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Foliar and fungal ^{15}N:^{14}N ratios reflect development of mycorrhizae and nitrogen supply during primary succession: testing analytical models

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Abstract Nitrogen isotopes (^{15}N/^{14}N ratios, expressed as δ^{15}N values) are useful markers of the mycorrhizal role in plant nitrogen supply because discrimination against ^{15}N during creation of transfer compounds within mycorrhizal fungi decreases the ^{15}N/^{14}N in plants (low δ^{15}N) and increases the ^{15}N/^{14}N of the fungi (high δ^{15}N). Analytical models of ^{15}N distribution would be helpful in interpreting δ^{15}N patterns in fungi and plants. To compare different analytical models, we measured nitrogen isotope patterns in soils, saprotrophic fungi, ectomycorrhizal fungi, and plants with different mycorrhizal habits on a glacier foreland exposed during the last 100 years of glacial retreat and on adjacent non-glaciated terrain. Since plants during early primary succession may have only limited access to propagules of mycorrhizal fungi, we hypothesized that mycorrhizal plants would initially be similar to nonmycorrhizal plants in δ^{15}N and then decrease, if mycorrhizal colonization were an important factor influencing plant δ^{15}N. As hypothesized, plants with different mycorrhizal habits initially showed similar δ^{15}N values (−4 to −6‰ relative to the standard of atmospheric N_2 at 0‰), corresponding to low mycorrhizal colonization in all plant species and an absence of ectomycorrhizal sporocarps. In later successional stages where ectomycorrhizal sporocarps were present, most ectomycorrhizal and ericoid mycorrhizal plants declined by 5–6‰ in δ^{15}N, suggesting transfer of ^{15}N-depleted N from fungi to plants. The values recorded (−8 to −11‰) are among the lowest yet observed in vascular plants. In contrast, the δ^{15}N of nonmycorrhizal plants and arbuscular mycorrhizal plants declined only slightly or not at all. On the forefront, most ectomycorrhizal and saprotrophic fungi were similar in δ^{15}N (−1 to −3‰), but the host-specific ectomycorrhizal fungus *Cortinarius tenebricus* had values of up to 7‰. Plants, fungi and soil were at least 4‰ higher in δ^{15}N from the mature site than in recently exposed sites. On both the forefront and the mature site, host-specific ectomycorrhizal fungi had higher δ^{15}N values than ectomycorrhizal fungi with a broad host range. From these isotopic patterns, we conclude:(1) large enrichments in ^{15}N of many ectomycorrhizal fungi relative to co-occurring ectomycorrhizal plants are best explained by treating the plant-fungal-soil system as a closed system with a discrimination against ^{15}N of 8–10‰ during transfer from fungi to plants, (2) based on models of ^{15}N mass balance, ericoid and ectomycorrhizal fungi retain up to two-thirds of the N in the plant-mycorrhizal system under the N-limited conditions at forefront sites, (3) sporocarps are probably enriched in ^{15}N by an additional 3‰ relative to available nitrogen, and (4) host-specific ectomycorrhizal fungi may transfer more N to plant hosts than non-host-specific ectomycorrhizal fungi. Our study confirms that nitrogen isotopes are a powerful tool for probing nitrogen dynamics between mycorrhizal fungi and associated plants.

Keywords Nitrogen concentration · Isotope ratios · Mycorrhizal · Nitrogen cycling · Primary succession · Soil

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Introduction

Fungi can be divided into two main types depending on their carbon sources, with saprotrophic fungi obtaining most of their carbon from the decay of dead organic

matter and biotrophic fungi, such as those forming mycorrhizal symbioses, obtaining their carbon from plant hosts with whom they form symbioses. Through their root-associated symbionts, mycorrhizal plants compete effectively against free-living microbes for essential nutrients, particularly phosphorus and nitrogen (N), and mycorrhizal fungi in return receive a large proportion of net primary production (Simard et al. 2002). Mycorrhizal symbioses are generally divided into several morphologically, taxonomically, and functionally distinct types. Of these, three types are relevant to the present study: ericoid mycorrhizae are prevalent on acidic, N-poor heathlands, ectomycorrhizae dominate in most temperate and boreal forests, and arbuscular mycorrhizae dominate in grasslands and many tropical forests (Read 1991). Understanding interactions between plants and their mycorrhizal fungi has always been difficult because those interactions are hidden within the soil matrix. One valuable technique has been to take the symbiosis out of the field and study it in the laboratory or greenhouse, but inferences derived from the laboratory should always be tested against field data (Trappe 1977; Read and Perez-Moreno 2003).

In field studies of the fungal role in N cycling, measuring the natural abundances of ^{15}N and ^{14}N has proven useful, as the ^{15}N:^{14}N ratio can be used to infer information about the forms of nitrogen used and the internal processing of nitrogen by plants and mycorrhizal fungi (Lilleskov et al. 2002; Trudell et al. 2004; Hobbie 2005). ^{15}N:^{14}N ratios are generally expressed as $\delta^{15}N$ values in which the $^{15}N/^{14}N$ of samples is compared against the $^{15}N/^{14}N$ of the standard, atmospheric N_2, according to Eq. (1), with R equal to the ^{15}N:^{14}N ratio.

$$\delta^{15}\mathrm{N}(‰) = \left(\frac{R_{\mathrm{sample}}}{R_{\mathrm{standard}}} - 1\right)1000 \quad (1)$$

Plants associated with ectomycorrhizal and ericoid mycorrhizal fungi are usually lower in $\delta^{15}N$ (^{15}N content) under N-limited conditions than non-mycorrhizal plants or plants associated with arbuscular mycorrhizal fungi (Michelsen et al. 1996, 1998; Schmidt and Stewart 1997). These patterns have been attributed to the retention of ^{15}N-enriched compounds by mycorrhizal fungi and transfer of ^{15}N-depleted compounds to host plants, with increased partitioning of N to the fungal symbiont leading to greater ^{15}N depletion in host plants (Hobbie et al. 1999). Because an isotopic mass balance must be preserved, greater ^{15}N depletion in the plant partner relative to that of available N probably indicates that more N is retained by the fungal partner in the mycorrhizal symbiosis (Hobbie and Colpaert 2003).

Different mathematical models have been created to explain isotopic partitioning in two-component systems that are considered to be open (exogenous inputs to the system) or closed (no inputs to the system) (Hayes 2002). In an open system (Fig. 1a), the proportion of N taken up by mycorrhizal fungi that is then passed on to host plants, termed the transfer ratio (T_r), can explain nitrogen isotope patterns in mycorrhizal plants according to the following equation (Hobbie et al. 2000):

$$\delta^{15}\mathrm{N}_{\mathrm{plant}} = \delta^{15}\mathrm{N}_{\mathrm{available\ N}} - (1 - T_r)\Delta \quad (2)$$

with the transfer ratio, T_r, between 0 and 1, and the discrimination against ^{15}N during the creation of transfer compounds (Δ) of 8–10‰. We previously used N budgets and ^{15}N differences among available N, non-mycorrhizal pines, and mycorrhizal pines under controlled conditions (Hobbie and Colpaert 2003) to estimate Δ at 9‰. The ^{15}N content of mycorrhizal fungi can then be calculated according to Eq. 3.

$$\delta^{15}\mathrm{N}_{\mathrm{fungi}} = \delta^{15}\mathrm{N}_{\mathrm{available\ N}} + T_r \cdot \Delta \quad (3)$$

If isotopic values are known, Eq. 2 can therefore be used to calculate the transfer ratio of a plant-mycorrhizal system, according to Eq. 4:

$$T_r = \frac{(\Delta + \delta^{15}\mathrm{N}_{\mathrm{plant}} - \delta^{15}\mathrm{N}_{\mathrm{available\ N}})}{\Delta} \quad (4)$$

In a closed system (Fig. 1b), Eqs. 5 and 6 can be used to calculate the isotopic signatures of plants and fungi, respectively. The quantity f is the proportion of original substrate transformed to product, and is equivalent to the transfer ratio (T_r) in Eqs. 2, 3, and 4.

$$\delta^{15}\mathrm{N}_{\mathrm{fungi}} = \delta^{15}\mathrm{N}_{\mathrm{available\ N}} - \Delta \ln(1 - f) \quad (5)$$

$$\delta^{15}\mathrm{N}_{\mathrm{plant}} = \delta^{15}\mathrm{N}_{\mathrm{available\ N}} + \Delta(\ln f)/(1 - f) \quad (6)$$

Although comparing ^{15}N patterns in mycorrhizal plants, non-mycorrhizal plants, mycorrhizal fungi, and saprotrophic fungi may indicate how mycorrhizal colonization influences plant and fungal $\delta^{15}N$, non-mycorrhizal plants are probably adapted to different N environments than are mycorrhizal plants and may consequently prefer different N forms than mycorrhizal plants (e.g., mineral N vs. organic, McKane et al. 2002). Ideally, mycorrhizal plants should be compared before and after colonization by mycorrhizal fungi. The paucity of fungal propagules in primary successional environments such as recently deglaciated forelands may present opportunities to observe mycorrhizal plants with no or only limited mycorrhizal colonization or without a great diversity of fungal associates (Helm et al. 1996; Jumpponen et al. 2002; Nara et al. 2003; Càzares et al. 2005). A few studies have reported plant $\delta^{15}N$ patterns from deglaciated forelands (Kohls et al. 1994; Hobbie et al. 2000), but these studies have not included nonmycorrhizal or ericoid mycorrhizal plants. In addition, the $\delta^{15}N$ of mycorrhizal and saprotrophic fungi have yet to be reported from very young primary successional sites, primarily because fungi produce few sporocarps under these low productivity conditions.

In this study, we investigated how mycorrhizal habit of the host plant and fungal life history strategy

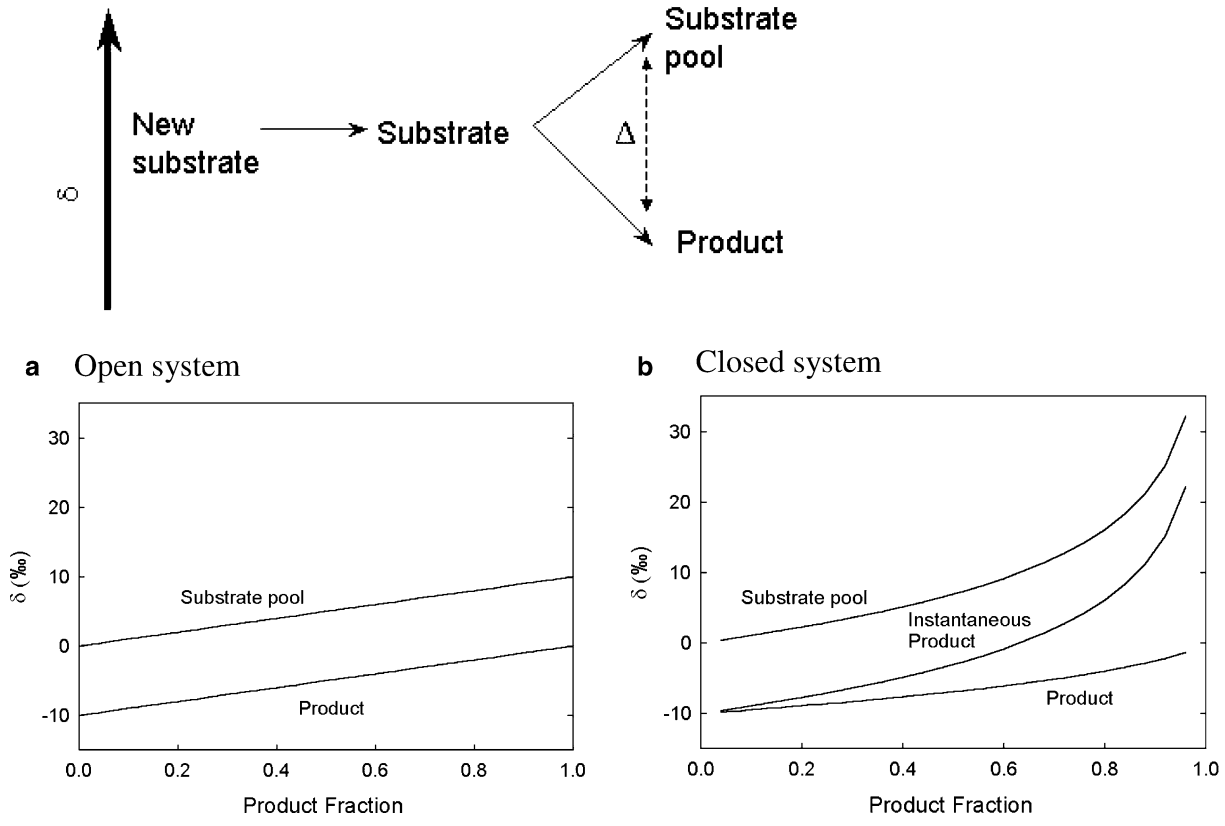


Fig. 1 Isotopic composition of substrate and product in an open system depends on the fraction (f) of substrate transformed to the product and the isotopic discrimination (Δ) of the reaction. Isotopic patterns for product (δ_p) and substrate (δ_s) for open (1a) and closed (1b) systems are shown as a function of f, Δ, and the initial isotopic content of substrate (δ_i). δ_i is set to 0‰ and Δ to 10‰ in these examples. In a closed system the flux of new substrate replenishing the substrate pool is zero. **a** Open system, $\delta_s = \delta_i + f\,\Delta$, $\delta_p = \delta_i - (1-f)\,\Delta$. **b** Closed system. $\delta_s = \delta_i - \Delta \ln(1-f)$, $\delta_p = \delta_s + \Delta\,(\ln f)/(1-f)$. The isotopic signature of the instantaneous product is also shown, which is simply $\delta_p = \delta_s - \Delta$

affected plant and fungal $\delta^{15}N$ signatures at a young (< 100 years) glacial forefront in western North America (Lyman Glacier). We then compared soil, foliar and fungal $\delta^{15}N$ between the forefront and adjacent mature alpine communities. To account for metabolic processes that might affect $\delta^{15}N$ and be specific to fungi, saprotrophic fungi were also sampled. Soil and foliar N concentrations were measured to indicate the relative N availability among different sites.

We hypothesized that plant $\delta^{15}N$ should follow the general pattern previously reported, with ericoid and ectomycorrhizal plants lower than arbuscular and non-mycorrhizal plants, but in very early succession when fully functional mycorrhizal symbioses may be limited, all plants should have similar $\delta^{15}N$ values. Because both non-mycorrhizal plants and saprotrophic fungi do not participate in transfer mechanisms that may discriminate against ^{15}N, they should be intermediate in ^{15}N composition between ectomycorrhizal fungi (high in ^{15}N) and ectomycorrhizal plants (low in ^{15}N). In addition, because the ^{15}N content of ecosystem pools increases with processing (Nadelhoffer and Fry 1994), we hypothesized that soil, plant, and fungal $\delta^{15}N$ from young, forefront sites should be relatively low compared to the adjacent mature site. By combining prior knowledge of patterns of discrimination against ^{15}N in culture studies with new information on field $\delta^{15}N$ patterns at Lyman Glacier, we tested whether treating field ^{15}N distributions as an open or a closed system provided a better fit to the available data. In this work, we sought to quantitatively relate nitrogen isotope patterns in ectomycorrhizal fungi and associated plants to nitrogen dynamics in the symbiosis.

Methods

Site description

The study area includes the forefront of Lyman Glacier and an adjacent mature site beyond the terminal moraine. Lyman Glacier is at 48°10′N, 120°53′W, at an elevation of 1,800 m in the Cascade Mountains of Washington, USA. The timing of glacial retreat has been determined from historical photographs and observations (Jumpponen et al. 1998, 2002). The sites were visited in late August 1999, and samples of soils, foliage and fungal sporocarps were collected.

Colonization patterns of the plant species sampled at Lyman Glacier are given in Table 1 and were extracted

Table 1 Colonization data for taxa at Lyman Glacier at forefront sites, as summarized from Cázares et al. (2005)

Taxa	NM (n)	AM	ECM	ERM	DS
Abies	–	–	1/5/3/1	–	4/0/0/0
Larix	–	–	1/0/0/1	–	–
Pice	–	–	0/2/0/0	–	–
Salix	(1)	0/1/0/0	2/5/5/3	–	5/4/2/3
Tsuga	(1)	–	0/5/6/1	–	4/5/1/0
Cassiope	–	–	–	2/0/1/3	1/3/0/1
Phyllodoce	(1)	–	–	4/8/6/4	11/0/2/1
Vaccinium	(1)	–	–	3/6/6/4	5/4/0/1
Epilobium	(8)	4/1/1/0	–	–	2/2/0/0
Luetkea	(1)	8/1/1/1	–	–	3/2/2/0
Luzula	(10)	2/0/0/0	–	–	8/0/0/0
Saxifraga	(22)	1/0/0/0	–	–	4/0/0/0

The procedure for calculating colonization intensity is given in the Methods section. Number of sampled individuals that were non-mycorrhizal is given in parentheses in the first column. The numbers of individuals for a given mycorrhizal type are listed as *w*/*x*/*y*/*z*, with "*w*", "*x*", "*y*", and "*z*" the number of individuals of colonization intensities of 1–25%, 26–50%, 51–75%, and 76–100%, respectively

NM, nonmycorrhizal; *AM*, arbuscular mycorrhizal; *ECM*, ectomycorrhizal; *ERM*, ericoid mycorrhizal; *DS*, dark septate fungi

from Cázares et al. (2005). Information is also included on root colonization by dark septate fungi, a poorly understood group of fungi commonly occurring in early succession environments (Jumpponen and Trappe 1998). Data are given on the range of colonization intensities for individuals (1–25%, 26–50%, 51–75%, and 76–100% of either feeder rootlets colonized for ectomycorrhizal and ericoid mycorrhizal fungi, or of root length colonized for dark septate and arbuscular fungi).

Sample collection

Forefront sites were located 100 m, 300 m, 450 m, 600 m, and 750 m from the glacial terminus. The corresponding ages since glacial retreat were approximately 20, 40, 50, 60, and 70 years. In forefront sites, two plant species of each of the four mycorrhizal types were selected. The non-mycorrhizal species were *Luzula piperi* (Juncaceae) and *Saxifraga ferruginea* (Saxifragaceae), the arbuscular mycorrhizal species were *Luetkea pectinata* (Rosaceae) and *Epilobium latifolium* (Onagraceae), the ectomycorrhizal species were *Abies lasiocarpa* (Pinaceae) and *Salix phylicifolia* (Salicaceae), and the ericoid mycorrhizal species were *Cassiope mertensiana* and *Phyllodoce empetriformis* (both Ericaceae) (Cázares et al. 2005). At each site, current-year foliage was collected from five plants of each species if present. Foliage from a few isolated individuals of *Alnus sinuata* (Betulaceae), *Lupinus latifolia* (Fabaceae), *Larix lyallii* (Pinaceae), *Picea engelmannii* (Pinaceae), *Tsuga mertensiana* (Pinaceae), and *Vaccinium deliciosum* (Ericaceae) were also collected. Tree and shrub species were generally less than 1 m in height. A "mature" site was located slightly beyond the terminal moraine, at about 1,100 m from the glacial terminus. The mature site has not recently been glaciated, as indicated by an undisturbed layer of Mt. Mazama tephra deposited $<7{,}000$ BP. Species collected at this site included ectomycorrhizal *Abies*, *Larix*, *Salix* and *Sorbus sitchensis* (Rosaceae); ericoid mycorrhizal *Phyllodoce* and *Vaccinium*; and arbuscular mycorrhizal *Luetkea*. No nonmycorrhizal plants were present. The mature site was dominated by *Abies* and *Larix* trees ranging up to 28 cm (*Abies*) or 63 cm (*Larix*) in diameter at breast height.

Five samples of surface soils (top 5 cm) from each site were also collected.

Sporocarps of mushrooms were collected along the forefront, and the distance from the glacial terminus for each sample recorded to the nearest 10 m. The most abundant saprotrophic mushrooms belonged to the genus *Galerina* ($n=21$) and the species *Omphalina obscurata* ($n=33$), and the most abundant ectomycorrhizal species was *Laccaria montana* ($n=40$). Smaller numbers of *Inocybe lacera* ($n=6$), *Cortinarius tenebricus* ($n=5$), and single individuals of *Russula fragilis*, *Hebeloma* sp., and *Entoloma* sp. were also collected. Because no mushrooms were fruiting at the mature site at the time of sampling, 17 specimens previously collected from the site and deposited in the Oregon State University Herbarium were also selected for isotopic analysis.

Sample processing and isotopic analyses

Fungal samples from the forefront and all foliar samples were analyzed for $\delta^{15}N$, %N, and %C on a Finnigan Delta-Plus isotope ratio mass spectrometer linked to a Carlo Erba NC2500 elemental analyzer (Finnigan MAT GmbH, Bremen, Germany) located at the U.S. Environmental Protection Agency, Corvallis, Oregon, USA. The internal standards for isotopic and concentration measurements were acetanilide and pine needles (NIST 1575). On the $\delta^{15}N$ scale, the $^{15}N/^{14}N$ ratio of the standard, atmospheric N_2, has a $\delta^{15}N$ value of 0‰, samples with lower $^{15}N/^{14}N$ than the standard have negative $\delta^{15}N$ values, and samples with higher $^{15}N/^{14}N$ have positive $\delta^{15}N$ values. The average difference of duplicate samples was 0.2‰ for $\delta^{15}N$. Samples with more of the heavy isotope are referred to as heavier, or enriched; samples with more of the light isotope are lighter, or depleted. Archived fungi and soils were analyzed with a PDZ Europa for %N and ^{15}N content at the Ecosystems Center, Marine Biological Laboratory, Woods Hole, Massachusetts, USA, whereas soils were analyzed for %C content at the Max Planck Institute for Biogeochemistry in Jena, Germany using a Finnigan Delta-Plus. The very low N content of forefront soils required sample sizes of about 90 mg. All soil samples were run in duplicate,

with an average difference of duplicate soil samples from the forefront of 0.3‰. Only 11 of the 25 soil samples from the forefront produced at least 7 micromoles of N, which was the cut-off for adequate sample size at the measuring laboratory.

Statistical analyses

We used the Statview statistical program for regressions, correlations, ANOVAs, and *t* tests (Abacus Concepts, Berkeley, California, USA). All results were considered significant at $P < 0.05$. We tested whether foliar %N and $\delta^{15}N$ differed among species or within species between forefront and mature sites. Soil %N, C/N, and $\delta^{15}N$ were also compared by regressing these values against a distance from the glacial terminus or comparing between mature and forefront sites. Values in the text are given ± standard error.

Results

Patterns of mycorrhizal colonization

Table 1 summarizes recent, published data on mycorrhizal colonization at the forefront sites for the plants analyzed isotopically (Cázares et al. 2005). Colonization was generally as expected, with coniferous species and *Salix* forming ectomycorrhizae, Ericaceae forming ericoid mycorrhizae, and the herbaceous plants *Epilobium* and *Luetkea* forming arbuscular mycorrhizae. Dark septate fungi also frequently occurred in many species, particularly in *Salix*, the three Ericaceae, and *Luetkea*. In addition, many species occasionally had no mycorrhizal colonization, particularly the facultatively mycorrhizal *Epilobium* and the generally non-mycorrhizal species *Luzula* and *Saxifraga*.

Soils

Forefront soils were extremely low in nitrogen, averaging from 50 to 190 ppm (Table 2), reflecting their early development and the near absence of nitrogen-fixing plants. Nitrogen content increased with increasing distance from the terminus ($r^2 = 0.21$, $n = 24$, $P = 0.024$). The mature soils contained significantly more nitrogen than forefront soils (unpaired *t* test, $P < 0.001$), but the high C:N ratio in mature soils suggested that nitrogen availability remained low. Forefront soils were low in $\delta^{15}N$ (−2 to 0‰) relative to more developed soils (Handley et al. 1999), but increased with increasing distance from the terminus (i.e., increasing time of exposure) ($r^2 = 0.72$, $n = 11$, $P < 0.001$). Soils increased to 3‰ in the mature site.

Table 2 Soil N concentrations, C:N ratios, and ^{15}N content for the top 5 cm of surface soil

Site distance from glacier (m)	Nitrogen (ppm)	C:N	$\delta^{15}N$ (‰)
100 ($n = 5$)	50 ± 10	41.6 ± 11.0	no data
300 ($n = 5$)	70 ± 30	45.9 ± 11.7	−1.8 ± 0.0
450 ($n = 4$)	120 ± 50	22.9 ± 2.9	−0.8 ± 0.3
600 ($n = 5$)	120 ± 40	31.8 ± 6.1	−0.6 ± 0.2
750 ($n = 5$)	190 ± 120	20.8 ± 3.2	−0.1 ± 0.2
Mature ($n = 5$)	4080 ± 270	20.6 ± 1.0	3.0 ± 0.1

Nitrogen concentration and $\delta^{15}N$ were significantly higher at the mature site than at the forefront, C:N did not differ significantly. Values are given ± standard error

Foliage

Foliar %N was low across all sites, reflecting the low soil N content (Table 3). The ectomycorrhizal species *Abies* and *Larix* were similar in %N between forefront and mature sites. In contrast, the Ericaceae (*Vaccinium, Cassiope*, and *Phyllodoce*) and the arbuscular mycorrhizal *Luetkea* were lower in %N at the forefront than at the mature site (unpaired *t* tests, $P = 0.016$, 0.002, 0.001, and 0.026, respectively).

Foliar $\delta^{15}N$ in forefront sites varied widely, ranging from −11.2 ± 0.5‰ for the ectomycorrhizal *Abies* at 450 m to 3.0 ± 0.5‰ for the facultatively arbuscular mycorrhizal *Epilobium* at 750 m (Table 2, Fig. 2). The $\delta^{15}N$ signatures in *Alnus sinuata* (−1.7‰) and *Lupinus* (−0.4‰) were characteristic for N_2-fixing plants (Högberg 1997). The ericoid plants *Cassiope*, *Phyllodoce*, and *Vaccinium* had low $\delta^{15}N$ values (−8 to −11‰) which were similar to isolated individuals of ectomycorrhizal *Picea* (−9.7‰ at 600 m) and *Tsuga* (−8.1‰ at 750 m).

Figure 2 clearly illustrates the potential of mycorrhizal colonization to control plant $\delta^{15}N$ in the forefront sites. Nonmycorrhizal plants and plants colonized by ericoid, arbuscular, or ectomycorrhizal fungi share similar $\delta^{15}N$ values adjacent to the glacial terminus (< 200 m), corresponding to low mycorrhization (Trowbridge and Jumpponen 2004; Cázares et al. 2005) and few or no mycorrhizal propagules in the soil (Jumpponen et al. 2002; Trowbridge and Jumpponen 2004). Subsequent colonization of plants by mycorrhizal fungi, as reported by Cázares et al. (2005) and suggested by first appearance of ectomycorrhizal sporocarps at 340 m from the present glacier terminus (Fig. 3), correlated with a 5–6‰ decline in ^{15}N content in ectomycorrhizal and ericoid mycorrhizal plants. Overall, foliar $\delta^{15}N$ was obviously related to mycorrhizal status, with arbuscular and non-mycorrhizal plants enriched in ^{15}N relative to ectomycorrhizal and ericoid mycorrhizal plants. In contrast to most mycorrhizal plants, $\delta^{15}N$ in the non-mycorrhizal plants *Luzula* and *Saxifraga* did not change along the chronosequence.

Table 3 Foliar %N and $\delta^{15}N$ vary with species and symbiont type at forefront and mature sites

Genus	Symbiont type	Forefront			Mature		
		%N ± SD	$\delta^{15}N$ ± SD (‰)	*n*	%N ± SD	$\delta^{15}N$ ± SD (‰)	*n*
Abies	ECM	1.50^{d} ± 0.25	-8.6^{a} ± 2.8	29	1.29^{a} ± 0.11	-4.6^{a} ± 2.4	11
Larix	ECM	2.51^{b} ± 0.29	-4.4^{bc} ± 1.4	4[1]	2.18^{d} ± 0.17	2.1^{b} ± 0.6	11
Salix	ECM	2.49^{b} ± 0.26	-5.5^{b} ± 2.4	24			
Cassiope	ERM	0.96^{ef} ± 0.08	-9.8^{a} ± 1.1	14	1.10^{a} ± 0.08	-5.0^{a} ± 2.2	5
Phyllodoce	ERM	1.22^{de} ± 0.20	-8.9^{a} ± 1.4	24	1.55^{b} ± 0.14	-4.5^{a} ± 3.0	5
Vaccinium	ERM	1.37^{cde} ± 0.28	-8.9^{ab} ± 0.9	3[2]	1.91^{c} ± 0.19	-2.8^{a} ± 1.4	5
Epilobium	AM	1.73^{c} ± 0.45	-1.7^{c} ± 3.2	19			
Luetkea	AM	0.97^{ef} ± 0.18	-6.7^{b} ± 1.1	24	1.17 ± 0.07	-4.0^{a} ± 0.5	5
Sorbus	AM	2.05^{cd} ± 0.07	-5.2^{a} ± 0.6	3			
Luzula	NM	1.35^{d} ± 0.30	-5.0^{b} ± 1.0	24			
Saxifraga	NM	0.69^{f} ± 0.33	-5.5^{b} ± 0.6	17			
Alnus	actinorrhizal[5]	3.24	−1.7	1[3]			
Lupinus	rhizobial[6]	3.95^{a} ± 0.35	-0.4^{c} ± 0.1	4[4]			

Within a column, mean %N and $\delta^{15}N$ differ significantly if not followed by the same letter. Because of large differences in sample size, values are given ± standard deviation (SD)

For symbiont type, *ECM* ectomycorrhizal, *ERM* ericoid mycorrhizal, *AM* arbuscular mycorrhizal, *NM* non-mycorrhizal, *actinorrhizal* N_2-fixing *Frankia*, and *rhizobial* N_2-fixing *Rhizobium*

[1]Two individuals collected at 450 m and two individuals collected at 750 m

[2]Collected at 750 m

[3]Collected at 900 m

[4]Collected at 450 m

[5]Tripartite symbiosis, associates with *Frankia* (N_2-fixing actinorrhizal bacteria) and ectomycorrhizal fungi

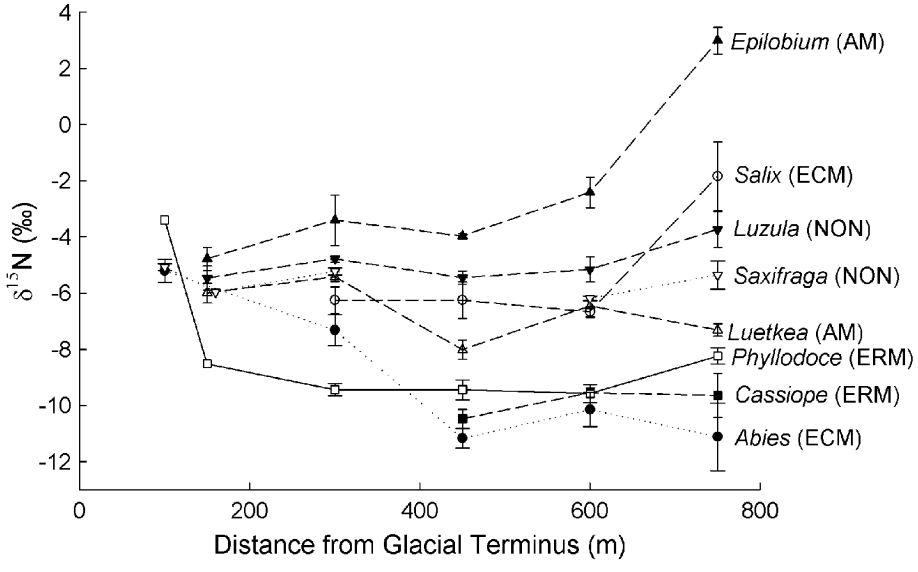
[6]Tripartite symbiosis, associates with *Rhizobium* (N_2-fixing rhizobial bacteria) and arbuscular mycorrhizal fungi

Our ability to directly compare the forefront and mature sites is somewhat limited, because non-mycorrhizal plants and two early colonizers, the arbuscular mycorrhizal *Epilobium latifolium* and the ectomycorrhizal *Salix phylicifolia*, were absent, the ectomycorrhizal *Larix lyallii* and *Abies lasiocarpa* dominated the plant community, and the tree *Sorbus sitchensis* became a minor component of the community. The species present in both forefront and mature sites were 3–6‰ enriched in ^{15}N at the mature site (Table 3), similar to the 3–5‰ enrichment in mature soils relative to forefront soils. Except for high $\delta^{15}N$ values for *Larix* (2.1 ± 0.2‰), all other species at the mature site, regardless of mycorrhizal association, ranged from −5 to −3‰.

Fig. 2 $\delta^{15}N$ patterns in plants after glacial retreat at Lyman Glacier, Washington, USA reflect development of mycorrhizae and nitrogen dynamics during primary succession. *x*-axis indicates distance from current glacial terminus, site ages range from 20 to 70 years (Jumpponen et al. 1998). Mycorrhizal habits of the plants are ericoid mycorrhizal (ERM), *Cassiope mertensiana* and *Phyllodoce empetriformis*; ectomycorrhizal (ECM), *Abies lasiocarpa* and *Salix phylicifolia*; arbuscular mycorrhizal (AM), *Epilobium latifolium* and *Luetkea pectinata*; and non-mycorrhizal (NON), *Luzula piperi* and *Saxifraga ferruginea*. Mycorrhizal habit is indicated besides each genus name. Values for plants are ± standard error (n = 5 typically)

Fungi

In the forefront, the first saprotrophic fungi appeared at 270 m and the first ectomycorrhizal fungi appeared at



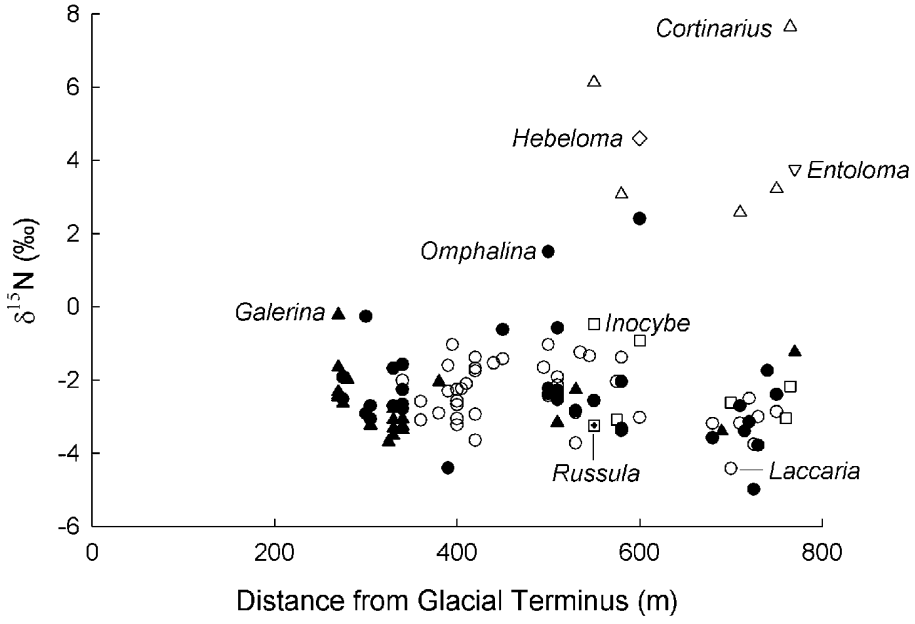


Fig. 3 $\delta^{15}N$ patterns in fungi after glacial retreat at Lyman Glacier, Washington, USA. *x*-axis indicates distance from current glacial terminus, site ages range from 20 to 70 years (Jumpponen et al. 1998). *Cortinarius tenebricus*, *Entoloma* sp., *Hebeloma* sp., *Inocybe lacera*, *Laccaria montana*, and *Russula fragilis* are ectomycorrhizal fungi, whereas *Galerina* sp. and *Omphalina obscurata* are saprotrophic fungi. *Clear symbols* are for ectomycorrhizal taxa and *filled symbols* for saprotrophic taxa. Symbols for specific taxa are: *Cortinarius*, *upright triangle*; *Entoloma*, *upside-down triangle*; *Hebeloma*, *diamond*; *Inocybe*, *square*; *Laccaria*, *circle*; *Russula*, *square* with *center cross*, *Galerina*, *upright filled triangle*; and *Omphalina*, *filled circle*. Values for fungi represent single specimens

Table 4 ^{15}N content in fungi from the mature site (archived specimens), showing clearly that host-specific ectomycorrhizal fungi are higher in $\delta^{15}N$ than generalist ectomycorrhizal fungi

Species	$\delta^{15}N$ (‰)	Associate	OSC #
Hypholoma capnoides	0.8	None (saprotrophic)	49236
Entoloma rhodopolium	1.5	Ectomycorrhizal	58916
Glomus convolutum	1.9	Arbuscular	49158
Laccaria bicolor	2.3	Ectomycorrhizal	58719
Russula emetica	2.4	Ectomycorrhizal	58949
Cortinarius violaceus	4.0	Ectomycorrhizal	49295
Entoloma rhodopolium	4.1	Ectomycorrhizal	58917
Inocybe lacera	4.3	Ectomycorrhizal	58728
Laccaria laccata	4.4	Ectomycorrhizal	49082
Thaxterogaster pingue	7.2	*Abies*-specific	49091
Cortinarius semisanguinea	9.4	Pinaceae-specific	60144
Fuscoboletinus ochraceoroseus	10.2	*Larix*-specific	49243
Fuscoboletinus aeruginascens	13.8	*Larix*-specific	49155
Rhizopogon subsalmonius	15.0	*Abies*-specific	49126
Suillus cavipes	15.2	*Larix*-specific	49166
Gautieria monticola	16.2	Pinaceae-specific	49237
Boletus calopus	16.6	Pinaceae-specific	49162

All except *Hypholoma* and *Glomus* are ectomycorrhizal fungi. "Associate" is the fungal life history strategy, designated as arbuscular for arbuscular mycorrhizal or ectomycorrhizal for taxa of broad host specificity. Host specificity taken from Molina et al. (1992). Genus or family associate is given for host-specific taxa. *OSC* from the Oregon State University herbarium.

340 m. The two saprotrophic taxa (*Galerina* sp. and *Omphalina obscurata*) and two of the ectomycorrhizal taxa (*Laccaria montana* and *Inocybe lacera*) had similar $\delta^{15}N$ values, averaging $-2.6 \pm 0.2‰$ ($n = 21$), $-2.4 \pm 0.2‰$ ($n = 33$), -2.4 ± 0.1 ($n = 40$), and $-2.1 \pm 0.5‰$ ($n = 6$), respectively. These values were about 3‰ enriched in ^{15}N relative to non-mycorrhizal and mycorrhizal plants sampled at 150 m from the present glacier terminus (Fig. 2, 3). In contrast, the ectomycorrhizal fungus *Cortinarius tenebricus* averaged $4.5 \pm 1.0‰$ ($n = 5$) and had $\delta^{15}N$ values of up to 7‰. *Cortinarius tenebricus* associates only with *Salix*, in contrast to *Laccaria montana* and *Inocybe lacera*, both of which lack host specificity.

At the mature site, ectomycorrhizal taxa of archived specimens ranged from 2 to 16‰ (Table 4), and were particularly high in ^{15}N for Boletaceae (*Boletus*, *Fuscoboletinus*, *Rhizopogon*, and *Suillus*) and a mat-forming fungus in the Gautieriaceae, *Gautieria*. A wood decay fungus (*Hypholoma*, $\delta^{15}N = 1‰$) and an arbuscular mycorrhizal sporocarp (*Glomus*, $\delta^{15}N = 2‰$) were both low in $\delta^{15}N$ compared to other archived specimens. Ectomycorrhizal fungi with potential host specificity (Table 4) were higher in ^{15}N content than fungi without host specificity (e.g., *Laccaria bicolor* and *Russula emetica*).

Discussion

Soils

Soil $\delta^{15}N$ initially results from the ^{15}N content of N inputs, but is subsequently modified by N cycling processes that remove ^{15}N-depleted or ^{15}N-enriched nitrogen from the soil pool. The $\delta^{15}N$ values for soils on the forefront of about $-1‰$ are among the lowest yet

observed, and are similar to values from soils developing on young lava flows on Hawaii (Vitousek et al. 1989; Martinelli et al. 1999). The low ^{15}N content of these young soils indicates that the initial inputs of N are depleted in ^{15}N. At the Lyman Glacier forefront, the four potential N sources to the developing soils include weathering from primary minerals (Holloway and Dahlgren 2003), inputs from atmospheric N deposition (Carrillo et al. 2002), biological N fixation, and transport by wind or animals of locally derived nitrogen. The large-scale surveys of the National Atmospheric Deposition Program indicate that inorganic N deposition currently averages only 1–2 kg N ha^{-1} $year^{-1}$, but may have averaged less than 1 kg N ha^{-1} $year^{-1}$ prior to the 1980s (http://nadp.sws.uiuc.edu).

Soil $\delta^{15}N$ was sharply higher at the mature site than at forefront sites by about 4‰ (Table 2). This is in agreement with the normal processes of soil development that increase the ^{15}N content of soils (Nadelhoffer and Fry 1994). We assume that the $\delta^{15}N$ of N available to plants increased in parallel to increases in bulk soil $\delta^{15}N$, as suggested by roughly similar increases between forefront and mature sites in total soil N (4‰) and in plant species (3–6‰, Table 3).

Foliage

The very low $\delta^{15}N$ signatures of −8 to −11‰ of many forefront specimens of *Cassiope*, *Phyllodoce* and *Abies* were remarkable. Such low values ($\sim < -8$‰) have never been reported for nonmycorrhizal plants, but have occasionally been reported from mycorrhizal plants in environments of low nutrient availability (Michelsen et al. 1996, 1998), particularly during early primary succession (Vitousek et al. 1989; Hobbie et al. 2000; Kohls et al. 2003). At Lyman Glacier forefront, such low values presumably arise because the baseline $\delta^{15}N$ of these young soils was low and N availability was very low, so most mycorrhizal plants depend heavily on ^{15}N-depleted N transferred from fungal symbionts. The low N availability leads to a low transfer ratio (T_r) of mycorrhizal fungi: only a small proportion of the N acquired by the mycorrhizal fungi is available for the transfer to the host plant after accounting for the proportion retained in fungal tissue. As a consequence of this high N retention, the N received by mycorrhizal plants is depleted in ^{15}N relative to the available soil N (Eq. 2). To maintain an isotopic mass balance in the plant-mycorrhizal system, high retention in the fungal partner of ^{15}N-enriched nitrogen results in low $\delta^{15}N$ in plants dependent on the mycorrhizal symbiosis for N uptake (Hobbie et al. 2000).

Numerous studies have attempted to relate foliar $\delta^{15}N$ patterns to the $\delta^{15}N$ of ammonium and nitrate in the soil (Högberg 1997; Schmidt and Stewart 1997; Hobbie et al. 2000). However, such efforts will not be successful if internal fractionations within the plant-mycorrhizal symbiosis (or uptake of organic N forms) largely control plant $\delta^{15}N$ patterns. Therefore, in this study we have used non-mycorrhizal plants as the most suitable reference point for comparisons of $\delta^{15}N$ signatures in mycorrhizal plants. The $\delta^{15}N$ of non-mycorrhizal plants functions as an integrated measure of the $\delta^{15}N$ of available N in the soil, without specifying the exact mix of assimilated N forms. For example, culture studies under carefully controlled conditions indicate that non-mycorrhizal pines have ^{15}N content similar to the supplied ammonium nitrate, whereas pines inoculated with the mycorrhizal fungi *Suillus luteus* or *Thelephora terrestris* are always depleted in ^{15}N relative to the N supply (Hobbie and Colpaert 2003).

At many forefront sites, the facultatively arbuscular mycorrhizal *Epilobium latifolium* and the ectomycorrhizal *Salix phylicifolia* did not conform to the expected pattern but had similar or higher $\delta^{15}N$ values than non-mycorrhizal plants. At the mature site, the deciduous ectomycorrhizal conifer *Larixlyalii* was relatively high in $\delta^{15}N$. For these species, other, poorly constrained mechanisms of ^{15}N partitioning may control $\delta^{15}N$ distribution. Such mechanisms include: (1) preferences for different N forms (McKane et al. 2002) that may differ in ^{15}N content; (2) discrimination against ^{15}N accompanying reduction of nitrate in roots or shoots (Evans et al. 1996), or (3) different modes of N uptake and transfer by plants and their mycorrhizal fungi (Bago et al. 2001). The mode of N transfer from arbuscular mycorrhizal fungi to plants may differ from that of ectomycorrhizal fungi (Bago et al. 2001), possibly explaining contrasting discrimination against ^{15}N (Fogel and Cifuentes 1993). The poorly characterized, dark septate fungi commonly associated with *Salix* and other taxa at the Lyman Glacier forefront (Trowbridge and Jumpponen 2004; Cázares et al. 2005) also probably differ from ectomycorrhizal fungi in their nutrient transfer to plants, given the different plant–fungal interfaces. Little is known of the enzymatic capabilities of dark septate fungi, aside from the presence of cellulases and other enzymes necessary for saprobic activity or necessary for bypassing plant defenses during colonization (Caldwell et al. 2000). More detailed studies on the preference for different forms of N, partitioning of ^{15}N within plants, and role of different mycorrhizae in plant N supply and ^{15}N partitioning are necessary to explain the high $\delta^{15}N$ values of *Epilobium*, *Salix*, and *Larix*.

Fungi

The general enrichment in ^{15}N of about 3‰ of most ectomycorrhizal and saprotrophic fungi in the forefront relative to nonmycorrhizal plants and relative to mycorrhizal plants collected at 150 m cannot be explained by fungal transfer of ^{15}N-depleted compounds to plants (Fig. 2, 3). Since both saprotrophic and mycorrhizal fungi were equally enriched in ^{15}N relative

to putatively nonmycorrhizal plants, these values may reflect a general enrichment of about 3‰ in ^{15}N of fungal sporocarps relative to assimilated N and vegetative mycelia. For example, Kohzu et al. (2000) reported that a mycorrhizal *Suillus granulatus* sporocarp was 2.7‰ enriched relative to its mycelium, whereas Handley et al. (1996) reported that caps, but not stipes, of a saprotrophic *Agrocybe* species were 1‰ enriched in ^{15}N relative to rhizomorphs. No other data directly comparing hyphae and sporocarps are available, although Hobbie and Colpaert (2003) calculated that fungal N in mycorrhizal roots was 3–5‰ enriched in ^{15}N relative to N in extraradical hyphae. Because of a consistent ^{15}N enrichment of protein relative to chitin of about 9‰ (Taylor et al. 1997), preferential export of protein-derived N to sporocarps and retention of chitin-bound N in hyphae may explain the ^{15}N enrichment in sporocarps.

Linking $\delta^{15}N$ patterns in mycorrhizal fungi to fungal functioning would be desirable. In the only published attempt to directly relate $\delta^{15}N$ to functional attributes, mycorrhizal taxa with proteolytic capabilities generally had high $\delta^{15}N$ signatures (Lilleskov et al. 2002). This implies that ectomycorrhizal fungi with high ^{15}N content in the forefront (*e.g.*, *Cortinarius tenebricus*), may possess greater proteolytic capabilities than fungi with low ^{15}N content (*e.g.*, *Laccaria montana*). *Laccaria* is a genus with usually limited proteolytic capabilities, whereas many species of *Cortinarius* have strong proteolytic capabilities (Lilleskov et al. 2002). Fungi high in ^{15}N that were collected from the mature site in this study may also have proteolytic capabilities (members of the family Boletaceae). Boletaceae have been reported to be high in ^{15}N content compared to other mycorrhizal families (Taylor et al. 2003).

Host-specific ectomycorrhizal fungi (*Cortinarius tenebricus, Fuscoboletinus ochraceoroseus*, and *Suillus cavipes* in this study) in the forefront and the mature site were invariably higher in ^{15}N content than ectomycorrhizal fungi adapted to colonize a wide variety of hosts (*Laccaria bicolor*, *Russula emetica*, and *Cortinarius violaceus* in this study). One possible explanation is that the success of host-specific fungi may be more closely tied to the fitness of their hosts than the non-host-specific fungi. If host-specific fungi transfer a higher proportion of assimilated N to their hosts than non-host-specific fungi (higher T_r), then the relative fitness of hosts colonized by host-specific fungi should exceed that of hosts colonized by non-host-specific fungi. When such transfers are accompanied by discrimination against ^{15}N, then host-specific fungi will be higher in $\delta^{15}N$ than non-host-specific fungi, according to Eq. 3. For example, in a study in Swedish boreal forests, the *Pinaceae*-specific *Suillus bovinus* was high in $\delta^{15}N$ relative to non-host-specific *Laccaria bicolor* (Taylor et al. 2003). Furthermore, Gorissen and Kuyper (2000) reported that *Suillus bovinus* transferred more N to *Pinus sylvestris* seedlings in a culture study than *Laccaria bicolor*.

Testing models of plant-mycorrhizal partitioning of ^{15}N

We can use this glacier forefront system to test different models of plant-mycorrhizal partitioning of ^{15}N. Our previous field and laboratory studies have assumed that plant-mycorrhizal systems can be treated as open (Fig. 1; Hobbie et al. 2000; Hobbie and Colpaert 2003), although many natural systems where substrate can be exhausted have been treated as closed systems when interpreting isotopic patterns (Högberg 1997; Evans and Belnap 1999; Hayes 2002). If we assume an open system and a value of Δ of 9‰, the 6‰ depletion of many ectomycorrhizal and ericoid mycorrhizal plants relative to non-mycorrhizal plants at Lyman Glacier forefront may indicate that only one-third of N taken up by mycorrhizal fungi is transferred to host plants. We cannot distinguish between open and closed systems based solely on these data. To do that, we must also consider ^{15}N patterns in fungi.

If we assume that the plant-mycorrhizal system is open (Fig. 1a), then it follows that the $\delta^{15}N$ in ectomycorrhizal fungi should have a maximum value of $\delta^{15}N_{\text{available N}} + T_r \times \Delta$. If $\delta^{15}N_{\text{available N}}$ is assumed to be −5‰ based on the average value for non-mycorrhizal plants, Δ is 9‰, and T_r is 0.5 or less, then $\delta^{15}N_{\text{fungi}}$ should be −0.5‰. Accordingly, the $\delta^{15}N$ values up to 7‰ for ectomycorrhizal sporocarps on the forefront cannot be explained based on an open system model, even if an additional enrichment in ^{15}N of 3‰ during the formation of sporocarps is included.

Some insight into how to treat ^{15}N patterns in fungal sporocarps can be gained by considering the processes of N mobilization and transport during sporocarp formation. Fruiting of fungi generally takes place only in response to specific environmental cues of moisture, temperature, nutrient exhaustion, or other factors (Deacon 1997). Upon initiation of fruiting, stored reserves are rapidly allocated for sporocarp formation. Nitrogen uptake during the period of fruiting is probably less than the N required to form sporocarps. Sporocarp formation therefore cannot be treated as a completely open system, particularly as fungi may use nutrient exhaustion to sense when resources should be shifted from somatic growth to the sexual growth of sporocarp formation. Under these circumstances, internal partitioning of N among labile and non-labile pools probably contributes to redistribution of ^{15}N among fungal storage compounds, thereby affecting the ultimate ^{15}N content of the resulting sporocarps. For ectomycorrhizal fungi with high $\delta^{15}N$, we propose that ^{15}N patterns could be better explained by treating fungi as the substrate in a closed system (Fig. 1b, Eq. 5). With Δ set to 9‰, a value of 0.4 for $1 - f$, the proportion of system N remaining in fungi, would increase fungal ^{15}N content by 15‰ relative to ectomycorrhizal plants. Treating the plant-mycorrhizal system as closed would account for the very high $\delta^{15}N$ values of ectomycorrhizal fungi reported in many studies (Kohzu et al. 1999, Henn

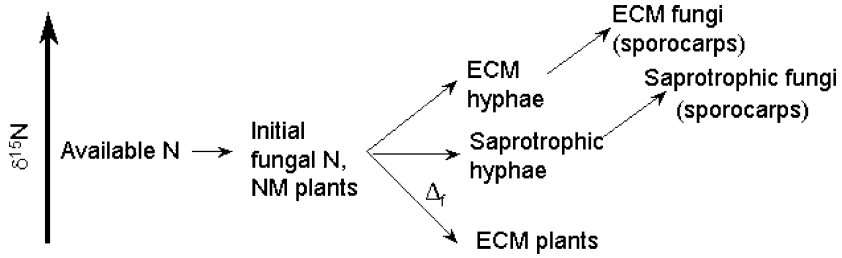


Fig. 4 Conceptual diagram of ^{15}N distribution patterns among available N, saprotrophic fungi, ectomycorrhizal (ECM) fungi, nonmycorrhizal plants (NM), and ectomycorrhizal plants. Fungi are separated into sporocarps and hyphae. ^{15}N enrichment occurs during sporocarp formation, ^{15}N depletion (designated Δ_f) occurs during creation of transfer compounds that are passed from mycorrhizal fungi to plants

and Chapela 2001; Hobbie et al. 2001; Trudell et al. 2004).

Our current understanding of ^{15}N distributions among non-mycorrhizal plants, mycorrhizal plants, mycorrhizal fungi, and saprotrophic fungi at Lyman Glacier is summarized in Fig. 4. Although we cannot currently explain why some plant $\delta^{15}N$ values are higher than those of bulk soil, this model fits most of the ^{15}N distributions observed in plants and fungi under N-limited conditions. Several areas require additional research, such as the influence of specific N sources on $\delta^{15}N$ patterns and the influence of nitrate reductase activity in leaves versus roots on $\delta^{15}N$ distributions within plants. Further insights will require specific information on the forms of N taken up by plants and fungi, as well as information on patterns of isotopic fractionation within organisms among different compound classes or tissues.

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