

Nitrogen preferences and plant-soil feedbacks as influenced by neighbors in the alpine tundra

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Abstract Plant resource partitioning of chemical forms of nitrogen (N) may be an important factor promoting species coexistence in N-limited ecosystems. Since the microbial community regulates N-form transformations, plant partitioning of N may be related to plant–soil feedbacks. We conducted a ^{15}N tracer addition experiment to study the ability of two alpine plant species, *Acomastylis rossii* and *Deschampsia caespitosa*, to partition organic and inorganic forms of N. The species are codominant and associated with strong plant–soil feedbacks that affect N cycling. We manipulated interspecific interactions by removing *Acomastylis* or *Deschampsia* from areas where the species were codominant to test if N uptake patterns varied in the presence of the other species. We found that *Deschampsia* acquired organic and inorganic N more rapidly than *Acomastylis*, regardless of neighbor treatment. Plant N uptake—specifically ammonium uptake—increased with plant density and the presence of an interspecific neighbor. Interestingly, this change in N uptake was not in the expected direction to reduce niche overlap and instead suggested facilitation of ammonium use. To test if N acquisition patterns were consistent with plant–

soil feedbacks, we also compared microbial rhizosphere extracellular enzyme activity in patches dominated by one or the other species and in areas where they grew together. The presence of both species was generally associated with increased rhizosphere extracellular enzyme activity (five of ten enzymes) and a trend towards increased foliar N concentrations. Taken together, these results suggest that feedbacks through the microbial community, either in response to increased plant density or specific plant neighbors, could facilitate coexistence. However, coexistence is promoted via enhanced resource uptake rather than reduced niche overlap. The importance of resource partitioning to reduce the intensity of competitive interactions might vary across systems, particularly as a function of plant-soil feedbacks.

Keywords Coexistence · Foliar nitrogen concentrations · Interspecific interactions · Neighbor effect · Nitrogen uptake patterns · Plant resource partitioning · Plant–soil feedback

Introduction

One widespread explanation for the maintenance of species diversity is that competition drives species to partition resources and occupy distinct niches (Hutchinson 1959; MacArthur and Levins 1967; Schoener 1989; Silvertown 2004). Competitive interactions with other organisms can cause a species' realized niche to be a subset of the environmental conditions that it can potentially tolerate (Ackerly 2003; Hairston 1980a, b; Hutchinson 1959; Schoener 1974), while facilitative interactions can broaden a fundamental niche (Brooker and Callaghan 1998; Travis et al. 2005). Patterns of resource utilization have been used

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as a measure of resource partitioning among species (e.g., Kahmen et al. 2006; MacArthur 1958; McKane et al. 1990). It is difficult, however, to discern whether present patterns of resource use reflect a reduction in the intensity of current competitive interactions (Silvertown 2004), as most tests assume. Resource utilization patterns could also reflect past adaptations to competitive interactions (Connell 1980) or the importance of facilitative interactions (Bertness and Callaway 1994). To date, there have been few empirical tests of the competitive mechanism assumed by niche models (Silvertown 2004), and the few that have been conducted have yielded conflicting results (Hairston 1980a, b; Jedlicka et al. 2006; Miller et al. 2007; Pacala and Roughgarden 1982; Thomson 2006).

In plant communities where numerous species co-occur despite having similar requirements for light, nutrients, and water (i.e., having similar fundamental niches), realized niches are often described as varying spatially (e.g., rooting depth) or temporally (e.g., phenology) among species (Anderson and Schelfhout 1980; Fargione and Tilman 2005; Parrish and Bazzaz 1976; Parrish and Bazzaz 1979). More recently, it has been suggested that realized niches may vary due to the chemical form of nitrogen (N) utilized by plants (Kahmen et al. 2006; McKane et al. 2002; Miller and Bowman 2002; Nadelhoffer et al. 1996). For example, in Arctic tundra, plant N-form preference correlates with abundance patterns, where the most abundant species uses the most abundant form of available N (McKane et al. 2002). While there is evidence that plants vary in their capacity to assimilate inorganic and organic N (Chapin et al. 1993; Miller and Bowman 2003; Weigelt et al. 2005), the prediction from niche theory that interactions with other species encourages specialization on a particular form of N is largely untested. By comparing N-form uptake in the absence or presence of neighbor species, we test whether plant resource partitioning of chemical forms of N follows the competitive mechanism assumed by niche models.

Plants can alter the availability of different chemical forms of N through microbially mediated feedbacks (Reynolds et al. 2003). While microbes are often more effective at taking up inorganic N than plants (Hodge et al. 2000; Jackson et al. 1989; Kaye and Hart 1997; Schimel and Bennett 2004), over time, plants can have strong effects on N cycling in soils via resource uptake patterns, N₂ fixation, litter quality, root exudation, and alterations of the soil microbial community (Aerts 1999; Ehrenfeld et al. 2005; Hobbie 1992). One mechanism by which plants can influence the availability of different N forms is through the production and exudation of extracellular enzymes (Dakora and Phillips 2002). Extracellular enzymes mediate the decomposition of litter, the breakdown of organic matter, and the mineralization of N and phosphorus (P)

(Sinsabaugh 1994) and can be produced directly by plant roots and associated mycorrhizae to acquire nutrients (Read and Perez-Moreno 2003). Plants and plant-associated soil microbes may also produce extracellular enzymes that act to increase pathogen resistance or act as signal inducers to increase microbial abundance and activity (Somers et al. 2004). Extracellular enzyme activity (EEA) is increasingly used as an assay of microbial community structure and function, decomposition dynamics, and nutrient limitation, yet few studies measure plant-associated activity and fewer still measure how this activity varies among species (Allison et al. 2006; Kourtev et al. 2002). By relating EEA to N-form preferences, we test whether EEA can affect N-form availability and facilitate partitioning of resources among plant neighbors.

Here we examine whether microbially mediated resource partitioning may reduce niche overlap of two dominant species of the alpine tundra: the rosaceous forb, *Acomastylis rossii* (R. Br) Greene, and the bunchgrass, *Deschampsia caespitosa* (L.) P. Beauv. While these species are found in approximately equal abundance in the moist meadow community of the central and southern Rocky Mountains, at about 30% cover, they affect N cycling and microbial activity very differently (Bowman et al. 2004; Steltzer and Bowman 1998; Suding et al. 2004). *Acomastylis* grows and acquires N relatively slowly and is characterized by very high levels of phenolic compounds (20–25% of dry mass) that it releases to the soil through litter decomposition (Bowman et al. 2004) and root exudation (C. Meier, unpublished data). This large input of labile carbon (C) to the soil increases microbial biomass and respiration under *Acomastylis* and, consequently, *Acomastylis* is associated with high N immobilization and low net N mineralization rates (Bowman et al. 2004; Steltzer and Bowman 1998). *Deschampsia*, on the other hand, grows more rapidly and is associated with high rates of net N mineralization and inorganic N uptake (Bilbrough et al. 2000; Monson et al. 2001; Steltzer and Bowman 1998; Theodose et al. 1996). Previous work has shown that the species compete with one another (Suding et al. 2004, 2006) and that the presence of *Acomastylis* litter reduces the growth of *Deschampsia* (Bowman et al. 2004; Suding et al. 2004).

Both *Acomastylis* and *Deschampsia* have the capacity to take up inorganic and organic forms of N (Miller and Bowman 2003), but it is unclear if their uptake preferences are influenced by other neighboring species. To examine N-form preferences of *Acomastylis* and *Deschampsia*, we followed three forms of ¹⁵N-labeled nitrogen (NH₄⁺, NO₃⁻, glycine) in plant, microbial, and soil pools. In combination with these tracer additions, we assessed how interspecific interactions affected N-use patterns by comparing tracer uptake in treatments where we removed either

Acomastylis or *Deschampsia* to treatments where both grew together. To test whether resource partitioning is mediated by the microbial community, we also assessed how the plant species (alone and together) influenced rhizosphere EEAs involved in N, C, and P acquisition. We asked two main questions:

1. Do *Acomastylis* and *Deschampsia* have preferences for different chemical forms of N when grown alone? When grown together, are N preferences altered to reduce niche overlap?
2. Are patterns of rhizosphere EEAs consistent with plant–soil feedbacks? Does rhizosphere EEA suggest that plants can enhance the production of the preferred form(s) of N? Is EEA production altered with the removal of interspecific neighbors?

Materials and methods

¹⁵N tracer experiment

We conducted this work in moist meadow tundra at Niwot Ridge, Colorado (40°03'N, 105°35'W; 3500 m a.s.l.), a Long-Term Ecological Research site and UNESCO Biosphere Reserve, during the 2005 growing season in an area co-dominated by *Acomastylis* and *Deschampsia*.

To test neighbor effects on N-form uptake, we manipulated interspecific interactions by removing one of the two focal species (three treatments: *Acomastylis* removed, *Deschampsia* removed, and neither removed) in all factorial combinations with ¹⁵N-labeled tracer addition (three treatments: labeled NH₄⁺, NO₃⁻, and glycine), for a total of nine treatment combinations.

Each neighbor × N-form treatment combination was replicated six times (two times in each of three blocks), for a total of 54 plots. The plots were 15 × 15 cm, separated by at least 15 cm, and were trenched to a depth of 15 cm. Because previous work has shown that *Acomastylis*-dominated and *Deschampsia*-dominated areas vary in N-pool size, soil moisture, and other abiotic factors (Steltzer and Bowman 1998), all plots originally included both *Acomastylis* and *Deschampsia* and then were randomly assigned to a removal treatment. By repeatedly clipping all non-focal species to ground level, we created plots with *Acomastylis* alone, *Deschampsia* alone, or both growing together. To standardize plant size, we clipped the remaining focal species to a constant size (approximately 25% cover). All removed biomass was collected, dried to a constant mass and weighed.

While removal experiments can have unintended disturbance effects, they avoid problems of confounding species-associated microenvironment effects as all

treatments start with a similar composition prior to removal (Diaz et al. 2003). In addition, the additive design (neighbor removed, neighbor present) is the most appropriate design for competition experiments in the field as it avoids problems of density and plant size that can be issues in substitutive competition designs (Gibson et al. 1999; Goldberg and Scheiner 2001). However, it is important to note that in additive designs the effects of density versus neighbor identity cannot be distinguished because both are manipulated simultaneously.

Three weeks after removal treatments were initiated (approximately at peak growing season), we added ¹⁵N tracers to the plots. The tracer addition consisted of the three N forms (NH₄⁺, NO₃⁻, and glycine) in combination, with one form ¹⁵N-labeled and the remaining two forms unlabeled (following methods described in Miller et al. 2007). Glycine was used as the organic form of N because it is the most abundant amino acid in these soils (Raab et al. 1996) and because plants appear to take up glycine easily relative to more complex forms (Lipson et al. 1999). Soils were injected with 0.6 mM solutions of ¹⁵NH₄⁺ (98% atom enrichment), ¹⁵NO₃⁻ (98% atom enrichment), or ¹⁵N-22(2)-[¹³C]glycine (98% atom enrichment ¹⁵N, 99% atom enrichment ¹³C-2), in combination with equal parts of 0.2 mM unlabeled N, for a total solution concentration of 1.0 mM N. We used a ¹⁵N-label that was threefold more concentrated than the unlabeled N to deliver enough ¹⁵N in the presence of unlabeled forms to detect N uptake, but at a low enough N concentration to avoid a fertilization effect (see Miller et al. 2007 for further discussion). The ¹⁵N tracer was injected to depth of 7.5 cm at eight points within a 10 × 5 cm rectangle around the target plants, delivering approximately 3.2 μg¹⁵N/g soil, or 3% of the total soil ¹⁵N pool. The relative availability of inorganic and organic N in the alpine tundra is highly spatially and temporally dynamic (Brooks et al. 1996; Miller and Bowman 2003; Raab et al. 1996). The tracer was added at an amount well within the range of natural variability (approx. 0.1–3.1 μgN/g soil, 0.1–6.5 μgN/g soil, and 0.9–256.8 μgN/g soil for NO₃⁻, glycine and NH₄⁺, respectively; after Miller and Bowman 2003) but at a low concentration to minimize fertilization effects. The amount of N added is only a small fraction of the annual N demand of *Acomastylis* and *Deschampsia* (7 and 6.1 mg N/g, respectively) (Bilbrough et al. 2000). Based on measured N pools adjacent to the labeling site (18.5 and 0.2 μgN/g dry soil NH₄⁺ and NO₃⁻, respectively), we estimated that >100% of the NO₃⁻ pool was labeled and approximately 17% of the soil NH₄⁺ pool was labeled. With each injection, the needle was slowly withdrawn to uniformly label the soil column. We added the label one block at a time and recorded the time of label addition for each block. To follow the fate of the ¹⁵N tracer in plant and soil pools, we harvested the plant and soils 6 h

after injection, keeping the time between label addition and harvest constant for all blocks. The 6 h period was chosen to minimize the potential for microbial transformation of the added N forms (NH_4^+ , glycine), as is standard in studies of organic N uptake by plants (e.g., Nordin et al. 2004).

Recovery of ^{15}N tracers

We followed the tracer in four pools: (1) exchangeable inorganic N (NH_4^+ and NO_3^-), (2) exchangeable dissolved organic N (DON), (3) CHCl_3 -labile microbial N, and (4) focal plant N. Soils were harvested in the field by removing the surrounding soil volume, including the target plants. After removal, the plugs were placed on ice and transported immediately to the laboratory. We separated the plant material from the soils and, in samples where *Acomastylis* and *Deschampsia* were both present, the species from each other. The plant material was then divided into live aboveground (leaves, stems, inflorescences) and belowground (root, rhizome) tissue. Plants were washed in tap water to remove remaining soil, immersed in 0.5 mM CaCl_2 + 1.0 mM KCl for 2–3 min to remove adsorbed ^{15}N , and rinsed well with deionized water. Dried and ground plant tissue was analyzed on a mass spectrometer (Europa Integra, Northwich, UK) for ^{15}N at the Stable Isotope Facility, University of California, Davis. Instrument precision was 0.4‰.

The soil samples were sieved through a 2-mm mesh screen to remove rocks and large debris. Soils were then extracted in 0.5 N K_2SO_4 for inorganic N and total nitrogen (TN). We measured inorganic N by colorimetry on a continuous flow autoanalyzer (Lachat QuikChem 8000; Lachat Instruments, Loveland, CO) and extractable TN and CHCl_3 -N colorimetrically as inorganic N following high-temperature persulfate digestion (Cabrera and Beare 1993). We calculated N concentrations and ^{15}N enrichments of extractable DON as the extractable TN minus extractable inorganic N. Chloroform-labile N was determined using the chloroform fumigation extraction technique, and soils were extracted with 0.5 N K_2SO_4 , as described above. We calculated microbial N as the CHCl_3 -labile N minus extractable TN, divided by a factor of 0.54 to correct for chloroform-labile N extraction efficiency (Brookes et al. 1985; Jenkinson et al. 2004). To measure the recovery of ^{15}N , we diffused the inorganic N, total N, CHCl_3 -N soil extracts for 5 days onto acidified disks (Brooks et al. 1989), which were analyzed using an automated Rittenberg apparatus-mass spectrometer (Mulvaney et al. 1990) at the ^{15}N Analysis Service of the University of Illinois (Champaign–Urbana, IL).

We used the concentration of ^{15}N in plant and soil pools to estimate uptake (F), calculated as $F = [T(A_S - A_B)]/A_F$ where T = the mass of N in the sample, A_S = atom

percentage of excess ^{15}N in sample, A_B = atom percentage of excess ^{15}N in the background, and A_F = atom percentage of excess ^{15}N in the tracer. The quantity F was divided by the sample mass to determine ^{15}N concentration in the sample (Hauck and Bremner 1976). Background enrichment was assumed to be 0.3663 atom% ^{15}N . Since this amount is negligible when compared to the amount in the tracer (98 atom% ^{15}N), the denominator of F is not corrected for this source. We calculated recovery of the ^{15}N label in soil pools from the ^{15}N concentration in the pool, the total mass of the pool, and the quantity of label added to each plot. To account for the possibility of our $^{15}\text{NH}_4^+$ label being diluted in the ambient N pool, we corrected uptake estimates by assuming that the NH_4^+ soil pool was 18.5 $\mu\text{gN/g}$ dry soil. We chose to primarily present the uncorrected results because the spatial and temporal variability in soil N pools is known to be large and, therefore, we assumed that the size of the N pool across the experiment was not consistent. Regardless, results are qualitatively similar and both are reported below. For estimated whole-plant recovery, we did not have accurate measures of biomass due to the difficulty in recovering all live roots. Instead, we harvested plants from near the study site to derive an estimate of the mass of *Deschampsia* and *Acomastylis* at 25% cover for the given plot size. We used the estimated biomass to scale up sample plant tissue ^{15}N data to total ^{15}N uptake in a similar manner as that described by Nordin et al. (2004). Since the total recoveries are estimated, we express plant ^{15}N uptake as the known plant tissue concentrations. We used the ^{15}N plant tissue concentrations to estimate niche breadth for each species in the presence and absence of the interspecific neighbor. Niche breadth was calculated using the proportional concentration of each N form. Calculations were done using the Shannon–Weaver information statistic (H') and the reciprocal of the Simpson's diversity index (B) where $H'_i = \sum_{j=1}^r p_{ij} \log p_{ij}$ and $B_i = 1/\sum_{j=1}^r p_{ij}^2$ and p = the proportional uptake of each N form and $r = 3$ (Levins 1968).

Extracellular enzyme assays

To measure functional differences in rhizosphere microbial community, we examined EEA in soils associated with *Acomastylis* and *Deschampsia* and in soils where both species were grown together. Soils were collected from areas adjacent to the ^{15}N experiment described above and within the same week in July 2005. To sample rhizosphere soils, we selected six replicate plants in the field with one of four growing conditions: *Acomastylis* growing only with conspecifics, *Deschampsia* growing only with conspecifics, *Acomastylis* with *Deschampsia* as the nearest neighbor, and *Deschampsia* with *Acomastylis* as the nearest neighbor. It is important to note that, due to logistical constraints, this

design is substitutive (as opposed to the N-uptake experiment, which is additive). While it does not involve potential disturbance effects associated with species removals, it has the potential to include species-associated microenvironment effects.

For each species combination, we removed the plant(s) and the 10-cm³ volume of soil surrounding the plant. We placed the soils and plants on ice and transported them back to the laboratory. Plants were removed from the sample, dried at 70°C, ground, and analyzed for foliar concentrations of C and N on an elemental analyzer. As plants were removed, soils surrounding and attached to the roots were shaken off, collected, sieved as above, and frozen until analysis. These rhizosphere soil samples were then assayed for activity of extracellular enzymes involved in the decomposition of simple and complex C compounds (α -glucosidase, β -glucosidase, β -D-cellobiohydrolase, β -xylosidase, phenol oxidase, and peroxidase), nitrogen [*N*-acetyl- β -glucosaminidase, leucine amino peptidase (LAP), urease] and phosphorus (acid phosphatase) activity using 4-methylumbelliferyl (MUB) α -D-glucopyranoside, 4-MUB β -D-glucopyranoside, 4-MUB β -D-cellobioside, 4-MUB β -D-xylopyranoside, L-3,4-dihydroxyphenylalanine (DOPA), 4-MUB *N*-acetyl- β -D-glucosaminide, L-leucine-7-amido-4-methylcoumarin hydrochloride, and 4-MUB phosphate, as substrates, respectively, following published protocols (Saiya-Cork et al. 2002; Sinsabaugh et al. 2003) that have been used successfully to measure enzyme activities in alpine soils (Grandy et al. 2007). Sample suspensions were prepared by placing 1.0 g rhizosphere soil in 125 ml acetate buffer (50 mM, pH 5) and homogenizing the mixture for 1 min using an electric mixer.

The urease, phenol oxidase, and peroxidase assays were conducted in clear 96-well microtiter plates. Sixteen replicate wells were used for each assay; eight additional wells were used as negative substrate controls, and another eight wells served as negative sample controls. The assay wells received 200 μ l of sample suspension; for the phenol oxidase and peroxidase assays, the assay well received an additional 50 μ l of 10 mM DOPA substrate. The negative sample control wells contained 200 μ l of sample and 50 μ l of acetate buffer. The negative substrate control wells received 200 μ l aliquots of acetate buffer and 50 μ l substrate. For peroxidase assays, each well also received 10 μ l of 0.3% H₂O₂. The plates were placed in an incubator at 13°C, for 18–24 h. For urease, prior to reading activity, 40 μ l salicylate and 40 μ l cynaurate were added to each well. Activity was measured spectrophotometrically at 460 nm using a VERSAmax plate reader (Molecular Devices, Sunnyvale, CA).

The remaining seven assays were conducted on black 96-well microtiter plates. The assay design was similar to that described above except that reference standards (eight

wells) and quench controls (eight wells per sample) were added to each plate. The reference standard for all enzymes except LAP was 10 μ l 4-methylumbelliferone; for LAP, 7-amino-4-methylcoumarin was used. Quench control wells contained 200 μ l of sample suspension and 50 μ l of reference standard. The assays were incubated as above, and reactions were terminated by adding 10 μ l 1.0 M NaOH to each well. Fluorescence was measured using a Labsystems Fluoroskan II spectrofluorometer (GMI, Ramsey, MI) with excitation filters set at 365 nm and emissions at 460 nm.

Statistical analyses

Differences in ¹⁵N uptake between *Acomastylis* and *Deschampsia*, and the effect of neighbors and N form on plant ¹⁵N concentrations were analyzed using ANCOVA in JMP ver. 5.1.2 (SAS Institute, Cary, NC), with species, N form and neighbor presence as main effects and biomass clipped from plots and the time required to process samples after ¹⁵N addition as covariates. The amount of biomass clipped from plots did not vary among species combinations ($F_{2,35} = 0.1$, $P = 0.917$) or N-label treatments ($F_{2,35} = 0.2$, $P = 0.653$); nevertheless we sometimes found clipped biomass to significantly covary with the recovery of ¹⁵N. Prior to analysis, plant ¹⁵N concentrations were log-transformed to meet assumptions of normality and heteroscedasticity. We tested for differences among N forms and response to neighbor presence using Student's *t* tests. Previous work suggests that these species can take up glycine directly (Miller and Bowman 2003; Miller et al. 2007), making it likely that ¹⁵N-glycine was taken up intact. However, since it is probable that microbial transformation of glycine into inorganic forms occurred within the 6-h period, we interpret glycine uptake more loosely as the uptake of ¹⁵N from ¹⁵N-glycine-labeled soils. Both measures of niche breadth were analyzed using an ANOVA with species and neighbor presence as main effects.

To examine the differences in the total concentrations of N and ¹⁵N in the soil inorganic N, dissolved organic N, and microbial N pools, we analyzed the data using an ANCOVA with main effects of species combination (*Acomastylis*, *Deschampsia*, or *Acomastylis* + *Deschampsia*) and N form, random effect of block, and biomass clipped from plots as a covariate. In cases where block was not significant, we dropped it from the model. To test how species combination and N form affected the proportion of ¹⁵N recovered in soil and plant pools, we determined the relative recovery of ¹⁵N label in each pool, arcsine-transformed the data to meet assumptions of normality, and then tested for species and N-form effects in a MANOVA using R (ver. 2.4.1). We use Wilk's λ statistic in the MANOVA, but results were identical using Pillai's trace and Roy's

greatest root statistic. We tested for the effects of plant species and the presence of an interspecific neighbor on all enzyme activities using MANOVA. To examine the influence of each of the nine EEAs on the results from the multivariate model, we tested for effects of species and neighbors with univariate ANOVAs for each enzyme. Activities were log-transformed prior to analyses to meet assumptions of normality and heteroscedasticity. Finally, we tested the effect of species and neighborhood on plant foliar carbon and N content using ANOVA.

Results

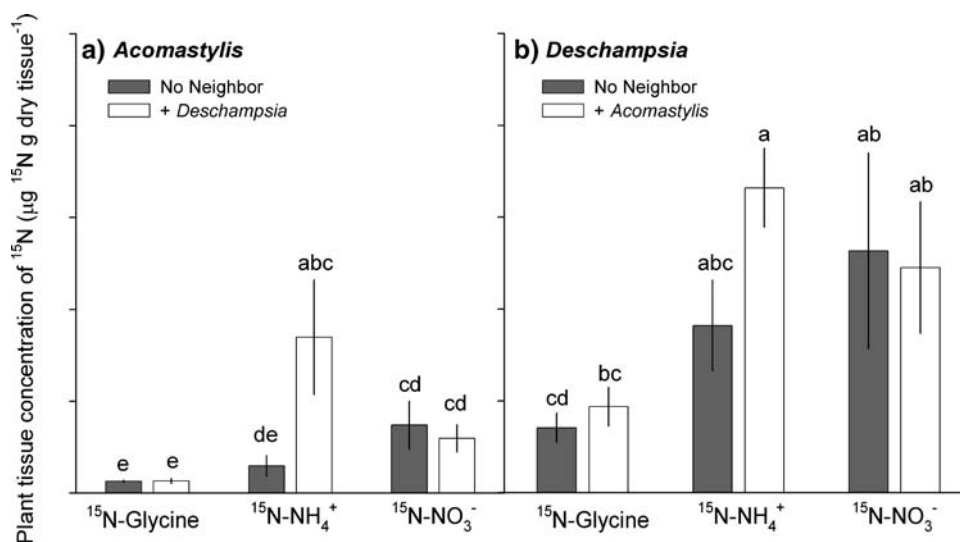
Deschampsia, the species associated with faster N-cycling rates, assimilated all forms of N at a faster rate than *Acomastylis* ($F_{1,44} = 41.3$, $P < 0.0001$; Fig. 1). However, the two species showed similar N preferences (non-significant species \times N-label interaction, $F_{1,44} = 0.4$, $P = 0.650$) and response to neighbors (non-significant species \times neighbor interaction, $F_{1,44} = 0.2$, $P = 0.680$). The amount of N assimilated by the plants varied with N form ($F_{2,44} = 14.2$, $P < 0.0001$; Fig. 1a) in that inorganic forms of N were preferred to glycine (post hoc tests, $F_{1,44} = 27.9$, $P < 0.0001$). Overall, uptake increased in the presence of the interspecific neighbor ($F_{1,44} = 4.6$, $P = 0.0378$; Fig. 1), and the presence of neighbors also caused a change in N-form preference (significant N label \times neighbor interaction, $F_{2,44} = 3.4$, $P = 0.0404$; Fig. 1). Neighbors caused an increase in NH_4^+ uptake ($F_{1,44} = 12.1$, $P = 0.001$) but no change in the uptake of NO_3^- or glycine ($F_{1,44} = 0.0001$, $P = 0.979$ and $F_{1,44} = 0.15$, $P = 0.700$, respectively) where the uptake of NH_4^+ by *Acomastylis* was more responsive to neighbors than *Deschampsia* (Fig. 1a). We found that neither biomass clipped from the

plots nor incubation time was significantly correlated with plant ^{15}N uptake ($F_{1,44} = 0.07$, $P = 0.799$ and $F_{1,44} = 0.31$, $P = 0.579$, respectively). When we adjusted the concentration estimates to account for dilution of the NH_4^+ label with the initial soil pool, uptake values increased by a factor of 6.6. When the statistical model was corrected for dilution, NH_4^+ was preferred to NO_3^- , and the inorganic forms were both preferred to glycine ($F_{2,44} = 71.4$, $P < 0.0001$; $P < 0.05$). There was no change in the qualitative pattern of species N-form in the effect of competition on plant preferences.

Similar to plant ^{15}N uptake, we found that niche breadth varied between the species for both H' and B ($F_{1,18} = 9.6$, $P = 0.006$; $F_{1,18} = 9.9$, $P = 0.006$, respectively). The N niche of *Deschampsia* ($H' = 0.92 \pm 0.04$, $B = 2.31 \pm 0.11$) was broader than that of *Acomastylis* ($H' = 0.72 \pm 0.05$, $B = 1.81 \pm 0.12$). However, niche breadth was not altered by the presence of a neighbor ($F_{1,18} = 0.2$, $P = 0.646$; $F_{1,18} = 0.3$, $P = 0.597$, respectively) for either species (neighbor \times species, $F_{1,18} = 0.5$, $P = 0.493$; $F_{1,18} = 0.8$, $P = 0.373$, respectively).

The ^{15}N concentrations found in the inorganic N pool varied among N forms ($F_{2,27} = 11.2$, $P < 0.001$; Fig. 2a) where, after 6 h, concentrations of $^{15}\text{NH}_4^-$ and ^{15}N -glycine were significantly higher than those of $^{15}\text{NO}_3^-$ (Student's t , $P < 0.05$). Concentrations of ^{15}N in the inorganic N pool only marginally varied by species presence ($F_{2,27} = 2.6$, $P = 0.09$; Fig. 2a), with the highest concentrations being found when both species were present (post hoc, $F_{1,27} = 4.6$, $P = 0.042$). However, there was also a significant interaction between neighbor presence and N form ($F_{4,27} = 3.3$, $P = 0.025$; Fig. 2a) that was primarily driven by higher concentrations of $^{15}\text{NH}_4^-$ and ^{15}N -glycine than $^{15}\text{NO}_3^-$ when both species were present. In addition, when *Deschampsia* was grown with a neighbor, the amount of

Fig. 1 The effect of a neighbor on nitrogen (N) uptake for *Acomastylis* (a) and *Deschampsia* (b) 6 h after labeling with different N forms. Different letters indicate that means significantly differ in the Student's t tests of label and neighbor treatments (mean \pm 1 SE, $n = 5$ –6 for all samples)



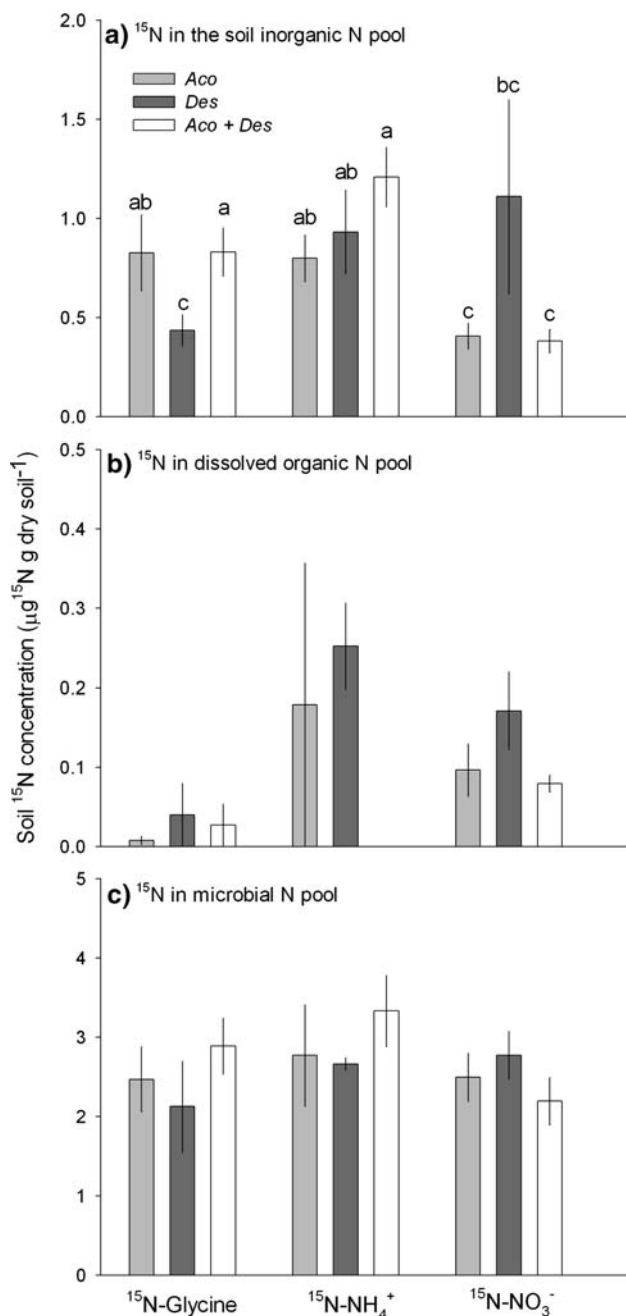


Fig. 2 The effect of species neighborhood on ^{15}N concentrations of soil inorganic N (a), dissolved organic N (b), and microbial N pools (c) labeled with three different N forms. Different letters indicate that means significantly differ in Student's t tests (mean ± 1 SE, $n = 5$ –6 for all samples). The concentration of ^{15}N in the inorganic N pool varied with neighbor and label treatments, but there were no differences in the microbial or dissolved organic N pool

^{15}N -glycine present in the inorganic pool increased (Fig. 2a). The amount of biomass clipped from the plots had a tendency to be negatively correlated with ^{15}N concentrations in the inorganic pool ($F_{1,27} = 4.1$, $P = 0.052$). When we considered the possibility of dilution of the NH_4^+ label, we found a difference among N forms ($F_{2,27} = 31.4$,

$P < 0.001$), where the concentration of $^{15}\text{NH}_4^+$ was significantly higher than those of both $^{15}\text{N-glycine}$ and $^{15}\text{NO}_3^-$ (Student's t , $P < 0.05$).

The ^{15}N enrichment of the dissolved organic N pool was small and did not vary between species ($F_{2,31} = 2.9$, $P = 0.127$; Fig. 2b) or N form ($F_{2,31} = 2.8$, $P = 0.087$; Fig. 2b). Similarly, ^{15}N enrichment of the microbial pool did not differ according to the form of N ($F_{2,30} = 0.5$, $P = 0.609$; Fig. 2c), nor did it differ among neighbor treatments ($F_{2,30} = 1.7$, $P = 0.206$; Fig. 2c). However, when the dilution of the NH_4^+ label was taken into account, microbial uptake of labels differed ($F_{2,30} = 202.4$, $P < 0.0001$), where NH_4^+ was preferred to the other forms ($P < 0.05$). The enrichment of DON was not altered by dilution ($F_{2,31} = 1.5$, $P = 0.241$), and the amount of biomass clipped was not correlated with ^{15}N concentration in the microbial pool ($F_{1,30} = 1.8$, $P = 0.179$). Finally, the size of the total unlabeled inorganic, dissolved organic, and microbial N pools did not vary by neighbor treatment ($F_{1,47} = 2.2$, $P = 0.147$; $F_{1,47} = 1.2$, $P = 0.288$; $F_{1,47} = 1.3$, $P = 0.253$, respectively), suggesting that any disturbance-related changes in pool sizes were minimal and that differences in density among neighbor treatments did not result in differences in N availability.

Among the plant and soil pools, the microbial N pool showed the greatest recovery of ^{15}N (75% of total) (Fig. 3). A smaller portion of the ^{15}N label was recovered in plant tissues (0.7%), with most of that being found in the roots (0.6%). Multivariate ANOVA results indicated significant species effects (Wilk's $\lambda = 0.52$,

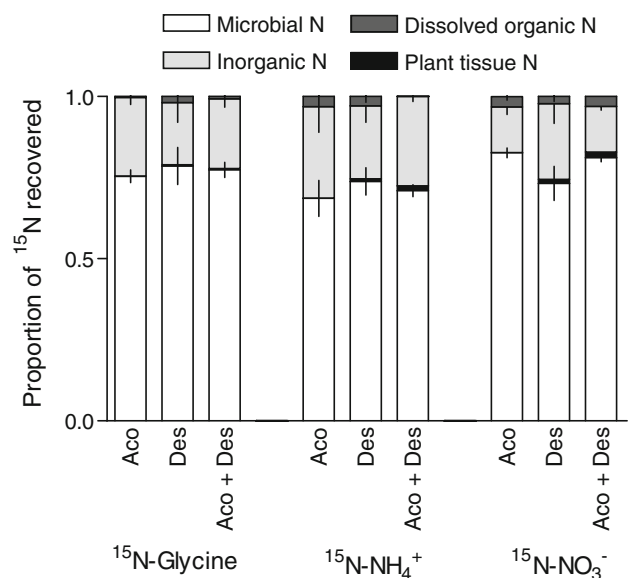


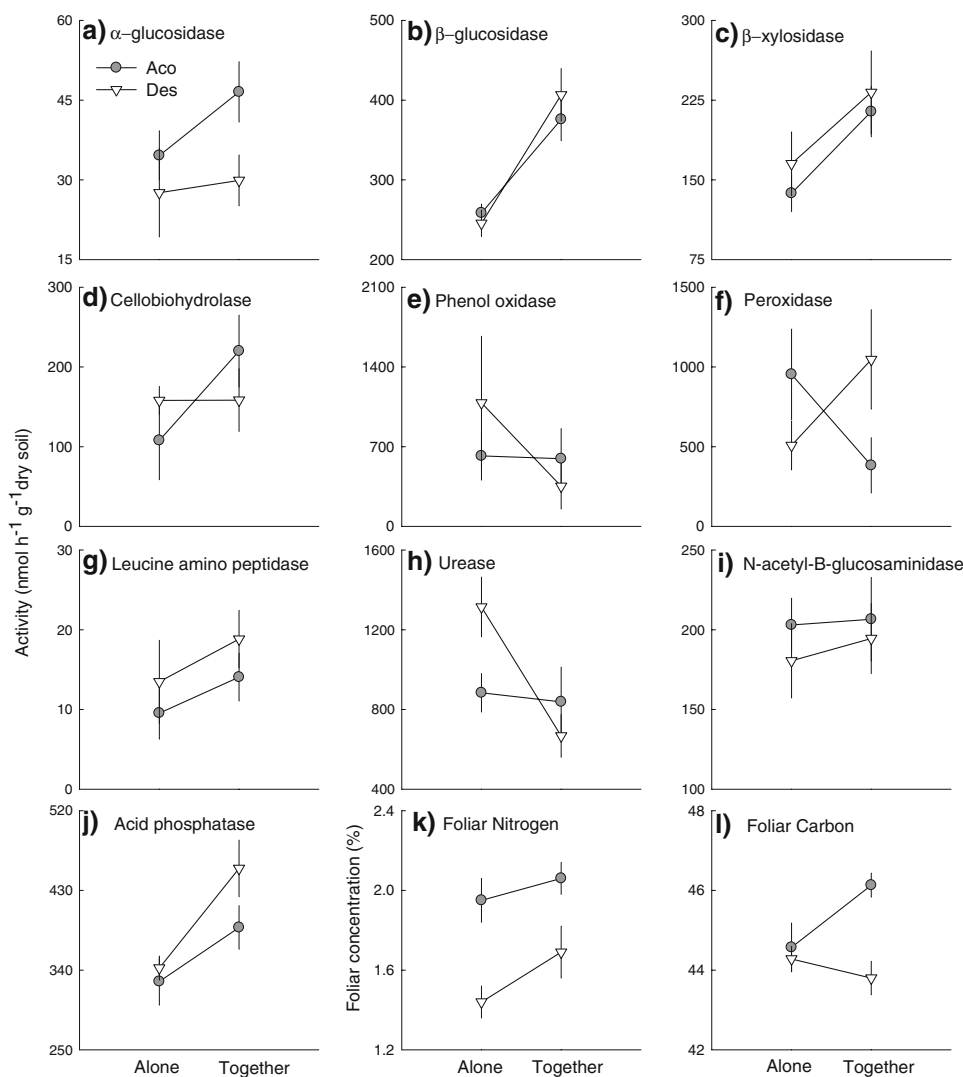
Fig. 3 The effect of plant species on the recovery of ^{15}N in plant and soil pools using labels of different N forms. Error bars are shown for each pool and represent ± 1 SE. There was greater a recovery in *Deschampsia* and a greater recovery of $^{15}\text{NO}_3^-$ in plant and microbial N

$F_{10,74} = 2.86$, $P = 0.0046$; Fig. 3), and a significant effect of N form on the recovery of ^{15}N (Wilk's $\lambda = 0.48$, $F_{10,74} = 3.32$, $P = 0.0013$; Fig. 3). The differences among species were largely due to increased recovery in plant tissues of *Deschampsia* compared to *Acomastylis* ($F_{2,35} = 6.7$, $P < 0.01$). The differences among N-form treatments were due to a greater recovery of $^{15}\text{NO}_3^-$ in plant ($F_{2,35} = 7.7$, $P < 0.01$) and microbial N pools ($F_{2,35} = 3.8$, $P < 0.05$).

Contrary to predictions, rhizosphere EEA did not differ between areas dominated by *Acomastylis* and areas dominated by *Deschampsia* (Wilk's $\lambda = 0.48$, $F_{10,10} = 1.07$, $P = 0.4582$). However, multivariate ANOVA results indicated that the EEA was significantly increased in areas where both species were present (Wilk's $\lambda = 0.14$, $F_{10,10} = 6.37$, $P = 0.0036$). The univariate tests revealed that the activity of five of the ten enzymes examined (α -glucosidase, β -glucosidase, β -xylosidase, leucine amino peptidase, and acid phosphatase) increased when

Deschampsia and *Acomastylis* were grown together ($F_{1,19} = 3.5$, $P = 0.075$; $F_{1,19} = 62.8$, $P < 0.0001$; $F_{1,19} = 11.4$, $P = 0.003$; $F_{1,19} = 12.0$, $P = 0.003$; $F_{1,19} = 5.0$, $P = 0.038$, respectively; Fig. 4a–c, g, j). In three cases (cellobiohydrolase, phenol oxidase, *N*-acetyl- β -glucosaminidase), neither species nor neighbor identity significantly affected activity (Fig. 4d, e, i, respectively). Peroxidase, an enzyme used to break down large C molecules particularly associated with polyphenol degradation, showed both species and neighbor dependence (significant interaction, $F_{1,19} = 5.9$, $P = 0.03$; Fig. 4f). In the *Acomastylis* rhizosphere, peroxidase activity significantly decreased when *Deschampsia* was present. Activity in the *Deschampsia* rhizosphere showed the opposite pattern, with an increase in peroxidase activity in the presence of *Acomastylis*. Finally, urease, an enzyme associated with N mineralization, decreased in activity when both species were grown together, particularly in the rhizosphere of *Deschampsia* ($F_{1,19} = 4.3$, $P = 0.05$, Fig. 4h).

Fig. 4 The effect of neighbor identity on *Acomastylis* (Aco) and *Deschampsia* (Des) rhizosphere extracellular enzyme activity (EEA) involved in the degradation of simple C compounds (a–d), complex C compounds (e–f), N compounds (g–i), P compounds (j), and foliar concentrations of N and C (k–l)



Consistent with the EEA data, foliar concentrations of N in *Acomastylis* and *Deschampsia* marginally increased in plants collected from areas where both species were present ($F_{1,20} = 3.2$, $P = 0.087$; Fig. 4k). *Acomastylis* foliar N content was significantly higher than that of *Deschampsia* ($F_{1,20} = 19.3$, $P < 0.001$, Fig. 4k), and it also had a higher foliar C concentration, likely due to high concentrations of phenolic compounds, which further increased when *Deschampsia* was present ($F_{1,20} = 5.6$, $P = 0.03$; Fig. 4l).

Discussion

To determine whether partitioning of N between two co-dominant alpine species follows the competitive mechanism assumed by niche models and whether microbial activity supports the acquisition of plant preferred N forms, we used a combination of ^{15}N tracers and neighbor manipulations to simultaneously track plant and microbial N uptake. In addition, we compared foliar N concentrations and EEA from undisturbed areas where the plants were dominant or co-dominant to determine if natural patterns were consistent with our experimental results. Contrary to our expectations, N-form preferences and niche breadth was not affected by interspecific interactions and, instead, plants increased N uptake in response to the presence of neighbors.

Our ^{15}N tracer studies indicated that the presence of a neighbor can drive plants to alter N-uptake patterns. We predicted that interspecific competition would cause the two species to specialize on different N sources, thereby decreasing niche overlap. Instead, we found that the two species had similar N-form preferences, and competition did not drive a change in niche breadth. Both plants preferred inorganic N forms to glycine, and neighbors caused an increase in ammonium uptake, particularly for *Acomastylis* (Fig. 1a). *Deschampsia* increased total N uptake by 30% in the presence of *Acomastylis*, but this increase was slightly more uniform across N forms (Fig. 1b). *Deschampsia* had a larger niche breadth than *Acomastylis*, but again the presence of a neighbor had no effect on niche breadth for either species. The difference in niche breadth can be explained by differences in proportional glycine uptake where it accounts for only 9% of the total N uptake in *Acomastylis* but 16% in *Deschampsia*. While *Acomastylis* can inhibit the growth of *Deschampsia* (Bowman et al. 2004; Suding et al. 2004), *Deschampsia*'s faster N uptake and a greater ability to use available N may contribute to its persistence and dominance in the alpine.

We expected that in addition to plant N uptake, plant–soil feedbacks would influence microbial N uptake and plant persistence. We hypothesized that plants would influence microbial N uptake to support production and

acquisition of the plant preferred N form. In particular, we predicted that the release of phenolic compounds from *Acomastylis* would alter rhizosphere EEA and microbial N uptake. Instead, we found that microbial N uptake was similar in the rhizosphere of *Acomastylis* and *Deschampsia*. We found microbes assimilated both inorganic and organic N rapidly regardless of the associated plant or the neighbor treatment (Fig. 2). Assuming the ammonium label was diluted in the ambient soil pool, we found that microbes preferred ammonium but showed no response to neighbor treatment. This is consistent with results from previous studies that have shown that microbes often preferentially assimilate ammonium (Jackson et al. 1989; Nordin et al. 2004).

The most striking result from this study is that, for both plant species, the presence of an interspecific neighbor caused an increase in plant N uptake, an increase in rhizosphere microbial enzyme activity, and a tendency to increase plant foliar N. These results are surprising because it is often thought that in N-limited systems competition would decrease the amount of resources taken up by each plant. For example, in a similar study in dry meadow tundra, plant N uptake was decreased by as much as 50% by the presence of a neighbor (Miller et al. 2007). It is possible, but unlikely, that our result is an artifact of the experimental treatments. An increase in the immobilization of N due to the disturbance of the neighbor removal could potentially decrease the N available when plants were grown alone. The removal treatment, however, consisted of a similar amount of biomass clipped from all plots, and the biomass removed was accounted for as a covariate in the analyses. Differences in mineralization rates among the removal treatments could also differentially dilute the ^{15}N label and change uptake estimates. However, our N-pool data showed no difference in available inorganic N across neighbor treatments, and accounting for dilution (Gilbert et al. 1982) did not change the relative uptake estimates across treatments. Furthermore, differences in density among neighbor treatments could have caused changes in plant N uptake, but the N-pool data suggest that density did not affect N availability. Most convincingly, the ^{15}N uptake results are consistent with the EEA data, taken in adjacent undisturbed plots, which showed moderate increases when both species were present. We also found that foliar N concentrations of plants from co-dominant areas were marginally higher than from areas where either species was dominant. While this could be explained by competitive release and greater nutrient use efficiency in the presence of intraspecific neighbors, it is consistent with our data suggesting that plant N uptake may be increased in the presence of interspecific neighbors.

One potential mechanism to explain increased N uptake may be provided by root foraging theory (de Kroon and

Mommer 2006). When nutrients are limiting, competition may drive plants to allocate more biomass to roots in a form of scramble competition for N (Craine 2006; Gersani et al. 2001). *Deschampsia* and *Acomastylis* may allocate more biomass to roots or increase root exudates when in association with each other, increasing their ability to take up N and decreasing the pool of N available to the other species and the microbial community. Plants can respond to nutrient stress by increasing their allocation to C exudates to promote the growth of mycorrhizae (Chapin 1980) and, in this case, the response to neighbors may be equivalent to a nutrient stress. However, this experiment was too short in duration to measure a difference in root biomass among treatments, and we did not measure C exudation or mycorrhizal infection, but it is possible that over longer periods, there is a shift in allocation patterns in response to neighbors. Alternatively, facilitation, an important mechanism in other alpine plant communities (Callaway et al. 2002), may play a role in structuring the moist meadow community. *Acomastylis* and *Deschampsia* may alter the microbial community and soil resource pool in a complementary manner by increasing the availability of inorganic N through faster N cycling (*Deschampsia*) while also increasing the retention of N through phenolic inputs (*Acomastylis*). Neighbors facilitating N uptake in other plant species has been demonstrated in studies where one species is an N-fixer (Fargione et al. 2007) or a hemiparasite (Bardgett et al. 2006); our data suggest that this may be a broader phenomenon.

If plants respond to the presence of a neighbor by increasing N uptake, increased plant diversity and/or density could result in increased N limitation of the microbial community. Our ^{15}N tracer experiment did not support this hypothesis, and we found that over the first 6 h, microbial N uptake rates were high and not limited by plants or neighbor treatments. However, we found that microbial EEA, which may be more reflective of long-term dynamics, showed patterns consistent with this hypothesis. The multivariate analyses revealed that EEA increased when both species were present. Rather than microbial limitation induced by the plants, it is also possible that an increased availability of C or N or, perhaps, the production and exudation of plant signaling compounds in the rhizosphere may have allowed for the production of more enzymes.

While the general trend of microbial EEA was to increase with the presence of neighbors, there were two interesting exceptions: urease (Fig. 4h) and peroxidase (Fig. 4f). Urease activity in the rhizosphere of *Deschampsia* was high, consistent with *Deschampsia*'s association with high N mineralization rates, but it decreased significantly when *Deschampsia* was grown in association with *Acomastylis*. It is interesting to note that urease activity decreased when both species were present,

suggesting that inorganic N availability in soils may be reduced. This is not consistent with our ^{15}N results that show no change in N pools and increased plant N uptake in the presence of neighbors. However, the link between enzyme activity and soil N availability can be complex, and further research is needed to understand the relationship between rhizosphere urease activity, soil N availability, and plant N uptake. Moreover, our EEA was limited to only one point in time, making it difficult to predict if these patterns are consistent over time. Peroxidase activity, an enzyme associated with the breakdown of complex aromatic compounds, was elevated in the rhizosphere of *Acomastylis* compared to *Deschampsia*. When the two species were grown together, however, peroxidase activity decreased in the rhizosphere of *Acomastylis* and increased in the rhizosphere of *Deschampsia*. Since *Acomastylis* has high phenolic concentrations (Bowman et al. 2004), high peroxidase activity in the *Acomastylis* rhizosphere is not surprising. It is unclear, however, why this activity decreases in the presence of *Deschampsia*. Previous studies have shown that the effect of phenolics on microbial activity and the production of polyphenol oxidase is complex and can depend on the availability of N and soil pH (Castells and Penuelas 2003). The presence of *Deschampsia* may dilute the response of *Acomastylis*, and the increase seen in the *Deschampsia* rhizosphere when neighbored by *Acomastylis* may be a response to higher phenolic concentrations in the soil. Alternatively, the presence of *Deschampsia* may increase the availability of simple C and N compounds to the soil and decrease the need for microbes to invest in the breakdown of larger C compounds.

In conclusion, our results indicate that plant neighborhood can influence N uptake and N-form preference of two co-dominant alpine plants, *Acomastylis* and *Deschampsia*. Interspecific interactions drove species to increase ammonium uptake rather than to partition N forms. While this is not what is predicted by competitive niche mechanisms typically invoked by resource partitioning, it points to a possible facilitative mechanism to explain the persistence of the two dominants in this community. Our results also suggest that increased EEA may be associated with the increase of plant N uptake, possibly due to the interactions between the rhizospheres of the two species. Further research is needed to understand how such rhizosphere interactions may influence N uptake and plant coexistence.

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