Oecologia (1999) 118:353–360

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Insights into nitrogen and carbon dynamics of ectomycorrhizal and saprotrophic fungi from isotopic evidence

Received: 7 January 1998 / Accepted: 9 November 1998

Abstract The successful use of natural abundances of carbon (C) and nitrogen (N) isotopes in the study of ecosystem dynamics suggests that isotopic measurements could yield new insights into the role of fungi in nitrogen and carbon cycling. Sporocarps of mycorrhizal and saprotrophic fungi, vegetation, and soils were collected in young, deciduous-dominated sites and older, coniferous-dominated sites along a successional sequence at Glacier Bay National Park, Alaska. Mycorrhizal fungi had consistently higher $\delta^{15}N$ and lower $\delta^{13}C$ values than saprotrophic fungi. Foliar $\delta^{13}C$ values were always isotopically depleted relative to both fungal types. Foliar $\delta^{15}N$ values were usually, but not always, more depleted than those in saprotrophic fungi, and were consistently more depleted than in mycorrhizal fungi. We hypothesize that an apparent isotopic fractionation by mycorrhizal fungi during the transfer of nitrogen to plants may be attributed to enzymatic reactions within the fungi producing isotopically depleted amino acids, which are subsequently passed on to plant symbionts. An increasing difference between soil mineral nitrogen $\delta^{15}N$ and foliar $\delta^{15}N$ in later succession might therefore be a consequence of greater reliance on mycorrhizal symbionts for nitrogen supply under nitrogen-limited conditions. Carbon signatures of mycorrhizal fungi may be more enriched than those of foliage because the fungi use isotopically enriched photosynthate such as simple sugars, in contrast to the mixture of compounds present in leaves. In addition, some ^{13}C fractionation may occur during transport processes from leaves to roots, and during fungal chitin biosynthesis. Stable isotopes have the potential to help clarify the role of fungi in ecosystem processes.

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Key words Nitrogen dynamics · Nitrogen isotope ratio · Carbon isotope ratio · Mycorrhizal fungi · Succession

Introduction

Life history strategies of mycorrhizal and saprotrophic fungi are distinguished primarily by how they obtain their energy. Mycorrhizal fungi colonize the roots of plants in a mutualistic symbiosis, providing water and nutrients to the plant and in return receiving photosynthates (Smith and Read 1996), whereas saprotrophic fungi obtain their energy and carbon directly by decomposing organic matter (Swift 1982).

Mycorrhizal fungi are an integral component of the carbon and nitrogen dynamics of forest ecosystems. Despite the difficulty of studying mycorrhizal processes, numerous investigations have shown that a large fraction of net primary production is often shunted belowground to fine-root growth and to maintaining mycorrhizal symbionts (Waring and Schlesinger 1985; Finlay and Söderström 1992). Read (1992) suggested that the ecology of mycorrhizal fungi is tightly coupled to their functioning in the acquisition of nitrogen and phosphorus. The ectomycorrhizal fungi dominant in temperate and boreal systems are thought to be particularly important in nitrogen nutrition (Read 1992).

In forested ecosystems, saprotrophic fungi are the most important decomposers. Their ubiquitous role in decomposition arises largely from their ability to degrade efficiently the structural polymer lignin (Dix and Webster 1995), which represents from 10 to 40% of woody plant tissues (Aber and Melillo 1991). After the lignin has been degraded, decomposition of the residue by other microbial fauna is enhanced (Kirk and Fenn 1982).

Stable isotope analysis is a potentially useful tool for analyzing the function of mycorrhizal and saprotrophic fungi because isotopic variations in plants, soils, and fungi reflect the net result of mechanisms of resource

acquisition, loss, and internal cycling. Because isotopic values are the results of many interacting processes, they can be difficult to interpret definitively (Handley and Scrimgeour 1997). However, isotopic patterns often point to particular mechanisms that can plausibly explain the data and can therefore guide us towards experimental tests of different hypotheses.

Previous studies have found significant differences in $\delta^{15}N$ among plants of differing mycorrhizal habit in arctic (Michelsen et al. 1998), subarctic (Michelsen et al. 1996), and tropical (Högberg 1990) ecosystems. Differences between saprotrophic and mycorrhizal strategies for resource acquisition suggest that stable isotope analysis could illuminate the mechanisms by which these two fungal classes interact with the environment. For example, Gleixner et al. (1993) utilized this approach to evaluate mechanisms of wood decomposition and carbon acquisition in saprotrophic fungi.

Sampling and identifying hyphae in field studies of fungal processes is problematic. For this reason, it would be convenient if isotopic measurements in aboveground fruiting bodies reflected isotopic signatures of belowground fungal hyphae. Definitive culture studies comparing $\delta^{15}N$ of hyphae and fruiting bodies in several fungal species have yet to be undertaken, although several studies suggest that this assumption may be valid. Handley et al. (1996) measured $\delta^{15}N$ of caps, stipes (stalks), and hyphae of the saprotrophic fungus *Agrocybe*, and reported all three tissues as within 1‰. In a recent review article, Högberg (1997) concluded that the $\delta^{15}N$ of mycorrhizal fruiting bodies in the field was similar to that of fungal tissue surrounding roots, based on isotopic results in ectomycorrhizal sheaths (Högberg et al. 1996) and fruiting bodies (Taylor et al. 1997). These studies suggest that isotopic signatures in easily measured fruiting bodies should be similar to hyphae, and therefore useful in the study of belowground carbon and nitrogen dynamics.

Large changes in carbon and nitrogen dynamics have been observed during forest succession. An area that has been extensively studied as a model system for successional processes is the primary successional sequence at Glacier Bay, Alaska, where glaciers have retreated approximately 100 km over ~250 years (Cooper 1923; Bormann and Sidle 1990; Chapin et al. 1994). The Glacier Bay system is characterized by rapid nitrogen cycling during the early successional dominance of the N-fixing tree *Alnus sinuata* (Regel) Rydb. (Sitka alder), and by slow nitrogen cycling during the late successional dominance of *Picea sitchensis* (Bong.) Carr. (Sitka spruce) (Chapin et al. 1994). Negative correlations have been observed between nitrogen availability and the degree of mycorrhizal colonization and development (Marx et al. 1977; Wallander and Nylund 1991). It is possible that in late succession at Glacier Bay, under conditions of slow N cycling, mycorrhizal fungi might be more important in supplying N to plants than under the conditions of rapid N cycling that prevail in early succession. In this ecosystem with soils of known age and successional stage, insights into the mechanistic role of fungi in C and N dynamics during succession are possible that would otherwise be obscured.

In this study, carbon and nitrogen isotope values were determined in mycorrhizal and saprotrophic fungi at sites along a successional sequence, where N dynamics in soils and vegetation had been studied previously (Hobbie et al. 1998). We addressed the following questions:

(1) Can nitrogen isotope measurements be used to distinguish between mycorrhizal and saprotrophic acquisition of nitrogen?
(2) Can carbon isotope measurements be used to distinguish between the different sources of mycorrhizal and saprotrophic carbon?
(3) Can nitrogen isotope measurements be used to decide how changing nitrogen dynamics during succession affect interactions between mycorrhizal fungi and host plants?

Materials and methods

Glacier Bay is located in southeast Alaska (59°N, 136°W), at the northern end of the temperate rainforest that stretches from Oregon to southern Alaska. Precipitation averages 183 cm yearly, with 60% falling from August to November. It has a cool maritime climate, with average temperatures of 12°C and −7°C for summer and winter, respectively. Under the early successional dominance of alder, soil O horizons are quite thin (~5 cm), whereas under the later successional dominance of spruce they increase to about 15 cm in thickness (Fastie 1994).

Five sites were sampled in summer 1995, representing the two major successional stages and a range of site ages (Table 1, Fig. 1). Sites 1 and 4 had been previously investigated by Fastie (1995) for successional history, and sites 2 and 5 were stem-mapped by Weishampel (1994) in a modeling study of forest dynamics.

At each site, a minimum of 15 sporocarps were collected for isotopic analyses. They were identified to genus and subsequently designated as mycorrhizal or saprotrophic, based on life history traits. In addition, foliage samples of alder and spruce were collected at each site. Seven soil cores of the O horizon were taken at each site (diameter = 4.2 cm) and all live roots less than 3 mm diameter were collected for analysis. Organic and mineral soil horizons at each site were also sampled ($n = 5$). Some wood cores from dominant spruce were also obtained.

All samples were oven-dried at 40–45°C. Vegetation samples were ground in a Wiley mill to pass through a 40 mesh screen prior

Table 1 Site descriptions, showing age, dominant vegetation, C/N of organic soil, and stem basal area of trees (at 137 cm height) at five sites

Site no, name (approximate number of years since deglaciation)	Dominant vegetation	C/N of organic soil	Basal area of trees ($m^2\ ha^{-1}$)
(1) Goose Cove (55)	*Alnus*	16.7	30.2
(2) Adams Inlet (90)	*Alnus*	17.2	27.9
(3) Muir Point (110)	*Alnus*, *Picea*	16.7	30.8
(4) Beartrack Cove (165)	*Picea*	29.0	60.6
(5) Bartlett Cove (225)	*Picea*	30.9	78.5

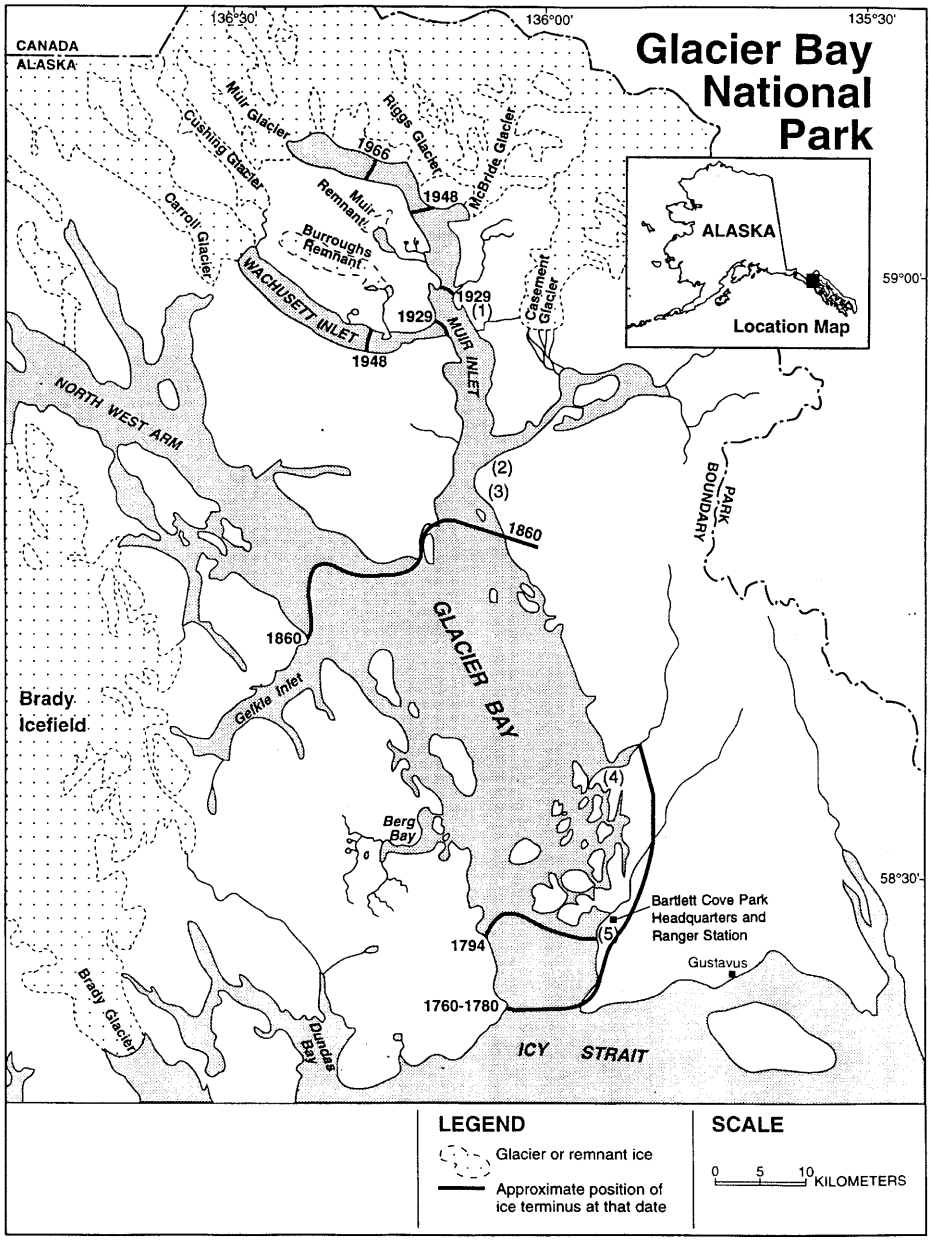

Fig. 1 Map of Glacier Bay National Park, showing dates of glacial retreat and site locations. Sites shown from youngest to oldest are *(1)* Goose Cove, *(2)* Adams Inlet, *(3)* Muir Point, *(4)* Beartrack Cove, *(5)* Bartlett Cove. Modified from Milner (1997), with permission of the publishers

to analysis. Soil horizons were separated from litter layers, passed through a 1-mm sieve and ground with a mortar and pestle.

Ammonium and nitrate in organic soil were analyzed for $\delta^{15}N$ according to the procedure of Velinsky et al. (1989). Only sites 1 and 2 were analyzed for nitrate ^{15}N. Soil samples of 75 g were extracted with 200 ml of a 1 N KCl solution and centrifuged; 5 ml of 5 N NaOH was then added to the supernatant, and the resulting ammonia was steam-distilled into an acidic solution. The pH of this solution was then adjusted to 5.5–6.0 and the ammonium bound by ion exchange to a zeolite molecular sieve (Union Carbide Ionsiv W-85). The nitrate in the remaining sample was reduced to ammonium with Devarda's alloy and a second steam distillation was carried out as before.

Samples for %C, %N, $\delta^{13}C$, and $\delta^{15}N$ were primarily determined in a continuous flow (CF) system on a Carlo Erba elemental analyzer coupled to a Micromass Optima mass spectrometer (Fisons/VG/Micromass, Manchester, UK). Flash combustion of samples under oxygen was followed by reduction of the gases on a Cu column at 550°C, chromatographic separation of the CO_2 and N_2, and subsequent isotopic analyses of the separate CO_2 and N_2 pulses. The large sample size necessary for isotopic analysis of mineral N, mineral horizon soil, and spruce wood required preparation using the Dumas sealed-tube method (Macko 1981). For the Dumas method, CO_2 and N_2 were first cryogenically separated and nitrogen and carbon stable isotope ratios subsequently determined on a VG Prism Series II isotope ratio mass spectrometer (IRMS) (Fisons/VG/Micromass).

Reproducibility of measurements on duplicate samples was $\pm 0.3‰$ using the CF-IRMS and $\pm 0.1‰$ using the Dumas sealed-tube method. The internal standards for CF-IRMS samples were acetanilide and orchard leaves (NIST 1572), calibrated against CO_2 and N_2 on the VG Prism Series II IRMS. Stable isotope abundances are reported as:

$$\delta^{15}N \text{ or } \delta^{13}C = (R_{sample}/R_{standard^{-1}}) \times 1000 \quad (1)$$

where $R = {}^{15}N/{}^{14}N$ or ${}^{13}C/{}^{12}C$ of either the sample or the reference standard (atmospheric N_2 for nitrogen, PeeDee belemnite for carbon). 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.

The statistical package Statview (Abacus Concepts, Berkeley, Calif.) was used to test for relationships among the data. Isotopic results for the different pools sampled were compared using a one-way, two-tailed ANOVA. Separation of means was performed using a Scheffé *F*-test at the 0.05 significance level. Means are reported ±1 SE. Correlation coefficients between isotopic results and site age were analyzed for a significant (0.05) difference from zero using Fisher's *r* to *z* transformation.

Results

It is helpful to examine the isotopic data from two perspectives. First, the broad patterns among individual ecosystem compartments are shown in the $\delta^{15}N$ and $\delta^{13}C$ averages for ecosystem pools across all five sites. Second, subsequent comparisons between the isotopic data at individual sites are then used to evaluate overall differences in ecosystem interactions between early and late succession. Patterns in isotopic composition among sites reflect how the interactions among ecosystem pools change as succession proceeds from rapid N cycling in early succession to slow N cycling in late succession.

Stable nitrogen isotopes

When pooled across all sites, large differences were observed among the various ecosystem pools and fungal taxa sampled, with the highest $\delta^{15}N$ values in mineral horizon soil and mycorrhizal fungi (Table 2), and the lowest in spruce foliage. Although ammonium $\delta^{15}N$ values from soil extracts averaged somewhat lower than $\delta^{15}N$ of the organic soil (Table 2), they were not statistically different ($P = 0.11$). Mycorrhizal fungi $\delta^{15}N$ values were clearly higher than those for saprotrophic fungi ($P < 0.0001$). Large differences in $\delta^{15}N$ were observed among different taxa of fungi, ranging from 8‰ for the mycorrhizal *Tricholoma*, to –5‰ for the saprotrophic *Fomitopsis* (Table 3). Within a given type of fungus (mycorrhizal or saprotrophic), the observed differences were smaller.

When compared by increasing site age (Figs. 2, 3), $\delta^{15}N$ of spruce foliage and fine roots generally declined during succession ($r^2 = 0.66$ and 0.67, respectively, $P < 0.0001$ for both), whereas $\delta^{15}N$ of ammonium in the soil, the N form preferred by spruce (Rygiewicz et al. 1984a,b), remained relatively constant ($r^2 = 0.17$, $P = 0.06$). Saprotrophic fungi also declined somewhat in $\delta^{15}N$ with increasing site age ($r^2 = 0.27$, $P = 0.003$), but mycorrhizal fungi did not ($r^2 = 0.03$) (Fig. 2). The $\delta^{15}N$ values for alder foliage and bulk soil pools did not vary greatly over succession (Fig. 3).

Carbon stable isotopes

Several distinct groupings were noted for carbon signatures among the taxa and ecosystem pools sampled (Table 2). Foliage, fine roots, and O horizon soils were

Table 2 Average nitrogen and carbon isotopic compositions at Glacier Bay for selected ecosystem pools. Means ± SE are given (with the number of samples across all five sites in parentheses). *Ammonium* signatures are from organic horizons at sites 1–5, *nitrate* signatures from organic horizons at sites 1 and 2. Values within a group followed by the same letter are not statistically different, based on Scheffé's post hoc comparison at $P = 0.05$

	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
Fungi		
Mycorrhizal fungi (67)	4.2 ± 0.4^{a}	-25.6 ± 0.2^{a}
Saprotrophic fungi (29)	-1.3 ± 0.5^{b}	-22.6 ± 0.2^{b}
Plant		
Alnus foliage (29)	-1.3 ± 0.2^{a}	-29.7 ± 0.3^{a}
Picea foliage (26)	-3.7 ± 0.6^{b}	-28.8 ± 0.4^{a}
Fine roots (31)	-2.0 ± 0.4^{ab}	-27.5 ± 0.2^{b}
Picea wood (13)	–	25.4 ± 1.1^{c}
Soil		
Mineral soil (11)	6.0 ± 2.5^{a}	-25.6 ± 0.6^{a}
Organic soil (25)	0.6 ± 0.3^{b}	-27.5 ± 0.2^{b}
Ammonium (21)	-0.7 ± 0.4^{b}	
Nitrate (6)	-0.1 ± 1.1^{b}	

Table 3 $\delta^{15}N$ and $\delta^{13}C$ for fungal taxa. Means ± SE are given (with the number of samples across all five sites in parentheses). Values within a group followed by the same letter are not statistically different, based on Scheffé's post hoc comparison at $P = 0.05$

	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
Mycorrhizal		
Cortinarius (23)	4.3 ± 0.9^{a}	-25.6 ± 0.2^{a}
Hygrophorus (8)	3.0 ± 0.7^{a}	-25.3 ± 0.3^{a}
Russula (16)	2.6 ± 0.6^{a}	-25.8 ± 0.2^{a}
Tricholoma (4)	8.0 ± 0.3^{a}	-24.8 ± 0.1^{a}
Saprophytic		
Coprinus (3)	0.6 ± 0.2^{a}	-23.1 ± 0.2^{a}
Fomitopsis (3)	-5.2 ± 0.9^{b}	-23.5 ± 0.0^{a}
Mycena (5)	-1.0 ± 1.1^{ab}	-22.3 ± 0.5^{a}
Polyporus (8)	-2.0 ± 0.8^{ab}	-22.5 ± 0.2^{a}

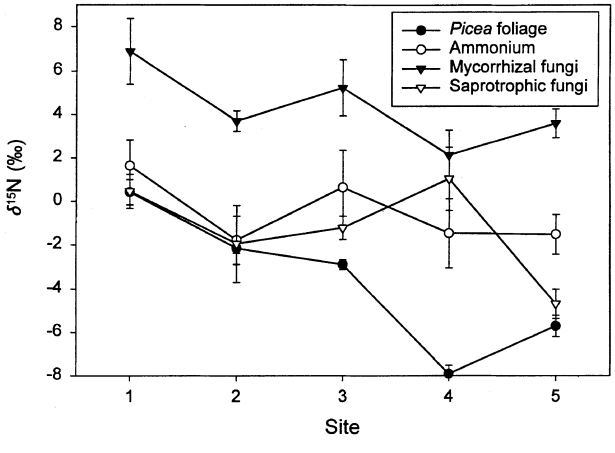

Fig. 2 $\delta^{15}N$ of *Picea* foliage, mycorrhizal fungi, saprotrophic fungi, and ammonium by site. *Error bars* represent the SE of the mean. Sites arranged from youngest to oldest

most depleted (−27.5‰ to −30‰), followed by wood, mineral horizon soils, and mycorrhizal fungi (~−25.5‰). Saprotrophic fungi were highly enriched relative to all other groups (~−23‰). In contrast to $\delta^{15}N$ results, mycorrhizal fungi were quite depleted in ^{13}C relative to saprotrophic fungi ($P < 0.0001$) (Fig. 4). Similarly classified taxa (mycorrhizal or saprotrophic) resembled each other in isotopic composition (Table 3).

Discussion

Nitrogen dynamics

One striking result of this study is the highly enriched $\delta^{15}N$ signature of mycorrhizal fungi relative to foliage throughout Glacier Bay, regardless of site age (Table 2). Studies of $\delta^{15}N$ of fruiting bodies elsewhere in Alaska (E. Lilleskov, personal communication), Japan and Malaysia (A. Kohzu, personal communication), Europe (Gebauer and Dietrich 1993; Taylor et al. 1997), Scotland and Australia (Handley et al. 1996) strongly suggest that this is a general pattern for mycorrhizal fungi. We

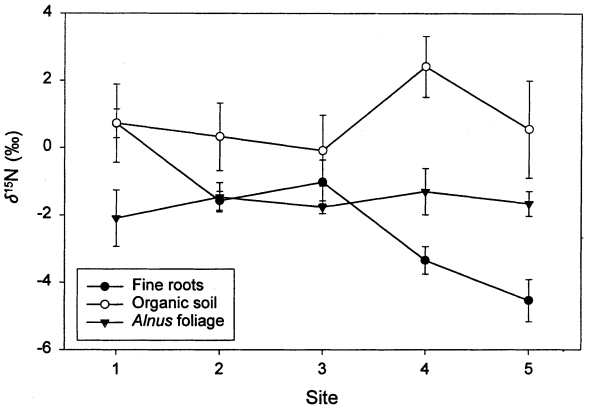

Fig. 3 $\delta^{15}N$ of *Alnus* foliage, fine roots, and organic horizon soil by site, arranged as in Fig. 2

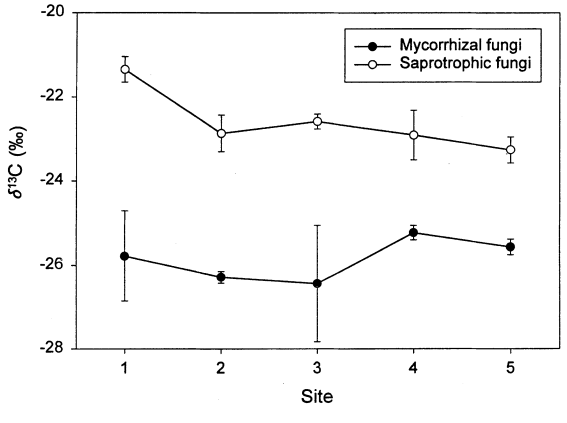

Fig. 4 $\delta^{13}C$ of mycorrhizal and saprotrophic fungi by site, arranged as in Fig. 2

hypothesize that biosynthetic processes within the mycorrhizal mycelium lead to the production of amino acids isotopically depleted relative to source N. For example, the transamination of glutamic acid to aspartic acid resulted in a 9‰ fractionation (Macko et al. 1986). If similar isotopically discriminating processes occur in fungi and if isotopically depleted amino acids are then passed on to the host plants, foliage would become isotopically depleted, and the mycorrhizal fungi would become isotopically enriched.

A logical test of this hypothesis would be compound-specific ^{15}N isotopic analysis of amino acids in mycorrhizal fungi. In fact, because N transfer in mycorrhizal fungi appears to operate via a glutamine-glutamate shuttle (Smith and Smith 1990), it would be desirable to know the $\delta^{15}N$ values of both the amino-N and amido-N of glutamine. A further test would be measuring the $\delta^{15}N$ of saprotrophic protein and chitin, as one might expect smaller differences between the two classes of compounds in saprotrophic fungi than were observed in mycorrhizal fungi (Taylor et al. 1997). Macko et al. (1990) reported that chitin was 3‰ depleted in $\delta^{15}N$ relative to whole-body N in the saprotrophic fungus *Agaricus*, whereas Taylor et al. (1997) recorded a 9‰ depletion in $\delta^{15}N$ between chitin and whole-body N in three species of ectomycorrhizal fungi. Handley et al. (1996) suggested that loss of isotopically depleted ammonia from fruiting bodies could also explain the high $\delta^{15}N$ values relative to plants. However, this will not explain why even young fruiting bodies are isotopically enriched relative to plants (Högberg 1997), why saprotrophic fruiting bodies were similar in $\delta^{15}N$ to plant foliage but mycorrhizal fungi were enriched (Handley et al. 1996), or why, in this study, saprotrophic and mycorrhizal fungi differed so dramatically in $\delta^{15}N$.

Ectomycorrhizal plants such as spruce are particularly reliant on mycorrhizal fungi for nitrogen nutrition (Read 1992). Both spruce and mycorrhizal fungi preferentially utilize ammonium (Ingestad 1979; Rygiewicz et al. 1984a,b; Smith and Read 1996), which is the dominant mineral form of nitrogen at Glacier Bay (Bormann and Sidle 1990). Rygiewicz et al. (1984a,b) measured ammonium uptake rates as 3.2 and 4.6 times faster than nitrate uptake in nonmycorrhizal and mycorrhizal sitka spruce, respectively. In the absence of fractionating processes, one would expect that ammonium $\delta^{15}N$ should be similar to either spruce foliage or mycorrhizal fungi $\delta^{15}N$. However, the actual pattern is for $\delta^{15}N$ of mycorrhizal fungi to be consistently higher than ammonium and for spruce foliage to be consistently lower (Fig. 2, Table 2). This again suggests that trees receive isotopically light N when they obtain nitrogen from their fungal symbionts. The N remaining in the symbionts, comprising the mycelium and the sporocarps, becomes isotopically heavy relative to the presumed source, the mineral N in the soil.

This mechanism can also explain the $\delta^{15}N$ trends observed for spruce foliage during succession (Fig. 2). In early succession (sites 1 and 2), with rapid cycling of

nitrogen, we would suggest that nitrogen is readily available in the soil for direct plant uptake without passing through mycorrhizal fungi, and therefore spruce foliage is isotopically similar to ammonium. In later succession (sites 4 and 5), with slower cycling of N, we would suggest that spruce is highly dependent on its mycorrhizal symbionts for its N supply, N is isotopically fractionated during its passage through mycorrhizal fungi, and therefore spruce foliage is isotopically depleted relative to ammonium (Fig. 5). Greater use of isotopically depleted nitrate by spruce in late succession than in early succession is not a tenable explanation for the decline in spruce $\delta^{15}N$ values, as soil nitrate concentrations in late succession at Glacier Bay are extremely low (Bormann and Sidle 1990).

Alternatively, mycorrhizal fungi and tree roots may draw from different soil N pools, with mycorrhizal fungi tapping an unknown, isotopically heavy organic N pool while trees acquire isotopically light ammonium and nitrate. However, the general pattern of enriched $\delta^{15}N$ signatures of mineral N when compared to foliage weighs heavily against this explanation. Measurements on the $\delta^{15}N$ of labile organic N compounds in the soil such as amino acids are needed to address this issue. The uptake of organic N by mycorrhizal fungi and vegetation could also be assessed through application in the field of ^{13}C- and ^{15}N-labeled amino acids (Näsholm et al. 1998).

Although not addressed in this study, variations in rooting depth among species can result in differences in the spatial pattern of N uptake (McKane et al. 1990), and could lead to isotopic differences among plant species. In the young soils of Glacier Bay, few roots are observed in the mineral soil, and therefore most N uptake probably occurs in the organic horizon and litter layer. This would minimize the influence of rooting depth differences on the isotopic composition.

Garten (1993) suggested that the amount by which soil $\delta^{15}N$ was greater than foliar $\delta^{15}N$ was an indicator of N saturation, with smaller differences indicating greater N saturation. More recently, Högberg et al. (1996) proposed that the difference between soils and roots could be a better indicator. We suggest that these differences primarily reflect the degree to which nitrogen uptake is mediated through mycorrhizal fungi, with relatively greater isotopic differences between plants and soil corresponding to a greater fungal role in N supply.

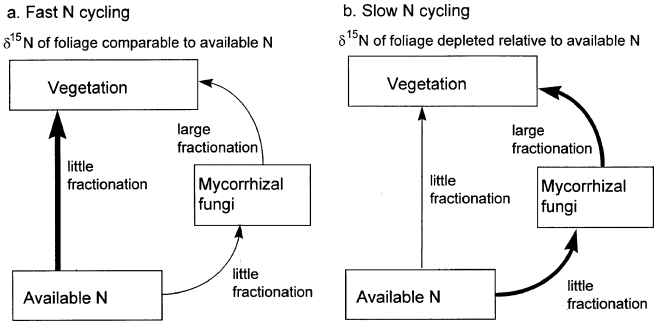

Fig. 5 Schematic diagram of hypothesized interactions among vegetation, soil available N, and mycorrhizal fungi under conditions of rapid N cycling (**a**), or slow N cycling (**b**)

Carbon dynamics

Some interesting implications arise from the patterns in $\delta^{13}C$ among soil pools, wood, foliage, and mycorrhizal and saprotrophic fungi. The very clear difference between mycorrhizal and saprotrophic fungi suggests that $\delta^{13}C$ measurements are a new and simple method for distinguishing mycorrhizal from saprotrophic fungi. This method even appears useful for distinguishing between mycorrhizal and saprotrophic life history strategies in closely related taxa (N. Weber, J. Trappe, E. Hobbie, unpublished data).

Some of these differences may result from isotopic fractionation during the synthesis of biochemicals, or from transport between different plant parts. For example, Gleixner et al. (1993) found a 2‰ enrichment during the formation of fungal chitin from wood cellulose. Wood cellulose is itself about 2‰ enriched relative to leaf cellulose, which should be isotopically similar to the simple sugars fixed during photosynthesis (Gleixner et al. 1993), and subsequently transported to mycorrhizal fungi via the roots. However, conflicting reports about isotopic differences among plant sugars, starches, and cellulose (Ehleringer 1991) indicate that more data about the $\delta^{13}C$ of potential source pools are needed to explain the cause of the clear isotopic differences between mycorrhizal and saprotrophic fungi.

The isotopic enrichment in ^{13}C of mycorrhizal fungi relative to plant foliage may arise from several causes. The large proportion of isotopically depleted lignins in foliage lowers the average isotopic signature of the bulk leaf. In contrast, mycorrhizal fungi receive primarily sugars from the host plant (Finlay and Söderström 1992). There may also be fractionation between the sugars fixed during photosynthesis and the sugars actually delivered to the mycorrhizal fungi, as root sugars were enriched about 2‰ compared to leaf sugars in one study of sugar beets (Gleixner et al. 1993). In the present study, the fine roots had an isotopic composition lower than mycorrhizal fungi and higher than foliage, which would suggest that both root-shoot and compound-specific fractionations are important. Compound-specific $\delta^{13}C$ analyses of plant and fungal sugars such as sucrose and trehalose would be useful to address these issues.

An alternative explanation for the enrichment of mycorrhizal fungi relative to plants is the respiration of isotopically light CO_2, as suggested by Boutton (1996). In general, heterotrophs are isotopically enriched 1‰ in ^{13}C relative to their diet (DeNiro and Epstein 1978). Several of the mechanisms discussed above may be operating simultaneously.

The isotopic enrichment in ^{13}C of saprotrophic fungi relative to wood is attributed primarily to a 2‰ enrichment during chitin formation (Gleixner et al. 1993), and secondarily to the acquisition of isotopically enriched cellulose during wood and litter degradation. Lignin, the other major constituent of wood, is always isotopically depleted relative to cellulose and the bulk wood (Benner et al. 1987). Although most wood-inhabiting fungi can degrade lignin, the isotopic evidence suggests to us that the actual incorporation of lignin degradation products into fungal tissues is probably not significant. Gleixner et al. (1993) did not report isotopic differences between white rot fungi that could degrade lignin and soft rot fungi that could not, which again suggests that both of these fungal types primarily incorporated breakdown products of cellulose.

The enrichment of $\delta^{13}C$ in mineral soil relative to the organic horizon has been observed in other studies (Nadelhoffer and Fry 1988). This isotopic enrichment has generally been attributed to discrimination against ^{13}C during decomposition and respiration. We believe that an additional mechanism that should be considered is the large mycorrhizal input of isotopically enriched carbon to soil below the litter layer. At the soil surface, carbon inputs are derived from isotopically light plant litter, whereas at greater soil depth, carbon inputs are comprised of root and mycorrhizal material, as well as inputs from higher in the soil profile.

Conclusions

We present isotopic evidence for possible differences between saprotrophic and mycorrhizal fungi in the processing of carbon and nitrogen. These differences should be very useful in providing an ecosystem perspective to the interactions among fungi, other organisms, and resources. In particular, the isotopic differences discussed reveal how mycorrhizal fungi influence plant N supply. It appears that the transfer of N from mycorrhizal fungi to plants is a fractionating process; enzymatic processes of amino acid biosynthesis within the fungal mycelia are suggested as one possible cause of this isotopic fractionation. The importance of mycorrhizal fungi to plant N supply appeared to increase as nitrogen became more limiting in later succession. Natural abundances of carbon and nitrogen isotopes could be a powerful tool in field studies to investigate the functioning of fungal taxa in C and N dynamics.

These results suggest that $\delta^{13}C$ analyses could be used to test for the relative contributions of soil organic matter and plant host carbon to mycorrhizal fungi. The mycorrhizal $\delta^{13}C$ signature should differ more from the signature of leaves as the saprotrophic capabilities increase. Studies of $\delta^{13}C$ have been useful in investigations of the incorporation of C_4 plant residues into soil organic matter in systems that have shifted from C_3 to C_4 plants (Balesdent and Mariotti 1996). Comparable studies could be used to clarify the relative contribution of litterfall and belowground inputs to the stable soil organic matter.

As discussed above, more detailed field and laboratory studies are necessary to examine the C and N fractionations that occur during N transfer from mycorrhizal fungi to plants, during C transfer from plants to mycorrhizal fungi, and during C uptake from soil organic matter and wood to mycorrhizal fungi and saprotrophs. These studies could reveal whether differences among taxa in isotopic signatures can be attributed to different strategies of resource acquisition or to different patterns of plant-fungal interactions.

Acknowledgements Ari Jumpponen, Erik Lilleskov, Paul Rygiewicz, Alison Hobbie, John Hobbie, and three anonymous reviewers gave helpful criticism that improved the manuscript. The fieldwork assistance of David Richardson and Mat Williams and the support of the staff of Glacier Bay National Park are gratefully acknowledged. This work was supported by NASA grant NAG5-2295 to Hank Shugart and several additional funding sources at the University of Virginia.

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