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Determinants of ¹⁵N natural abundance in leaves of co-occurring plant species and types within an alpine lichen heath in the Northern Caucasus

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Abstract

Several factors may have interactive effects on natural ^{15}N abundance of plant species. Some of these effects could be associated with different plant functional types, including mycorrhizal association type. Due to its high taxonomic and functional diversity, the alpine heath community in the Caucasus is a suitable object for studying ^{15}N natural abundance of plants in relation to different functional/mycorrhizal groups, contrasting with the limited numbers of plant groups or species considered in previous studies of individual communities. The N concentration and $\delta^{15}N$ were determined in leaves of 25 plant species from 8 functional/mycorrhizal groups from an alpine lichen heath in the Teberda Reserve, Northern Caucasus, Russia. Functional groups were represented by ericoid mycorrhizal species (ERI), ectomycorrhizal species (ECT), arbuscular mycorrhizal forbs (AM–FORB), arbuscular mycorrhizal grasses (AM–GRA), arbuscular mycorrhizal nodulated legumes (FAB–N), non-mycorrhizal graminoids (sedges and rushes) (NOM–GRA), non-mycorrhizal hemiparasites (NOM–SP), and orchids (ORC). We can summarize our results in two rankings for leaf N concentration (FAB–N > ORC > AM–FORB, ECT > NOM–SP, ERI ≥ NOM–GRA, AM–GRA) and leaf $\delta^{15}N$ signature (ORC > NOM–GRA, FAB–N > ECT ≥ ERI ≥ AM–FORB, NOM–SP, AM–GRA) of alpine heath species. We conclude that, within the alpine lichen heath in the Northern Caucasus, the $\delta^{15}N$ signature of plant foliage is a relevant indicator of plant functional groups with relatively high ^{15}N content (ORC, FAB-N, NOM-GRA), while the absence of a significant difference between relatively ^{15}N-depleted groups (AM, ERI, and ECT species) isn't clear and may result from both processes, as the increased N isotope fractionation by arbuscular mycorrhizal fungi as the decreased role of ecto- and ericoid mycorrhizal fungi in the flux of N.

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Introduction

Plant growth in alpine communities is often nitrogen (N) limited, and the various adaptations of plant species to cope with such limitation may be reflected in interspecific variation in leaf N concentrations and leaf $\delta^{15}N$ signature, which give some information about N uptake and conservation strategies in infertile environments (Michelsen et al., 1998; Cornelissen et al., 2001; Quested et al., 2003; Hobbie and Hobbie, 2008). However, several underlying factors may have interactive effects on natural ^{15}N abundance of plant species. Such factors include (1) the difference in $\delta^{15}N$ of nitrogen sources (NH_4^+, NO_3^-, N_2, organic N), (2) the difference in $\delta^{15}N$ of the same sources in different soil horizons (as related to plant rooting depth), (3) isotope discrimination during N uptake and assimilation, and (4) the influence of mycorrhiza (Schulze et al., 1994; Nadelhoffer et al., 1996; Högberg, 1997; Evans, 2001; Robinson, 2001; Miller and Bowman, 2002; Craine et al., 2009). Some of these effects could be associated with different plant functional types, including mycorrhizal association type, and have been convincingly confirmed for co-occurring species in field studies.

The most evident differentiation in leaf $\delta^{15}N$ has been shown for plants with different types of mycorrhiza. ^{15}N depletion of ectomycorrhizal and ericoid mycorrhizal species was found for tundra, alpine, and forest plants from different northern regions and was initially attributed to the influence of soil nitrogen sources, that is, fungal uptake of ^{15}N-depleted organic N compounds (Schulze et al., 1994; Nadelhoffer et al., 1996; Michelsen et al., 1996, 1998). However, subsequent studies indicated that ectomycorrhizal and ericoid mycorrhizal species are ^{15}N-depleted by preferred transfer of ^{14}N from mycorrhizal fungi to their host plants (Högberg et al., 1999; Hobbie et al., 2000; Emmerton et al., 2001; Hobbie and Colpaert, 2003).

All other effects (different nitrogen sources, source localization in the soil, and isotope fractionation during nitrogen uptake) have been accompanied with no or very special empirical field observations. For instance, the influence of N sources on ^{15}N concentration in plant leaves has been indicated for spatially separated plants in agricultural studies where $\delta^{15}N$ of plants directly reflected utilization of isotopically different N fertilizers (Choi et al., 2003; Yun et al., 2006). Another example is the differences in $\delta^{15}N$ for plants of different communities along a geochemical gradient for which a clear effect of ^{15}N abundances of N sources was observed (Garten, 1993). However, for co-occurring species within a community, the effect of different nitrogen sources was observed in special cases only, that is, legumes that obtain atmospheric N_2 through fixation by symbiotic bacteria (Bowman et al., 1996; Michelsen et al., 1996; Nadelhoffer et al., 1996; Körner, 2003; Hobbie et al., 2005) or orchids that obtain N from their mycorrhizal fungi by transfer from hyphae (Gebauer and Meyer, 2003; Zimmer et al., 2007).

The influence of rooting depth has been confirmed experimentally even less. Earlier assumptions that higher $\delta^{15}N$ values of arbuscular mycorrhizal *Calamagrostis canadensis* (Schulze et al., 1994) and non-mycorrhizal *Eriophorum vaginatum* (Nadelhoffer et al., 1996) in central and northern Alaska ecosystems was a result of deeper roots seem incorrect because these species were compared with ericoid mycorrhizal and ectomycorrhizal species in which ^{15}N depletion is a function of metabolic N fractionation by mycorrhizal fungi (Högberg et al., 1999; Hobbie et al., 2000; Emmerton et al., 2001). This difference could be also connected with different preferential uptake of N compounds. Furthermore, our recent study indicated that plants with deeper root systems can probably consume lighter rather than heavier NH_4^+–N (Makarov et al., 2008).

Due to its high taxonomic and functional diversity, the alpine heath community in the Caucasus is a suitable object for studying ^{15}N natural abundance of plants in relation to different functional/mycorrhizal groups, contrasting with the limited numbers of plant groups or species considered in previous studies of individual communities. We hypothesized that $\delta^{15}N$ of plant leaves within the alpine heath community is the result of several interacting drivers associated with their functional/mycorrhizal status. The objectives of this study were (1) to compare N concentration and $\delta^{15}N$ in leaves of co-occurring species within an alpine heath community, and (2) to investigate if variation in N concentration and ^{15}N natural abundance is characteristic for different plant functional/mycorrhizal groups.

Material and Methods

SITE DESCRIPTION

The research was conducted at the Teberda Biosphere Reserve (Northwestern Caucasus, Russia). The study site was located at Mount Malaya Khatipara (43°27′N, 41°42′E) at 2750 m a.s.l. in an alpine lichen heath, which has been the subject of diverse ecological studies during more than 20 years (Onipchenko, 2004). The climate of the area is characterized by low air temperatures (mean annual temperature is –1.2 °C, mean July temperature is 7.9 °C) and high annual precipitation (1400 mm). The lichen heath covers snow-free wind-exposed ridges and upper slopes in the alpine landscape. The plant community is dominated by fruticose lichens (mainly *Cetraria islandica*), which cover about 30%–60% of the area. Vascular plants are represented by more than 40 species. The most common are *Anemone speciosa, Antennaria dioica, Trifolium polyphyllum, Festuca ovina, Carex sempervirens, Carex umbrosa, Campanula tridentata,* and *Vaccinium vitis-idaea*. Detailed description of the site plant community, including species' nomenclature, is published elsewhere (Onipchenko, 2002).

STUDIED SPECIES

Twenty-five species from eight functional/mycorrhizal groups (three species per group, except legumes which were represented by three nodulated and one non-nodulated [*Trifolium polyphyllum*] species) were studied. The different functional groups with respect to nutrient uptake strategy were represented by ericoid mycorrhizal species (ERI), ectomycorrhizal species (ECT), arbuscular mycorrhizal forbs (AM–FORB), arbuscular mycorrhizal grasses (AM–GRA), arbuscular mycorrhizal nodulated legumes (FAB–N), non-mycorrhizal graminoids (sedges and rushes) (NOM–GRA), non-mycorrhizal hemiparasites (NOM–SP), and orchids (ORC) (Table 1). Type of mycorrhiza and mycorrhizal infection rate for arbuscular mycorrhizal species (proportion of thin root mesoderm cells occupied by fungi) were studied earlier (Onipchenko and Zobel, 2000; Onipchenko, 2004).

Foliage from these plant species was sampled during one week at the beginning of August in five replications from a sampling site of 30 × 30 m in size. In general, leaves from five separate plants per species were collected, although in some cases several individuals of a species were pooled to make one replicate sample.

CHEMICAL ANALYSES

Total N was determined by dry combustion on an Elementar Vario EL elemental analyzer. Natural ^{15}N abundance of plant samples was determined by dry combustion on a Carlo Erba NC 2500 elemental analyzer coupled with a Delta^{plus} continuous-flow isotope ratio mass spectrometer (Thermo Finnigan, Bremen, Germany) and expressed as δ values, which were defined as:

$$[(\text{atom\% } ^{15}N_{sample} - \text{atom\% } ^{15}N_{standard}) / \text{atom\% } ^{15}N_{standard}] \times 1000. \quad (1)$$

STATISTICS

Nested design ANOVAs in general linear models were applied to estimate differences in nitrogen content and $\delta^{15}N$ for plant species and functional types, with three species nested in each functional type (and excluding the non-nodulated legume). In case of significant effects, post hoc Tukey HSD test was used for comparison of means between individual species or functional groups. The calculations were made in Statistica 6.0 for Windows.

To compare nitrogen content and $\delta^{15}N$ between three nodulated legumes and the non-nodulated legume *Trifolium polyphyllum,* one-way ANOVA was used. Correlation coefficients were calculated to test for a relationship between mycorrhizal infection rate and nitrogen content or $\delta^{15}N$ for AM forbs and grasses.

Results

NITROGEN CONCENTRATION

The N concentration varied significantly and widely among alpine plant species and functional groups of lichen heath (Tables 2 and 3, Fig. 1). *Festuca ovina* (AM–GRA) had the lowest N concentration (0.97%), whereas *Astragalus levieri* (FAB–N) and *Traunsteinera globosa* (ORC) were the most N-rich (3.30% and 3.34%, respectively). The groups of N_2-fixing legumes, orchids, arbuscular mycorrhizal forbs, and ectomycorrhizal species generally had higher N concentrations than other groups. Non-mycorrhizal hemiparasites and ericoid mycorrhizal species tended to have a somewhat higher N concentration among the four groups with relatively low N concentrations. Graminoids, irrespective of their mycorrhizal status, had the lowest N leaf concentration. So, we may represent the following ranking of the leaf N concentration among alpine heath species:

TABLE 1

Mycorrhizal and functional types of the studied plant species.

Species	Family	Mycorrhiza	Functional type
Anemone speciosa	Ranunculaceae	AM	AM-FORB
Anthyllis vulneraria	Fabaceae	AM	FAB-N
Astragalus levieri	Fabaceae	AM	FAB-N
Bromus variegatus	Poaceae	AM	AM-GRA
Campanula tridentata	Campanulaceae	AM	AM-FORB
Carex sempervirens	Cyperaceae	NO	NOM-GRA
Carex umbrosa	Cyperaceae	NO	NOM-GRA
Carum caucasicum	Apiaceae	AM	AM-FORB
Coeloglossum viride	Orchidaceae	ORC	ORC
Euphrasia ossica	Scrophulariaceae	NO	NOM-SP
Festuca ovina	Poaceae	AM	AM-GRA
Gymnadenia conopsea	Orchidaceae	ORC	ORC
Helictotrichon versicolor	Poaceae	AM	AM-GRA
Juniperus communis	Cupressaceae	ECT	ECT
Luzula spicata	Juncaceae	NO	NOM-GRA
Oxytropis kubanensis	Fabaceae	AM	FAB-N
Pedicularis caucasica	Scrophulariaceae	NO	NOM-SP
Pedicularis comosa	Scrophulariaceae	NO	NOM-SP
Polygonum viviparum	Polygonaceae	ECT	ECT
Rhododendron caucasicum	Ericaceae	ERI	ERI
Salix kazbekensis	Salicaceae	ECT	ECT
Traunsteinera globosa	Poaceae	ORC	ORC
Trifolium polyphyllum	Fabaceae	AM	FAB-NON
Vaccinium myrtillus	Ericaceae	ERI	ERI
Vaccinium vitis-idaea	Ericaceae	ERI	ERI

Notes: Mycorrhiza (AM = arbuscular, ECT = ecto, ERI = ericoid, ORC = orchid types, NO = non-mycorrhizal species). Functional types: (AM-GRA = arbuscular mycorrhizal grasses, AM-FORB = arbuscular mycorrhizal forbs, ECT = ectomycorrhizal species, ERI = ericoid mycorrhizal species, FAB = arbuscular mycorrhizal legumes [N = nodulated, NON = without nodules], NOM-GRA = non-mycorrhizal graminoids (sedges and rushes), NOM-SP = non-mycorrhizal hemiparasites, ORC = orchids).

TABLE 2

Results of nested ANOVA (species are nested in functional type) for nitrogen concentration and $\delta^{15}N$.

	SS	Degrees of freedom	MS	*F*	*p*
N (%)					
Intersept	520.70	1	520.70	12695	<0.0001
Species (type)	18.197	16	1.14	27.7	<0.0001
Type	40.23	7	5.75	140.1	<0.0001
Error	3.81	93	0.041		
$\delta^{15}N$ (‰)					
Intercept	539.92	1	539.92	835.0	<0.0001
Species (type)	93.41	16	5.84	9.03	<0.0001
Type	264.90	7	37.84	58.52	<0.0001
Error	60.14	93	0.647		

TABLE 3

Leaf N concentration and $\delta^{15}N$ for studied alpine species from different functional types (see Table 1 for abbreviations).

Species	Functional type	N (%) Mean	N (%) SE	$\delta^{15}N$ (‰) Mean	$\delta^{15}N$ (‰) SE
Anemone speciosa	AM-FORB	2.40	0.10	–2.20	0.28
Campanula tridentata	AM-FORB	2.14	0.09	–4.40	0.36
Carum caucasicum	AM-FORB	2.68	0.09	–3.58	0.34
Bromus variegatus	AM-GRA	1.64	0.12	–5.52	0.41
Festuca ovina	AM-GRA	0.97	0.04	–3.60	0.34
Helictotrichon versicolor	AM-GRA	1.43	0.01	–2.22	0.49
Juniperus communis	ECT	1.48	0.12	–3.14	0.37
Polygonum viviparum	ECT	3.12	0.09	–2.30	0.35
Salix kazbekensis	ECT	2.61	0.13	–1.59	0.38
Rhododendron caucasicum	ERI	1.64	0.10	–4.38	0.38
Vaccinium myrtillus	ERI	2.23	0.11	–1.55	0.86
Vaccinium vitis-idaea	ERI	1.25	0.04	–2.76	0.20
Anthyllis vulneraria	FAB-N	2.64	0.07	–1.86	0.10
Astragalus levierii	FAB-N	3.30	0.10	–0.71	0.20
Oxytropis kubanensis	FAB-N	3.21	0.11	–0.81	0.36
Trifolium polyphyllum	FAB-NON	2.05	0.02	–2.62	0.37
Carex sempervirens	NOM-GRA	1.46	0.12	–1.47	0.38
Carex umbrosa	NOM-GRA	1.23	0.05	–1.17	0.38
Luzula spicata	NOM-GRA	1.75	0.03	–0.20	0.43
Euphrasia ossica	NOM-SP	2.24	0.06	–3.48	0.29
Pedicularis caucasica	NOM-SP	1.34	0.05	–4.17	0.23
Pedicularis comosa	NOM-SP	1.66	0.10	–2.96	0.31
Coeloglossum viride	ORC	2.81	0.16	0.07	0.39
Gymnadenia conopsea	ORC	2.21	0.06	–0.02	0.39
Traunsteinera globosa	ORC	3.34	0.10	2.33	0.27

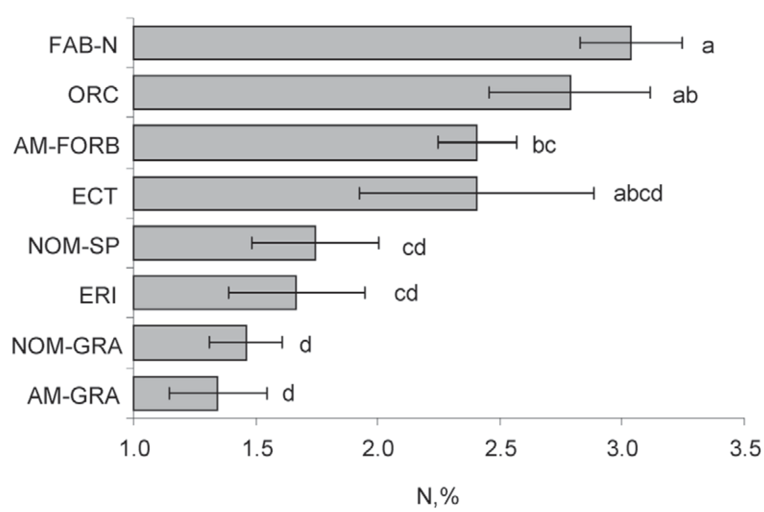

FIGURE 1. Leaf N concentration for different functional types of alpine plants (mean and standard error) (see Table 1 for abbreviations). Significant ($p < 0.05$) differences are shown by different letters.

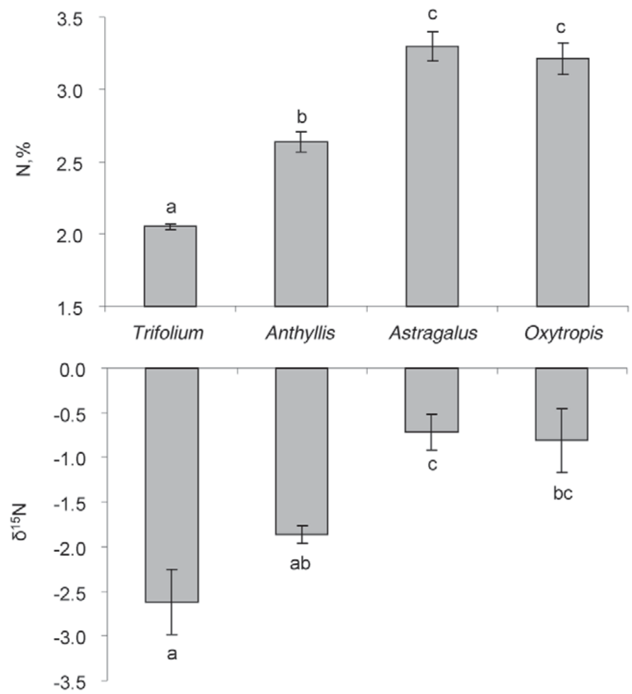

FIGURE 2. Leaf N concentration and $\delta^{15}N$ for 3 nitrogen fixing and one non-fixing (*Trifolium polyphyllum*) legumes in the alpine lichen heath. Significant (p < 0.05) differences are shown by different letters.

FAB–N > ORC > AM–FORB, ECT > NOM–SP,
ERI ≥ NOM–GRA, AM–GRA.

However, within separate functional groups the variation was also high, and in each group there were species differing significantly in N concentration from each other. For example, ectomycorrhizal *Polygonum viviparum* had more than twice the N concentration (3.12%) of another ectomycorrhizal species—*Juniperus communis* (1.48%). Within other groups, pronounced differences were also observed (Table 3). A separate one-way ANOVA for legumes indicated that leaf N concentration in non-fixing *Trifolium polyphyllum* was significantly lower than the N concentrations for other legumes (Fig. 2).

There was a highly significant positive correlation (r^2 = 0.70) for six AM species (forbs and grasses) between N leaf concentration and rate (degree) of AM infection (Fig. 3). The same tendency is apparent within three species of grasses, but not within forbs.

^{15}N ABUNDANCE

Natural abundance of ^{15}N in the plant leaves also varied widely among species within the lichen heath community, ranging from –5.5‰ in *Bromus variegatus* (AM–GRA) to +2.3‰ *Traunsteinera globosa* (ORC). All orchid species had about zero or positive $\delta^{15}N$; their mean of +0.8‰ was significantly higher than that in any other group, excluding the marginally ^{15}N-depleted non-mycorrhizal graminoids (sedges and rushes) and nodulated legumes (Table 3, Fig. 4). In contrast, there was no single most ^{15}N-depleted group. Pronounced ^{15}N depletion was typical for arbuscular mycorrhizal forbs and grasses, non-mycorrhizal hemiparasites, and ericoid mycorrhizal species, with $\delta^{15}N$ values ranging from –3.8‰ to –2.9‰ and not significantly differing from each other. Ectomycorrhizal species were intermediate. We can summarize these results in the following ranking of leaf ^{15}N enrichment among alpine heath species:

ORC > NOM–GRA, FAB–N > ECT ≥ ERI ≥ AM–FORB,
NOM–SP, AM–GRA.

Similar to N concentration, $\delta^{15}N$ varied widely within separate functional groups, often by more than 2‰. For example, *Helictotrichon versicolor* and *Bromus variegatus* (AM-GRA species) has $\delta^{15}N$ values of –2.2‰ and –5.5‰, respectively (Table 3). Within all legumes, non-nodulated *Trifolium polyphyllum* had the lowest $\delta^{15}N$ (–2.6‰), which was close to the values for the ERI and ECT mycorrhizal groups (Fig. 4). This value was significantly lower than $\delta^{15}N$ of two other legume species—*Oxytropis kubanensis* and *Astragalus levieri*—but there was no significant difference between $\delta^{15}N$ for *Trifolium polyphyllum* and *Anthyllis vulneraria* (Fig. 2).

In contrast to N concentration, $\delta^{15}N$ did not show any relationships with mycorrhizal infection rate for six AM species.

Across all samples, foliar $\delta^{15}N$ increased with increasing N concentrations (r^2 = 0.22, P < 0.001) (Fig. 5, part a). The group of non-mycorrhizal sedges, being strongly N-depleted

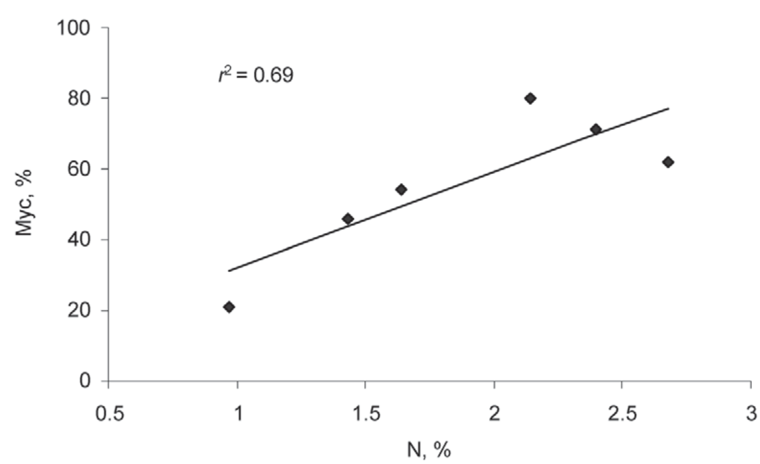

FIGURE 3. Relationship between N leaf concentration and rate of mycorrhizal infection (%) for 6 arbuscular mycorrhizal (AM) plant species.

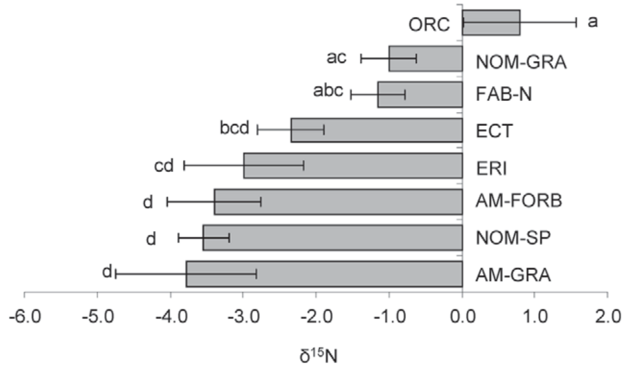

FIGURE 4. $\delta^{15}N$ for different functional types of alpine plants (mean and standard error) (see Table 1 for abbreviations). Significant ($p < 0.05$) differences are shown by different letters.

and relatively ^{15}N-enriched, was the main functional group that decreased the strength of this correlation. A highly significant correlation ($r^2 = 0.83$, $P < 0.001$) between N concentration and $\delta^{15}N$ was observed within the group of legume species (Fig. 5, part b).

Discussion

NITROGEN CONCENTRATION IN ALPINE PLANTS

Leaf N concentrations in our study for all but four of the studied species were lower than the mean values for alpine plants in the Alps (2.87%) and the northern Scandes (3.18%) (Körner, 1989). The mean value for the Caucasian species was 2.11%, which is lower than the N concentration in leaves of plant species even from low altitude in the Alps (2.40%) (Körner, 1989). Monson et al. (2001) reported mean foliar N concentration in alpine forbs in Niwot Ridge (Colorado) of about 3.1%, which is higher than our data for AM forbs (2.4%). Relatively low N concentrations in the species of the lichen heath may be explained by a general tendency of decreasing N concentration in alpine plants with decrease of latitude (Körner, 2003). Also, nitrogen availability in lichen heath is the lowest among the plant communities in the same region (Makarov et al., 2003), as evidenced by a doubling of productivity after N fertilization (Soudzilovskaia et al., 2005).

Hemiparasitic plants usually have higher N and other nutrient concentrations than their hosts (Press et al., 1999). Our results for three Scrophulariaceae species (annual *Euphrasia ossica* and perennial *Pedicularis comosa* and *P. caucasica*) showed that N leaf concentration was lower than those values for AM-FORB but higher than for NOM-GRA (both groups can serve as hosts for

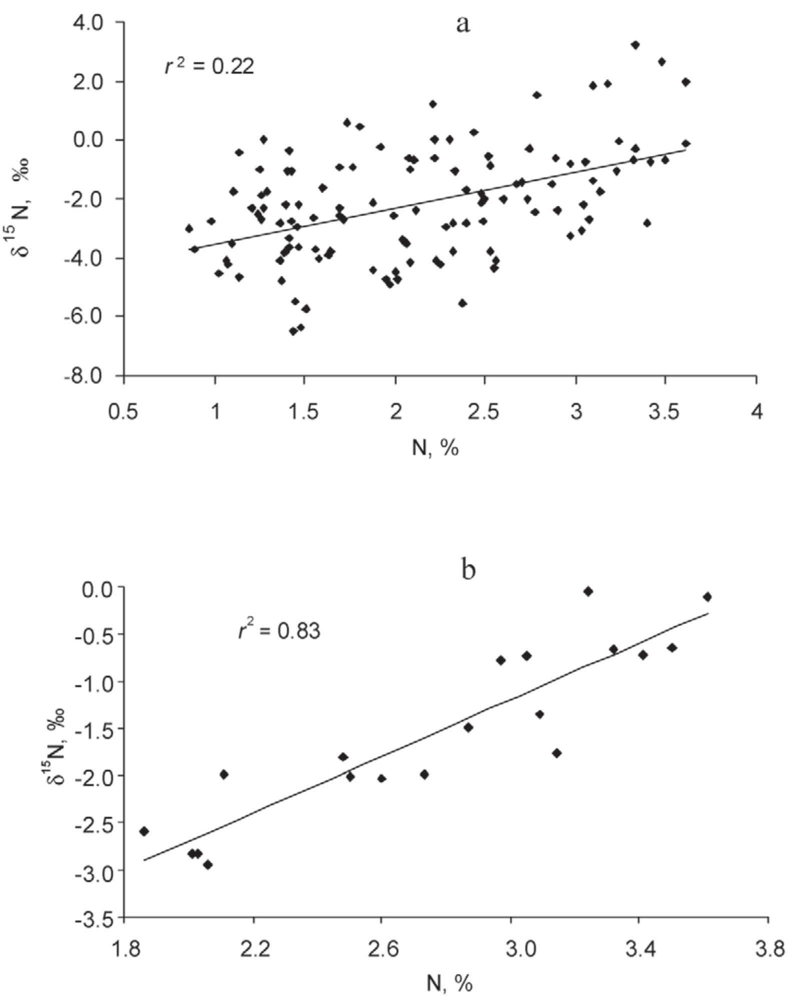

FIGURE 5. Relationships between N concentration and $\delta^{15}N$ in alpine plants: (a) all species; (b) legume species.

studied hemiparasites [Popova, 1966]). We can suppose that the results indicate relatively low or facultative root parasitic activity of the studied species.

Nitrogen concentration in leaves of *Trifolium polyphyllum* was significantly lower (2.05%) than in other studied legumes (2.64%–3.30%). This result confirms our visual observations of the absence of nodules in the root system of *Trifolium polyphyllum*. The non-nitrogen fixing status of *Trifolium polyphyllum* was also confirmed by its positive responses to N fertilization (Soudzilovskaia et al., 2005; Soudzilovskaia and Onipchenko, 2005; Soudzilovskaia et al., 2012) and by the acetylene reduction method (Makarov et al., 2011). *Trifolium polyphyllum* is therefore the first known temperate non-fixing legume besides previously reported tropical examples (Sprent, 2005). The N concentrations of the three other legume species that do have root nodules correspond to those reported from high mountain ecosystems in Colorado and the Alps (Bowman et al., 1996; Jacot et al., 2000), confirming the adaptiveness of this strategy at high altitude.

Arbuscular mycorrhizal fungi are known to provide plant access to organic soil phosphates and improve mainly phosphate acquisition of plants (Cavagnaro et al., 2003; Smith and Read, 2008). Nitrogen capture from organic material and transferring N to plants via AM is a recently discovered phenomenon (Nakano et al., 2001; Jin et al., 2005; Leigh et al., 2009). However, to our knowledge there is no example in the literature of positive relationships between rate of AM infection and leaf N concentration. For example, the alpine species *Ranunculus adoneus* had no relationship between AM root colonization and leaf N concentration (Monson et al., 2001). In spite of the limited number of AM species in the present research, the strong positive correlation between these parameters (Fig. 3) is consistent with AM fungi influencing plant nitrogen uptake as well.

^{15}N NATURAL ABUNDANCE IN ALPINE PLANTS

The δ^{15}N variation among plant species could be due to several factors. Many surveys highlighted the influence of the mycorrhizal status of species (Schulze et al., 1994; Michelsen et al., 1996, 1998; Nadelhoffer et al., 1996; Högberg et al., 1999; Hobbie et al., 2000; Emmerton et al., 2001; Hobbie and Colpaert, 2003), while other factors (species-specific differences in isotopic fractionation, differences in plant preference for N forms and root placement at different soil depths) could also be important (Schulze et al., 1994; Emmerton et al., 2001; Miller and Bowman, 2002).

Relatively high ^{15}N abundances of plant leaves of ORC, FAB-N, and NOM-GRA species from an alpine lichen heath correspond to previously published results. For instance, the highest δ^{15}N values of orchids were also observed in forest and some grassland sites in Bavaria and southern France and were explained by utilization of fungi-derived ^{15}N-enriched nitrogen (Gebauer and Meyer, 2003).

N_2-fixing legumes that partly acquire atmospheric N_2 have higher δ^{15}N (close to naught) relative to non-fixing species growing in N-limited conditions of tundra and alpine ecosystems (Bowman et al., 1996; Michelsen et al., 1996; Nadelhoffer et al., 1996; Körner, 2003; Hobbie et al., 2005). This difference in ^{15}N signature between N_2-fixing and non-fixing species is often used to assess the degree of N_2 fixation by symbiotic bacteria using the dual-sources model (Shearer and Kohl, 1986). Although this method requires care in selecting non-fixing reference plants, which ideally should be characterized by the same preference of soil N and the same temporal and spatial N uptake pattern as N-fixing species, it was successfully applied for alpine ecosystems (Bowman et al., 1996). The studied alpine lichen heath provides a unique test case for revealing N_2-fixation with the δ^{15}N method. The non-nodulated legume *Trifolium polyphyllum* provides a taxonomically close reference species for N_2-fixing legumes. The lowest N concentration and δ^{15}N in *Trifolium polyphyllum* leaves (Fig. 2) and highly significant correlation between N concentration and δ^{15}N for all legume species (Fig. 5, part b) allow us to conclude that the ^{15}N natural abundance method can provide an acceptable estimation of N_2-fixation. Applying the dual-source model and the result of Bowman et al. (1996) that ^{15}N enrichment of the legume grown solely with atmospheric N_2 is close to 0‰, we calculated that the percentage of plant N that is fixed from the atmosphere varied from 27% in *Anthyllis vulneraria* to 69% and 73% for *Oxytropis kubanensis* and *Astragalus levieri*, respectively (Makarov et al., 2011). The latter results are in close correspondence with the large proportions of atmospherically fixed N_2 (from 70% up to 100%) to satisfy N requirements of legumes in other mountains (Bowman et al., 1996; Jacot et al., 2000).

The ^{15}N enrichment of non-mycorrhizal graminoids (sedges and rushes) of alpine lichen heath corresponds to the high ^{15}N content in plant species from this functional group within two plant communities in northern Swedish Lapland where *Carex* spp. and *Luzula arcuata* had δ^{15}N values between –0.5‰ and +2.5‰ (Michelsen et al., 1996). Non-mycorrhizal graminoids in Alaskan tundra (*Eriophorum vaginatum* and *Carex bigelowii*) and in Niwot Ridge alpine tundra (*Kobresia myosuroides, Carex rupestris, Luzula spicata*) were also among the species with the highest δ^{15}N (up to +2.3‰) (Nadelhoffer et al., 1996; Miller and Bowman, 2002; Körner, 2003). According to Körner (2003), a probable reason for high ^{15}N enrichment of Cyperaceae species (up to +3.5‰) could be their access to ^{15}N-enriched stable forms of organic N. However, the more recent finding of considerable ^{15}N enrichment of DON (Pörtl et al., 2007) indicated that the reason could also be access to labile forms of organic N. In particular, Raab et al. (1996, 1999) demonstrated that non-mycorrhizal alpine Cyperaceae species had uptake rates for glycine similar to or substantially greater than those for inorganic N.

Isotopic nitrogen fractionation during ammonium and nitrate plant assimilation should essentially complicate the measure of the δ^{15}N of available N in the soil (Emmerton et al., 2001). However, ^{15}N discrimination during nitrogen uptake will only be observed when plant nitrogen demand is relatively low, compared with the nitrogen available in the solution, and will probably decrease to negligible under conditions of low N concentration and highly efficient N uptake typical for most natural ecosystems. Hence, in natural N-limited ecosystems the δ^{15}N of non-mycorrhizal plants is a good approximation of δ^{15}N of the available N (Högberg et al., 1999; Hobbie et al., 2005). This assumption was confirmed by very similar δ^{15}N values of non-mycorrhizal plants (between –2‰ and +2‰) (Michelsen et al., 1996) and of NH_4^+-N (between –1.5‰ and +1.4‰) (Makarov et al., 2008) from the surface soil horizon in tundra ecosystems in northern Sweden. Similar δ^{15}N were also found for non-mycorrhizal plants, total soil N, ammonium, and nitrate N in the arctic tundra ecosystem in Alaska (Hobbie and Hobbie, 2006).

Conditions of N-limitation are quite applicable to the alpine lichen heath where inorganic N concentrations in the soil solution are typically low at about 0.05 mg L^{-1} both N-NH_4^+ and N-NO_3^- (Makarov et al., 2003). However, in our case, alpine non-mycorrhizal sedges were much more ^{15}N-enriched (between –0.2‰ and –1.5‰) than the dominant form of inorganic soil N—

exchangeable NH_4^+-N (between –2.6‰ and –5.1‰) (Makarov et al., 2008). It seems likely that the ^{15}N abundance of alpine non-mycorrhizal plants is the result of uptake of a mixture of N sources.

^{15}N AND TYPE OF MYCORRHIZA

In spite of the prevalent opinion that there is the great difference between ericoid mycorrhizal and ectomycorrhizal species on the one hand (more negative $\delta^{15}N$ values), and non-mycorrhizal or arbuscular mycorrhizal species on the other hand (less negative $\delta^{15}N$ values) (Schulze et al., 1994; Nadelhoffer et al., 1996; Michelsen et al., 1996, 1998; Körner, 2003; Craine et al., 2009), it was not confirmed in our study in an alpine heath community, where ^{15}N abundance of ericoid mycorrhizal and especially ectomycorrhizal species tended to be rather higher than that of arbuscular mycorrhizal forbs and grasses (Fig. 4). Some other previous results also did not correspond to this prevalent pattern. For example, the isotopic similarity within ecto- and arbuscular mycorrhizal species in forest sites in Bavaria was explained by utilization of isotopically similar N compounds (Gebauer and Meyer, 2003). Also, Hobbie et al. (2005) demonstrated that some arbuscular mycorrhizal species (*Luetkea pectinata*) had similar $\delta^{15}N$ values with ecto- and ericoid mycorrhizal plants in the Cascade Mountains of Washington, U.S.A. Last, our study of AM species in subarctic meadow communities in the Khibiny Mountains (NW Russia) and Abisko region (N Sweden) indicated that these plants were characterized by low $\delta^{15}N$ values close to ERI and ECT species (not published).

These findings could be connected with increased N isotope fractionation by arbuscular mycorrhizal fungi and preferential transfer of ^{15}N-depleted compounds from fungi to host plants in a similar fashion to the well-established transfer in ectomycorrhizal symbioses (Hobbie and Hobbie, 2008; Craine et al., 2009). However, it remains unclear why it occurs in meadow communities where nitrogen is probably more available to plants in comparison with heaths for which much higher $\delta^{15}N$ values of AM species are typical (Schulze et al., 1994; Nadelhoffer et al., 1996; Michelsen et al., 1996, 1998). In addition, culture studies investigating the effects of arbuscular mycorrhizal colonization on plant $\delta^{15}N$ indicated only modest changes and sometimes resulted in ^{15}N enrichment rather than depletion (Handley et al., 1993, 1999; Azcon-Aguilar et al., 1998).

On the other hand, the rate of ^{15}N fractionation by ecto- and ericoid mycorrhizal fungi probably depends on N availability. In conditions of strongly pronounced N limitation, plants take up the most part of N through mycorrhiza and thus become ^{15}N-depleted, while for ecosystems with higher N availability and lower importance of mycorrhiza in plant N nutrition, similarity between ecto-, ericoid, and arbuscular mycorrhizal species is more typical (Hobbie et al., 2000, 2005). The alpine lichen heath, while being the most N limited among alpine ecosystems in the Teberda Reserve, is probably not as limited as arctic and subarctic tundra ecosystems, because annual net N mineralization rate in the heath soil (0.6 g m^{-2}) (Makarov et al., 2003) is higher than in soils of Arctic ecosystems (0.05–0.5 g m^{-2}) (Giblin et al., 1991; Schmidt et al., 2002). Probably this is the reason of relatively high $\delta^{15}N$ values of ERI and ECT plant species, which isn't comparable with values close to –8‰ or –9‰, as it was observed when studying subarctic communities (Schulze et al., 1994; Nadelhoffer et al., 1996; Michelsen et al., 1996, 1998).

Therefore we assume that the reason of absence of a significant difference between AM, ERI, and ECT species in the Caucasian alpine heath community isn't clear and may result from both processes: the increased N isotope fractionation by arbuscular mycorrhizal fungi and the decreased role of ecto- and ericoid mycorrhizal fungi in the flux of N.

VARIATION IN $\delta^{15}N$ VALUES WITHIN FUNCTIONAL GROUPS

The variability of $\delta^{15}N$ within separate plant functional groups, as discussed above for legumes, can be due to species-specificity in isotopic fractionation (Emmerton et al., 2001), differences in plant preference for N forms (Schulze et al., 1994; Miller and Bowman, 2002), and their root placement at different soil depths (Schulze et al., 1994; Nadelhoffer et al., 1996).

We have no experimental data to evaluate the first two determinants, while the effect of rooting depth was not confirmed in our study, because $\delta^{15}N$ varied widely among species of the same functional group with similar rooting depths. For example, arbuscular mycorrhizal grasses *Helictotrichon versicolor* and *Bromus variegatus*, both have rooting depths mainly within the upper 5–10 cm of soil but very different $\delta^{15}N$ values of –2.2‰ and –5.5‰, respectively. Similarly, *Anemone speciosa* and *Campanula tridentata* (AM-FORB), both with deeper (15–20 cm) root systems (Onipchenko, 1987), have $\delta^{15}N$ values of –2.2‰ and –4.4‰, respectively.

The dependence of $\delta^{15}N$ of plant leaves on rooting depth is one of the least confirmed in field observations. We do not know of any studies that experimentally demonstrate any relationships between $\delta^{15}N$ of plant leaves and rooting depth, although Schulze et al. (1994) and Nadelhoffer et al. (1996) hypothesized that deep rooting system can be responsible for the $\delta^{15}N$ increase of forest and Arctic tundra plant species (see Introduction). Similar to our study, considerable foliar $\delta^{15}N$ differences within functional groups were found for forbs, grasses, and sedges (rushes) in two dry meadow alpine tundra sites in the Rocky Mountains, Colorado (Miller and Bowman, 2002), while there were no differences in the $\delta^{15}N$ of bulk soils within the upper 15 cm where roots of dry meadow species are concentrated. Therefore, the authors concluded that the observed variation in the foliar $\delta^{15}N$ was due to factors other than rooting depth and was attributed to the acquisition of different N forms. At the same time, $\delta^{15}N$ of non-mycorrhizal and ectomycorrhizal fine roots in European forest soils usually increased with soil depth (Högberg et al., 1996), and the idea of Taylor et al. (1997) that $\delta^{15}N$ values of ectomycorrhizal fungi may be a function of the soil depth at which they utilize the N compounds was subsequently confirmed by Wallander et al. (2004) for the ectomycorrhizal mycelia, the $\delta^{15}N$ of which increased with soil depth in two forest types.

Though we assumed that low $\delta^{15}N$ values of the studied AM species may result from the increased N isotope fractionation by arbuscular mycorrhizal fungi, it also remains unclear whether arbuscular mycorrhizal fungi contribute to variation in foliar $\delta^{15}N$, because we did not find any relationships with mycorrhizal colonization rate for six AM species.

RELATION BETWEEN FOLIAR N AND $\delta^{15}N$

Positive relationships between foliar $\delta^{15}N$ and N concentration were reported at the global (Craine et al., 2009) and landscape (regional) scale (Hobbie et al., 2000), indicating the effect of N availability. Hobbie et al. (2000) determined $\delta^{15}N$ in plant materials across six sites representing different postdeglaciation ages at Glacier Bay, Alaska. When compared across all sites and species, the foliar $\delta^{15}N$ values and N concentrations were generally lower at sites of low N availability, suggesting either an increased fraction of N obtained from mycorrhizal uptake, or a reduced proportion of mycorrhizal N transferred to vegetation. However, within our alpine lichen heath, higher foliar N concentrations and $\delta^{15}N$ values are mostly connected with atmospheric N_2 fixation by legumes (see also Hobbie et al., 2000, for Glacier Bay) or utilization of fungi-derived ^{15}N-enriched N by orchids and accompanying species.

Conclusion

We conclude that, within the alpine lichen heath in the Northern Caucasus, the $\delta^{15}N$ signature of plant foliage is a relevant indicator of plant functional groups with relatively high ^{15}N content (ORC, FAB-N, NOM-GRA), while the lack of clear difference between relatively ^{15}N-depleted groups (AM, ERI and ECT species) isn't clear and may result from both processes: the increased N isotope fractionation by arbuscular mycorrhizal fungi and the decreased role of ecto- and ericoid mycorrhizal fungi in the flux of N. These findings are important pieces of the large puzzle of understanding belowground competition and complementarity in N-limited ecosystems.

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