Myxococcaceae	arctica	0.006	-	8	5
Proteobacteria	Deltaproteobacteria	Myxococcales	Myxococcaceae	compressa	0.001	-	9	4
Proteobacteria	Deltaproteobacteria	Myxococcales	Myxococcaceae	nemoralis	0.001	-	10	4
Proteobacteria	Deltaproteobacteria	Myxococcales	Polyangiaceae	alpina	<0.001	-	13	4
Proteobacteria	Deltaproteobacteria	Myxococcales	Polyangiaceae	arctica	<0.001	-	16	2
Proteobacteria	Deltaproteobacteria	Myxococcales	Polyangiaceae	compressa	<0.001	-	15	4
Proteobacteria	Deltaproteobacteria	Myxococcales	Polyangiaceae	glauca	<0.001	-	15	3
Proteobacteria	Deltaproteobacteria	Myxococcales	Polyangiaceae	nemoralis	<0.001	-	19	3
Proteobacteria	Deltaproteobacteria	Myxococcales	Polyangiaceae	pratensis	0.003	-	10	4
Proteobacteria	Deltaproteobacteria	Myxococcales	Polyangiaceae	reflexa	0.004	-	11	4
Proteobacteria	Deltaproteobacteria	Myxococcales	unclassified	glauca	0.005	-	5	2
Proteobacteria	Deltaproteobacteria	Myxococcales	unclassified	nemoralis	<0.001	-	8	2
Proteobacteria	Deltaproteobacteria	Spirobacillales	unidentified	glauca	0.008	-	3	1
Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophobacteraceae	arctica	<0.001	-	22	14
Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophobacteraceae	glauca	0.002	-	20	14
Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophobacteraceae	pratensis	0.002	-	21	13
Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophobacteraceae	reflexa	0.007	-	20	13
Proteobacteria	Gammaproteobacteria	unidentified	unidentified	pratensis	0.005	-	8	3
Proteobacteria	Gammaproteobacteria	unidentified	unidentified	reflexa	0.01	+	7	17
Proteobacteria	Gammaproteobacteria	Legionellales	unclassified	alpina	0.001	-	6	1
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	pratensis	0.004	-	79	60
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	alpina	<0.001	+	56	121
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	arctica	<0.001	+	64	126
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	compressa	<0.001	+	60	117
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	glauca	<0.001	+	67	110

Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	nemoralis	<0.001	+	60	126
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	pratensis	0.004	+	67	129
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	reflexa	<0.001	+	64	109
Proteobacteria	unclassified	unclassified	unclassified	alpina	0.001	-	5	2
Proteobacteria	unclassified	unclassified	unclassified	arctica	<0.001	-	7	2
Proteobacteria	unclassified	unclassified	unclassified	pratensis	0.004	-	5	2
Tenericutes	Mollicutes	Anaeroplasmatales	Anaeroplasmataceae	arctica	0.008	+	12	27
Tenericutes	Mollicutes	Anaeroplasmatales	Anaeroplasmataceae	glauca	<0.001	+	11	25
Tenericutes	Mollicutes	Anaeroplasmatales	Anaeroplasmataceae	nemoralis	0.003	+	6	26
Tenericutes	Mollicutes	Anaeroplasmatales	Anaeroplasmataceae	pratensis	0.01	+	11	28
Tenericutes	Mollicutes	Anaeroplasmatales	Anaeroplasmataceae	reflexa	0.008	+	6	20
Verrucomicrobia	[Pedosphaerae]	[Pedosphaerales]	unidentified	glauca	0.012	+	24	34
Verrucomicrobia	[Pedosphaerae]	[Pedosphaerales]	Ellin517	compressa	0.006	+	17	28
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	arctica	0.006	-	5	2
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	glauca	0.01	-	4	2