Ecology, 87(4), 2006, pp. 816–822

^{15}N IN SYMBIOTIC FUNGI AND PLANTS ESTIMATES NITROGEN AND CARBON FLUX RATES IN ARCTIC TUNDRA

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Abstract. When soil nitrogen is in short supply, most terrestrial plants form symbioses with fungi (mycorrhizae): hyphae take up soil nitrogen, transport it into plant roots, and receive plant sugars in return. In ecosystems, the transfers within the pathway fractionate nitrogen isotopes so that the natural abundance of ^{15}N in fungi differs from that in their host plants by as much as 12‰. Here we present a new method to quantify carbon and nitrogen fluxes in the symbiosis based on the fractionation against ^{15}N during transfer of nitrogen from fungi to plant roots. We tested this method, which is based on the mass balance of ^{15}N, with data from arctic Alaska where the nitrogen cycle is well studied. Mycorrhizal fungi provided 61–86% of the nitrogen in plants; plants provided 8–17% of their photosynthetic carbon to the fungi for growth and respiration. This method of analysis avoids the disturbance of the soil–microbe–root relationship caused by collecting samples, mixing the soil, or changing substrate concentrations. This analytical technique also can be applied to other nitrogen-limited ecosystems, such as many temperate and boreal forests, to quantify the importance for terrestrial carbon and nitrogen cycling of nutrient transfers mediated by mycorrhizae at the plant–soil interface.

Key words: Alaska; arctic tundra; carbon flux; mycorrhizae; ^{15}N; nitrogen-limited ecosystems; plant nitrogen; soil nitrogen; plant–fungal symbioses; soil–microbe–root relationships.

INTRODUCTION

It is increasingly recognized that the enzymatic breakdown of large organic molecules to simple organic compounds may be a more important part of the soil nitrogen (N) cycle (Schimel and Bennett 2004) than the microbial production of inorganic N such as NH_4^+ and NO_3^-. In this view, the resulting labile organic N, such as amino acids, may be used by either plants or microbes. However, the importance to the plants of direct uptake of amino acids by plant roots has been recently questioned by Jones et al. (2005), who concluded that much of the measured plant uptake of intact amino acids measured in the field may be due to the mycorrhizal symbiont rather than to root uptake per se. In a recent review of N cycling, Schimel and Bennett (2004) stressed the possible key role of mycorrhizal fungi in supplying N to plants under N-limited conditions. Here we add to this developing picture by quantifying the role of mycorrhizal fungi in N cycling through measurements of the natural abundances of ^{15}N. We conclude that mycorrhizal fungi are the main conduit of N between the soil organic matter and plants.

The importance of mycorrhizal fungi in the N nutrition of plants has been studied for over a century (Frank 1894 [as cited in Smith and Read (1997)]). Plants obtain N through the fungi and, in return, provide fungi with sugars from photosynthesis (Read 1991). Although it would be highly desirable to quantify carbon and nitrogen fluxes in the symbiosis, difficulties in studying the symbiosis in the field thus far have limited the information on how much N actually moves through this pathway into plants. Evidence of the importance of the symbiosis in N-limited ecosystems comes from several sources. Almost all trees in boreal and temperate forests, including oaks, birches, willows, and most conifers, are symbiotic with ectomycorrhizal fungi, and ericaceous plants are symbiotic with ericoid mycorrhizal fungi. These fungi often possess strong hydrolytic capabilities to aid in the breakdown of complex N-containing organic polymers such as protein or chitin (Read and Perez-Moreno 2003), whereas the third main class of symbiotic fungi, arbuscular mycorrhizal fungi, do not appear to possess such capabilities. Mycorrhizal fungi form sheaths of hyphae around fine roots of ectomycorrhizal plants, or form hyphal coils within cortical root cells of ericaceous plants, thereby making it likely that soil N in the form of NH_4^+, NO_3^-, and organic molecules passes through the fungi prior to reaching the plant roots (Read 1991, Wallenda et al. 2000). Ending N limitation by deliberate fertilization (Rühling and Tyler 1991) or by increased atmospheric N deposition decreases carbon allocation belowground, fungal fruiting, and growth of extraradical hyphae in ectomycorrhizal systems (Wallenda and Kottke 1998). Estimates of the transfer rates of N into plants come

Manuscript received 8 August 2005; revised 18 November 2005; accepted 28 November 2005. Corresponding Editor: B. B. Casper.

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from short-term measurements, in laboratory or field studies, of the incorporation by plants of added ^{15}N-labeled compounds. Although these methods identify pathways and processes, they also alter soil chemistry, disturb microbe–soil–root relationships, and are restricted to specific N forms. Accordingly, these methods are not ideal for quantifying N dynamics in nature. For example, microbial uptake of nutrients may result in diffusion gradients, with very low nutrient concentrations actually available to roots and microbes. These available concentrations may be much lower than concentrations estimated from extractions of bulk soil samples, and may therefore lead to overestimates of uptake when ^{15}N compounds are added in tracer experiments. In addition, measurements of concentrations of organic compounds may be flawed because extractions of soil samples may disrupt fine roots, root hairs, and fungal hyphae and thereby release large amounts of dissolved organic N (Jones et al. 2005).

Using the natural abundance of ^{15}N in the environment as a marker of N cycling avoids the problems of disturbing the microenvironment, but as yet there is no generally accepted way to interpret field data. Based on low ^{15}N content of ectomycorrhizal and ericoid mycorrhizal plants and high ^{15}N content of ectomycorrhizal fungal fruiting bodies, several authors have suggested that mycorrhizal fungi alter the isotopic composition of the N that they take up from the soil and transfer to plants (Högberg 1990, Schmidt and Stewart 1997, Hobbie et al. 1999). The transfer compound between ectomycorrhizal fungi and plants is probably the amino acid glutamine (Smith and Smith 1990); evidence from laboratory and field studies indicates that transamination during the formation of glutamine strongly fractionates against ^{15}N (summarized in Hobbie and Colpaert [2003]).

We have devised a method to quantify N transfers by using a mass balance of ^{15}N in plants, fungal fruiting bodies, and the soil. This is very similar to the method of Robinson et al. (1998), which did not include mycorrhizae. In addition, given the close links between carbon and N cycling in mycorrhizal symbioses, we can also estimate the carbon fluxes from the plant to support fungal growth. Here we analyze ^{15}N data from a well-studied tundra site in Alaska and ask if the results are ecologically consistent and reasonable.

Methods

Samples were collected at the Arctic LTER (Long Term Ecological Research) site near Toolik Lake, Alaska (68°38′ N, 149°34′ W), in the northern foothills of the Brooks Range. The well-described collection sites (Shaver and Chapin 1991, Chapin et al. 1993) are in the moist, acidic tussock tundra south and east of Toolik Lake, where the pH of the soil is 3.5, NO_3^- concentrations are extremely low, and nitrification rates are slow. Whole-plant and soil samples were collected in 1992 as the natural abundance controls of a ^{15}N-addition experiment (McKane et al. 2002) south of Toolik Lake. The previously unpublished ^{15}N data are included here; a plant leaf and soil collection east of Toolik Lake (Nadelhoffer et al. 1996) yielded ^{15}N results very similar to those reported here. Fungal fruiting bodies and NH_4^+ and NO_3^- for isotope analysis were collected in 2003 from the Shaver and Chapin (1991) site east of Toolik Lake.

Fungal fruiting bodies were dried at 50°C and were analyzed at the Marine Biological Laboratory (MBL), Woods Hole, Massachusetts, USA, using a PDZ Europa 20–20 continuous-flow isotope ratio mass spectrometer. Analysis of N isotopes in soil and in leaves and stems of plants has been described previously (McKane et al. 2002). Stable isotope abundances are reported as δ^{15}N in parts per mille (‰) expressed as $(R_{sam}/R_{std} - 1)1000$, where R is the ^{15}N/^{14}N ratio of either the sample (sam) or the reference standard (std; atmospheric N_2 for nitrogen). Samples with more of the heavy isotope are referred to as enriched; samples with more of the light isotope are referred to as depleted (Hobbie et al. 2000).

To collect enough inorganic N from soil water for ^{15}N analysis, cation and anion exchange resins were deployed for seven weeks at a depth of 5 cm in the soil in bags made with nylon stocking material. Each bag contained 8 mL of either IONAC C-267 cation exchange or IONAC ASB-1P anion exchange resin (IONAC Chemical Company, Birmingham, New Jersey, USA). Four bags were deployed for NO_3^- and eight for NH_4^+. For analysis, the resin from four bags was pooled into a composite sample. The bags were removed from the soil on 18 August 2003 and were extracted (Giblin et al. 1994) with 2 mol/L KCl. For dissolved NO_3^-, we used an alkaline headspace diffusion procedure after first reducing NO_3^- to NH_4^+ with Devarda's alloy (Sigman et al. 1997). For NH_4^+, a diffusion method (Holmes et al. 1998) was modified by using 7-day incubation and no salt adjustment. Analyses for ^{15}N were conducted at the MBL as described previously.

Results and Discussion

^{15}N in Toolik soils, plants, and fungal fruiting bodies

The δ^{15}N values (Table 1, mean ± SEM) for non-mycorrhizal plants (2.2‰ ± 0.5‰, 3.7‰ ± 1.9‰) and arbuscular mycorrhizal plants (2.2‰ ± 0.8‰, 2.0‰) are very similar to those of the bulk soil (1.2‰ ± 0.5‰), NH_4^+ (1.5‰ ± 0.5‰), and NO_3^- (1.0‰). These plants evidently take up and incorporate N from the soil without fractionation (Hobbie et al. 2000). In contrast to these values, the ectomycorrhizal and ericoid mycorrhizal plants are depleted (−2.4‰ ± 0.5‰ to −6.7‰ ± 0.5‰) and the ectomycorrhizal fungi are enriched (2.5‰ to 8.1‰ ± 0.5‰) relative to bulk soil and inorganic N. Fractionation against ^{15}N during the formation of transfer compounds inside the hyphae of ectomycorrhizal and ericoid mycorrhizal fungi leads to host plants receiving N as ^{15}N-depleted amino acids. The N remaining in the hyphae is enriched in ^{15}N and

TABLE 1. Mean $\delta^{15}N$ of roots, leaves, and stems of plants and of soil nitrogen and fungal fruiting bodies at the moist tussock sites at Toolik Lake, Alaska, USA.

Source	$\delta^{15}N \pm$ SE (‰)
A) Plants	
Non-mycorrhizal	
Eriophorum vaginatum	3.7 ± 1.9
Carex bigelowii	2.2 ± 0.5
Arbuscular mycorrhizal	
Rubus chamaemorus	2.2 ± 0.8
Polygonum bistorta	2.0†
Ectomycorrhizal	
Salix sp.	−2.4 ± 0.5
Betula nana	−5.6 ± 0.5
Ericoid mycorrhizal	
Vaccinium uliginosum	−4.0†
V. vitis-idaea	−6.7 ± 0.5
Empetrum sp.	−4.3 ± 0.01
Ledum palustre	−4.3 ± 0.4
Hemiparasitic	
Pedicularis sp.	−5.4†
B) Soil and pore water N	
Soil N between and below tussocks	1.2 ± 0.7
NH_4^+ in pore water	1.4 ± 0.5
NO_3^- in pore water	1.0†
C) Fungal fruiting bodies	
Laccaria sp.	2.5†
Cortinarius sp.	8.1 ± 0.5
Leccinum sp.	5.7†
Russula sp.	7.3†
Lactarius sp.	6.5 ± 0.2

Notes: Data are means of 2–5 composite samples except where noted. Plant and soil data (see *Methods*) are the previously unpublished controls of the McKane et al. (2002) experiment. The NH_4^+, NO_3^-, and fungal fruiting-body samples were collected in 2003 at the same sites (see details in *Methods*).

† Sample size $n = 1$.

ultimately forms the fungal fruiting bodies. The low $\delta^{15}N$ (2.5‰) of one fungus, *Laccaria* sp., presumably reflects its known inability to degrade protein, in agreement with prior reports linking ^{15}N content and proteolytic capabilities (Lilleskov et al. 2002).

This analysis assumes that N isotopes are not fractionated during the uptake of N compounds into hyphae and roots from the soil solution. Field studies presented here (Table 1) confirm this. That is, the similar $\delta^{15}N$ of non-mycorrhizal plants, of arbuscular mycorrhizal plants, and of soil organic and inorganic N indicates that no fractionation had taken place during uptake of N into these types of plants at the concentration of N compounds present. It is likely, therefore, that N isotopes were not fractionated during uptake by roots and fungi at these same sites. Additional field data come from measurements of filamentous algae (Peterson et al. 1993) in a nearby stream. The algae had a $\delta^{15}N$ of 3‰ when the concentration of NH_4^+ was <1 μmol/L; fertilization of this stream to 7 μmol/L NH_4^+ caused a fractionation against ^{15}N of 10‰ during uptake and assimilation.

Laboratory studies of isotopic fractionation of inorganic N upon uptake by fungi, algae, and higher plants have concluded that their fractionation is minimal at low substrate concentrations for non-mycorrhizal plants, mycorrhizal plants, and algae (Fogel and Cifuentes 1993, Hobbie and Colpaert 2003). At high concentrations of 2–4 mmol/L NH_4^+, Emmerton et al. (2001) and Henn and Chapela (2004) found that laboratory cultures of mycorrhizal fungi fractionated against ^{15}N upon uptake, but these concentrations are not found in N-limited systems. For example, concentration of NH_4^+ in soil waters was only 10 μmol/L at our site (Chapin et al. 1993). In theory, fractionation upon uptake of amino acids should also be negligible because of the mass of the molecule. This was confirmed by laboratory studies of fungal uptake of amino acids at high concentration (Emmerton et al. 2001). We conclude from these several lines of evidence that N compounds are not isotopically fractionated when taken up by hyphae or roots in N-limited systems.

^{15}N pathways and mass balance calculations

The pathways of N from soil to mycorrhizal fungi and associated plants are shown in Fig. 1A. One pathway is the direct uptake of N from soil to plant roots. Another pathway begins with the uptake of inorganic and organic forms of N by hyphae. Within the hyphae, ^{15}N is fractionated against during reactions, and ^{15}N-depleted amino acids are subsequently transferred to the plant. The N remaining in the hyphae is accordingly enriched in ^{15}N.

In the following equations, Eq. 1 links isotopic signatures of available N ($\delta^{15}N_{av}$), fungal N ($\delta^{15}N_{fun}$), and N transferred from fungi to plants ($\delta^{15}N_{tr}$), where T is the percentage of available N entering hyphae that is transferred to the plant and $(100 - T)$ is the percentage that remains in the fungi; Eq. 2 expresses the plant isotope signature ($\delta^{15}N_{pl}$) as a function of $\delta^{15}N_{tr}$, $\delta^{15}N_{av}$, and f, the percentage of plant N coming from mycorrhizal fungi; and Eq. 3 describes Δ as the fractionation against ^{15}N during the creation of transfer compounds:

$$100(\delta^{15}N_{av}) = (100 - T)(\delta^{15}N_{fun}) + (T)(\delta^{15}N_{tr}) \quad (1)$$

$$100(\delta^{15}N_{pl}) = (f)(\delta^{15}N_{tr}) + (100 - f)(\delta^{15}N_{av}) \quad (2)$$

$$\Delta = \delta^{15}N_{av} - \delta^{15}N_{tr}. \quad (3)$$

In these equations, $\delta^{15}N_{av}$, $\delta^{15}N_{fun}$, and $\delta^{15}N_{pl}$ are all known. This leaves four unknowns ($\delta^{15}N_{tr}$, T, f, and Δ) in three simultaneous equations. Therefore, specifying any of the four unknowns allows us to calculate the other three.

As an example of the transfers and fractionations of N isotopes in the arctic tundra, we analyze a situation with $\delta^{15}N$ values of 1‰ and 2‰ for available soil N, of −5‰ for plants, and of 7‰ for mycorrhizal fungi (Fig. 1A). The

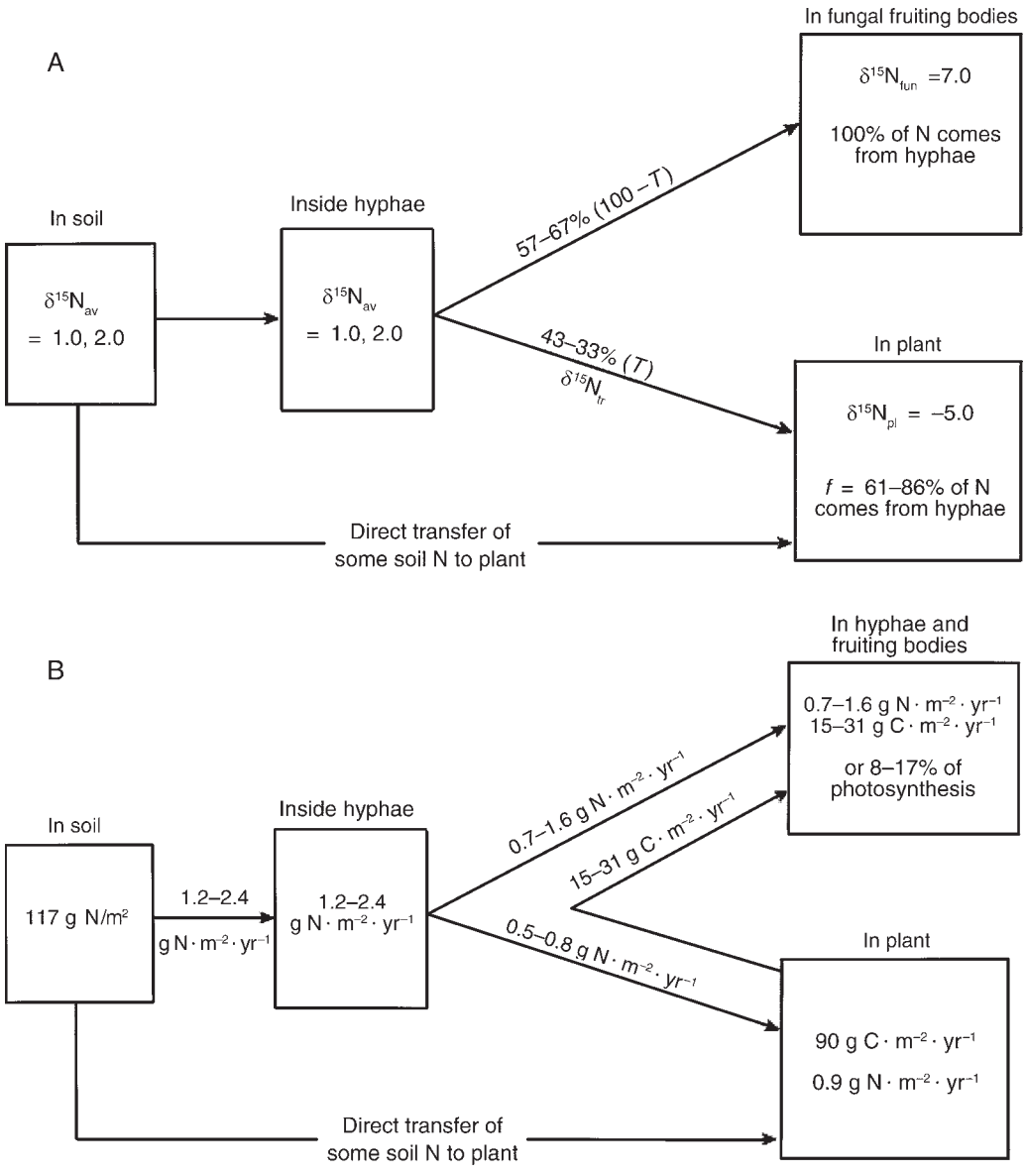

FIG. 1. Fluxes of ^{15}N, N, and C in the soil, mycorrhizal hyphae, mycorrhizal plants, and fungal fruiting bodies. (A) Calculations are based on Eqs. 1–3 for values of $\delta^{15}N_{av}$ (available) in soil of 1.0‰ and 2.0‰, for $\delta^{15}N_{pl}$ (plants) of −5.0‰, and for $\delta^{15}N_{fun}$ (fungi) of 7.0‰; f is the percentage of plant N supplied by mycorrhizal fungi. (B) Calculations are based on plant productivity. The amounts of C and N in the plant box are plant production as C and the amount of N needed for production from the soil each year. This N divided by the transfer percentage (T) in Fig. 1A and Table 2 gives the N available inside the hyphae. This quantity multiplied by 100 − T is the amount of N in hyphae and fruiting bodies. This N multiplied by the C:N ratio (10) and divided by the growth efficiency (50%) is the C needed for hyphal growth. This C divided by the GPP (180 g C·m^{-2}·yr^{-1}) is the percentage of GPP used by mycorrhizal fungi.

mycorrhizal fungi value is the mean of the four species in Table 1 omitting *Laccaria* sp. The values for ectomycorrhizal and ericoid mycorrhizal plants have been weighted by correcting for the plant productivity at this site (Shaver et al. 2001). Thus, 32% of the production was by *Betula,* 3% by *Salix*, and 65% by ericaceous evergreen plants, with *Ledum* and *Vaccinium* the most productive.

The simultaneous equations produce a number of solutions. However, two constraints exist on solutions for the mass balance values in Table 2. First, no more than 100% of plant N can come from mycorrhizal hyphae (f). Second, values for fractionation against ^{15}N during creation of transfer compounds (Δ) must be at least 8‰ to 10‰, based on values for amino acid transamination (Macko et al. 1986), peptide hydrolysis (Werner and Schmidt 2002), and chitin formation from amino acids (Taylor et al. 1997). With these constraints, solutions 2, 3, 5, and 6 in Table 2 are most likely.

TABLE 2. Solutions to the ^{15}N mass balance (Eqs. 1–3) of the tundra at Toolik Lake, Alaska.

	Solution					
Variable	1	2	3	4	5	6
$\delta^{15}N_{av}$ (‰)	1.0	1.0	1.0	2.0	2.0	2.0
T (%)	50	43	38	42	38	33
$100 - T$ (%)	50	57	62	58	62	67
$\delta^{15}N_{tr}$ (‰)	−5.0	−6.9	−8.8	−4.9	−6.2	−8.1
Δ (‰)	6.0	7.9	9.8	6.9	8.2	10.1
f (%)	100	75	61	101	86	69

Notes: For this case, $\delta^{15}N$ values are as follows: fungal fruiting body ($\delta^{15}N_{fun}$), 7‰; plant ($\delta^{15}N_{pl}$), −5.0‰; available soil nitrogen ($\delta^{15}N_{av}$), 1‰ or 2‰. T is the percentage of available N entering the hyphae that is transferred ($\delta^{15}N_{tr}$) to the plant, $100 - T$ is the percentage that remains in the fungi, f is the percentage of plant N supplied by mycorrhizal fungi, and Δ is the isotopic fractionation during the creation of transfer compounds.

Therefore, 57–67% of the N taken up by hyphae remains in the fungus and 33–43% is transferred to the plant, where it makes up 61–86% of foliar N.

The $\delta^{15}N$ analysis sheds light on how plants obtain N from the soil at this arctic site. One group, non-mycorrhizal plants such as *Eriophorum* and *Carex*, obtain inorganic N and perhaps organic N from soil solution through their roots (Chapin et al. 1993). The second group, *Rubus* and *Polygonum*, forms arbuscular mycorrhizal associations. Because their $\delta^{15}N$ values are close to 2‰, no conclusions can be made about their N sources. The third group, *Salix, Betula, Ledum, Vaccinium*, and *Empetrum,* form ectomycorrhizal or ericoid mycorrhizal associations and obtain most of their N from mycorrhizal fungi. Although it is unknown if the fungi use organic sources, inorganic sources, or both, organic N is probably a significant component of assimilated N, given the predominance of mycorrhizal fungi of known proteolytic ability and the low availability of inorganic N. A fourth group, represented by the hemiparasitic plant *Pedicularis,* obtains N by directly tapping into the root phloem of other plants; here, based on its similar ^{15}N content, *Betula* is a possible N source for *Pedicularis*.

Ectomycorrhizal and ericoid mycorrhizal fungi depend upon plant carbohydrates to fuel metabolic activity, including chitin and protein synthesis. We estimate (Fig. 1B) the N needed for the symbiosis from the following available data and assumptions. Annual productivity (NPP) of ectomycorrhizal and ericoid mycorrhizal plants at this site (Shaver and Chapin 1991, Nadelhoffer et al. 2002) is 90 g C/m^2, requiring a soil input of 0.9 g N/m^2 when corrected for N stored in the plant (G. Shaver, *personal communication*, emphasizes that the exact value of stored N that contributes to annual plant growth is not known, but that 0.9 g N is a reasonable estimate). Thus 1.3–2.0 molecules of N remain in the hyphae and fruiting bodies for every 1.0 molecule transported to the plant (i.e., a $100 - T$ of 57–67%). The amount of N contributing to the annual growth of the fungal hyphae is 0.7–1.6 g N/m^2. Soil N is 117 g N/m^2 (Shaver and Chapin 1991).

We estimate the C needed for the symbiosis from the following data and assumptions: the mass ratio of C:N for hyphae is 10 (Lodge 1987) and the efficiency of fungal growth (Ek 1997) is 50%. Applying these ratios to the N in the annual growth of fungal hyphae yields an annual carbon demand for mycorrhizal fungal growth of 15–31 g C/m^2. With gross primary productivity (GPP) about twice NPP (Schlesinger 1991), then GPP is 180 g $C \cdot m^{-2} \cdot yr^{-1}$. The fungal growth uses 8–17% of the total carbon productivity of these plants. This range is similar to the 2.5–14% estimated in field studies of the carbon allocation to ectomycorrhizal fungi as a percentage of GPP (Vogt et al. 1982, Fogel and Hunt 1979) and to the 1–11% estimated in culture studies (Hobbie 2006).

The sensitivity of these estimates to variability in the $\delta^{15}N$ values of plants and mycorrhizal fungi is an important issue. Values of standard error of the mean (SEM) for $\delta^{15}N$ measurements for ectomycorrhizal plants, ericoid mycorrhizal plants, and ectomycorrhizal fungi in Table 1 are all <0.5‰. However, only two of the five fungi have associated standard errors. Standard errors of other samples of ^{15}N in fungi in Alaska are also low, as follows (Lilleskov et al. 2002): *Russula*, ±0.4‰, *Laccaria,* ±0.6‰, *Lactarius*, ±0.2‰, and *Cortinarius*, ±0.6‰. We conclude that the Toolik samples are not atypical. To test the impact of variability on our calculations, we have estimated how varying the $\delta^{15}N$ values for fungi and plants by ±1‰ alters the percentage of plant N supplied by fungi (f; Table 2) and the percentage of GPP allocated to fungi. These ranges capture almost all of the range for $\delta^{15}N$ in our samples (Table 1). The calculations were made with the values in Fig. 1A for T and $100 - T$ and in Fig. 1B for the plant C and N. In one set of calculations, $\delta^{15}N$ values were −5‰ ± 1‰ for plants and 7‰ for fungi and in the other set they were −5‰ for plants and 7‰ ± 1‰ for fungi. The results widen the range of "f" from 61–86% to 51–98% and of the percentage of GPP allocated to fungi from 8–17% to 7–20%. These values do not change the conclusions of this paper.

In our calculations, we have used concepts and constants from the rather limited literature on ^{15}N in soils, mycorrhizal fungi, and plants. What are the next steps for research? First, we need to know the time scale over which N in fungal fruiting bodies has been assimilated from the soil. Do foliar and fungal ^{15}N contents represent comparable time periods of soil N assimilation? Second, we need to know if winter storage of N in plant roots and the subsequent reallocation to foliage fractionates N isotopes. The few studies to date (Näsholm 1994, Hobbie et al. 2001) show no isotopic effect of reallocation. Third, our calculations indicate that non-mycorrhizal pathways by plants account for 14–39% of the total N uptake. We need to estimate more directly the extent of direct root uptake by mycorrhizal plants. Fourth, we measured ^{15}N in the inorganic soil N

pools and in the total soil N pool. Given the hypothesized importance of organic N uptake by mycorrhizal fungi in N-limited soils, we need to measure the ^{15}N of the labile soil organic N as well. Finally, we need to understand ^{15}N discrimination processes in arbuscular mycorrhizal fungi. Does the similar ^{15}N content of non-mycorrhizal and arbuscular mycorrhizal plants indicate that arbuscular mycorrhizal fungi are not important to plant N supply in our system, or just that the different N transfer process in arbuscular mycorrhizal fungi (Bago et al. 2001) reduces the opportunities for discrimination?

For this arctic site, we conclude that the model relating ^{15}N measurements of ectomycorrhizal and ericoid mycorrhizal plants to soil and fungi produces an internally consistent mass balance of ^{15}N that accounts for ^{15}N-depleted plants and ^{15}N-enriched fungi. The results imply that the key fractionation against ^{15}N takes place inside mycorrhizal hyphae when amino acids are synthesized for transfer to the plant, and that the same type of fractionation during synthesis takes place in both ectomycorrhizal and ericoid mycorrhizal fungi.

This quantification of the N cycle, based upon natural abundance values of ^{15}N and the measured plant production data, is ecologically reasonable for this arctic site. Mycorrhizal fungi supply most of the N to mycorrhizal plants while using a significant amount of photosynthate.

In N-poor boreal and temperate ecosystems, the relative distribution of δ^{15}N values is similar to that in the arctic (Högberg 1997). Therefore, we suggest that N cycling through mycorrhizal fungi can explain many isotopic observations in a variety of systems.

1) In culture, the δ^{15}N in pine seedlings declined with increasing biomass of mycorrhizal fungi because at higher fungal colonization more system N was sequestered as ^{15}N-enriched fungal tissue (Hobbie and Colpaert 2003).

2) The δ^{15}N of needles of Sitka spruce decreased in forests as N availability declined over time (Hobbie et al. 1999). As N limitation increased, mycorrhizal fungi provided a greater percentage of plant N or sequestered a higher percentage of system N as ^{15}N-enriched fungal tissue.

3) The δ^{15}N of plants decreased with doubled CO_2 concentrations in Free Air CO_2 Enrichment experiments (BassiriRad et al. 2003). The doubling probably increased sugar supply to mycorrhizal fungi and increased the retention of ^{15}N-enriched N by these fungi. Because an isotopic mass balance must be preserved, the N transferred to the plant was therefore increasingly depleted in ^{15}N.

Conclusions

The method of using the natural abundance of ^{15}N to quantify the contribution of N from mycorrhizal fungi to host plants produced ecologically reasonable data and explained the depletion of ^{15}N in plants and the enrichment of ^{15}N in mycorrhizal fungi. Because the key fractionation step very likely acts in all ectomycorrhizal and ericoid mycorrhizal fungi, the method of quantification of the N transfer should work in all N-limited forests. The paradigm of N cycling in soils being driven by the enzymatic breakdown of N-containing organic molecules should include the paramount importance of mycorrhizal fungi in transferring organic molecules containing N from soils to their symbiotic plant partners.

Acknowledgments

We acknowledge with gratitude the ideas and expertise provided by Gary Laursen, mycologist at the University of Alaska Museum, Peter Ray of the University of Alaska Fairbanks, and James McClelland, Jerry Melillo, Marshall Otter, Bruce Peterson, Ed Rastetter, and Gaius Shaver of the Ecosystems Center. We thank Yuriko Yano of the Ecosystems Center for extracting and preparing inorganic N from resin bags for isotope analysis, and Robert McKane of the USEPA, Corvallis, for ^{15}N data for soil and plants. David Robinson of the University of Aberdeen provided helpful comments during review. This research was funded by National Science Foundation grants to J. E. Hobbie (DEB-9810222, OPP-9911278) and E. A. Hobbie (DEB-0235727).

Literature Cited

Bago, B., P. Pfeffer, and Y. Shachar-Hill. 2001. Could the urea cycle be translocating nitrogen in the arbuscular mycorrhizal symbiosis? New Phytologist **149**:4–8.

BassiriRad, H., J. V. H. Constable, J. Lussenhop, B. A. Kimball, R. J. Norby, W. C. Oechel, P. B. Reich, W. H. Schlesinger, S. Zitzer, and H. L. Sehtiya. 2003. Widespread foliage δ^{15}N depletion under elevated CO_2: inference for the nitrogen cycle. Global Change Biology **9**:1582–1590.

Chapin, F. S., III., L. Moilanen, and K. Kielland. 1993. Preferential use of organic nitrogen for growth by a non-mycorrhizal arctic sedge. Nature **361**:150–154.

Ek, H. 1997. The influence of nitrogen fertilization on the carbon economy of *Paxillus involutus* in ectomycorrhizal association with *Betula pendula*. New Phytologist **135**:133–142.

Emmerton, K. S., T. V. Callaghan, H. E. Jones, J. R. Leake, A. Michelsen, and D. J. Read. 2001. Assimilation and isotopic fractionation of nitrogen by mycorrhizal fungi. New Phytologist **151**:503–511.

Fogel, M. L., and L. A. Cifuentes. 1993. Isotope fractionation during primary production. Pages 73–100 *in* M. H. Engel and S. A. Macko, editors. Organic geochemistry: principles and applications. Plenum, New York, New York, USA.

Fogel, R., and G. Hunt. 1979. Fungal and arboreal biomass in a western Douglas-fir ecosystem: distribution patterns and turnover. Canadian Journal of Forest Research **9**:245–256.

Frank, A. B. 1894. Die Bedeutung der Mykorrhiza-pilze für die gemeine Kiefer. Forstwissenschaftliches Centralblatt **16**:185–190.

Giblin, A. E., J. A. Laundre, K. J. Nadelhoffer, and G. R. Shaver. 1994. Measuring nutrient availability in arctic soils using ion exchange resins: a field test. Soil Science Society of America Journal **58**:1154–1162.

Henn, M. R., and I. H. Chapela. 2004. Isotopic fractionation during ammonium assimilation by basidiomycete fungi and its implications for natural nitrogen isotope patterns. New Phytologist **162**:771–781.

Hobbie, E. A. 2006. Carbon allocation to ectomycorrhizal fungi correlates with total belowground allocation in culture studies. Ecology **87**:563–569.

Hobbie, E. A., and J. V. Colpaert. 2003. Nitrogen availability and colonization by mycorrhizal fungi correlate with nitrogen isotope patterns in plants. New Phytologist **157**:115–126.
Hobbie, E. A., S. A. Macko, and H. H. Shugart. 1999. Insights into nitrogen and carbon dynamics of ectomycorrhizal and saprotrophic fungi from isotopic evidence. Oecologia **118**: 353–360.
Hobbie, E. A., S. A. Macko, and M. Williams. 2000. Correlations between foliar $\delta^{15}N$ and nitrogen concentrations may indicate plant–mycorrhizal interactions. Oecologia **122**: 273–283.
Hobbie, E. A., D. M. Olszyk, P. T. Rygiewicz, M. G. Johnson, and D. T. Tingey. 2001. Foliar nitrogen concentrations and natural abundance of ^{15}N suggest nitrogen allocation patterns of Douglas-fir and mycorrhizal fungi during development in elevated carbon dioxide concentration and temperature. Tree Physiology **21**:1113–1122.
Högberg, P. 1990. ^{15}N natural abundance as a possible marker of the ectomycorrhizal habit of trees in mixed African woodlands. New Phytologist **115**:483–486.
Högberg, P. 1997. ^{15}N natural abundance in soil–plant systems. New Phytologist **137**:179–203.
Holmes, R. M., J. McClelland, D. M. Sigman, B. Fry, and B. J. Peterson. 1998. Measuring ^{15}N-NH_4 in marine, estuarine, and fresh waters: an adaptation of the ammonia diffusion method for samples with low ammonium concentrations. Marine Chemistry **60**:235–243.
Jones, D. L., J. R. Healey, V. B. Willett, J. F. Farrar, and A. Hodge. 2005. Dissolved organic nitrogen uptake by plants—an important N uptake pathway? Soil Biology and Biochemistry **37**:413–427.
Lilleskov, E. A., E. A. Hobbie, and T. J. Fahey. 2002. Ectomycorrhizal fungal taxa differing in response to nitrogen deposition also differ in pure culture organic nitrogen use and natural abundance of nitrogen isotopes. New Phytologist **154**:219–231.
Lodge, D. J. 1987. Nutrient concentrations, percentage moisture and density of field-collected fungal mycelia. Soil Biology and Biochemistry **19**:727–733.
Macko, S. A., M. L. Fogel Estep, M. H. Engle, and P. E. Hare. 1986. Kinetic fractionation of stable nitrogen isotopes during amino acid transamination. Geochemica et Cosmochemica Acta **50**:2143–2146.
McKane, R. B., L. C. Johnson, G. R. Shaver, K. J. Nadelhoffer, E. B. Rastetter, B. Fry, A. E. Giblin, K. Kielland, B. L. Kwiatkowski, and J. A. Laundre. 2002. Resource-based niches provide a basis for plant species diversity and dominance in arctic tundra. Nature **415**:68–71.
Nadelhoffer, K. J., L. Johnson, J. Laundre, A. E. Giblin, and G. R. Shaver. 2002. Fine root production and nutrient content in wet and moist arctic tundras as influenced by chronic fertilization. Plant and Soil **242**:107–113.
Nadelhoffer, K., G. Shaver, B. Fry, A. Giblin, L. Johnson, and R. B. McKane. 1996. ^{15}N natural abundances and N use by tundra plants. Oecologia **107**:386–394.
Näsholm, T. 1994. Removal of nitrogen during needle senescence in Scots pine (*Pinus sylvestris* L.). Oecologia **99**: 290–296.
Peterson, B., B. Fry, L. Deegan, and A. Hershey. 1993. The trophic significance of epilithic algal production in a fertilized tundra river ecosystem. Limnology and Oceanography **38**: 872–878.
Read, D. J. 1991. Mycorrhizas in ecosystems. Experientia **47**: 376–391.
Read, D. J., and J. Perez-Moreno. 2003. Mycorrhizas and nutrient cycling in ecosystems—a journey towards relevance? New Phytologist **157**:475–492.
Robinson, D., L. L. Handley, and C. M. Scrimgeour. 1998. A theory for $^{15}N/^{14}N$ fractionation in nitrate-grown vascular plants. Planta **205**:397–406.
Rühling, Å., and G. Tyler. 1991. Effects of simulated nitrogen deposition to the forest floor on the macrofungal flora of a beech forest. Ambio **20**:261–263.
Schimel, J. P., and J. Bennett. 2004. Nitrogen mineralization: challenges of a changing paradigm. Ecology **85**:591–602.
Schlesinger, W. H. 1991. Biogeochemistry: an analysis of global change. Academic Press, San Diego, California, USA.
Schmidt, S., and G. R. Stewart. 1997. Waterlogging and fire impacts on nitrogen availability and utilization in a subtropical wet heathland (wallum). Plant Cell and Environment **20**:1231–1241.
Shaver, G. R., S. Bret-Harte, M. H. Jones, J. Johnstone, L. Gough, J. Laundre, and F. S. Chapin, III. 2001. Species composition interacts with fertilizer to control long-term change in tundra productivity. Ecology **82**:3163–3181.
Shaver, G. R., and F. S. Chapin, III. 1991. Production: biomass relationships and element cycling in contrasting arctic vegetation types. Ecological Monographs **61**:1–31.
Sigman, D. M., M. A. Altabet, R. Michener, D. C. McCorkle, B. Fry, and R. Holmes. 1997. Natural abundance-level measurement of nitrogen isotopic composition of oceanic nitrate: an adaptation of the ammonia diffusion method. Marine Chemistry **57**:227–242.
Smith, S. E., and D. J. Read. 1997. Mycorrhizal symbiosis, 2nd Edition. Academic Press, New York, New York, USA.
Smith, S. E., and F. A. Smith. 1990. Structure and function of the interfaces in biotrophic symbioses as they relate to nutrient transport. New Phytologist **114**:1–38.
Taylor, A. F. S., L. Högbom, M. Högberg, A. J. E. Lyon, T. Näsholm, and P. Högberg. 1997. Natural ^{15}N abundance in fruit bodies of ectomycorrhizal fungi from boreal forests. New Phytologist **136**:713–720.
Vogt, K. A., C. Grier, C. E. Meier, and R. L. Edmonds. 1982. Mycorrhizal role in net primary production and nutrient cycling in *Abies amabilis* ecosystems in western Washington. Ecology **63**:370–380.
Wallenda, T., and I. Kottke. 1998. Nitrogen deposition and ectomycorrhizas. New Phytologist **139**:169–187.
Wallenda, T., C. Stober, L. Högbom, H. Schinkel, E. George, P. Högberg, and D. J. Read. 2000. Nitrogen uptake processes in roots and mycorrhizas. Pages 122–143 *in* E.-D. Schultz, editor. Carbon and nitrogen cycling in European forest ecosystems. Ecological Studies 142. Springer-Verlag, Berlin, Germany.
Werner, R. A., and H. L. Schmidt. 2002. The in vivo nitrogen isotope discrimination among organic plant compounds. Phytochemistry **61**:465–484.