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

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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.

Key words: aluminum toxicity; *Carex rupestris;* diversity; ectomycorrhizal fungi; *Kobresia myosuroides;* nitrogen deposition; Niwot Ridge, USA plant–soil interactions.

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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 et al. 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 et al. 2009, Dickson and Foster 2011), changes in plant–microbial

interactions (Johnson et al. 2008, Suding et al. 2008), and soil acidification (Houdijk et al. 1993, Roem et al. 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 et al. 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 et al. 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 et al. 1993, Mills and Bever 1998), or indirectly via microbial alteration of the supply of plant resources (Hodge et al. 2000, Schimel and Bennett 2004, Suding et al. 2008). Additionally, these processes can involve feedback loops that influence the plant species responses to N deposition (Bezemer et al. 2006, Sigüenza et al. 2006, Kardol et al. 2007, Kulmatiski et al. 2008). 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 et al. 1983, Bowman et al. 2008, Stevens et al. 2009). Increased Al mobility is detrimental because Al is toxic to plants and soil microorganisms (Thompson and Medve 1984, Delhaize and 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). 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 drive 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 dry mass of soil; Lieb et al. 2011). Aluminum toxicity in plants usually occurs when soil pH drops below pH 5.5

(Vitorello et al. 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 concurrently 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 belowground 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 dry meadow plant community the long-term N addition plots are located within.

The experiment simulated a range of N deposition rates in a species-rich dry meadow alpine community on Niwot Ridge, Colorado, for 17 yr before these data were collected. Five replicate 1×1.5 m plots receive N fertilizer at rates of 0, 20, 40, or 60 kg $N{\cdot}ha^{-1}{\cdot}yr^{-1}$, 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 ~6 kg $N{\cdot}ha^{-1}{\cdot}yr^{-1}$ (Sievering 2001). Soils are cryumbrepts with granitic parent material and contain $28.8 \pm 1.5\%$ 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 rhizosphere 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 3-cm fragments, rinsed with water, and surveyed for ectomycorrhizal fungi using a 10× 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 and 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 0.25 g of soil from 40 samples stratified across blocks and N treatments using the Mo Bio PowerSoil DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, California, 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, Carlsbad, California, USA). Normalized samples were pooled for PCR cleanup (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

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 m 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. Twelve replicates per Al treatment level were made for each sedge species, and pot locations were randomized among pot racks. 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. 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 Applied Research Laboratories (ARL) inductively coupled plasma emission spectrophotometer (ICP-AES, Thermo Electron, Waltham, Massachusetts, USA).

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 min 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 fitted 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 among samples) exhibited a significant correlation in relative abundance of sequences with increasing N level. This was done to determine whether any bacteria taxa may be candidates to further investigate as potential 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 were presumed to have negligible effects on plant roots. Additionally, by reducing the number of correlations tests performed we lowered the probability of making a type I statistical error. Bonferroni corrections were made on common families and genera to avoid type II 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 heights 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, four plants averaged per plot per species; Fig. 1). *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

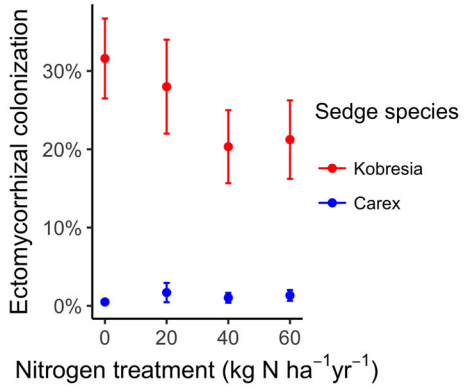


Fig. 1. Colonization by ectomycorrhizal fungi (percent of infected root tips per plant) showing means and standard error of the means on *Carex rupestris'* and *Kobresia myosuroides*' root tips for each N level (n = 5 plots per treatment level).

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 <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 the four N levels ($P = 0.12$, $R^2 = 0.09$), but did differ between the two sedge species ($P < 0.01$, $R^2 = 0.06$) 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* (Fig. 2). Bacteria OTU frequencies grouped by higher

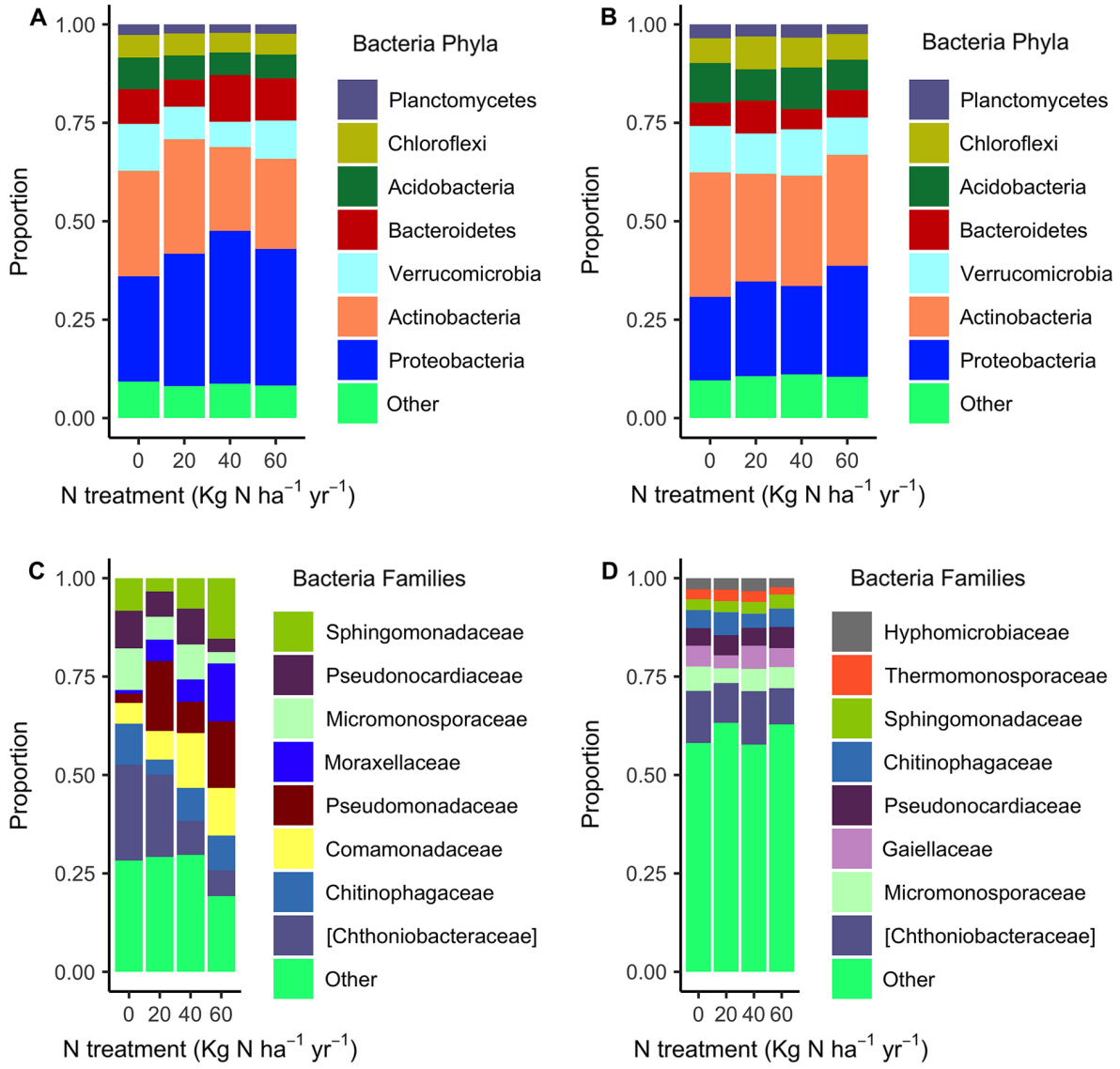


Fig. 2. Relative proportions of bacterial OTUs within each N treatment. Taxa shown are the seven most abundant phyla on *Kobresia myosuroides'* roots (A) and *Carex rupestris'* roots (B), and the eight most abundant bacteria families are also shown for *Kobresia* (C), and *Carex* (D).

taxonomic levels (family and genus) did not reveal any shifts in composition to indicate directional changes in abundance of OTUs within families ($P = 0.75$, $R^2 = 0.04$), or genera ($P = 0.76$, $R^2 = 0.04$) with increasing N level. Correlation tests on the 67 common families by N treatment resulted in no individual families varying significantly with N level ($n = 67$, $P > 0.05$). Correlation tests for the 120 common genera also did not reveal any taxa varying significantly in relative abundance with N treatment ($n = 120$, $P > 0.05$). The lack of directional changes in abundance of bacteria across N levels suggested that no specific taxonomic group needed to be investigated further for potential pathogenic effects on *Kobresia* or mutualisms with *Carex*.

The Al treatments had no negative effects on sedge growth for either species (Fig. 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. 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 (standard error of the mean [SEM]) 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 yr 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

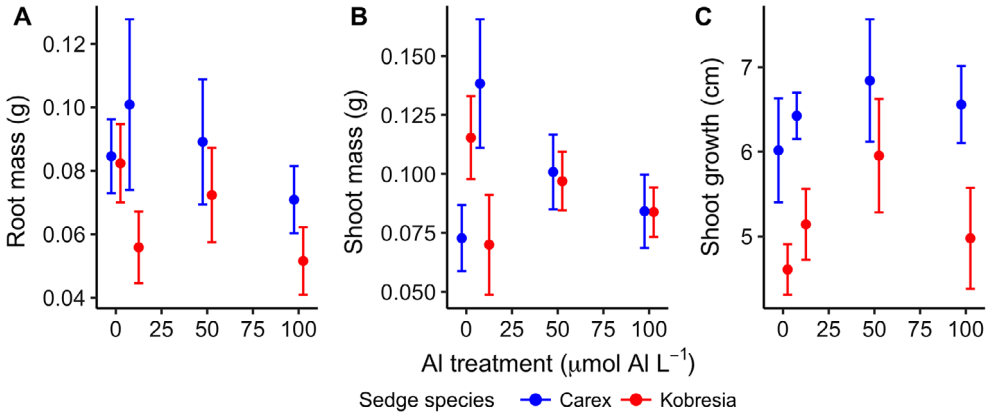


Fig. 3. Root dry mass (A), shoot dry mass (B), and shoot growth (change in shoot length of the longest blade per plant from onset of Al additions) (C) for *Kobresia myosuroides* and *Carex rupestris* plants subjected to three levels of Al with a control treatment (tap water). Data are means and standard error of the means ($n = 5$ plots per treatment level).

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 and Kottke 1998, Treseder 2008, Suding et al. 2005, Shantz et al. 2015). *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 are typically limiting resources in this system besides N (Bowman et al. 1993, Fan et al. 2016). A 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 and Helgason 2018, Treseder et al. 2018). Recent research that characterizes plant–mycorrhizal relationships with N addition (Lilleskov et al. 2011, Treseder et al. 2018) provides additional plant–mycorrhizal species pairs to investigate functional relationships.

Carex appears to be a superior competitor in this community under elevated N deposition. *Kobresia's* and *Carex*'s cover was negatively correlated in the high N level field plots and the relationship increases in magnitude over time (W. D. 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 these species take several years to show directional responses to experimental manipulation (Bowman et al. 2018); however, changes in competition may very well be both a consequence of changes in resource availability and 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 bacterial taxa had enhanced pathogenic effects with elevated N that could contribute to *Kobresia*'s decline in cover. Additional correlation tests on common bacterial families and genera associated with *Kobresia* in N treatment plots confirmed that there was little change in the relative abundances of taxa with N addition for each individual family and genus examined. This result provides no candidate taxa to examine for pathogenic effects in high N conditions, and we therefore believe it is unlikely that pathogens are involved in *Kobresia's* decline. This null result differs from field fertilization experiments that have demonstrated shifts in abundance of bacterial taxa with added N (Allison and Martiny 2008, Nemergut et al. 2008, Ramirez et al. 2012, Coolon et al. 2013). More recently, a few studies have reported no change in bacterial community composition in N addition experiments (Jing et al. 2016, McHugh et al. 2017). Differences in experimental designs among studies likely contribute to differences in the magnitude of the treatment effects. First, experimental manipulations differ greatly in C:N of soil used, as well as the dosage and duration of N fertilization (ranging from 10 to 800 kg $N \cdot ha^{-1} \cdot yr^{-1}$; Ramirez et al. 2010). Even when N additions are applied at a constant rate within experiments, N does not often have a consistent effect on bulk soil bacteria biomass (Treseder 2008) or composition (Leff et al. 2015) among 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.

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 and Mulder 1995). While Niwot Ridge alpine soils are relatively high in organic matter, 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. Because 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 and 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 that can occur with elevated N deposition. It may also be the case that mature plants, such as those transplanted in our experiment, may be less sensitive to low pH and Al toxicity than young or stressed plants. It should also be noted 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. 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 impact 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 among different ecosystems.

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