

PLANT UPTAKE OF INORGANIC AND ORGANIC NITROGEN: NEIGHBOR IDENTITY MATTERS

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Abstract. The importance of interspecific competition as a cause of resource partitioning among species has been widely assumed but rarely tested. Using neighbor removals in combination with ¹⁵N tracer additions in the field, we examined variation among three alpine species in the uptake of ¹⁵N-NH₄⁺, ¹⁵N-NO₃⁻, and ¹⁵N-¹³C-[2]-glycine in intact neighborhoods, when paired with a specific neighbor, and when all neighbors were removed. Species varied in the capacity to take up ¹⁵N-labeled NH₄⁺, NO₃⁻, and glycine in intact neighborhoods and in interspecific pairs. When interspecific neighbor pairs were compared with no neighbor controls, neighbors reduced ¹⁵N uptake in target species by as much as 50%, indicating competition for N. Furthermore, neighbor identity influenced the capacity of species to take up different forms of N. Thus, competition within interspecific neighbor pairs often caused reduced uptake of a particular form of N, as well as shifts to uptake of an alternative form of N. Such shifts in resource use as a result of competition are an implicit assumption in studies of resource partitioning but have rarely been documented. Our study suggests that plasticity in the uptake of different forms of N may be a mechanism by which co-occurring plants reduce competition for N.

Key words: alpine dry meadow community; ammonium; ¹⁵N uptake; glycine; neighbor removals; nitrate; nutrient limitation; plant competition; resource partitioning; species coexistence.

INTRODUCTION

The maintenance of species diversity under conditions of resource limitation is often attributed to nonequilibrium processes (e.g., spatial or temporal variation in resource availability), neutral processes (e.g., competitive equivalence), or complementary patterns of resource use through niche separation (e.g., resource partitioning). Any one of these processes could slow rates of competitive displacement and/or facilitate species coexistence (Chesson 2000, Loreau et al. 2001, Silvertown 2004), thus enabling the community to support a greater number of species. Nitrogen (N) is a key limiting resource to primary productivity in many terrestrial communities, and yet species diversity in these communities is often high (Gough et al. 2000). Because of this apparent disconnect between nutrient limitation and species diversity, there have been many studies examining the potential for partitioning of soil N spatially and temporally (McKane et al. 1990, Gebauer and Ehleringer 2000, Duke and Caldwell 2001), and via the uptake of different chemical forms of N (Näsholm et al. 1998, Nordin et al. 2001, McKane et al. 2002, Miller and Bowman 2002, Kahmen et al. 2006). To date, the

strongest evidence for plant partitioning by N form has been that species differ in their uptake of different chemical forms of N, either when surrounded by neighbors in the field (e.g., Näsholm et al. 1998, McKane et al. 2002, Kahmen et al. 2006), or when isolated in the laboratory (Kielland 1994, Raab et al. 1999, Miller and Bowman 2002). A basic assumption underlying these studies is that species are reducing competition for the resource by taking up different forms of N.

While variation among species in the capacity to take up different forms of N suggests that resource partitioning could occur, this variation alone is not sufficient to explain species coexistence via a competitive mechanism. First, species that overlap in the use of a particular resource may not be limited by that resource, either because the resource is not in short supply or because the growth or fitness of the species is limited by some other factor, such as disturbance. Thus, similarities among species in resource use do not necessarily imply that interspecific competition for that resource should occur (Strong 1983). Second, current resource utilization patterns could be the product of past competition (Connell 1980), and might have little impact on current competitive interactions.

One of the most robust ways to show separation in resource use among species is to manipulate the density of individual competitors and test whether this manipulation alters the resource use patterns of other species

Manuscript received 6 June 2006; revised 19 October 2006; accepted 8 January 2007. Corresponding Editor: J. M. Richards.

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in the neighborhood (Silvertown 2004). A comparison of two species' resource use patterns, singly and in competition with each other, tests for divergence in resource use due to competition. In essence, this comparison can show shifts in resource use between a species' fundamental and realized niches, which is an important prediction in competition theory.

To date, research related to N partitioning by plants has focused on the potential outcome of such partitioning; i.e., differences among species in the uptake of different N forms in situ (e.g., McKane et al. 2002) and in the laboratory (e.g., Kielland 1994). Alternatively, researchers have examined the partitioning of N between plants and microbes (e.g., Cheng and Bledsoe 2004, Nordin et al. 2004). To our knowledge, no other study has used neighbor removals to establish whether a shift in resource (N) use has occurred in response to competition between neighboring plants. To test whether competition might be driving N partitioning, we first examined short-term N uptake dynamics of three alpine dry meadow species growing with neighbors in intact neighborhoods. We then used neighbor removals to compare the N uptake response of individuals in interspecific pairs with those in isolation. We used ^{15}N tracer additions (^{15}N -labeled NH_4^+ , NO_3^- , and glycine) to examine species' N uptake patterns in each of these environments (intact neighborhood, interspecific neighbor pairs, and no neighbors) and addressed the following questions: (1) To what extent do co-occurring species overlap in their resource use in the presence of neighbors? (2) Does separation in resource use, if evident, appear to be the result of competition? and (3) Are competitive effects dependent on neighbor identity? We used glycine 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 compete well for glycine relative to more complex forms of N (Lipson et al. 1999a).

METHODS

Field methods

We examined early-season ^{15}N uptake over two consecutive years at Niwot Ridge, Colorado (40°03' N, 105°35' W; 3500 m), a Long-Term Ecological Research site and UNESCO Biosphere Reserve, to quantify variation among species in the capacity to take up, and compete for, different chemical forms of N. Production of alpine dry meadow has been shown to be N-limited (Bowman et al. 1993), soil N concentrations are typically low during the growing season (Raab et al. 1996, Miller and Bowman 2002), and competition for N has been inferred from both species removal and N fertilization experiments (Theodose and Bowman 1997a, b). *Kobresia myosuroides* (Vill.) Fiori & Paol., a tussock-forming sedge, comprises nearly 50% of cover in the dry meadow community (Theodose and Bowman 1997a). Nitrogen-fixing clovers (*Trifolium* spp.) are

present but comprise <1% cover (Theodose and Bowman 1997a).

Two sites, "East Knoll" and "T-Van," were established in representative areas of dry meadow tundra. The sites were located ~0.5 km apart on a low-angle (<10°), south-facing slope and were characterized by similar soils and species composition. The study species included two common forbs (*Artemisia scopulorum* Gray (Compositae), and *Acomastylis rossii* (R. Br.) Greene ssp. *turbinata* (Rydb.) W.A. Weber (Rosaceae)), and a sedge (*Carex rupestris* All. ssp. *drummondiana* (Cyperaceae)), all of which co-occur. *Artemisia*, *Acomastylis*, and *Carex* comprise 1–3%, 6–8%, and 12–13% of total cover, respectively, at the two sites (A. Miller, unpublished data). The forbs are AM-colonized (Miller and Bowman 2002), while *Carex* is nonmycorrhizal. Below-ground biomass is concentrated in the top 10 cm of the soil profile (Webber and May 1977), with the majority of N uptake occurring in the top 5 cm (A. Miller, unpublished data). Previous ^{15}N uptake studies have shown that the majority of N uptake occurs early in the growing season (Theodose et al. 1996), but that the three study species might vary in their N preferences (Miller and Bowman 2003). Natural abundance $\delta^{15}\text{N}$ values from unlabeled plants in the field have shown a separation of ~2‰ between the two most disparate species (*Artemisia* and *Acomastylis*), likewise suggesting the potential for differences in N uptake among species (Miller and Bowman 2002).

Here we report on two ^{15}N uptake experiments conducted over the two-year period. We used the first ^{15}N tracer experiment (2000) to identify variation among species in the capacity to take up different forms of N in an intact neighborhood. Approximately three weeks following snowmelt, we applied one of three ^{15}N -labeled forms of N (NH_4^+ , NO_3^- , or glycine) to each of the three study species (*Artemisia*, *Acomastylis*, *Carex*) in a split-plot design at the T-Van site ($n = 4$ blocks). Each block consisted of three 1 × 1 m plots labeled separately with each form of N. Within each plot, two individuals per species were labeled, for a total of eight individuals per species × N form. In order to determine the N preferences of species, we injected the three N forms in combination, with one form ^{15}N -labeled and the remaining two forms unlabeled (cf. Nordin et al. 2001). We used a 0.6 mmol/L solution of ^{15}N - NH_4^+ (98 atom % ^{15}N), ^{15}N - NO_3^- (98 atom % ^{15}N), or ^{15}N - ^{13}C -[2]-glycine (98 atom % ^{15}N , 99 atom % ^{13}C -2), in combination with equal volumes of the remaining two N forms as 0.2 mmol/L unlabeled N solution. The total concentration of the combined solutions was 1.0 mmol/L N. We used a ^{15}N -labeled N source that was three times more concentrated than the unlabeled N because we wanted to deliver enough ^{15}N in the presence of unlabeled forms to detect N uptake, but at a low enough overall N concentration (≤ 1 mmol/L) to avoid a fertilization effect. While equal concentrations of ^{15}N -labeled and unlabeled N solutions have been used

previously for identifying species' N preferences in the field (e.g., Nordin et al. 2001, 2004), the total N concentrations used in those studies, and the total amount of N added to soil, were markedly higher. We found in a pilot study that a lower concentration of ^{15}N -labeled solution (0.33 mmol/L), in combination with equal concentrations of unlabeled N, would not result in measurable ^{15}N recovery over the limited uptake period.

We injected the ^{15}N tracer to a depth of 10 cm, at six points of 1 mL each within a 2 cm radius of the target plant. With each injection, the needle was slowly withdrawn, uniformly labeling the soil column. The tracer addition delivered $\sim 0.8 \mu\text{g } ^{15}\text{N/g}$ soil to each target individual, a dosage equivalent to $\sim 3\%$ of the soil ^{15}N pool. At the time of labeling, this addition represented $\sim 10\text{--}17\%$ of the extractable inorganic N pool.

Plants were harvested six hours after the tracer addition and were separated into live aboveground (leaves, stems, inflorescences) and belowground (root, rhizome) tissue. We used the six-hour incubation time to minimize the potential for microbial transformation of the added N forms (NH_4^+ , glycine-N), as is standard in studies of organic N uptake by plants (e.g., Näsholm et al. 1998, Nordin et al. 2004). Fine roots were washed in tap water to remove soil, immersed in 0.5 mmol/L $\text{CaCl}_2 + 1.0 \text{ mmol/L KCl}$ for 2–3 minutes to remove adsorbed ^{15}N and ^{13}C , and rinsed well with deionized water.

Dried and ground root tissue was analyzed for ^{15}N and ^{13}C at the Stable Isotope Facility (University of California, Davis, California, USA). Instrument precision was 0.04%. We used ^{15}N concentrations in fine roots and aboveground tissue to estimate ^{15}N uptake into the total plant pool (micrograms of ^{15}N), following the equations of Hauck and Bremner (1976): ^{15}N uptake = $[T(A_S - A_B)]/A_F$, where T = mass of N in sample, A_S = atom % excess ^{15}N in sample, A_B = atom % excess ^{15}N in background, and A_F = atom % excess ^{15}N in the tracer. The ^{15}N uptake was divided by the sample mass to determine ^{15}N concentration in the sample tissue. Plant ^{15}N pools were calculated as the sum of above- and belowground tissue ^{15}N concentrations multiplied by the above- and belowground mass of the labeled individuals. Background concentrations of ^{15}N and ^{13}C were calculated from control plants that had received an equivalent dose of unlabeled N. Enrichment in aboveground tissue was undetectable in 2000, and so ^{15}N pool estimates for that year were based only on belowground ^{15}N concentrations.

The second ^{15}N uptake experiment (2001) was conducted to determine whether the form of N taken up by an individual plant was influenced by the identity of its neighbor. Approximately three weeks following snowmelt, we applied one of three ^{15}N -labeled forms of N to each of six species–neighbor combinations (3 focal species \times 2 neighbor treatments) at the T-Van and E-Knoll sites, for a total of 18 treatment combinations.

Each experimental block consisted of three 2×3 m plots labeled separately with each form of N ($n = 3$ blocks per site, for a total of 6 blocks). Within each plot, two individuals per species–neighbor combination were labeled. Neighbor treatments consisted of isolated individuals (“no neighbor”) and species pairs (“neighbor”) of similar biomass and phenology. Species were the same as in 2000, but in all pairwise combinations. Neighbor treatments were established by clipping all surrounding vegetation to the ground within a 7.5 cm radius of the target(s) in order to reduce belowground competition for nutrients (cf. Caldwell et al. 1987). In this way, target plant N uptake in species pairs was standardized to uptake without a neighbor. Because the focus of this study was niche separation, i.e., to quantify the response of targets without neighbors to those with neighbors of another species, we did not use same-species pairs. Same-species pairs provide a measure of the relative intensity of intra- vs. interspecific competition, but fail to address differences between the fundamental niche of a species (in isolation, without competition) and the realized niche (with interspecific competition) (Goldberg 1996, Gibson et al. 1999).

Neighbor removals were initiated 10 days prior to the ^{15}N tracer addition, with repeated clipping to control regrowth. Clipped vegetation from the initial harvest was sorted to species (*Artemisia*, *Acomastylis*, *Carex*, *Trifolium*, Other) or categorized as litter, and was dried and weighed. We analyzed for effects of removed vegetation on N uptake in target species and included N_2 -fixing *Trifolium* species as a separate group because they had the potential to influence the background $\delta^{15}\text{N}$ signature of adjacent species (cf. Handley and Scrimgeour 1997) and to increase inorganic N concentrations in soil (Thomas and Bowman 1998).

We applied the ^{15}N tracer as three forms of N in combination, as described in the preceding paragraphs. Due to low ^{15}N recoveries and high sample variance in 2000, we modified our ^{15}N application in 2001 as follows: we increased the number of replicate blocks to six (described in the preceding paragraphs), and we increased the amount of ^{15}N added by a factor of four over 2000 levels, injecting the label to a depth of 5 cm on each side of the paired (neighbor) or individual (no neighbor) plants. Each set of paired or individual plants received $\sim 3.2 \mu\text{g } ^{15}\text{N/g}$ soil, or 10% of the total soil ^{15}N pool. Based on early-season exchangeable N concentrations of 7.8, 1.4, and $1.0 \mu\text{g N/g}$ soil for NH_4^+ , NO_3^- , and total amino acid N, respectively (Miller and Bowman 2003), we estimate that $>100\%$ of soil NO_3^- and glycine pools, and $\sim 40\%$ of the NH_4^+ pool, could have been labeled in the 2001 experiment. However, water-extractable amino acid concentrations of up to $\sim 6.5 \mu\text{g N/g}$ soil have been measured in this system (Miller and Bowman 2003), and the highest concentrations in most years occur shortly after snowmelt (Lipson et al. 1999b), suggesting that the ^{15}N -labeled fraction could have been substantially less. A correction for the

dilution of the $^{15}\text{N-NH}_4^+$ tracer in the soil NH_4^+ pool did not affect our estimates of N uptake in 2001, but could increase our estimate of $^{15}\text{N-NH}_4^+$ uptake in 2000 by nearly 10-fold, when only 10% of the NH_4^+ pool was labeled.

We used six injection points of 1 mL each in 2001, as in 2000, but the points were arranged in two parallel rows of three injections, each within 2 cm of either side of the neighbor pair or no-neighbor control. We did not attempt to inject between paired plants, as the separation between pairs was often ≤ 2 cm and to do so could have damaged both above- and belowground tissue. Consequently, we cannot discount the possibility that uptake of ^{15}N by plants outside of the 7.5 cm clipped radius contributed to variability in ^{15}N uptake by targets. Plants were harvested after six hours and processed as in 2000. Enrichment in above- and belowground tissue was used to calculate plant ^{15}N pools.

Statistical analyses

Plant belowground and total ^{15}N pools were log-transformed to meet assumptions of normality and homoscedasticity. Within-species treatment (N form [2000, 2001]; neighbor identity [2001 only]) effects were analyzed separately for the 2000 and 2001 data using a mixed model (PROC MIXED) in SAS (SAS Institute 2004) with block as a random factor. Variance components were estimated using Type 3 sums of squares, with degrees of freedom determined by the Satterthwaite approximation. A posteriori differences among N form and neighbor identity treatments were analyzed using differences of least-square means. Incubation time (the time required to process samples following harvest), target biomass, and neighbor biomass were tested as covariates in each model. A comparison of covariance structures using the finite-population corrected AIC (AICC) indicated that target biomass alone resulted in the most parsimonious model. Due to differences in the methods used in each year, we cannot directly compare the results of the 2000 and 2001 ^{15}N uptake experiments, but we provide the 2000 results as an indication of the species' behavior (N preferences) in an unmanipulated, low-N environment.

In 2001, we used the natural-log-transformed response ratio (ln RR) of ^{15}N pools and belowground ^{15}N concentration, averaged for all individuals in each block, to quantify the response of a target individual to the presence (target with neighbor) or absence (target control) of a specific neighbor (Hedges et al. 1999): $\ln \text{RR} = \ln(\text{target with neighbor} \div \text{target control})$.

The logarithm normalizes the ratio and produces values that are symmetric around zero (Hedges et al. 1999, Suding and Goldberg 2001). Thus, $\ln \text{RR} > 0$ indicates facilitation and $\ln \text{RR} < 0$ indicates competition between neighbors for N. We used a mixed model to test for the effects of target species and N form on ln RR, using neighbor biomass as a covariate. Post hoc

comparisons on least-square means of fixed effects (N form, target species, neighbor treatment, neighbor identity) were made using Bonferroni-adjusted significance levels. A Dunnett's one-sided test was used within N form treatments to determine whether ^{15}N uptake in targets was significantly reduced by neighbors, relative to the no neighbor control (i.e., whether ln RR differed significantly from zero). Niche separation along an axis of N form use would be demonstrated if neighborhood manipulations (2001) altered resource use by the target species.

RESULTS

Species varied in the capacity to take up ^{15}N -labeled NH_4^+ , NO_3^- , and glycine in an intact neighborhood (2000), and when neighbor removals were used to establish interspecific pairs (2001) (Fig. 1). Plants from intact neighborhoods did not necessarily show the same N preferences as those from species pairs (Fig. 1), suggesting that neighborhood characteristics (e.g., neighbor density) or, potentially, rates of N supply (cf. Weigelt et al. 2003) could shape species' uptake patterns. For example, *Acomastylis* took up NH_4^+ to a greater extent than other N forms when the neighborhood was intact ($P < 0.05$; Fig. 1), but took up more NO_3^- than other N forms when paired with *Artemisia* ($P < 0.001$). In addition, *Acomastylis* was the only species to show marked differentiation in N uptake in the intact neighborhood (Fig. 1), perhaps due to its capacity to exploit more abundant forms of N (e.g., NH_4^+) when neighbor densities were high. The remaining two species, *Carex* and *Artemisia*, did not show marked preferences for a particular form of N until neighbor densities were reduced, in 2001. We found no effect of the removed biomass, including *Trifolium* biomass, on N uptake by the remaining target species.

All species showed significant ^{13}C enrichment in fine root tissue ($P < 0.05\text{--}0.001$; data not shown) in the $^{15}\text{N}\text{-}^{13}\text{C}$ -glycine treatments in 2000 and 2001, and ^{13}C recoveries that were generally comparable to ^{15}N recoveries, regardless of neighbor treatment. Regressions between the percentage recovery of ^{13}C and ^{15}N were significant for all species in 2001 (*Artemisia*: $r^2 = 0.11$, $P < 0.05$; *Acomastylis*: $r^2 = 0.18$, $P < 0.01$; *Carex*: $r^2 = 0.50$, $P < 0.001$). In addition, regressions between molar excess ^{13}C and ^{15}N in belowground tissue were significant for *Carex* (Fig. 2; cf. Näsholm and Persson 2001), suggesting that at least 8% of the glycine tracer was taken up intact. However, because of low ^{13}C recovery and uncertainties regarding the interpretation of the regression results (e.g., Nordin et al. 2001, Weigelt et al. 2003), we interpret "glycine uptake" in general terms as uptake of N derived from a glycine source. As such, glycine uptake accounted for $\sim 10\text{--}18\%$ of total N taken up by plants in 2000 and 2001. Uptake of NH_4^+ ranged from $\sim 10\text{--}60\%$, and NO_3^- from $25\text{--}85\%$, of total N uptake (Fig. 1). In both years, $< 1\%$ of the

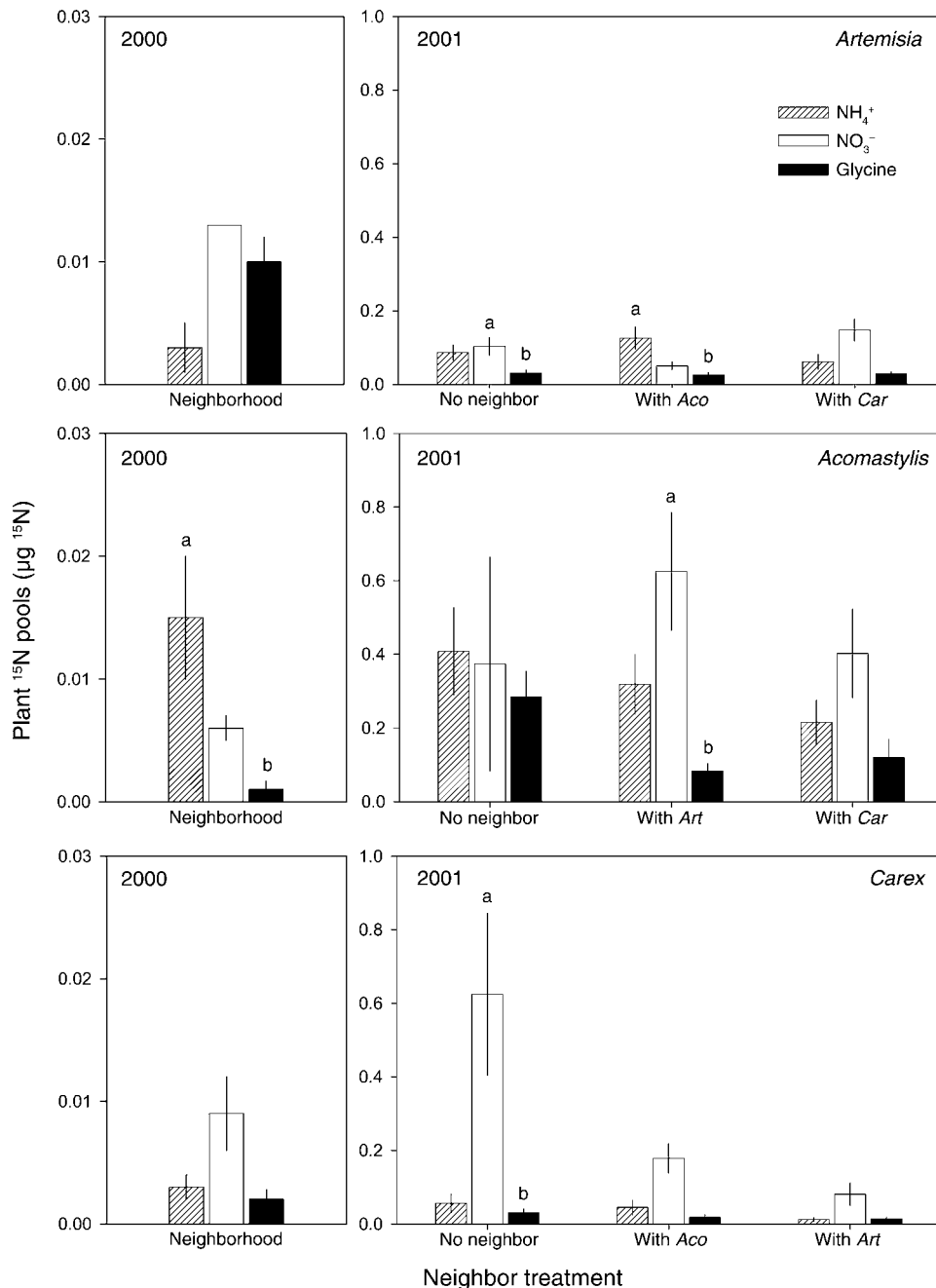


FIG. 1. Effect of neighbors on $^{15}\text{N-NH}_4^+$, NO_3^- , and glycine uptake (above + belowground ^{15}N pools) in three alpine dry meadow species. Lowercase letters above the error bars denote significant differences ($P < 0.05$) among N form within neighbor treatment (backtransformed means \pm SE). "Neighborhood" denotes individuals labeled in an intact neighborhood (2000). Neighbor pairs (2001) are denoted as "with *Art*" (paired with *Artemisia*); "with *Aco*" (paired with *Acomastylis*); and "with *Car*" (paired with *Carex*). Note differences in the scale of the y-axes between experiments in 2000 and 2001 due to differences in ^{15}N application rate.

experimentally added ^{15}N was recovered in plants six hours after the label was applied.

In 2001, neighbors reduced ^{15}N uptake in *Acomastylis* (17%) and *Carex* (50%; $P < 0.05$) across all N form treatments. However, no one species was the best competitor for all forms of N over the course of the

incubation, and in roughly two-thirds of the pairs the rate of N uptake was not influenced by neighbor presence. Neighbor identity influenced ^{15}N uptake in *Carex* ($P < 0.10$; Table 1), whereas in *Artemisia*, the effect of neighbor identity was dependent on the form of N supplied ($P < 0.10$). Differences of least-square means

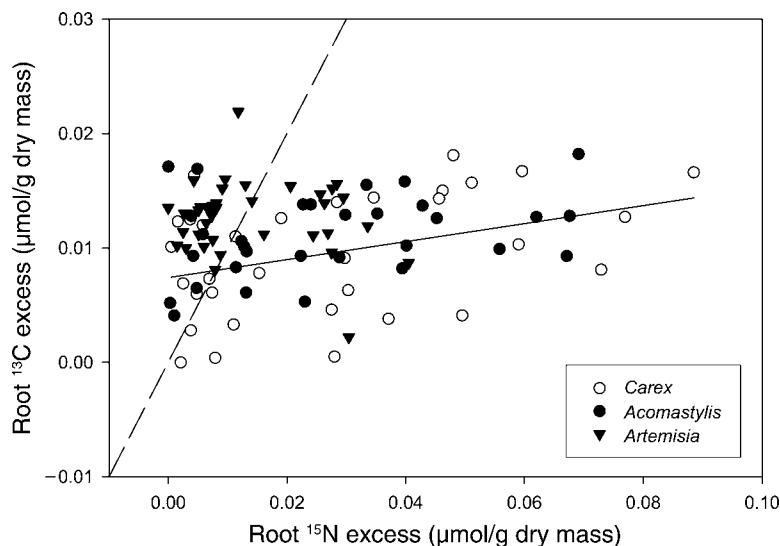


FIG. 2. Relationship between excess ^{13}C and excess ^{15}N in belowground tissue of plants labeled with ^{15}N - ^{13}C -[2]-glycine in 2001. Each symbol represents one sample. The solid line indicates a linear regression between ^{13}C and ^{15}N excess for *Carex* (slope = 0.08, $r^2 = 0.14$, $P < 0.05$). The broken line indicates the molar ^{13}C : ^{15}N ratio of the tracer (1:1).

indicated that *Acomastylis* and *Carex* had significantly different effects on N uptake by *Artemisia* ($P < 0.001$), and that *Acomastylis* and *Artemisia* likewise differed in their effects on *Carex* ($P < 0.10$). Neighbor biomass affected N uptake in *Artemisia* ($P < 0.05$; data not shown), but not in the other two species. *Acomastylis* had greater belowground biomass than *Artemisia* and *Carex* and a greater capacity for ^{15}N uptake in isolation (Fig. 1), but *Acomastylis* reduced ^{15}N uptake only in *Artemisia* (Fig. 3), and by an amount comparable to its uptake in isolation. In contrast, *Artemisia* disproportionately reduced ^{15}N pools in both *Carex* ($^{15}\text{N}\text{-NO}_3^-$) and *Acomastylis* (^{15}N -glycine) (Fig. 3), decreasing ^{15}N uptake in neighbors by an amount equivalent to 4.3–5.7 times its uptake in isolation. Thus, while *Acomastylis* was a better competitor for $^{15}\text{N}\text{-NO}_3^-$ than *Artemisia* in *Acomastylis*–*Artemisia* pairs, it was a less effective competitor than *Artemisia* when both species were paired with *Carex*.

Acomastylis and *Artemisia* showed marked flexibility in their uptake of N, with each species shifting its

allocation to a different form of N when paired with the other (Fig. 1), and each competing with the other for a different form of N (Fig. 3). *Carex* took up less ^{15}N when neighbors were present, but its capacity for $^{15}\text{N}\text{-NO}_3^-$ uptake remained high across all neighbor treatments (~65–85% of total N uptake; Fig. 1). As a result, the proportion of $^{15}\text{N}\text{-NO}_3^-$ taken up by *Carex* was similar whether it was paired with a single neighbor (2001) or labeled within an intact neighborhood (2000), indicating the potential for relatively fixed resource requirements in the field.

DISCUSSION

Our results demonstrate that co-occurring species in an N-limited community differ in their capacity for N uptake, depending on the form of N supplied. Species-specific N uptake preferences have been described in other N-limited ecosystems (Näsholm et al. 1998, McKane et al. 2002, Nordin et al. 2004), and have been hypothesized to contribute to the partitioning of a small resource pool. However, our results also demonstrate

TABLE 1. Effect of neighbor identity (Neighbor) and N form on ^{15}N uptake (above + belowground pools) in alpine dry meadow species paired with interspecific neighbors (2001).

Source of variation	<i>Artemisia</i>		<i>Carex</i>		<i>Acomastylis</i>	
	df	F	df	F	df	F
Neighbor	1, 60	1.41	1, 60	3.25†	1, 59	0.12
N form	2, 60	6.67**	2, 60	12.05***	2, 59	8.67***
Neighbor × N	2, 60	2.53†	2, 60	0.62	2, 59	0.45
Target biomass	1, 60	8.51**	1, 60	11.06***	1, 59	6.54*
Block	1, 1	4.38	1, 1	0.36	1, 59	322.48***
Block × neighbor	1, 60	0.61	1, 60	1.09	1, 59	0.00

Notes: The F ratio and significance level are given for each factor. Model results using target biomass as the covariate are shown. † $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

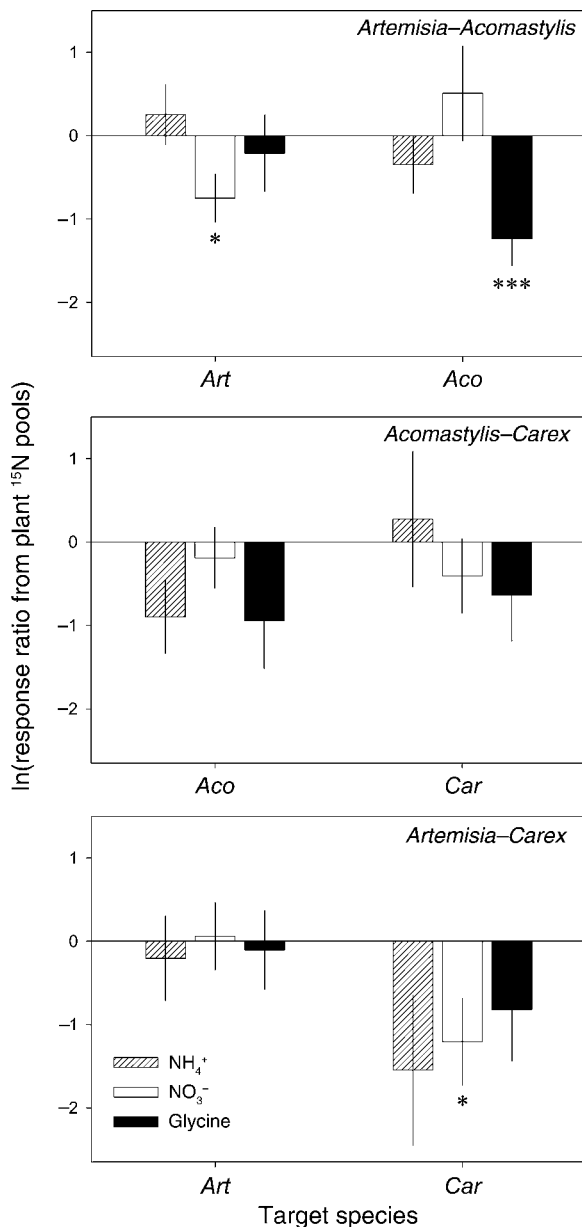


FIG. 3. Response ratios (means \pm SE) calculated from ^{15}N - NH_4^+ , NO_3^- , and glycine uptake (above + belowground ^{15}N pools) for the three interspecific neighbor pairs (2001). Target species within each pair are shown on the x-axis. Significant negative departures from zero (no neighbor control) are denoted by asterisks (* $P < 0.05$; *** $P < 0.001$) and indicate competition for N between neighboring plants.

that the presence of a neighbor can alter the pattern of N uptake in a target species, and more importantly, that neighbor identity influences the capacity of targets to take up different forms of N. The effect of neighbors in 2001 was often twofold. Neighbors reduced the uptake of a particular form of N by targets, but also induced uptake of an alternative form. Such shifts in resource use as a result of neighbor presence are an implicit

assumption in studies of resource partitioning due to competition, but have rarely been documented.

In 2001, all species showed reductions in ^{15}N uptake due to neighbor presence. The capacity of plants to take up N in the absence of neighbors (an approximation of the fundamental niche) often appeared to differ from their resource use patterns in intact neighborhoods (the realized niche), although direct comparisons between the 2000 and 2001 data are not possible. No one species was a superior competitor for all forms of N in the short term, suggesting that if the observed N uptake patterns held over longer time periods, segregation on this resource axis could occur (cf. Silvertown 2004). We hypothesized that if competition was important in structuring species' patterns of N uptake during the six-hour incubation period, we should see a shift in N preference in at least one species of a pair, relative to its preference in no-neighbor controls. The interaction between *Artemisia* and *Acomastylis*, in which *Artemisia* reduced ^{15}N - NO_3^- uptake when paired with *Acomastylis*, and *Acomastylis* reduced glycine uptake when paired with *Artemisia* (Fig. 3), suggests that competition could drive the partitioning of soil N between these two species. Similarly, interactions between *Artemisia* and *Carex* indicated asymmetric competition: *Artemisia* was not affected by *Carex*, but it reduced the uptake of all forms of N in *Carex*, particularly ^{15}N - NO_3^- (Fig. 3). Thus, *Artemisia* appeared to be the superior competitor for N in the short term, but did not alter its relative uptake of the different forms of N when grown with *Carex*.

A number of recent studies (e.g., Dudley and Schmitt 1995, 1996, Van Kleunen and Fischer 2001) suggest that morphological and/or physiological plasticity among species can reduce competition and, by extension, increase the probability of species coexistence (Callaway et al. 2003). The alpine species in our study were able to take up all forms of ^{15}N in combination with unlabeled N, demonstrating that they have the potential to use these N forms when they are available in the soil. In some cases, competition had little influence on short-term resource use. In other cases, however, species' resource use patterns depended upon neighbor identity, and the form of N supplied. Flexibility in resource use appeared to buffer the effects of neighbors and facilitate partitioning of soil N. Species that were able to vary the forms of ^{15}N that they took up maintained ^{15}N pools roughly comparable to controls in the presence of neighbors, and the one species that did not (*Carex*) showed reduced ^{15}N uptake overall rather than compensatory uptake of other N forms. Species and functional group identity have been found to explain both absolute N uptake at the ecosystem level, and patterns of similarity between N availability and N uptake (Kahmen et al. 2006). Our results suggest that species' N preferences could be mediated by neighbor identity and that, depending upon the characteristics of

the neighborhood, different species might rely on different N pools to meet their N requirements.

It has been argued that partitioning by N form could be based more upon differences in soil N availability than upon species-specific preferences (Nordin et al. 2004). Our data suggest that, at least in some cases, species' N preferences could be independent of N availability (e.g., uptake of NO_3^- by *Carex*). However, the effect of neighbors on target plants could also be manifested through the direct depletion of soil resources (e.g., D'Antonio and Mahall 1991, Jackson and Caldwell 1996), or through indirect effects on resource availability, potentially mediated by microbial interactions (Reynolds et al. 1997, 2003). Although we found no effect of removed vegetation on ^{15}N uptake in our study, we did not test for microsite differences associated with each neighbor pair. We therefore have little information regarding plant–soil feedbacks on N availability. However, previous work has indicated that foliar N concentrations in *Acomastylis* are positively associated with soil NH_4^+ but not NO_3^- concentrations ($r^2 = 0.32$, $P < 0.001$; W. D. Bowman, unpublished data), and that *Acomastylis* litter may promote microbial immobilization of N, reducing N availability to neighbors (Bowman et al. 2004). In addition, a comparison of soil N concentrations under *Carex* and *Acomastylis* grown in isolation suggests that exchangeable NO_3^- , while still negligible compared to NH_4^+ , is greater under *Carex* ($P < 0.01$; I. Ashton, unpublished data), and that NH_4^+ is greater under *Acomastylis* ($P < 0.10$). These data are consistent with our finding that *Acomastylis* has a high capacity for NH_4^+ uptake under unmanipulated field conditions (2000), and that *Artemisia* and *Carex* increase NH_4^+ uptake when in association with *Acomastylis* (2001). Similarly, they suggest that increased NO_3^- uptake by *Artemisia* and *Acomastylis* when paired with *Carex* (2001) could be due to increased availability of NO_3^- under *Carex*, or simply that *Carex* is a weaker competitor for this preferred form of N.

The extent to which short-term measures of competitive ability will translate to long-term, fitness-related outcomes of competition for N in the field cannot be determined from this study, as our ^{15}N uptake measurements do not address the long-term impact of species on the resource pool, variation in the timing, duration and rate of supply of different forms of N, or the capacity of these species to translate N uptake into growth and reproduction. A species could be a poor competitor for N in the short term, but have a greater capacity for N uptake in the long term, e.g., as N availability declines. Nevertheless, even short-term competitive interactions between plants have been shown to markedly affect resource acquisition over longer time periods (Caldwell et al. 1987), suggesting their potential importance in ecosystems characterized by a variable resource supply.

Factors affecting the microbial community and plant–microbe interactions could also shift the balance of

competition among species. The low (<1%) ^{15}N recoveries that we observed in plant tissue likely reflect high microbial demand for N in our system and the capacity of these soils to immobilize N (Holland 2006). Similar labeling experiments in the Arctic have resulted in comparable ^{15}N recoveries, suggesting that plants compete poorly with microbes for N in the short term (Schimel and Chapin 1996, Nordin et al. 2004). Regardless, the alpine dry meadow species in our study had the capacity to use all forms of N available, and more often than not utilized N forms normally present only in low concentrations (e.g., NO_3^- , glycine).

How species diversity can be maintained under conditions of resource limitation is a long-standing question in ecology. Our results are significant in that they demonstrate the uptake of different forms of N as a short-term partitioning mechanism, potentially driven by interspecific competition. By comparing N uptake in species with and without neighbors, we showed that competitive interactions among neighbors have the potential to drive short-term patterns of resource use, and that depending upon neighbor identity, the outcome of these interactions may differ substantially.

ACKNOWLEDGMENTS

This work was supported by a grant from the Andrew W. Mellon Foundation and by the Niwot Ridge LTER. We thank D. Harris, University of California–Davis, and C. Seibold, Mountain Research Station, University of Colorado, Boulder, for sample analyses. We are grateful to J. Larsen, H. Bechtold, A. Townsend, L. Turner, T. Forbis, L. Krukenburg, C. Snyder, and E. Thorsos for field and laboratory assistance, and to H. Reynolds, G. Shaver, I. Ashton, and several anonymous reviewers for helpful comments on an earlier version of this manuscript.

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