Oecologia (2002) 130:609–616
DOI 10.1007/s00442-001-0838-8

ECOSYSTEMS ECOLOGY

Amy E. Miller · William D. Bowman

Variation in nitrogen-15 natural abundance and nitrogen uptake traits among co-occurring alpine species: do species partition by nitrogen form?

Received: 9 May 2001 / Accepted: 21 September 2001 / Published online: 21 November 2001

Abstract In the N-limited alpine tundra, plants may utilize a diversity of N sources (organic and inorganic N) in order to meet their nutritional requirements. To characterize species-level differences in traits related to N acquisition, we analyzed foliar $\delta^{15}N$, nitrate reductase activity (NRA) and mycorrhizal infection in co-occurring alpine species during the first half of the growing season and compared these traits to patterns of N uptake using a ^{15}N (^{15}N-NH_4^+, ^{15}N-NO_3^-) or ^{13}C,^{15}N ([1]-^{13}C-^{15}N-glycine) tracer addition in the greenhouse. ^{13}C enrichment in belowground tissue indicated that all species were capable of taking up labeled glycine, although only one species showed uptake of glycine potentially exceeding that of inorganic N. Species showing the most depleted foliar $\delta^{15}N$ and elevated NRA in the field also tended to show relatively high rates of NO_3^- uptake in the greenhouse. Likewise, species showing the most enriched foliar $\delta^{15}N$ also showed high rates of NH_4^+ uptake. The ratio of NO_3^-:NH_4^+ uptake rates and growth rate explained 64% and 72% of the variance in foliar $\delta^{15}N$, respectively, suggesting that species differ in the ability to take up NO_3^- and NH_4^+ in the field and that such differences may enable species to partition soil N on the basis of N form.

Keywords Alpine tundra · Ammonium · Nitrate · Organic nitrogen · Soil nitrogen-15 natural abundance

Introduction

Several mechanisms may facilitate species coexistence within N-limited systems, including variation in resource availability, variation in species' resource requirements, and the partitioning of N in space and time. An additional mechanism by which species may partition a limited soil N pool is through the uptake of different chemical forms of N, organic and inorganic. Given that species differ in their ability to take up different forms of N, such partitioning may enable plants to efficiently utilize available soil N (Chapin et al. 1993; Leadley et al. 1997; Näsholm et al. 1998; Lipson et al. 2001). Alternatively, if partitioning by N form does not occur, the majority of species should show no difference in the uptake of different N forms and should take up either the most prevalent form of N, or multiple forms of N. The latter mechanism, i.e. flexibility by all species in the uptake of different forms of N, may likewise enable co-occurring species to exploit a limited soil N pool.

The alpine dry meadow community provides an excellent system for examining plant patterns of N uptake because N is limiting to production (Bowman et al. 1993; Bowman et al. 1995; Theodose and Bowman 1997), species densities are among the highest reported for herbaceous communities (Gough et al. 2000), and the potential for spatial and temporal partitioning of N by plants appears to be fairly constrained. Roots are confined to a relatively narrow zone (0–10 cm) within the soil profile (Webber and May 1977), and plants appear to acquire the majority of their N during the first half of the growing season (Jaeger et al. 1999), coincident with the pulse of N that follows snowmelt (Lipson et al. 1999). Total organic N pools are large relative to inorganic N pools in dry meadow soils (Fisk and Schmidt 1996), and species capable of utilizing organic sources of N, primarily amino acid N, may face little N limitation (Lipson et al. 2001). However, the positive response of a number of dry meadow species to long-term N fertilization suggests that at least some species may be poorly equipped to utilize organic N and are limited instead by the availability of inorganic N (Bowman et al. 1993; Bowman et al. 1995; Theodose and Bowman 1997). Thus, all three mechanisms of N acquisition previously described: (1) specialization among species on different forms of N (partitioning), (2) specialization by all species on one,

A.E. Miller (✉) · W.D. Bowman
Mountain Research Station,
Institute of Arctic and Alpine Research,
and Department of Environmental,
Population and Organismic Biology, University of Colorado,
Boulder, Colorado 80309-0334, USA
e-mail: amym@colorado.edu
Fax: +1-303-4928699

common form of N, and (3) flexibility by all species in the uptake of different forms of N, seem possible for plants within the dry meadow tundra.

The goals of our research were to determine whether individual species differed in the ability to take up inorganic (NH_4^+, NO_3^-) and organic (glycine) N, and whether the species as a group would show different patterns of N uptake. 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 previous work with a dominant dry meadow species, *Kobresia myosuroides*, has yielded uptake rates for glycine comparable to those for inorganic N (Raab et al. 1996, 1999). We combined results of a greenhouse ^{15}N uptake experiment with field measurements of foliar and soil $\delta^{15}N$ and in vivo nitrate reductase activity (NRA) to infer potential N sources used by plants in the field.

Materials and methods

Study sites and experimental design

We sampled foliar tissue and soils from two dry meadow alpine tundra sites, Niwot Ridge (Front Range, Boulder County, Colorado), and the headwaters of the Snake River (Tenmile Range, Summit County, Colorado) located approximately 50 km apart on the Continental Divide of the Rocky Mountains, Colorado. The replicate sites are referred to as "Niwot Ridge" and "Summit," respectively. Niwot Ridge (40°03′N, 105°35′W; 3,500 m), a Long-Term Ecological Research site and UNESCO Biosphere Reserve, and Summit (39°31′N, 105°50′W; 3,750 m) are characterized by a low mean annual temperature (–3°C), a springtime precipitation maximum (mean 900–1,000 mm), most of which falls as snow, and a short (3 month) growing season. Soils at both sites are Cryocrepts underlain by granitic parent material, with high organic matter contents (13%), low N (1.0%) and mean pH of 5.5 (Theobald et al. 1963; Burns 1980; Fisk and Schmidt 1995; Heuer et al. 1999).

At each site, we selected representative areas of homogeneous dry meadow tundra approximately 25 m^2, within which we sampled soils and foliar tissue from four 1-m^2 patches per sampling date. Sampling was conducted at 2-week intervals during the 6-week period between snowmelt and peak biomass (mid June and late July 1999).

Field collections: soil N

We measured natural abundance ^{15}N-organic (total) N [$\delta^{15}N$-organic (total) N] and natural abundance ^{15}N-NH_4^+ ($\delta^{15}N$-NH_4^+) of soil N pools in mineral soil and water extracts of soil, respectively, at both Niwot Ridge (1999–2000) and Summit (1999), and $\delta^{15}N$-NO_3^- in water extracts of soil at Niwot Ridge (2000). Exchangeable NH_4^+, NO_3^-, and dissolved organic N (DON) concentrations were measured concurrently with $\delta^{15}N$. Soil cores (33 mm diameter, n=4–8) were collected to a 10 cm depth, transported to the laboratory on ice, composited and sieved to 2 mm. A 500- to 600-g (fresh weight) soil sample was extracted in HPLC-grade water at 3°C, filtered through a pre-combusted Whatman GF/F (0.7 µm) filter, acidified with concentrated HCl, and stored at –40°C until analysis for NH_4^+, NO_3^-, and DON. Exchangeable NH_4^+-N and NO_3^--N were analyzed following methods outlined in Bowman et al. (1993). Total dissolved N was determined by colorimetric analysis following persulfate oxidation, and DON was calculated by difference. Subsamples of soil were oven-dried at 60°C for determination of gravimetric soil moisture.

$\delta^{15}N$-NH_4^+ was measured in acidified soil extracts following the methods of Stark and Hart (1996), as modified by Holmes et al. (1998). One hundred and twenty milliliter samples were diffused at 22°C for 6 days and NH_3 was collected onto pre-combusted, acidified glass fiber (Whatman GF/D) disks enclosed in teflon tape. Diffused samples and bulk (total N) soils were analyzed for $\delta^{15}N$ on a Europa Scientific Hydra 20/20 IRMS (PDZ Europa, Crewe, Cheshire, UK) connected to an on-line combustion system at the Stable Isotope Facility, University of California, Davis. Recovery of ^{15}N-NH_4^+ from diffusions ranged from 88% to 98% and was used to correct for fractionation, following the equations of Holmes et al. (1998). The precision of the $\delta^{15}N$ measurements was 0.25‰ (n=4–8).

Natural abundance ^{15}N-NO_3^- ($\delta^{15}N$-NO_3^-) was determined by collecting NO_3^- from composited (n=2) soil extracts onto anion-exchange columns following pre-treatment with a cation column, as described by Chang et al. (1999). Columns were capped with HPLC-grade water and analyzed for $\delta^{15}N$-NO_3^- on a Micromass Isochrom mass spectrometer connected to an on-line combustion system (Micromass, Manchester, UK) at the Environmental Isotope Laboratory, University of Waterloo, Ontario, Canada.

Field collections: foliar $\delta^{15}N$, NRA, and mycorrhizal infection

Foliar $\delta^{15}N$ and in vivo NRA were measured in leaf tissue of ten species collected at the same sampling dates, and from the same 1-m^2 patches, as soil samples. Study species included five forbs [*Acomastylis rossii* (R. Br.) Greene ssp. *turbinata* (Rydb.) W.A. Weber (Rosaceae), *Potentilla ovina* Macoun. (Rosaceae), *Bistorta bistortoides* (Pursh) Small (Polygonaceae), *Artemisia scopulorum* Gray (Compositeae), *Mertensia lanceolata* (Pursh) A. DC. (Campanulaceae)]; two grasses [(*Festuca brachyphylla* Schult. (Gramineae), *Trisetum spicatum* (L.) Richt. ssp. *spicatum* (Gramineae)]; and three sedges/rushes [*Carex rupestris* All. spp. *drummondiana* (Cyperaceae), *Kobresia myosuroides* (Vill.) Fiori and Paol. (Cyperaceae); *Luzula spicata* (L.) DC. (Juncaceae)]. An additional grass, *Calamagrostis purpurascens* R. Br. (Gramineae) was collected at Niwot Ridge, but was not present at Summit. Nomenclature for all species follows Weber (1976), and species are hereafter referred to by genus. Community dominants at both sites include the sedges *Kobresia* (45–47% cover) and *Carex* (13–20% cover), and the forb, *Acomastylis* (7–10% cover) (Table 1).

We collected foliar tissue from plants of similar phenological stage, eliminating stems, petioles, and inflorescences. Where possible, tissue was taken from two or more different species growing in close proximity (1–10 cm) to one another. Where clovers (*Trifolium* spp.) were present (Summit site), samples were collected at least 1 m from *Trifolium* patches to minimize the impact of N_2 fixation on foliar and soil $\delta^{15}N$ isotopic signatures. Foliar samples (n=8) were collected between 9:30 and 12:00 a.m. to reduce the possible effect of diurnal variation in NRA and were transported on ice to the laboratory. Approximately 0.2 g fresh weight of material was analyzed for in vivo NRA following the methods of Hageman and Hucklesby (1971) and Jaworski (1971), as modified by Atkin and Cummins (1994). Tissue blanks were produced by destabilizing the enzyme for 5 min at 100°C. NO_2^- evolution was assayed following Snell and Snell (1959).

A subsample of foliar tissue collected for NRA on the first and last sampling dates (corresponding with snowmelt and peak biomass) was oven-dried at 55°C, ground to a fine powder, and analyzed for %N and $\delta^{15}N$ at the Stable Isotope Facility. Internal standards for EA-MS (calibrated against N_2) were ammonium sulfate and sucrose, both of which were cross-calibrated against the international standards, IAEA (NIST 8547) and NBS22 Oil (NIST 8539). The precision for foliar $\delta^{15}N$ measurements was 0.27‰.

Root samples from an additional set of plants were harvested at peak biomass, fixed in FAA, cleared and stained (Phillips and Hayman 1970), and examined for ectomycorrhizal (ECM), arbuscular mycorrhizal (AM) and endosymbiotic dark septate (EDS) infection. Infection rates were estimated as the proportion of fungal intercepts on 1-cm root segments from 1–3 cm below the root crown. The frequency of arbuscules was estimated from the proportion of arbuscules intercepted in 1-cm root segments, as above.

Table 1 Summary of species' characteristics related to N uptake and utilization. Means are ±1 SE. Percent cover (n=71), foliar $\delta^{15}N$ (n=8), foliar nitrate reductase activity (*NRA*) (n=8), and frequency (%) of arbuscules (n=6) recorded at peak biomass, Niwot Ridge. Maximum potential growth rates (n=10) were measured in the greenhouse over a 6- to 8-week growth period. NO_3^-:NH_4^+ uptake ratios in *italics* represent differences between NO_3^- and NH_4^+ uptake rates that were significant at α=0.05. *dw* dry weight

Species	Growth form	Cover	$\delta^{15}N$ (‰)	NRA (μmol NO_2 g^{-1} dw h^{-1})	Arbuscules (%)	Growth rate (g dw day^{-1})	NO_3^-:NH_4^+ uptake
Mertensia	Forb	0.3±0.1	–1.41±0.41	9.5±1.1	16.7±6.8	–	–
Festuca	Grass	1.3±0.3	–1.07±0.19	1.8±0.2	1.3±0.8	0.030±0.002	*1.48 (NH_4^+<NO_3^-)*[c]
Potentilla	Forb	0.2±0.3	–0.76±0.23	2.8±0.7	6.6±3.3	–	–
Trisetum	Grass	1.1±0.3	–0.70±0.26	2.3±0.2	2.3±13	–	–
Artemisia	Forb	0.8±0.3	–0.51±0.14	3.3±0.3	6.0±2.1	0.026±0.002	*1.47 (NH_4^+<NO_3^-)*
Bistorta	Forb	1.0±0.3	–0.37±0.30	6.6±0.7	2.5±0.9	–	–
Kobresia	Graminoid	47.6±2.2	0.21±0.28	1.0±0.1	0.0[a]	0.002±0.001	0.99 (NH_4^+=NO_3^-
Carex	Graminoid	12.7±0.8	0.52±0.34	1.2±0.2	0.0[b]	0.004±0.001	0.79 (NH_4^+=NO_3^-)
Calamagrostis	Grass	0.6±0.5	0.77±0.38	0.9±0.2	1.5±1.5	0.025±0.003	1.32 (NH_4^+<NO_3^-)
Acomastylis	Forb	6.6±1.3	1.74±0.28	2.8±0.4	8.8±2.4	0.001±0.000	*0.45 (NH_4^+>NO_3^-)*
Luzula	Graminoid	<0.1±0.0	2.34±0.31	2.0±0.3	3.7±1.4	0.002±0.000	0.63 (NH_4^+>NO_3^-)

[a] *Kobresia*: ectomycorrhizal
[b] *Carex*: non-mycorrhizal
[c] Ratio of uptake rates calculated per unit tissue N (μg ^{15}N mg^{-1} N h^{-1}); all other ratios calculated per unit biomass (μg ^{15}N mg^{-1} N h^{-1})

Greenhouse study: plant ^{15}N uptake

Seven of the species examined in the field (*Acomastylis, Artemisia, Calamagrostis, Festuca, Carex, Kobresia, Luzula*) were included in a greenhouse study of inorganic and organic (amino acid) N uptake. Cuttings were collected at Niwot Ridge immediately following snowmelt, trimmed to 1 cm below the root crown, and transplanted into an autoclaved 5:2:1 sand:topsoil:vermiculite medium at the University of Colorado's alpine greenhouse (16-h photoperiod, mean daily temperature 15°C). Each pot contained one cutting of uniform size of a single species. Initial fresh weights were recorded and initial dry weights (dws) estimated from fresh weight:dw regressions (n=5). Within a species, pots were randomly assigned to one of three 1-mM N treatments [14 mg N l^{-1} as either KNO_3, $(NH_4)_2SO_4$, or glycine (n=20) plus the following micronutrients: 0.005 mg MoO_3 l^{-1}, 0.01 mg $CuSO_4.5H_2O$ l^{-1}, 0.025 mg $ZnSO_4.7H_2O$ l^{-1}, 0.25 mg H_3BO_3 l^{-1}, 0.25 mg $MnCl_2.4H_2O$ l^{-1}, 0.25 mg FeEDTA l^{-1}] and were irrigated 3 times per week until water drained freely from pots. Approximately 8 h following each fertilization treatment, pots were flushed with tap water (NH_4^+ and NO_3^- concentrations undetectable) to minimize microbial transformation of residual N.

Following 6–8 weeks of growth on N treatments, a subset of plants (n=10) received 200 ml of a 1-mM ^{15}N (98 atom %^{15}N-NH_4^+ or NO_3^-) or ^{15}N, [1]-^{13}C (98 atom %^{15}N, 99 atom %^{13}C-glycine) tracer, corresponding to the respective fertilization regime. Plants were harvested between 6 and 14 h following the ^{15}N tracer addition, rinsed with tap water, and separated into above and belowground material. Aboveground live tissue, aboveground senescent tissue, and belowground (root, rhizome) tissue were weighed separately. Foliar tissue was dried at 60°C weighed, ground, and analyzed for %C, %N, and ^{15}N and ^{13}C enrichment using a Carlo Erba elemental analyzer (NA1500, Series 1; Carlo Erba Instrumentazione, Milan) coupled with a SIRA Series II IRMS (Micromass) at the Duke University Phytotron. ^{15}N enrichment in foliar tissue was calculated using the equations of Hauck and Bremner (1976), as cited in Knowles and Blackburn (1993): $F=[T(A_S–A_B)]/A_F$, where F is the weight of N derived from the ^{15}N tracer, T is the total weight of N in the sample, A_S is atom% excess ^{15}N in the labeled sample, A_B is atom% excess ^{15}N in the control (background) sample, and A_F is atom% excess in the ^{15}N tracer. Uptake rates were calculated on a per unit aboveground live biomass (μg ^{15}N g^{-1} dw h^{-1}) and per unit tissue N (μg ^{15}N mg^{-1} N h^{-1}) basis to reflect differences in foliar N pools and efficiency of N uptake among species. Maximum potential growth rates (g dw day^{-1}, Table 1) were calculated for each species at the first harvest.

Due to the possible retention of added N within the potting medium, potential nitrification in NH_4^+- and NO_3^--treatment soils was estimated over a 24-h period at the final harvest (12 weeks), using incubations of soil in either a 50 mM $(NH_4)_2SO_4$ or K_2SO_4 substrate (Hart et al. 1994). While we likely overestimated maximum potential nitrification rates, these rates were negligible when compared to species' N uptake rates (generally <5% of plant uptake). Neither trends in, nor significance of, N treatment effects varied with our nitrification-corrected ^{15}N uptake rates, and thus we report only the measured ^{15}N uptake rates.

Statistical analyses

Differences in soil $\delta^{15}N$ and exchangeable soil N concentrations were examined across sites and sampling dates using the GLM procedure in SAS (SAS Institute, Carey, N.C.). Foliar $\delta^{15}N$ values (field) were ranked among species within each sampling area at each sampling date and the frequency of ranks examined to determine whether the relationship among species was consistent across sites. Differences in foliar $\delta^{15}N$ among species were tested within site and sampling date using a one-way ANOVA (PROC GLM) with species as the categorical variable. A Tukey's studentized range (honestly significant difference) test was used to examine a posteriori differences among species' means at a Bonferroni-adjusted significance level of α=0.01.

Within-species differences in the response to N treatment (greenhouse) were evaluated using one-way ANOVAs with treatment as the categorical variable. Relative uptake of NO_3^- and NH_4^+ was expressed as a ratio of uptake rates (NO_3^-:NH_4^+ uptake) within a species. We used Pearson correlation analyses to examine relationships among NO_3^-:NH_4^+ uptake, foliar $\delta^{15}N$ and growth rate across species, and relationships between foliar $\delta^{15}N$ and NRA within species.

Results

Soil N

Soil $\delta^{15}N$-organic (total) N was enriched relative to NH_4^+ and NO_3^- pools (Fig. 1a), while $\delta^{15}N$-NH_4^+ was enriched relative to $\delta^{15}N$-NO_3^- at the first sampling date ($F_{1,9}$=20.06, P<0.01). Exchangeable soil N was highest

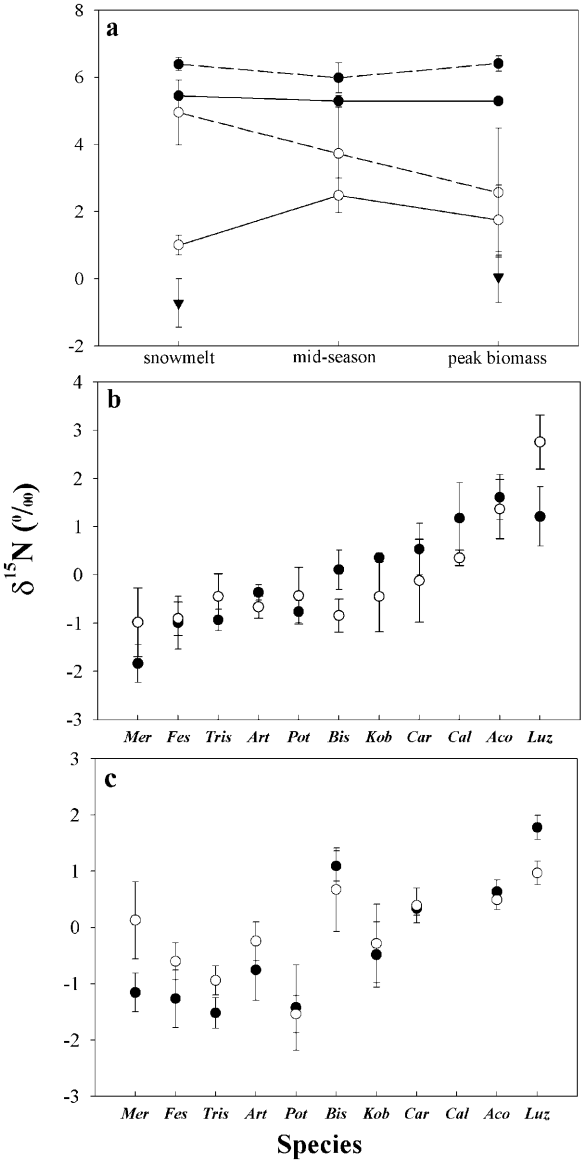

Fig. 1 **a** Soil $\delta^{15}N$ for bulk (total) soil N, NH_4^+ and NO_3^- pools. *Closed circles* represent $\delta^{15}N$-organic (total) N, *open circles* represent $\delta^{15}N$-NH_4^+, and *closed inverted triangles* represent $\delta^{15}N$-NO_3^-. $\delta^{15}N$ values for Niwot Ridge are connected by *dashed lines* (exception: $\delta^{15}N$-NO_3^-), and values for Summit are connected by *solid lines*. Means are ±1.0 SE (n=4–8). **b** Seasonal variation in foliar $\delta^{15}N$, Niwot Ridge. *Closed circles* denote early season (snowmelt) and *open circles* denote late-season (peak biomass) sampling dates. Means are ±1 SE (n=8). Forbs: *Acomastylis* (*Aco*), *Artemisia* (*Art*), *Bistorta* (*Bis*), *Mertensia* (*Mer*), *Potentilla* (*Pot*). Grasses: *Calamagrostis* (*Cal*), *Festuca* (*Fes*), *Trisetum* (*Tris*). Sedges/rushes: *Carex* (*Car*), *Kobresia* (*Kob*), *Luzula* (*Luz*). **c** Seasonal variation in foliar $\delta^{15}N$, Summit site. Means are ±1 SE (n=8). Symbols and species abbreviations are as in **b**

approximately 3–4 weeks following snowmelt and was consistently higher at Summit than at Niwot Ridge. At both sites, DON concentrations (35–70 µg N g^{-1} dw) were approximately 3–5 times greater than NH_4^+ concentrations (12–30 µg N g^{-1} dw), and approximately 10 times greater than NO_3^- concentrations (6–14 µg N g^{-1} dw).

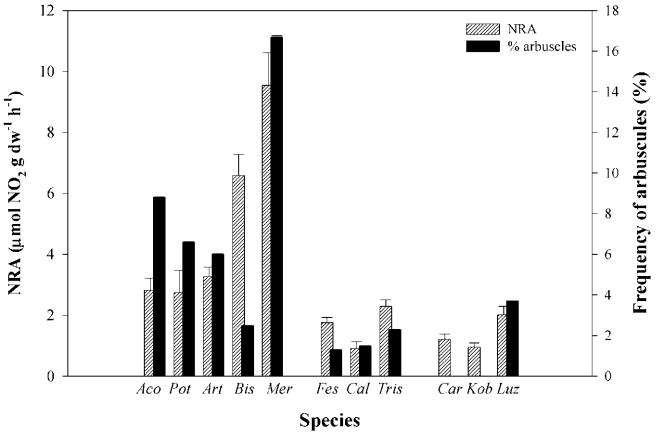

Fig. 2 In vivo nitrate reductase activity (*NRA*) and frequency of arbuscules (%) for Niwot Ridge species. Means are ±1.0 SE (n=8–16). Species abbreviations are as in Fig. 1

Soil NH_4^+ and DON concentrations did not differ between sites, but seasonal soil moisture ($F_{1,23}$=5.75, P<0.05) and NO_3^- concentrations ($F_{1,23}$=15.95, P<0.001) were greater at the Summit site.

Foliar $\delta^{15}N$ signatures

Species differed in foliar $\delta^{15}N$ when pooled across site and sampling date ($F_{10,152=}$13.77, P<0.0001; Fig. 1b, c), and species ranks in within-patch foliar $\delta^{15}N$ were highly concordant across sites ($F_{1,127}$=14.982, P<0.001; Fig. 1b, c). The consistency in rank (approximately 30–60%) among several of the species [*Luzula* (rush); *Acomastylis* (forb); *Calamagrostis, Festuca, Trisetum* (grasses)] suggests that patterns of foliar $\delta^{15}N$ are relatively fixed among co-occurring individuals, particularly the grasses. However, we found no relationship between within-patch foliar $\delta^{15}N$ and soil $\delta^{15}N$-organic (total) N or $\delta^{15}N$-NH_4^+ across species.

Nitrate reductase activity (NRA)

Species differed in in vivo NRA, primarily in association with growth form, but showed little intraspecific variation in NRA. Forbs (*Mertensia, Bistorta*) showed the highest NRAs, while the sedges (*Carex, Kobresia*) showed the lowest NRAs (Fig. 2). We found no relationship between foliar $\delta^{15}N$ and either foliar N concentration or NRA across species, although species-specific, inverse correlations between $\delta^{15}N$ and NRA were found in *Carex*, a sedge (r^2=0.18; $F_{1,27}$=6.857; P<0.05), and *Luzula*, a rush (r^2=0.23; $F_{1,29}$=9.671; P<0.01).

Mycorrhizal infection rates

With the exception of the sedges *Kobresia* (ECM) and *Carex* (non-mycorrhizal), all species showed measurable

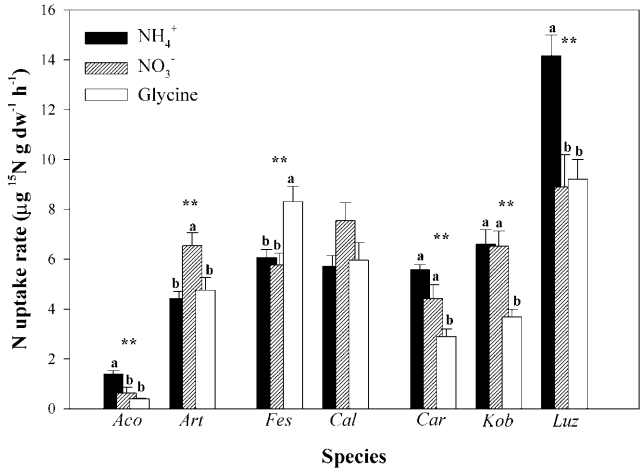

Fig. 3 Aboveground ^{15}N uptake calculated on a per unit dry mass basis (μg ^{15}N g^{-1} dw h^{-1}). Means are ±1 SE (n=10). *Lower case letters* indicate differences among treatment means significant at α=0.01. **P<0.01, ***P<0.001, from one-way ANOVAs, with initial plant biomass as a covariate. Species abbreviations are as in Fig. 1

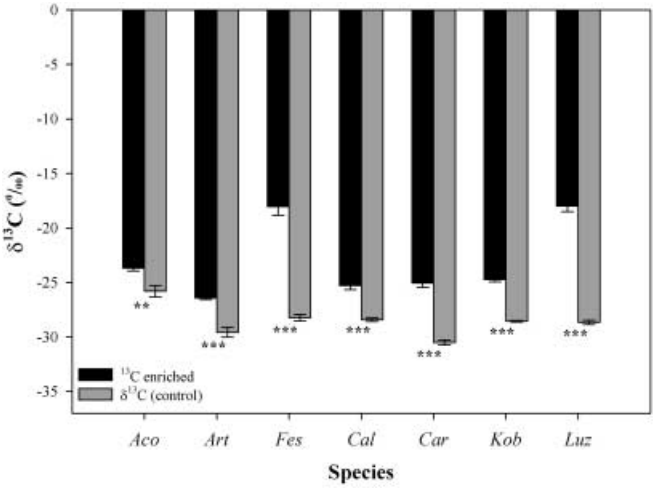

Fig. 4 ^{13}C enrichment in foliar tissue (‰). Means are ±1 SE (n=10). **P<0.01, ***P<0.001, from one-way ANOVAs with treatment as the categorical variable. Species abbreviations are as in Fig. 1

(30–70%) AM and EDS infection, and one forb, *Mertensia*, showed >15% infection by arbuscules alone (Fig. 2). AM infection was not associated with foliar $\delta^{15}N$, but the frequency of arbuscules (% infection) was positively correlated with NRA (r^2=0.55, $F_{1,10}$=13.45, P<0.01).

Plant ^{15}N uptake

Most alpine species differed in the ability to take up different forms of ^{15}N (NH_4^+, NO_3^-, glycine) under greenhouse conditions (Fig. 3), although community dominants *Kobresia* and *Carex* (sedges) did not differ in the

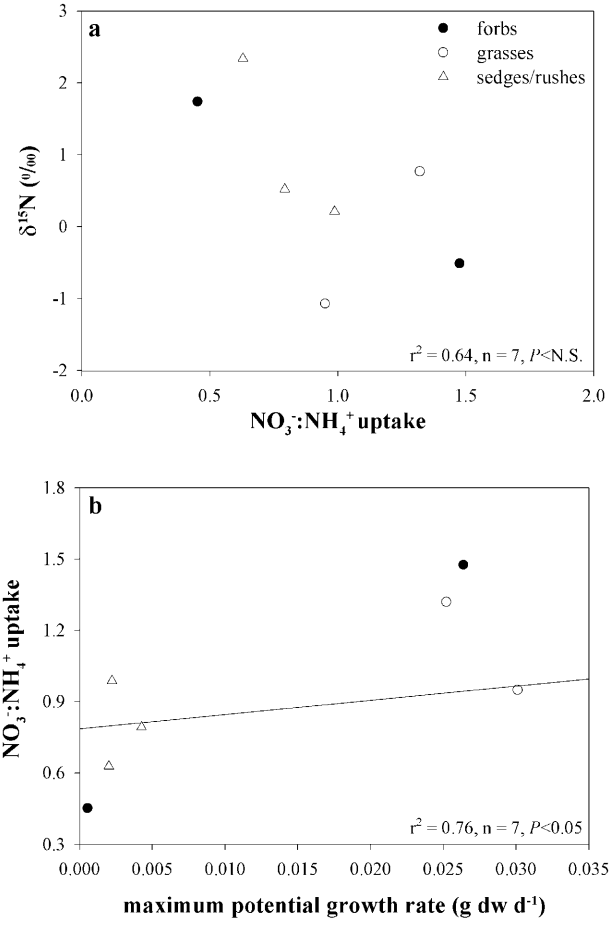

Fig. 5 Relationship between NO_3^-:NH_4^+ uptake ratio and **a** foliar $\delta^{15}N$, and **b** maximum potential growth rate for species collected at Niwot Ridge. Forbs: *Acomastylis, Artemisia*. Grasses: *Festuca, Calamagrostis*. Sedges/rushes: *Carex, Kobresia, Luzula*

ability to take up NH_4^+ and NO_3^-. *Festuca* (grass) showed no difference between NH_4^+ and NO_3^- uptake when the uptake rate was calculated on a per unit aboveground biomass basis (Fig. 3), but showed greatest uptake of NO_3^- when uptake was calculated on a per unit tissue N basis ($F_{2,29}$=10.69, P<0.001; data not shown).

All species showed significant ^{13}C enrichment of shoot tissue with uptake of ^{13}C-^{15}N-labeled glycine (Fig. 4). Glycine uptake ranged from approximately 35% to >100% of NH_4^+ uptake, but only *Festuca* showed glycine uptake exceeding that of both NH_4^+ and NO_3^-. Mass balance calculations (molar ratio of recovered ^{15}N:^{13}C) indicated that at least two species, *Festuca* and *Luzula*, took up between 50% and 70% of ^{15}N as intact glycine (r^2=0.31, P<0.06; r^2=0.76, P<0.001 for *Festuca* and *Luzula*, respectively).

We used correlation analyses to examine relationships among species in foliar $\delta^{15}N$ (field), N uptake rate (greenhouse) and potential growth rate (greenhouse), using within-species' means from both the field and greenhouse. NO_3^-:NH_4^+ uptake rates explained 64% of the variation in foliar $\delta^{15}N$ among species, with species showing the greatest NO_3^- uptake rates also having the

most depleted foliar $\delta^{15}N$ (Fig. 5a). While this relationship was not statistically significant, the correlation between NO_3^-:NH_4^+ uptake and growth rate was significant (Fig. 5b), and the correlation between foliar $\delta^{15}N$ and growth rate nearly so (r^2=0.72, n=7, P<0.07; data not shown).

Discussion

Differential uptake of N: implications for plant partitioning

Our results from the field and greenhouse are consistent with the hypothesis that co-occurring alpine species may be differentially utilizing soil NH_4^+, NO_3^- and organic N (glycine). All species were able to take up all forms of N in the greenhouse, but species were not uniformly flexible in their resource (N) use. If we assume that species' N uptake characteristics in the greenhouse are representative of their uptake patterns in the field, we see that no single pattern of N uptake predominated among species: some species appeared to preferentially take up a single form of N, while others showed no difference in the ability to take up different forms of N.

While it is difficult to demonstrate that plants are actually partitioning soil N on the basis of chemical form, our foliar $\delta^{15}N$ and ^{15}N uptake results are more consistent with this scenario than with the alternative of no N partitioning among species, for which we would predict no difference among species in either foliar $\delta^{15}N$ (field) or in the ability to take up different forms of N (greenhouse). However, because the species in this study differed in foliar $\delta^{15}N$ and in the ability to take up NH_4^+, NO_3^- and glycine, both of which may be indicators of the form of N taken up, we argue that these plants have the potential to partition soil N on the basis of N form. Furthermore, the constancy in rank of foliar $\delta^{15}N$ across sites of varying N availability suggests that most of these species may exhibit relatively fixed traits with respect to N utilization, regardless of site characteristics.

Our field NRA and foliar $\delta^{15}N$ data corroborate the results of the greenhouse ^{15}N-tracer experiment. While species were able to take up all forms of N in the greenhouse, our results suggest that some species may show a disproportionate uptake of one or more forms of N in the field. NRA has been shown to be correlated with NO_3^- uptake in plants (Högberg et al. 1986; Stewart et al. 1993), and the elevated NRAs in several of our dry meadow species provide additional evidence that these plants may have recently taken up NO_3^-. We found species-specific, inverse relationships between $\delta^{15}N$ and NRA, suggesting that species with depleted foliar $\delta^{15}N$ signatures may rely on NO_3^- for their N nutrition to a greater degree than NH_4^+ and organic N.

Most species showed a greater ability to take up inorganic N than glycine, despite the relatively high concentrations of total free amino acids, particularly glycine, measured in dry meadow tundra soils (Raab et al. 1996). Several of the study species appeared to utilize NO_3^- disproportionately well, even though net nitrification rates in dry meadow soils are low during the growing season (0.9 g m^{-2}; Fisk and Schmidt 1995; Fisk et al. 2001) and exchangeable NO_3^- concentrations are frequently an order of magnitude lower than NH_4^+ and DON. The N-treatment concentration of 1 mM used in the greenhouse was 4–10 times greater than exchangeable N concentrations in dry meadow soil and thus may have influenced uptake kinetics in the study species. However, the results of the greenhouse tracer experiment are consistent with both the foliar $\delta^{15}N$ and NRA data and with preliminary field ^{15}N-tracer uptake results (A. E. Miller and W. D. Bowman, unpublished data), suggesting that the uptake patterns we observed in the greenhouse reflect potential patterns of N use in the field.

Factors controlling foliar $\delta^{15}N$

Implicit in our interpretation of foliar $\delta^{15}N$ is the assumption that foliar $\delta^{15}N$ reflects source (soil N) $\delta^{15}N$ to some degree. Foliar $\delta^{15}N$ should reflect the $\delta^{15}N$ of soil N sources when N is limiting (Högberg 1997; Högberg et al. 1999), and as N is processed within the soil, N sources should become progressively depleted in $\delta^{15}N$ if denitrification rates are low (i.e., $\delta^{15}N$-NO_3^- < $\delta^{15}N$-NH_4^+ ≤ $\delta^{15}N$-organic N; Nadelhoffer and Fry 1988; Högberg 1997). We used soil $\delta^{15}N$ to bracket foliar $\delta^{15}N$ values and to show that $\delta^{15}N$-NH_4^+ > $\delta^{15}N$-NO_3^-, from which we have inferred, qualitatively, the inorganic forms of N that may have been taken up by plants. However, $\delta^{15}N$ cannot be used as a tracer of N source, as a number of other factors including mycorrhizal infection, internal fractionations, and plant rooting depth may also contribute to variation in foliar $\delta^{15}N$ (Handley and Scrimgeour 1997; Högberg 1997; Hobbie et al. 1999; Evans 2001).

All but two of the species in our study were AM infected, and fractionation associated with mycorrhizal uptake and/or transfer of isotopically depleted N may have contributed to depleted foliar $\delta^{15}N$ values (Handley et al. 1993; Azcón-G-Aguilar et al. 1998; Hobbie et al. 1999). When ratios of N_{uptake}:$N_{supplied}$ are high, however, fractionation may be minimal (Handley et al. 1993; Högberg et al. 1999). In our study, foliar $\delta^{15}N$ was not correlated with mycorrhizal infection, but frequency of arbuscules and NRA were positively correlated across species, suggesting enhanced uptake of NO_3^- with AM infection (Azcón and Tobar 1998; Subramanian and Charest 1999).

We estimated the degree to which fractionation associated with mycorrhizal uptake and transfer of N could contribute to variation in foliar $\delta^{15}N$ using a model outlined by Hobbie et al. (2000). When we set mycorrhizal fractionation, Δ_f, equal to 10‰ and mycorrhizal transfer ratio, T_r, equal to 0.2 (cf. Hobbie et al. 2000) and used estimates of mycorrhizal infection and $\delta^{15}N_{available(soil)N}$ from Niwot Ridge, we found that the best approxima-

tions of $\delta^{15}N_{plant}$ for species at each end of the isotopic range (*Mertensia*, *Luzula*; Fig. 1b) were derived from the $\delta^{15}N_{available\ N}$ of soil NO_3^- and NH_4^+, respectively, assuming 100% uptake of each N form. Hence, where mycorrhizal infection rates are relatively low, $\delta^{15}N_{available\ N}$ may contribute substantially to foliar $\delta^{15}N$.

As with N uptake and transfer by mycorrhizal fungi, internal fractionations associated with plant assimilation of NH_4^+ and NO_3^- may be minimal when external N concentrations are low (Evans et al. 1996). However, fractionation associated with NO_3^- assimilation in the roots may account for significant enrichment in leaves (Yoneyama and Kaneko 1989; Evans et al. 1996). Such variation may confound the use of foliar $\delta^{15}N$ as a proxy for whole plant $\delta^{15}N$, but we found no difference between root and shoot $\delta^{15}N$ in the species at Niwot Ridge (W. D. Bowman, unpublished data).

Differences among species in foliar $\delta^{15}N$ may also reflect differences in rooting depth, as soil $\delta^{15}N$ tends to become enriched with depth (Nadelhoffer and Fry 1988; Koba et al. 1998; Hobbie et al. 2000). However, roots of dry meadow species are concentrated within the upper 15 cm of soil (Webber and May 1977), and we found no difference in the $\delta^{15}N$ of bulk soils collected from the dry meadow at 5 cm and 15 cm (A. E. Miller and W. D. Bowman, unpublished data). In addition, a subset of species used in this study were found to take up a greater proportion of N from depths of 5 cm than from 15 cm (A. E. Miller and W. D. Bowman, unpublished data), suggesting that the observed variation in foliar $\delta^{15}N$ is due to factors other than rooting depth.

Conclusion

The intraspecific differences that we observed in foliar $\delta^{15}N$ and plant uptake of NO_3^- and NH_4^+, and the variation among species in the uptake of these N forms, suggest that dry meadow species have the potential to partition soil N on the basis of N form. With a shift in N economy (e.g., via fertilization) some species may be able to alter the forms of N taken up, whereas others may show little flexibility in N use (Nadelhoffer et al. 1996; Frank and Evans 1997; Nielsen et al. 1998). The alpine species in our study were relatively flexible in their N use, although species showing the highest growth rates and greatest uptake of inorganic N, notably NO_3^- [e.g., *Artemisia* (forb); *Calamagrostis*, *Festuca* (grasses)], should be favored by increasing inputs of inorganic N. In fact, *Calamagrostis* and *Festuca* have already shown an approximately 15–40% increase in relative cover in response to 10 years of N fertilization at Niwot Ridge (K. Suding and W. D. Bowman, unpublished data). An understanding of the species-specific patterns of NH_4^+, NO_3^-, and glycine uptake under a range of field conditions may therefore increase our understanding of the mechanisms responsible for species coexistence in this and other N-limited systems, and may enable us to predict how these systems will respond to additional N inputs.

Acknowledgements Thanks to David Harris, Stable Isotope Facility, University of California, Davis; Christine Seibold, University of Colorado Mountain Research Station; and Tom Lemieux, University of Colorado. Trevor Popp and R. Scott Cornman helped with field and laboratory work, and Russell Monson, Katherine Nash Suding, and Ruth Ley provided helpful comments on early drafts. This work was supported by a grant from the Andrew W. Mellon Foundation to W. D. Bowman and by the Niwot Ridge Long-Term Ecological Research program.

References

Atkin OK, Cummins R (1994) The effect of root temperature on the induction of nitrate reductase activities and nitrogen uptake rates in arctic plant species. Plant Soil 159:187–197

Azcón R, Tobar RM (1998) Activity of nitrate reductase and glutamine synthetase in shoot and root of mycorrhizal *Allium cepa*: effect of drought stress. Plant Sci 133:1–8

Azcón-G-Aguilar R, Handley LL, Scrimgeour CM (1998) The $\delta^{15}N$ of lettuce and barley are affected by AM status and external concentration of N. New Phytol 138:19–26

Bowman WD, Theodose TA, Schardt JC, Conant RT (1993) Constraints of nutrient availability on primary production in two alpine tundra communities. Ecology 74:2085–2097

Bowman WD, Theodose TA, Fisk MC (1995) Physiological and production responses of plant growth forms to increases in limiting resources in alpine tundra: implications for differential community response to environmental change. Oecologia 101:217–227

Burns SF (1980) Alpine soil distribution and development, Indian Peaks, Colorado Front Range. Dissertation. University of Colorado, Boulder, Colo.

Chang CY, Langston J, Riggs M, Campbell DH, Silva SR, Kendall C (1999) A method for nitrate collection for $\delta^{15}N$ and $\delta^{18}O$ analysis from waters with low nitrate concentrations. Can J Fish Aquat Sci 56:1856–1864

Chapin FS III, Moilanen L, Kielland K (1993) Preferential use of organic nitrogen for growth by a non-mycorrhizal arctic sedge. Nature 361:150–153

Evans RD (2001) Physiological mechanisms influencing plant nitrogen isotope composition. Trends Plant Sci 6:121–128

Evans RD, Bloom JA, Sukrapanna SS, Ehleringer JR (1996) Nitrogen isotope composition of tomato (*Lycopersicon esculentum* Mill. cv. T-5) grown under ammonium and nitrate nutrition. Plant Cell Environ 19:1317–1323

Fisk MC, Schmidt SK (1995) Nitrogen mineralization and microbial biomass nitrogen dynamics in three alpine tundra communities. Soil Sci Soc Am J 59:1036–1043

Fisk MC, Schmidt SK (1996) Microbial responses to nitrogen additions in alpine tundra soil. Soil Biol Biochem 28:751–755

Fisk MC, Brooks PD, Schmidt SK (2001) Nitrogen cycling. In: Bowman WD, Seastedt TR (eds) Structure and function of an alpine ecosystem. Oxford University Press, New York, pp 237–253

Frank DA, Evans DR (1997) Effects of native grazers on grassland N cycling in Yellowstone National Park. Ecology 78:2238–2248

Gough L, Osenberg CW, Gross KL, Collins SL (2000) Fertilization effects on species density and primary productivity in herbaceous plant communities. Oikos 89:428–439

Hageman RH, Hucklesby DP (1971) Nitrate reductase from higher plants. In: San Pietro A (ed) Methods in enzymology, vol 23. Academic Press, New York, pp 491–503

Handley LL, Scrimgeour CM (1997) Terrestrial plant ecology and ^{15}N natural abundance: the present limits to interpretation for uncultivated systems with original data from a Scottish old field. Adv Ecol Res 27:133–212

Handley LL, Daft MJ, Wilson J, Scrimgeour CM, Ingleby K, Sattar MA (1993) Effects of the ecto- and VA-mycorrhizal fungi *Hydnagium carneum* and *Glomus clarum* on the $\delta^{15}N$ and $\delta^{13}C$ values of *Eucalyptus globulus* and *Ricinus communis*. Plant Cell Environ 16:375–382

Hart SC, Stark DM, Davidson EA, Firestone MK (1994) Nitrogen mineralization, immobilization, and nitrification. In: Weaver RW, Angle JS, Bottomley PS (eds) Methods of soil analysis. Part 2. Microbiological and biochemical properties. Soil Science Society of America book series, no. 5. Soil Science Society of America, Madison, Wis. pp 985–1018

Hauck RD, Bremner JM (1976) Use of tracers for soil and fertilizer nitrogen research. Adv Agron 28:219–260

Heuer K, Brooks PD, Tonnessen KA (1999) Nitrogen dynamics in two high elevation catchments during spring snowmelt 1996, Rocky Mountains, Colorado. Hydrol Process 13:2203–2214

Hobbie EA, Macko SA, Shugart HH (1999) Insights into nitrogen and carbon dynamics of ectomycorrhizal and saprotrophic fungi from isotopic evidence. Oecologia 118:353–360

Hobbie EA, Macko SA, Williams M (2000) Correlations between foliar $\delta^{15}N$ and nitrogen concentrations may indicate plant-mycorrhizal interactions. Oecologia 122:273–283

Högberg P (1997) Tansley review no. 95. ^{15}N natural abundance in soil-plant systems. New Phytol 137:179–203

Högberg P, Granström A, Johansson T, Lundmark-Thelin A, Näsholm T (1986) Plant nitrate reductase activity as an indicator of availability of nitrate in forest soils. Can J For Res 16:1165–1169

Högberg P, Högberg MN, Quist ME, Ekblad A, Näsholm T (1999) Nitrogen isotope fractionation during nitrogen uptake by ectomycorrhizal and non-mycorrhizal *Pinus sylvestris*. New Phytol 142:569–576

Holmes RM, McClelland JW, Sigman DM, Fry B, Peterson BJ (1998) Measuring $^{15}N\text{-}NH_4^+$ in marine, estuarine and fresh waters: an adaptation of the ammonia diffusion method for samples with low ammonium concentrations. Mar Chem 60:235–243

Jaeger CH, Monson RK, Fisk MC, Schmidt SK (1999) Seasonal partitioning of nitrogen by plants and soil microorganisms in an alpine ecosystem. Ecology 80:1883–1891

Jaworski EG (1971) Nitrate reductase in intact plant tissue. Biochem Biophys Res Commun 43:1274–1279

Knowles R, Blackburn TH (1993) Nitrogen isotope techniques. Academic Press, New York, pp 263–268

Koba K, Tokuchi N, Yoshioka T, Hobbie EA, Iwatsubo G (1998) Natural abundance of nitrogen-15 in a forest soil. Soil Sci Soc Am J 62:778–781

Leadley PW, Reynolds JF, Chapin FS III (1997) A model of nitrogen uptake by *Eriophorum vaginatum* roots in the field: ecological implications. Ecol Monogr 67:1–22

Lipson DA, Schmidt SK, Monson RK (1999) Links between microbial population dynamics and nitrogen availability in an alpine ecosystem. Ecology 80:1623–1631

Lipson DA, Raab TK, Schmidt SK, Monson RK (2001) An empirical model of amino acid transformations in an alpine soil. Soil Biol Biochem 33:189–198

Nadelhoffer KJ, Fry B (1988) Controls on natural nitrogen-15 and carbon-13 abundances in forest soil organic matter. Soil Sci Soc Am J 52:1633–1640

Nadelhoffer K, Shaver G, Fry B, Giblin A, Johnson L, McKane R (1996) ^{15}N natural abundances and N use by tundra plants. Oecologia 107:386–394

Näsholm T, Ekblad A, Nordin A, Giesler R, Högberg M, Högberg P (1998) Boreal forest plants take up organic nitrogen. Nature 392:914–916

Nielson R, Hamilton D, Wishart J, Marriot CA, Boag B, Handley LL, Scrimgeour CM, McNicol JW, Robinson D (1998) Stable isotope natural abundances of soil, plants and soil invertebrates in an upland pasture. Soil Biol Biochem 30:1773–1782

Phillips JM, Hayman DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans Br Mycol Soc 55:158–161

Raab TK, Lipson DA, Monson RK (1996) Non-mycorrhizal uptake of amino acids by roots of the alpine sedge *Kobresia myosuroides*: implications for the alpine nitrogen cycle. Oecologia 108:488–494

Raab TK, Lipson DA, Monson RK (1999) Soil amino acid utilization among species of the Cyperaceae: plant and soil processes. Ecology 80:2408–2419

Snell FD, Snell CT (1949) Nitrites. In: Snell FD, Snell CT (eds) Colorimetric methods of analysis. Van Nostrand, Princeton, pp 802–807

Stark JM, Hart SC (1996) Diffusion technique for preparing salt solutions, Kjeldahl digests, and persulfate digests for nitrogen-15 analysis. Soil Sci Soc Am J 60:1846–1855

Stewart GR, Pate JS, Unkovich M (1993) Characteristics of inorganic nitrogen assimilation of plants in fire-prone Mediterranean-type vegetation. Plant Cell Environ 16:351–363

Subramanian KS, Charest C (1999) Acquisition of N by external hyphae of an arbuscular mycorrhizal fungus and its impact on physiological responses in maize under drought-stressed and well-watered conditions. Mycorrhiza 9:69–75

Theobald PK Jr, Lakin HW, Hawkins DB (1963) The precipitation of aluminum, iron and manganese at the junction of Deer Creek with the Snake River in Summit County, Colorado. Geochim Cosmochim Acta 27:121–132

Theodose TA, Bowman WD (1997) Nutrient availability, plant abundance, and species diversity in two alpine tundra communities. Ecology 78:1861–1872

Webber PJ, May DE (1977) Magnitude and distribution of below ground plant structures in alpine tundra of Niwot Ridge, Colorado. Arct Alp Res 9:157–174

Weber WA (1976) Rocky Mountain flora: a field guide for the identification of the ferns, conifers, and flowering plants of the southern Rocky Mountains from Pikes Peak to Rocky Mountain National Park and from the plains to the Continental Divide. University Press of Colorado, Niwot, Colo.

Yoneyama T, Kaneko A (1989) Variations in the natural abundance of 15 N in nitrogenous fractions of komatsuna plants supplied with nitrate. Plant Cell Physiol 30:957–962