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Nitrogen isotope fractionation during nitrogen uptake by ectomycorrhizal and non-mycorrhizal *Pinus sylvestris*

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SUMMARY

An experiment was performed to find out whether ectomycorrhizal (ECM) fungi alter the nitrogen (N) isotope composition, $\delta^{15}N$, of N during the transport of N from the soil through the fungus into the plant. Non-mycorrhizal seedlings of *Pinus sylvestris* were compared with seedlings inoculated with either of three ECM fungi, *Paxillus involutus*, *Suillus bovinus* and *S. variegatus*. Plants were raised in sand in pots supplied with a nutrient solution with N given as either NH_4^+ or NO_3^-. Fractionation against ^{15}N was observed with both N sources; it decreased with increasing plant N uptake, and was larger when NH_4^+ was the source. At high ratios of $N_{uptake}/N_{supplied}$ there was no (NO_3^-), or little (NH_4^+), fractionation. There seemed to be no difference in fractionation between ECM and non-mycorrhizal plants, but fungal rhizomorphs were sometimes enriched in ^{15}N (up to 5‰ at most) relative to plant material; they were also enriched relative to the N source. However, this enrichment of the fungal material was calculated to cause only a marginal decrease (−0.1‰ in *P. involutus*) in $\delta^{15}N$ of the N passing from the substrate through the fungus to the host, which is explained by the small size of the fungal N pool relative to the total N of the plant, i.e. the high efficiency of transfer. We conclude that the relatively high ^{15}N abundance observed in ECM fungal species should be a function of fungal physiology in the ECM symbiosis, rather than a reflection of the isotopic signature of the N source(s) used. This experiment also shows that the $\delta^{15}N$ of plant N is a good approximation of $\delta^{15}N$ of the available N source(s), provided that N is limiting growth.

Key words: $\delta^{15}N$, ectomycorrhiza, nitrogen, stable isotopes, trees.

INTRODUCTION

The ratio between the two stable isotopes of N, ^{15}N and ^{14}N, differs between compartments of ecosystems because of isotope fractionations during the N cycle (Shearer & Kohl, 1986; Handley & Scrimgeour, 1997; Högberg, 1997). Fractionation against ^{15}N during N uptake is influenced by the balance between plant N uptake rate (demand) and the external N supply rate; in other words, there is strong evidence from studies of non-mycorrhizal plants that fractionation against ^{15}N becomes more strongly expressed at higher N supply rates (Hoch *et al.*, 1994) and that this fractionation occurs during the uptake of both NO_3^- (Mariotti *et al.*, 1982) and NH_4^+ (Yoneyama *et al.*, 1991). Under conditions of strong N limitation, a plant should take up virtually all of the N supplied, which should leave no possibility of a potential fractionation (Nadelhoffer & Fry, 1994; Evans *et al.*, 1996).

However, under natural conditions most plants are mycorrhizal and N from the soil is often taken up through the fungal symbionts (Smith & Read, 1997). Several studies report differences in ^{15}N natural abundance between co-existing plants with different types of mycorrhiza (Högberg, 1990; Michelsen *et al.*, 1996, 1998). Such differences might primarily reflect (i) the use of N sources with different isotopic signatures (organic N, NH_4^+, NO_3^-), (ii) that N is taken up from different soil horizons (Högberg *et al.*, 1996) or different sub-sites, (iii) differences in fractionation of N isotopes during uptake, or (iv) that fungi and plants differ in their N physiologies. Compartmentation and recycling of N, as well as losses of N, might also cause differences between

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species and plant parts in $\delta^{15}N$ as secondary effects after N uptake (Handley & Scrimgeour, 1997; Högberg, 1997). Of particular interest is the fact that sporocarps of ectomycorrhizal (ECM) fungi are often enriched in ^{15}N by 5–10‰ relative to their alleged host plants (Gebauer & Dietrich, 1993; Handley *et al.*, 1996; Taylor *et al.*, 1997) and that Högberg *et al.* (1996) found that the fungal sheaths in ECMs were enriched in ^{15}N by 2.4–6.4‰ relative to the remaining fine root cores from which they were stripped. The latter observation excludes (ii) above, but suggests two possibilities: (1) that ECM fungi use sources of N with a high $\delta^{15}N$ that are not used by or transferred to the host plants (Gebauer & Dietrich, 1993) or (2) that ECM fungi become enriched in ^{15}N, whereas the N passed on to the hosts becomes depleted in ^{15}N relative to source N.

The experiment reported here was conducted to determine whether the presence of the fungus in ECM symbiosis alters the isotopic signature of N during its transport from the soil to the plant. No published experiment has addressed this question directly, although significant differences in $\delta^{15}N$ have been reported between ECM and non-mycorrhizal pines (Bardin *et al.*, 1977) and between vesicular–arbuscular mycorrhizal (VAM) and non-mycorrhizal castor bean (Handley *et al.*, 1993). Those differences were small and the absence of total N isotope budgets made interpretation difficult (Högberg *et al.*, 1994); for example, data on $\delta^{15}N$ of fungal material were not presented. Our experiment was therefore designed to provide N isotope data on both plants and fungi.

MATERIALS AND METHODS

A factorial experiment with eight treatments was conducted, in which non-mycorrhizal or ECM *Pinus sylvestris* L. (using three different fungal symbionts) were exposed to either NH_4^+ or NO_3^- as the N source. There were 10 replicates of each treatment.

Seeds of *P. sylvestris* (provenance Harastorp, south Sweden) were surface-sterilized (10 min in H_2O_2, followed by rinsing three times in water for 5 min), and sown on a moist sand bed. After 2 wk, seedlings (6 cm in height, apical root length 6 cm) were planted in individual 0.5 l plastic pots filled with quartz sand (98.8% quartz, grain size 0.35–2.00 mm, with a mean size of 0.87 mm). In each pot in the inoculated ECM treatments, ten 0.3 cm^3 agar plugs with isolates of the fungi *Paxillus involutus* (Batsch) Fr., *Suillus bovinus* (L.) Kuntze or *S. variegatus* (Swartz ex Fr.) O. Kuntze were placed in the sand below the pine roots at planting. No fungal inoculum was introduced in the non-mycorrhizal treatment.

The pots were placed in a climate chamber (16 h daylength, photon flux density 500 μmol m^{-2} s^{-1} in the interval 400–700 nm at plant height, 22°C : 10°C day: night temperatures, 75% : 100% day : night rh). Twice a week the positions of the pots were shifted to avoid any influence of gradients within the chamber.

A nutrient solution (Table 1) with the proportions between macronutrients as recommended for pine by Ingestad (1979) was added twice a week at a volume of 100 ml per pot for the first 5 wk, and thereafter at 150 ml. Nitrogen was added as NH_4Cl or $NaNO_3$, and the amount of P in the ECM treatments was only one-third of the dose given in the non-mycorrhizal treatment, to account for the higher uptake capacity in the ECM plants. The pH of the nutrient solution was 6.0, but pH became approx. 2 units lower in the NH_4^+ treatment and 1.4 units higher in the NO_3^- treatment in the respective solutions draining from the pots.

Plants were harvested after 88 d. Shoots and roots were separated, and any sand adhering to roots was washed away with tap water; if necessary, sand was removed manually by forceps. The degree of ECM colonization was assessed visually under a dissecting microscope ($\times 40$). The degree of colonization was determined by the percentage of root tips colonized; the following eight classes were used: 0, 0–1%, 1–5%, 5–10%, 10–25%, 25–50%, 50–75% and 75–100%. Samples of fungal rhizomorphs were taken whenever found. The plant material was dried at 70°C for 24 h and ground to a fine powder in a ball mill. Pine seeds and isolates of the fungi from the agar plates were also dried and ground for further analyses.

Subsamples of shoots, roots, seeds, fungal isolates and whole samples of rhizomorphs, as well as samples of NH_4Cl and $NaNO_3$, were analysed for their natural abundances of %N and ^{15}N as described by Högberg *et al.* (1996). Results are expressed in $\delta^{15}N$ (‰) deviations from the standard atmospheric N_2:

$$\delta^{15}N(‰) = ((R_{sample}/R_{standard}) - 1) \times 10^3$$

(R denotes the ratio $^{15}N/^{14}N$). The standard deviation of repeated samples was ± 0.3‰. The $\delta^{15}N$ of the N taken up was calculated with the use of a mixing-model analysis that took into account the contribution from seed N (Högberg, 1997):

$$\delta^{15}N_{uptake} = ((\delta^{15}N_{plant} \times N_{plant}) - (\delta^{15}N_{seed} \times N_{seed}))/(N_{plant} - N_{seed})$$

(N_x is the amount of N in compartment x). This equation works well, except when $\delta^{15}N_x$ closely approaches 0‰. This was not observed here, and the isotopic source effect of the seed was small (5.1‰ and 0.02 mmol N). In any case, the problem of δ values very close to 0‰ can be handled by transforming data to atom% (A) and using that unit in the above equation. We take this opportunity to correct equation (2) in Högberg (1997) for conversion from δ to A:

$$A = 100/(1/(\delta/1000 + 1)R_{standard} + 1).$$

Table 1. *Concentrations of elements (mmol l^{-1}) in the nutrient solutions used*

Element	Concentration (mmol l^{-1})	Comments
N	1.43	As NH_4^+ or NO_3^-
K	0.24	
P	3.55×10^{-2} or 10.7×10^{-2}	With and without fungal inoculation, respectively
Mg	4.94×10^{-2}	
Ca	2.25×10^{-2}	
Na	0.00087 or 1.43	With NH_4^+ or NO_3^-, respectively
Cl	0.046 or 1.47	With NO_3^- or NH_4^-, respectively
S	9.04×10^{-2}	
Fe	3.6×10^{-4}	
Mn	1.1×10^{-4}	
Cu	7.8×10^{-6}	
Zn	1.5×10^{-5}	
B	6.5×10^{-4}	
Mo	9.9×10^{-8}	
Co	2.4×10^{-7}	

Nitrogen was added as NH_4Cl or $NaNO_3$, which resulted in different concentrations of Cl and Na in the two N treatments. The concentration of P in the treatments with ectomycorrhizal fungi was one third of the concentration of P in the non-mycorrhizal treatment to account for the higher capacity for nutrient uptake in mycorrhizal plants.

As a more objective estimate of fungal colonization, we measured the chitin content of roots by using an HPLC method (Ekblad & Näsholm, 1996). The results were initially analysed statistically by ANOVA, which was followed by Tukey's test. Because we suspected a close relationship between isotope fractionation and the ratio $N_{uptake}/N_{supplied}$, we tested the significance of regressions between $\delta^{15}N$ in plants and rhizomorphs against the amount of N taken up (the amount of N supplied was the same in all treatments). We also used *t* tests to examine the differences between $\delta^{15}N$ values in rhizomorphs and plants.

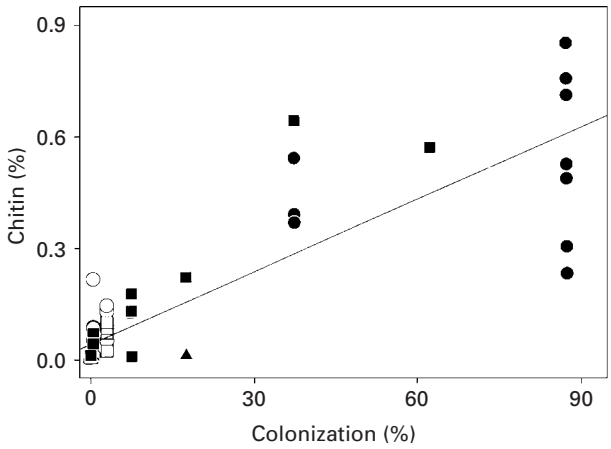

Fig. 1. Relation between the concentration of chitin and visual estimates of the degree of ectomycorrhizal colonization. Circles, squares and triangles represent plants inoculated with *Paxillus involutus*, *Suillus bovinus* and *S. variegatus*, respectively. Open symbols, plants given NO_3^-; filled symbols, plants given NH_4^+.

RESULTS

There was a correlation ($r^2 = 0.72$, $P < 0.001$) between the visual estimates of mycorrhizal colonization levels and the levels of chitin (Fig. 1), in accordance with a previous study (Ekblad *et al.*, 1998). According to both the visual estimates and the chitin analysis, the inoculation with *S. variegatus* was unsuccessful, whereas mycorrhizal roots were common in plants inoculated with *P. involutus* in particular, and in plants inoculated with *S. bovinus*. Colonization levels were higher in plants given NH_4^+ compared with those in plants given NO_3^- (Fig. 1, Table 2).

The aim was not to make a classical comparison between small non-mycorrhizal plants and large ECM plants, but to study the effects of N supply and mycorrhiza on the $\delta^{15}N$ of plants. The low P level supplied in the ECM treatments was used to reduce their size relative to that of non-mycorrhizal plants, and resulted in non-mycorrhizal plants becoming larger than ECM plants (Table 2). The largest plants were the non-mycorrhizal plants given NH_4^+, followed by those inoculated with *P. involutus* and given the same N source (Table 2). Those two, plus plants inoculated with *S. bovinus* and given NH_4^+, had a significantly higher shoot:root ratio than plants in the other treatments (Table 2). Thus, among the (ECM) plants supplied with the low level of P, those inoculated with *S. variegatus* showed very low levels of mycorrhizal colonization and were also the smallest, which indicated a positive effect of mycorrhizal colonization on growth and nutrient uptake in

Table 2. *Total biomass (g d. wt), shoot : root ratio, amount of N taken up (mmol), and chitin concentration in roots (mg g^{-1}) of non-mycorrhizal and ectomycorrhizal (ECM) seedlings of* Pinus sylvestris *at harvest (n = 9 or 10)*

N source	Treatment	Total biomass	Shoot : root ratio	N uptake	Chitin (in roots)
NH_4^+	Non-mycelial	2.10 (0.62) a	2.4 (0.4) a	3.2 (0.5) a	0.1 (0.0) a
	Paxillus involutus	1.42 (0.29) b	2.3 (0.5) a	2.4 (0.3) b	5.2 (2.0) b
	Suillus bovinus	1.00 (0.34) bc	1.9 (0.8) a	2.0 (0.5) b	1.9 (2.3) a
	S. variegatus	0.74 (0.14) c	1.3 (0.4) b	1.4 (0.3) c	0.1 (0.0) a
NO_3^-	Non-mycelial	0.87 (0.21) c	1.2 (0.2) b	1.2 (0.2) cd	0.1 (0.0) a
	Paxillus involutus	0.84 (0.17) c	1.1 (0.1) b	0.8 (0.1) d	1.2 (0.5) a
	Suillus bovinus	0.92 (0.10) c	1.1 (0.2) b	0.8 (0.1) d	0.5 (0.5) a
	S. variegatus	1.07 (0.26) bc	1.3 (0.3) b	0.9 (0.2) d	0.1 (0.0) a

ECM seedlings were inoculated with one of three fungal species, namely *Paxillus involutus*, *Suillus bovinus* and *S. variegatus*. Values are means, with SD in parentheses. Mean values not significantly different (column by column) at the $P<0.05$ level are followed by the same letter (ANOVA followed by Tukey's test).

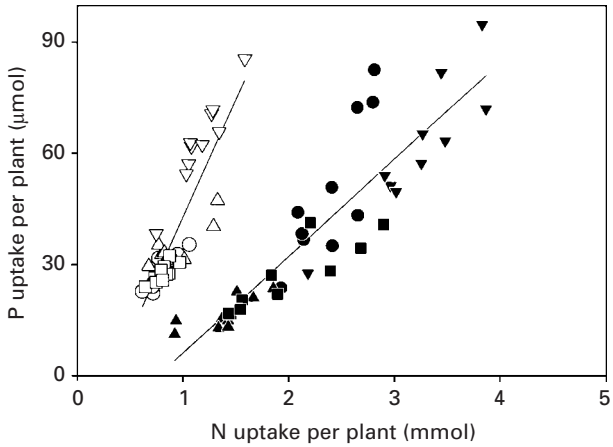

Fig. 2. Relation between the amounts of N and P taken up by non-mycorrhizal plants (triangles, apex downwards) and by plants inoculated with the ectomycorrhizal fungi *Paxillus involutus* (circles), *Suillus bovinus* (squares) and *S. variegatus* (triangles, apex upwards). Open symbols, plants given NO_3^-; filled symbols, plants given NH_4^+.

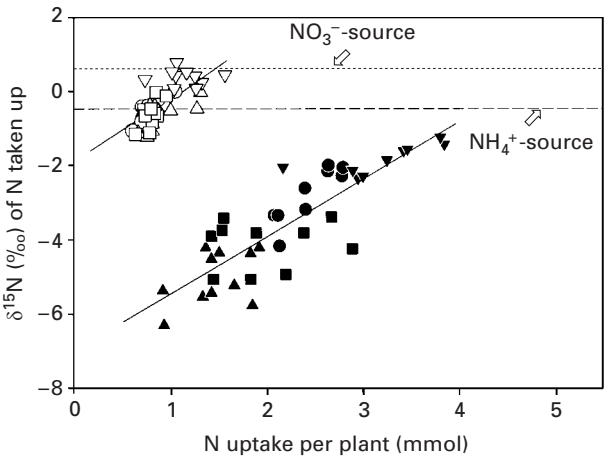

Fig. 3. Relation between the $\delta^{15}N$ of the N taken up by non-mycorrhizal and ectomycorrhizal pine seedlings and the amount of N taken up (4.6 mmol N were supplied to all treatments). There were positive correlations between the $\delta^{15}N$ of N taken up and the amount of N taken up for both NH_4^+ ($r^2 = 0.73$, $P<0.001$) and NO_3^- ($r^2 = 0.49$, $P<0.001$). Symbols as in Fig. 2.

the other ECM treatments (Table 2, Figs 1, 2). The uptake of N was strongly correlated with the uptake of P in both N treatments (NH_4^+, $r^2 = 0.80$, $P<0.0001$; NO_3^-, $r^2 = 0.74$, $P<0.0001$), but the P : N (w/w) ratios were approx. 0.04 in the NH_4^+ treatment in comparison with approx. 0.10 in the NO_3^- treatment (Fig. 2). Ratios of P : N of less than 0.08 are considered to be an indication of P limitation, whereas ratios from 0.08 to 0.10 are thought to indicate an optimal balance between the two elements (Ericsson & Ingestad, 1988).

Shoots of plants given NO_3^- became light yellow-green, whereas those given NH_4^+ became dark green. Meanwhile, the roots of plants given NO_3^- became thinner than in plants given NH_4^+. The total amount of N added to each pot during the period of growth was 4.6 mmol. This compares with the observed amounts of 0.6–4.0 mmol N taken up per plant. Plants given NO_3^- each took up 0.6–1.5 mmol N, that is, between 12% and 33% of the N supplied, whereas plants given NH_4^+ each took up 0.9–4.0 mmol N, that is, between 18% and 86% of the N supplied (Table 2, Figs 2, 3).

There were large and significant differences between the $\delta^{15}N$ values of source N and in the different compartments of ECM plants, but we consider that these should be analysed in relation to the ratio $N_{uptake}/N_{supplied}$ to provide meaningful information because of the presumed influence of this ratio on isotope fractionation during uptake. Irrespective of the source of N, there was indeed a positive correlation between the calculated $\delta^{15}N$ of the N taken up and the amount of N taken up (Fig. 3); that is, $\delta^{15}N$ was dependent on the ratio $N_{uptake}/N_{supplied}$. When NO_3^- was the source, the fractionation against ^{15}N varied between 0‰ and 1.7‰ in the material observed, and the intercept with the *y*-axis based on linear regression was

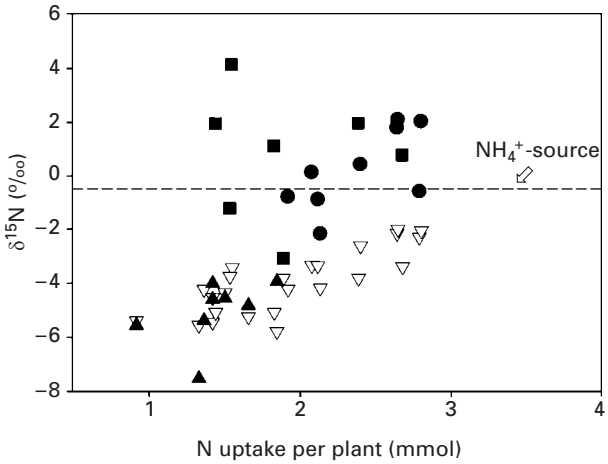

Fig. 4. Relation between the ^{15}N abundance of rhizomorphs and the N taken up and the amount of N taken up by plants given NH_4^+. Rhizomorphs on plants inoculated with the ectomycorrhizal fungi *Paxillus involutus* (closed circles), *Suillus bovinus* (closed squares) and *S. variegatus* (closed triangles); plant N (open triangles) (placed at the same position along the *x*-axis as the corresponding rhizomorph sample).

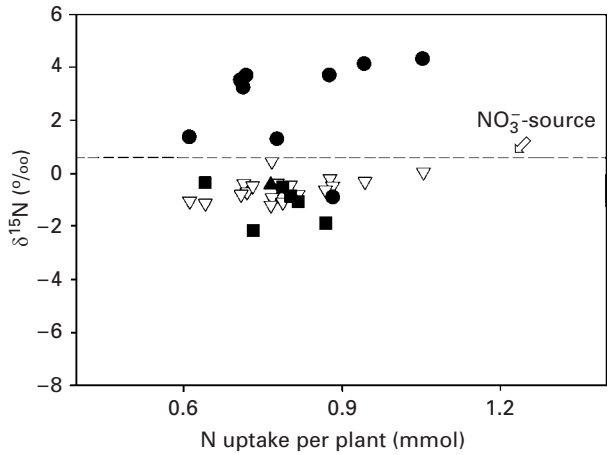

Fig. 5. Relation between the ^{15}N abundance of rhizomorphs and the N taken up and the amount of N taken up by plants given NO_3^-. Symbols as in Fig. 4.

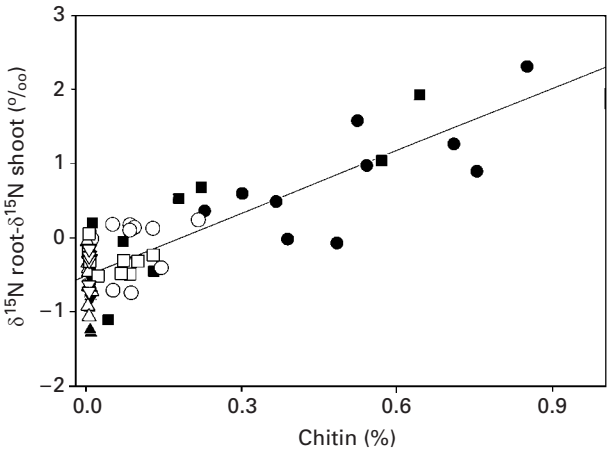

Fig. 6. Relation between the difference in ^{15}N abundance between roots and shoots and the mycorrhizal colonization expressed as the concentration of chitin in roots. Symbols as in Fig. 2.

$-2.0‰$. Taking into account that the source was $0.6 \pm 0.0‰$ (mean $\pm$ SE), the maximal fractionation should be $-2.6‰$. When NH_4^+ was the source, the fractionation against ^{15}N varied between 0.9‰ and 5.8‰ in this material; the intercept with the *y*-axis based on linear regression was $-7.0‰$. Taking the $\delta^{15}N$ of the source, $-0.5 \pm 0.0‰$, into account, resulted in a predicted maximal fractionation of $-6.5‰$. However, these relations should be asymptotic, and nonlinear regressions would produce slightly lower intercepts with the *y*-axes.

Rhizomorphs of ECMs were significantly enriched in ^{15}N relative to the N in shoot tissues (which do not contain mycorrhizal hyphae); that is, by $4.9 \pm 1.0‰$ ($P < 0.01$, mean $\pm$ SE) in plants inoculated with *S. bovinus* and given NH_4^+, and by $3.3 \pm 0.3‰$ ($P < 0.001$) and $2.8 \pm 0.5‰$ ($P < 0.001$) in plants inoculated with *P. involutus* and given NH_4^+ and NO_3^-, respectively. In plants inoculated with *S. variegatus* and given NH_4^+ and in plants inoculated with *S. bovinus* and given NO_3^-, the fungi were not enriched relative to the N in the shoot. Only one plant inoculated with *S. variegatus* and supplied with NO_3^- had a rhizomorph. In Fig. 4 (NH_4^+ treatment) and Fig. 5 (NO_3^- treatment), the $\delta^{15}N$ values of fungal and plant N are plotted against the amount of N taken up by each plant. In ECM plants given NH_4^+, the amount of N taken up per plant was weakly positively correlated with the difference in $\delta^{15}N$ between rhizomorphs and shoots ($r^2 = 0.18$, $P < 0.05$), suggesting that rhizomorphs attached to plants that had taken up more N were more enriched in ^{15}N. It is evident that fungal N can be enriched in ^{15}N relative to both plant N and source N (Figs 4, 5). The strong correlation ($r^2 = 0.75$, $P < 0.001$) between the difference in ^{15}N abundances between root and shoot and the concentration of chitin in roots (Fig. 6) also suggested that the fungal component must be enriched in ^{15}N relative to the plant.

DISCUSSION

In many field studies, the $\delta^{15}N$ of plants, notably of leaves, is used as a proxy for the $\delta^{15}N$ of available N in the soil to make inferences about which N species that plants take up from the soil. In agreement with Nadelhoffer & Fry (1994) and Evans *et al.* (1996), we observed small or negligible fractionation of N isotopes when the plants took up a high proportion of the N supplied (Fig. 3). However, under conditions of low uptake in relation to supply, fractionation was considerable, especially that of NH_4^+. The larger isotope effect thus observed for NH_4^+ than for NO_3^- is to be expected, because of the larger relative difference in mass if ^{15}N replaces ^{14}N in NH_4^+ compared with that in NO_3^- (1.056 in NH_4^+, compared with 1.016 in NO_3^-).

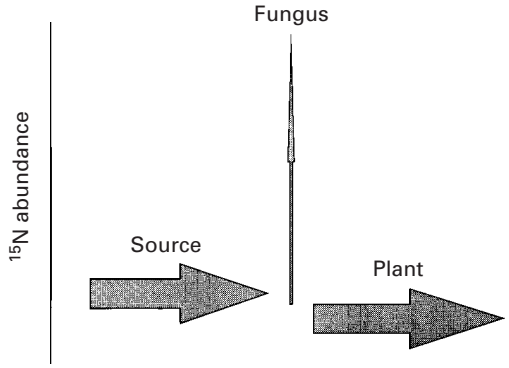

Fig. 7. Schematic drawing of changes in the natural ^{15}N abundance during the flux of soil-derived N (source) to the plant through an ectomycorrhizal fungus.

The most important question here is whether the passage of N through the fungal component on its way into the plant alters significantly the δ^{15}N of the N taken up from the source. We argue that our results suggest that this potential mechanism is of a marginal importance for the δ^{15}N of the plant (Fig. 7). As is clear from Fig. 3, the non-mycorrhizal and ECM plants fall on the same regression of δ^{15}N of the N taken up against the amount of N taken up; the same is true for plots of δ^{15}N of shoots against the amount of N taken up (results not shown). Moreover, our argument is further supported by the fact that plants inoculated with *P. involutus* and *S. bovinus* are positioned along this same regression between the non-mycorrhizal plants and those inoculated with *S. variegatus*, which, according to the analysis of chitin, were in reality also non-mycorrhizal (compare also Fig. 1 and Table 2). Furthermore, the regression analysis supports the notion that fractionation during N uptake by the mycorrhizal plant would be essentially zero if more of the N had been taken up. However, the fungal component evidently becomes enriched relative to the plant in many cases (compare Figs 4 and 5), and this phenomenon was also observed under a wide range of N supply rates in the field (Högberg *et al.*, 1996). We did not measure the biomass and N concentration of the total extramatrical mycelium. However, if this compartment retained the missing N, that is, the difference between $N_{supplied}$ and N_{uptake}, it would on average have a δ^{15}N value higher than that of the mycelium (rhizomorphs) sampled. Moreover, because these ECM plants fell on the same regression of δ^{15}N against amount of N taken up as did non-mycorrhizal plants (Fig. 3), it does not seem probable that the retention of N by the extramatrical mycelium compromises our interpretation significantly.

We used our results from seedlings mycorrhizal with *P. involutus* and given NH_4^+ to calculate the effect of the fungus on the δ^{15}N of the N passed on to the host plant. We used this symbiosis because it was the only one that had a high enough percentage of ECM colonization to be comparable to plants in the field. Using the conversion factor derived from an analysis of chitin in pure cultures of *P. involutus* (Ekblad & Näsholm, 1996), we calculated from the results on chitin that 5% of total ECM root biomass was fungal material. We then found that fungal material contained only 3% of the N in the mycorrhizal plants (root + shoot), on the basis of the assumption that the N concentration of the fungus is approx. 4%, whereas it is 2% in the plant root (Högberg *et al.*, 1996). This value of 3% and the 3.3‰ enrichment of ^{15}N in rhizomorphs relative to the N taken up (Fig. 4) were used to calculate that the passage of N through the ECM fungus would, at most, decrease the δ^{15}N of N passed on to the plant by 0.1‰. This effect would increase if the proportion of fungal biomass increased, its N concentration increased, or if the relative enrichment in ^{15}N of fungal N increased; in other words, it relates to the efficiency of transfer through the fungus. For example, increasing fungal biomass to 10% of ECM root biomass, and increasing the difference in δ^{15}N between fungal N and the total N taken up to 10‰, would lead to a depletion of plant N by 0.6‰ relative to the N taken up. The δ^{15}N of 10‰ for fungal N relative to plant N is taken from Taylor *et al.* (1997) for northern coniferous forests. A higher contribution of fungal material to total root biomass than a few percent is unlikely in temperate mycorrhizas, although it might be up to 20–40% in ECM tips (Harley & Smith, 1983). This decrease in of δ^{15}N 0.6‰ is small in comparison to differences in δ^{15}N between plant species observed in the field (Högberg, 1990; Schulze *et al.*, 1994; Nadelhoffer *et al.*, 1996; Michelsen *et al.*, 1996, 1998).

Do our simple mixing-model calculations based on laboratory-grown seedlings have any bearing on the situation in the field? We do not know, because of a lack of relevant data. In particular, it is unclear whether the efficiency of transfer of N through the fungus to the plant is the same in the field. We observe, however, that ECM roots were at the most enriched approx. 2‰ relative to non-mycorrhizal material (shoots) in our experiment (Fig. 6), which was the average difference observed between ECM and non-mycorrhizal roots in the field across a range of conditions in Europe (Högberg *et al.*, 1996).

There are large differences in the contribution of fungal material to roots in mycorrhizal symbioses. For example, for ericoid mycorrhizas (ERMs) the fungal component might be up to 70% of the finest roots (Harley & Smith, 1983), whereas for ECM a range of 20–40% is often discussed (Smith & Read, 1997), and values of $<20\%$ are reported for arbuscular mycorrhizas (AMs) (Smith & Read, 1997). On the basis of the observation of ^{15}N enrichment of the fungal component, one could speculate that the δ^{15}N of shoots of these three groups of plants would rank in the order ERM < ECM < AM if they were using the same N

source and if the efficiencies of transfer of N through the fungal compartments were similar. However, results from the field are contradictory: Michelsen *et al*. (1996, 1998) reported evidence in support of this pattern, whereas Schulze *et al*. (1994) did not. The cause of $\delta^{15}N$ in plants in the field is complex, especially because there is often a steep gradient of increasing ^{15}N down the soil profile (Gebauer & Schulze, 1991; Högberg *et al*., 1996). Högberg *et al*. (1996) therefore compared roots of different species by horizon and found, under conditions of strong N limitation, that roots of ERM, ECM and AM species did not differ in ^{15}N abundance by more than 1‰. However, on plots that had received approx. 100 kg N ha^{-1} yr^{-1} over two decades, roots of these species differed by as much as 5‰, and the $\delta^{15}N$ values of plants increased in the order ERM < ECM < AM. We note that this pattern might be caused by several factors in addition to a decrease in the contribution of fungal N to total plant N, as explained above. For example, it might also be caused by differences in fractionation of N isotopes and reflect an increase in the ratio $N_{uptake}/N_{supplied}$; the ERM *Vaccinium* spp. studied have inherent slow growth rates relative to the other species (Chapin, 1980) and would express a low demand in relation to the N supplied in the N-addition treatment.

The possibility of a high enrichment of the fungal component seems to be an analogue of that sometimes observed in root nodules of legumes (Reinero *et al*., 1983; Yoneyama, 1988; Kohl & Shearer, 1995) and actinorhizal plants (Yoneyama & Sasakawa, 1991). The root nodules also represent a relatively small pool of N in relation to the total plant N of the symbiotic plant, but a large amount of N is passed through these into the host root and shoot. Under such conditions fractionation against ^{15}N, for example during transaminations (Macko *et al*., 1986) during the synthesis of N transport compounds, could result in that small compartment becoming highly enriched in relation to the N exported. This enrichment should be correlated with the amount of N that has passed through this compartment relative to the size of the N pool of the compartment; this is at least weakly supported by our results (Fig. 4).

The high abundance of ^{15}N in ECM fungal material compared with that of plant material is interesting with regard to the N physiology of ECM symbiosis (Martin & Botton, 1993) and to soil profile development (Högberg *et al*., 1996), and could possibly be a useful tracer in food web studies.

In conclusion, our results show that N isotope fractionation is small in both non-mycorrhizal and ECM plants provided that a large proportion of the N supplied is taken up (Fig. 3), which is likely in most situations in the field. Our results, based on laboratory-grown seedlings, showed that the ECM fungus can become highly enriched relative to the plant, but because of the small N content of this compartment it does not lead to a significant decrease in $\delta^{15}N$ of the N that is passing through the fungus on its way from the soil to the plant (Fig. 7). In the field, the efficiency of transfer of N through the fungus might be different, which could lead to a larger or smaller shift in $\delta^{15}N$ than in our model experiment. However, this remains to be tested. Also, our results suggest that the $\delta^{15}N$ of plant N can be a good proxy for the $\delta^{15}N$ of available N in the soil, provided that the N supply rate is low in relation to plant demand.

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