Tansley review

Nitrogen isotopes link mycorrhizal fungi and plants to nitrogen dynamics

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Summary

In this review, we synthesize field and culture studies of the $^{15}N/^{14}N$ (expressed as $\delta^{15}N$) of autotrophic plants, mycoheterotrophic plants, parasitic plants, soil, and mycorrhizal fungi to assess the major controls of isotopic patterns. One major control for plants and fungi is the partitioning of nitrogen (N) into either ^{15}N-depleted chitin, ammonia, or transfer compounds or ^{15}N-enriched proteinaceous N. For example, parasitic plants and autotrophic hosts are similar in $\delta^{15}N$ (with no partitioning between chitin and protein), mycoheterotrophic plants are higher in $\delta^{15}N$ than their fungal hosts, presumably with preferential assimilation of fungal protein, and autotrophic, mycorrhizal plants are lower in ^{15}N than their fungal symbionts, with saprotrophic fungi intermediate, because mycorrhizal fungi transfer ^{15}N-depleted ammonia or amino acids to plants. Similarly, nodules of N_2-fixing bacteria transferring ammonia are often higher in $\delta^{15}N$ than their plant hosts. N losses via denitrification greatly influence bulk soil $\delta^{15}N$, whereas $\delta^{15}N$ patterns within soil profiles are influenced both by vertical patterns of N losses and by N transfers within the soil–plant system. Climate correlates poorly with soil $\delta^{15}N$; climate may primarily influence $\delta^{15}N$ patterns in soils and plants by determining the primary loss mechanisms and which types of mycorrhizal fungi and associated vegetation dominate across climatic gradients.

I. Introduction

Nitrogen (N) commonly limits plant growth in terrestrial ecosystems (Tamm, 1991; Vitousek & Howarth, 1991) and how plants obtain N influences both vegetational responses to elevated CO_2 (Finzi *et al.*, 2007) and competitive interactions within plant communities (Wilson & Tilman, 1991). Most plants rely on symbiotic (mycorrhizal) fungi to supply them with N. These fungi function at the interface between plants and the soil from which plants derive their nutrients, with much of the carbon (C) flux from plants to the soil mediated by mycorrhizal fungi. Because of the increased surface area for nutrient absorption of fungal hyphae and

the extensive enzymatic capabilities of many mycorrhizal fungi, the dominant plants in most ecosystems are mycorrhizal.

Mycorrhizal fungi can be separated into three major groups, arbuscular mycorrhizal, ectomycorrhizal, and ericoid mycorrhizal fungi. These fungal types differ considerably in their spatial extent, C demands, type of host plant, species diversity, enzymatic capabilities to access different forms of N, and association with different N dynamics (Read & Perez-Moreno, 2003; Smith & Read, 2008; He *et al.*, 2009). In general, arbuscular mycorrhizal fungi are adapted to sites with high rates of N cycling and lack proteolytic capabilities, ericoid mycorrhizal fungi are adapted to organic-rich ecosystems with low rates of N cycling and have good proteolytic and litter-degrading capabilities, and ectomycorrhizal fungi vary greatly in their enzymatic capabilities. Ectomycorrhizal taxa with hydrophilic ectomycorrhizas often lack proteolytic capabilities and assimilate soluble N forms after release from free-living microbes, whereas other ectomycorrhizal taxa with hydrophobic ectomycorrhizas have proteolytic capabilities and are adapted to N-limited conditions (Lilleskov *et al.*, 2011). Rates of N decomposition may therefore determine the extent to which plants rely on N mobilized by mycorrhizal enzymes for N supply. Many saprotrophic fungi and many ectomycorrhizal fungi with hydrophobic ectomycorrhizas also form aggregated hyphae (rhizomorphs) for long-distance transport, which is presumably an adaptation for patchily distributed resources (Peay *et al.*, 2011).

One common technique for assessing N dynamics, measuring N isotope ratios ($^{15}N/^{14}N$) in plants and other ecosystem pools, has proved useful because it reflects the sum total of inputs, outputs, and fractionation processes. In addition, interpretations of N isotope ratios at natural abundance do not need to consider experimental artifacts, which may be a problem in N isotope tracer studies. Despite these advantages, the interpretation of N isotope patterns in nature remains an inexact science because of the range of fluxes potentially influencing specific pools, difficulties in studying the soil processes that control many key N transformations, and the uncertainty of isotopic fractionation during many of these processes (Robinson, 2001). As a result, although quantitative and analytical relationships among $\delta^{15}N$ of different ecosystem components have been proposed, scientists have lacked underlying theories to explain N isotope patterns that are as widely accepted as those used to interpret plant isotope patterns of C (Farquhar *et al.*, 1982), hydrogen, and oxygen (Roden *et al.*, 2000).

Mycorrhizal fungi appear to be key mediators of N movement in the plant–soil system that can influence isotopic patterns. Mycorrhizal fungi provide plants with access to organic N forms that are usually higher in ^{15}N than the inorganic forms generally considered available to plants. Mycorrhizal fungi also retain ^{15}N-enriched N and transfer ^{15}N-depleted N to plant hosts. They also influence N availability by competing against saprotrophic (free-living) fungi and bacteria for N and assimilating ammonium and organic N before it can be mineralized or nitrified. Mycorrhizal fungi therefore influence ecosystem N loss rates via denitrification and nitrification, processes that discriminate strongly against ^{15}N. We accordingly propose that interactions between plants and mycorrhizal fungi greatly influence both plant and soil $\delta^{15}N$. Many ectomycorrhizal fungi (but not other mycorrhizal fungi) produce

macroscopic reproductive structures (sporocarps) that can be readily sampled for isotopic analyses, providing additional insights into their functioning.

A previous Tansley review by Högberg (1997) laid out both general principles and numerous case studies on N isotope patterns from individual systems. Since then, several broad surveys have been published of $\delta^{15}N$ patterns in foliage, soil, and fungi (Handley *et al.*, 1999; Amundson *et al.*, 2003; Craine *et al.*, 2009; Hobbie & Ouimette, 2009; Mayor *et al.*, 2009). In addition, several reviews have addressed different aspects of N isotope interpretation, with general overviews by Evans (2001), Robinson (2001), and Adams & Grierson (2001), the biochemical basis for N isotope patterns in Werner & Schmidt (2002), the controls over fungal $\delta^{15}N$ by Hobbie (2005), and using natural abundance and tracer ^{15}N to assess transfers of N in fungal–plant networks by He *et al.* (2009).

In this review, we combine insights from culture studies and site-specific field studies with data from broad surveys to assess whether mycorrhizal fungi drive N isotope patterns in terrestrial plants and soils. We also explore the links between functional characteristics and $\delta^{15}N$ in different taxa of ectomycorrhizal fungi. The $\delta^{15}N$ patterns in mycoheterotrophic plants (reliant on fungi for their C and nutrients), parasitic plants (tapping into the xylem or phloem of other plants), and N_2-fixing plants are also examined for potential additional insights into mechanisms governing isotopic patterns in symbioses.

II. Background on isotopes

Nitrogen has two stable isotopes, ^{15}N (0.3663% of total) and ^{14}N (99.6337% of total). Most physical, chemical and biochemical processes favor the initial incorporation of the lighter isotope in the product, leaving the substrate enriched in the heavy isotope. The magnitude of this 'isotopic fractionation' differs depending on the compounds involved and the specific reaction mechanism. Isotopically fractionating processes therefore result in differences in the isotopic ratios between the substrate and the product. These ratios depend on the isotopic ratio of the substrate, the proportion of substrate transformed to product, and whether the system is open or semi-closed (Fig. 1). Isotope effects during reactions can be expressed mathematically in several ways. In one convention, isotopic fractionation is expressed as the rate reaction or equilibrium constant of the light isotope divided by that for the heavy isotope, such that $\alpha = {}^{14}K/{}^{15}K$. The reverse convention of $\alpha = {}^{15}K/{}^{14}K$ is occasionally reported.

Natural abundance studies use isotopic differences among different ecosystem pools and compounds to understand the sources and fluxes of many biologically important compounds. Because differences in isotopic ratios are very small, they are measured using the 'δ' notation, as deviations in parts per mil (‰) from a standard ratio, according to Eqn 1.

$$\delta^{15}N(‰) = (R_{sample}/R_{standard} - 1) \times 1000‰ \qquad \text{Eqn 1}$$

In Eqn 1, *R* equals the molar abundance of the heavy isotope divided by the light isotope ($^{15}N/^{14}N$). The isotopic standard
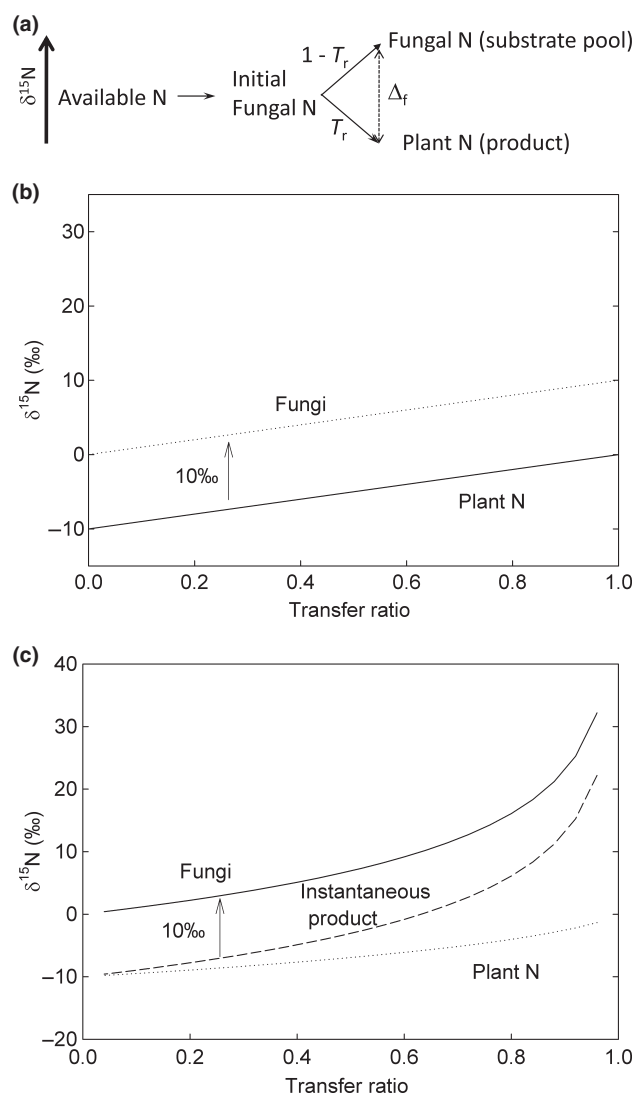
(a)

(b)

(c)

Fig. 1 Nitrogen (N) isotope patterns in an open vs a semi-closed system. In this example, the substrate, available N, is taken up by fungi, then a fraction (transfer ratio, T_r) is transferred to host plants (product pool) with a fractionation of Δ_f during creation of transfer compounds (a). At a value for available N of 0‰ and for Δ_f of 10‰, this leads to the isotopic patterns shown in open systems (b), and semi-closed systems (c), as T_r is varied from 0 to 1. The remaining fraction $(1 - T_r)$ is used to construct fungal biomass. Modified from Hobbie & Agerer (2010). With kind permission from Springer Science and Business Media.

for N is atmospheric N_2 ($^{15}N/^{14}N = 0.0036765$; Mariotti, 1983; Hoefs, 1997). For $\delta^{15}N$, pools in terrestrial ecosystems are usually between 20 and -10‰. In comparisons among samples, samples with more of the heavy isotope are commonly referred to as isotopically enriched, or heavy, and samples with less of the heavy isotope are referred to as isotopically depleted, or light. Isotopic fractionation in per mil is expressed as Δ, with

$$\Delta = (\alpha - 1) \times 1000‰ \qquad \text{Eqn 2}$$

If a steady state is assumed, isotopic fractionation (Δ) for reactions in open systems can be calculated based on the isotopic signature of the source and product according to Eqn 3.

$$\Delta = (\delta^{15}N_{source} - \delta^{15}N_{product})/(1 + \delta^{15}N_{product}) \qquad \text{Eqn 3}$$

An important distinction in interpreting isotopic patterns is whether kinetic or equilibrium isotopic effects dominate in a reaction. In kinetic isotopic effects, the light isotope reacts more rapidly than the heavy isotope, so that the resulting product is depleted in the heavy isotope relative to the substrate. Isotopic effects during irreversible reactions are governed by kinetic isotopic effects. By contrast, in equilibrium reactions the back-reaction from product to substrate also occurs, and therefore the kinetic isotope effects for both the forward and back reactions must be considered. In equilibrium reactions the heavy isotope generally concentrates in the compound with stronger bonds (Bigeleisen, 1965). For example, in the equilibrium reaction between ammonia and ammonium, the ammonium ion accumulates 19–21‰ more ^{15}N than ammonia, whereas the kinetic isotope effect of the forward reaction is *c.* 27‰. Similarly, the equilibrium isotope effect in a reaction is the sum of the forward and the backward kinetic isotopic effects; for example, for the reaction glutamate + oxalo-acetate $\rightarrow$ alpha-oxoglutarate + aspartate as catalyzed by oxaloacetate:glutamate aminotransferase, the forward reaction has a kinetic isotope effect (designated α) of 1.0083 and the back reaction a kinetic effect of 1.0017, so that the equilibrium isotope effect is 1.0083/1.0017 = 1.0066, or a 6.6‰ discrimination against ^{15}N (Macko *et al.*, 1986).

Information about patterns of ^{15}N discrimination in biochemical reactions can be useful. However, translating such information into insights into the behavior of bulk samples is not easy. Both kinetic and equilibrium isotopic effects are often reported, with equilibrium effects equivalent to adding the kinetic effects for the forward and the back reactions of an equilibrium reaction. Some of the most important reactions for isotopic distributions are presented in Table 1. Equilibrium isotope effects were summarized in Rishavy & Cleland (1999) and kinetic isotope effects were summarized in Werner & Schmidt (2002). An additional factor to consider when applying isotopic discrimination factors is whether to treat a system as open or closed, as for the same isotopic discrimination factor the resulting distribution of isotopic values will differ. Metabolic branch points, where different metabolic products are produced from a common precursor substrate, are also required for isotopic differences among different pools, as pointed out for C isotope distributions by Hayes (2001).

Different mathematical models have been created to explain isotopic partitioning in two-component systems that are considered to be open (exogenous inputs to the system) or closed (no inputs to the system; Hayes, 2001). As an example of an open system (Fig. 1b), the proportion of N taken up by mycorrhizal fungi that is then passed on to host plants, termed the transfer ratio (T_r), can explain N isotope patterns in mycorrhizal plants according to the following equation (Hobbie *et al.*, 2000):

$$\delta^{15}N_{plant} = \delta^{15}N_{available\ nitrogen} - (1 - T_r) \times \Delta \qquad \text{Eqn 4}$$

with the transfer ratio, T_r, between 0 and 1, and the discrimination against ^{15}N during the creation of transfer compounds (Δ) of 8–10‰. To explore isotope patterns among plants, available N,

Table 1 ^{15}N fractionation during biochemical reactions or ^{15}N enrichment between protein and chitin

Reaction	Value (‰)	Source	References
Nitrogen fixation	0–2	–	Högberg (1997); Clarkson *et al.* (2005)
Denitrification	13.2	Field	Houlton *et al.* (2006)
Denitrification	13–30	Field	Pérez *et al.* (2000)
Mineralization	2.4	Field	Koba *et al.* (2003)
Nitrification	11.4	Field	Koba *et al.* (2003)
Protein – chitin	9.9	Field	Taylor *et al.* (1997)
Protein – chitin	8–10	Field	Hobbie & Colpaert (2003)
Protein – chitin	7, 15	Field	Hobbie *et al.* (2012)
Polypeptide cleavage	10[1], 24[2]	Laboratory	Medina & Schmidt (1982)
Urea hydrolysis	8	Laboratory	Werner & Schmidt (2002)
Arginine hydrolysis	10	Laboratory	Werner & Schmidt (2002)
Ammonium assimilation	9–20	Laboratory	Handley & Raven (1992)
Transamination	8.3[3], 1.7[4]	Laboratory	Werner & Schmidt (2002)

Here, laboratory studies were all of kinetic isotope effects, whereas field studies were of net measured fractionations.
[1]Chymotrypsin.
[2]Papain.
[3]Glutamate → aspartate.
[4]Aspartate → glutamate.

and mycorrhizal fungi in an open system, Eqn 3 can be rearranged, with $\delta^{15}N_{plant} = \delta^{15}N_{product}$, and $\delta^{15}N_{available\ nitrogen} = \delta^{15}N_{substrate}$. As a simplification, the denominator $(1 + \delta^{15}N_{plant})$ can be dropped, as with a value of $\delta^{15}N_{plant}$ usually between −10 and 10‰, the denominator is generally between 0.99 and 1.01. We previously used N budgets and ^{15}N differences among available N, nonmycorrhizal pines and mycorrhizal pines under controlled conditions (Hobbie & Colpaert, 2003) to estimate Δ at 9‰. The ^{15}N content of mycorrhizal fungi can then be calculated according to Eqn 5.

$$\delta^{15}N_{fungi} = \delta^{15}N_{available\ nitrogen} + T_r \times \Delta \qquad \text{Eqn 5}$$

In an intermediate case, the semi-closed system, the fungal pool is not replenished by additional N from the available N pool. In this system (Fig. 1c), Eqns 6 and 7 can be used to calculate the isotopic signatures of plants and fungi, respectively (modified from Hobbie *et al.*, 2005). The quantity f is the proportion of original substrate transformed to product, and is equivalent to the transfer ratio (T_r) in Eqns 4 and 5.

$$\delta^{15}N_{fungi} = \delta^{15}N_{available\ nitrogen} - \Delta \times \log_e(1 - f) \qquad \text{Eqn 6}$$

$$\delta^{15}N_{plant} = \delta^{15}N_{available\ nitrogen} + \Delta \times (\log_e f)/(1 - f) \qquad \text{Eqn 7}$$

This approach can be used directly on data from laboratory settings, where $\delta^{15}N_{available\ nitrogen}$ is known. In field settings, the multiple N sources have dynamically varying isotopic signatures, which restricts the direct application of these equations.

III. Patterns of soil δ^{15}N

1. Bulk soil

Bulk soil δ^{15}N in a steady state must reflect the input and output δ^{15}N signatures (Amundson *et al.*, 2003). Thus, if mycorrhizal type or soil age correlates with soil δ^{15}N, then this presumably reflects parallel correlations between these parameters and the proportion of N lost via highly fractionating reactions, such as denitrification and nitrification. Houlton *et al.* (2009) showed that streamwater nitrate was similar to bulk soil, and denitrification losses of N_2 and N_2O must therefore be the dominant influence on system ^{15}N balances.

Large-scale databases for soil δ^{15}N were compiled by Martinelli *et al.* (1999), Handley *et al.* (1999) and Amundson *et al.* (2003). Bulk soil δ^{15}N averages between 2 and 6‰ in most cases, with soil δ^{15}N values commonly increasing with greater depth in the soil profile. Amundson *et al.* (2003) integrated δ^{15}N values for the top 50 cm of soil, and then regressed these values against site temperature and precipitation for both their entire data set and a subset of their data set (termed the climosequence data) for which mountain sites were selected specifically along gradients of precipitation and temperature. This climosequence regression (adjusted $r^2 = 0.36$; $n = 30$) was then applied to global patterns of precipitation and temperature to create a global map of estimated soil δ^{15}N. Subsequent work has applied this regression to calculate global patterns of trace gas fluxes from soil, under the assumption that soil δ^{15}N is at steady state, and reflects the fractionation against ^{15}N of the major loss terms, and that the calculated regression is a reasonable representation of the influence of precipitation and climate on patterns of N losses and global patterns of soil δ^{15}N (Houlton *et al.*, 2009).

Soil δ^{15}N was poorly correlated with climate for the complete data set (adjusted $r^2 = 0.16$; $n = 48$), and very poorly correlated with the data that were not part of the climosequence data (adjusted $r^2 = 0.05$; $n = 18$). Thus, the regression model of Amundson *et al.* (2003) predicted soil δ^{15}N reasonably well for the specified mountain locations, but lacked predictive power outside of these sites. Accordingly, using this regression to estimate global patterns of δ^{15}N and trace gas fluxes (denitrification in Houlton *et al.*, 2009) is not valid in our view. We conclude that factors other than climate must influence the long-term fluxes of ^{15}N-depleted N from soil.

Because mycorrhizal type varies strongly with climate (Table 2) and is correlated with patterns of N cycling (Read & Perez-Moreno, 2003), the hidden influence of mycorrhizal association is a potentially confounding factor. We reanalyzed the climosequence data of Amundson *et al.* (2003) by adding mycorrhizal type as an additional variable, with sites classified as dominated by nonmycorrhizal, ectomycorrhizal, or arbuscular mycorrhizal plants, and a few savannah sites classified as mixed arbuscular mycorrhizal/ectomycorrhizal. The regression analyses on the Amundson *et al.* (2003) data are presented in Table 3. Adding mycorrhizal type to the model increased the strength of the regression, with the adjusted r^2 increasing from 0.35 to 0.52 for climosequence data and from 0.16 to 0.27 for the entire data set. Temperature explained the

Table 2 Mean annual temperature (MAT; °C) and precipitation (MAP; mm) ± SE for sites where foliage of different mycorrhizal types was collected in the Craine *et al.* (2009) database of foliar $\delta^{15}N$

Mycorrhizal type	MAT	MAP	*n*
Nonmycorrhizal	1.3 ± 0.5	439 ± 14	702
Arbuscular mycorrhizal	18.5 ± 0.1	1162 ± 9	8609
Ectomycorrhizal	4.5 ± 0.2	983 ± l3	1824
Ericoid mycorrhizal	0.7 ± 0.4	604 ± 19	692

n is the number of each mycorrhizal type in the database.

smallest amount of variance. Thus, precipitation and mycorrhizal type are more important than temperature in influencing the $\delta^{15}N$ of bulk soil over climatic gradients.

Age may be an additional factor influencing bulk soil $\delta^{15}N$. For example, tropical ultisols and inceptisols in Costa Rica and Panama (apparently dominated by arbuscular mycorrhizal trees) were depleted by 4–5‰ in ^{15}N relative to old and highly weathered Brazilian oxisols (Pérez *et al.*, 2000; Corre *et al.*, 2010). In general, older soils in chronosequence studies have higher $\delta^{15}N$ values (Brenner *et al.*, 2001; Menge *et al.*, 2011), presumably as a consequence of N limitation decreasing and losses of nitrate, N_2O, and N_2 increasing as phosphorus availability decreases.

The effects of denitrification and nitrification on soil ^{15}N enrichment were explored by Houlton *et al.* (2006, 2007) in Hawaii in two detailed studies. In these studies, N isotope patterns in plants, soil, and inorganic N across a precipitation gradient from 2200 to 5000 mm indicate that sites with higher rainfall may allow essentially all produced nitrate to be denitrified, leading to no ^{15}N enrichment of soils. By contrast, with partial denitrification at lower rainfall, ^{15}N-enriched residual substrate was reassimilated, leading to ^{15}N enrichment of the resulting soils.

2. Soil profiles

Bulk $\delta^{15}N$ generally increases within soil profiles and provides information about what processes control N movement and retention. This increase varies with the dominant mycorrhizal type; $\delta^{15}N$ increased with depth an average of 9‰ in ectomycorrhizal

systems and 4‰ in arbuscular mycorrhizal systems (measured from the litter to 50 cm depth; Hobbie & Ouimette, 2009). In 40% of arbuscular mycorrhizal systems, soil $\delta^{15}N$ increases initially and then declines at greater depths. This presumably reflects the importance in some arbuscular mycorrhizal systems of ^{15}N-depleted products, such as dissolved organic N or nitrate, which can be reassimilated at greater depths. In addition, the loss of ^{15}N-depleted N_2O and N_2 may enrich the remaining N in ^{15}N primarily at depths favorable for denitrification. When mycorrhizal type is ignored, mean annual temperature (MAT) and precipitation (MAP) weakly correlate (adjusted $r^2 = 0.17$) with the difference in $\delta^{15}N$ between soil at 50 cm depth and litter (Hobbie & Ouimette, 2009) according to the following equation, with MAP measured in millimeters:

$$\delta^{15}N_{soil-litter} = -0.161 \times MAT - 7.88 \cdot 10^{-4} \times MAP + 11.3$$
$$(P < 0.001, n = 85)$$

Eqn 8

However, when soils are classified by mycorrhizal type into arbuscular mycorrhizal (AM), ectomycorrhizal (ECM), and mixed arbuscular/ectomycorrhizal systems, temperature and precipitation no longer influence the ^{15}N enrichment of soil at 50 cm relative to surface litter and the strength of the regression increases to an adjusted r^2 of 0.46 (Table 4).

$$\delta^{15}N_{soil-litter} = -3.29 \times [AM] + 1.18 \times [ECM] + 9.07$$
$$(P < 0.001, n = 85)$$

Eqn 9

Thus, ^{15}N enrichment in soil profiles is *c.* 4.5‰ higher in systems dominated by ectomycorrhizal symbioses than in systems dominated by arbuscular mycorrhizal symbioses. Transfer of ^{15}N-depleted N to ectomycorrhizal plants and retention of ^{15}N-enriched N by ectomycorrhizal fungi appears to drive ^{15}N depletion in surficial litter layers and ^{15}N enrichment in deeper soil horizons, as also suggested in several previous studies (Högberg *et al.*, 1996; Billings & Richter, 2006; Lindahl *et al.*, 2007; Hobbie

Table 3 Mycorrhizal type and climate correlate with the $\delta^{15}N$ of bulk soil (0–50 cm)

Parameter	Climosequence No mycorrhizal	Climosequence Mycorrhizal	All Samples No mycorrhizal	All Samples Mycorrhizal
Adjusted r^2	0.360 (< 0.001)	0.527 (< 0.001)	0.159 (0.008)	0.267 (0.002)
Intercept	4.4 ± 1.1	4.9 ± 1.3	3.9 ± 1.1	2.8 ± 1.3
MAT	0.17 ± 0.06 (0.010)	0.15 ± 0.09 (0.136)	0.20 ± 0.07 (0.006)	0.14 ± 0.07 (0.057)
MAP	$-0.7 ± 0.4e^{-3}$ (0.002)	$-1.6 ± 0.3e^{-3}$ (< 0.001)	$-1.2 ± 0.3e^{-3}$ (0.073)	$-1.01 ± 0.38e^{-3}$ (0.010)
AM	–	1.67 ± 0.64 (0.015)	–	2.65 ± 0.87 (0.004)
AM/ECM	–	–	–	-2.12 ± 0.73 (0.007)
ECM	–	-0.22 ± 1.20 (0.858)	–	1.54 ± 1.05 (0.151)

Climate and bulk soil data are from Amundson *et al.* (2003); mycorrhizal type is based on dominant vegetation, and classified as arbuscular mycorrhizal (AM) (32), ectomycorrhizal (ECM) (11), mixed arbuscular/ectomycorrhizal (AM/ECM) (4), and nonmycorrhizal (1) (Supporting Information Table S1). Regressions include either the complete data set (*n* = 48) or just climosequence data (*n* = 30) from mountain gradients of temperature or precipitation. Regressions were done with or without mycorrhizal type as an explanatory variable. Mycorrhizal type significantly correlated with bulk $\delta^{15}N$ for the climosequence (*P* = 0.009) and the complete data set (*P* = 0.033). Significance values indicated in parentheses after coefficients (± SE) for different variables. $e^{-3} = 10^{-3}$. Regressions were done using JMP 9.0 (SAS, Cary, NC, USA). MAT, mean annual temperature (°C); MAP, precipitation (mm).

Table 4 Regressions of climate and mycorrhizal type against ^{15}N enrichment between surface litter and deep soil

Parameter	Value ± SE	P
Adjusted r^2	0.456	< 0.001
Intercept	9.1 ± 1.1	< 0.001
MAT	−0.028 ± 0.057	0.624
MAP	3.1 ± 2.6e-4	0.222
Mycorrhizal type	–	< 0.001
AM	−3.29 ± 0.51	< 0.001
ECM	1.18 ± 0.55	0.037

Climate, soil data, and mycorrhizal type are from Hobbie & Ouimette (2009). Mycorrhizal type is based on dominant vegetation, and classified as arbuscular mycorrhizal (AM) (32), ectomycorrhizal (ECM) (47), and mixed arbuscular/ectomycorrhizal (6) (Supporting Information Table S2). MAT, mean annual temperature (°C); MAP, precipitation (mm).

& Ouimette, 2009). For example, in chronosequences of boreal forests the lowest part of the surficial organic mor layer becomes increasingly enriched in ^{15}N with age (Wallander *et al.*, 2009). In these systems, correlations of ^{15}N with fungal biomass and low pH (limiting nitrification) led the authors to conclude that redistribution of N isotopes resulted from the ectomycorrhizal symbiosis rather than from N losses.

3. Forms of nitrogen

Although isotopic measurements of bulk N are common, measurements of other N forms are scarce. Ammonification discriminates slightly against ^{15}N (Koba *et al.*, 2003), whereas the discrimination against ^{15}N during nitrification and denitrification is larger (Table 1). The relative fluxes of these three processes will accordingly affect the isotopic relationships among bulk soil, ammonium, and nitrate (Houlton *et al.*, 2007). Nitrate is usually 1–6‰ depleted in ^{15}N relative to ammonium in N-limited systems. Such patterns have been reported from coniferous forests (Choi *et al.*, 2005; Takebayashi *et al.*, 2010) and Arctic tundra (Yano *et al.*, 2009). Significant nitrification can lead to ^{15}N enrichment of ammonium relative to the bulk soil and significant further denitrification can lead to ^{15}N enrichment of nitrate relative to ammonium (Koba *et al.*, 1998; Houlton *et al.*, 2007).

The $\delta^{15}N$ of organic forms of N are sometimes measured in soil. In general, dissolved organic N appears similar to bulk N values (Houlton *et al.*, 2007), although Yano *et al.* (2009) reported low $\delta^{15}N$ for dissolved organic N relative to other measured pools in Arctic tundra. According to models and data across soil density gradients (Sollins *et al.*, 2006, 2009), dense, mineral-associated organic N that derived from proteinaceous microbial residues was particularly high in ^{15}N, with a maximum enrichment of 4.8‰ between the lightest and the heaviest mineral fractions (< 1.65 to > 2.55 g cm^{-3}).

IV. Patterns of fungal $\delta^{15}N$

Mayor *et al.* (2009) compiled the available isotopic data on fungi and determined that little relationship existed between climatic variables and N isotope patterns in saprotrophic and ectomycor-

rhizal fungi. Ectomycorrhizal fungi averaged 6.7‰ higher in ^{15}N than saprotrophic fungi. Other studies have reported that litter decay fungi are 1.6–2.4‰ higher in ^{15}N than wood decay fungi (Kohzu *et al.*, 1999; Hobbie *et al.*, 2001). Some of the ^{15}N enrichment of ectomycorrhizal fungi relative to saprotrophic fungi undoubtedly arises because saprotrophic fungi are active higher in the soil profile than ectomycorrhizal fungi (Lindahl *et al.*, 2007), but physiological differences, particularly the transfer of ^{15}N-depleted N from mycorrhizal fungi to host plants (Hobbie & Colpaert, 2003), are also important.

1. Patterns among taxa

Functional attributes may correlate with N isotope patterns in ectomycorrhizal fungi. Lilleskov *et al.* (2002) and Trudell *et al.* (2004) suggested that rhizomorph abundance and $\delta^{15}N$ could be linked, with taxa with thick rhizomorphs (aggregated hyphae for long-distance transport) such as *Cortinarius* and *Tricholoma* generally higher in $\delta^{15}N$ than other taxa. Hobbie & Agerer (2010) analyzed data from two previous studies of sporocarp isotope patterns (Taylor *et al.*, 2003; Trudell *et al.*, 2004) to confirm that N isotopes correlated with functional attributes of ectomycorrhizal fungi; specifically with how they explore the soil (termed exploration type; Agerer, 2006) and with the hydrophobicity of ectomycorrhizas. The ectomycorrhizal exploration types separated into two groups isotopically, with exploration types with hydrophobic ectomycorrhizas averaging 3–4‰ higher in ^{15}N than exploration types with hydrophilic mycorrhizas. In Table 5 we have used the data of Mayor *et al.* (2009) to report average values for ectomycorrhizal fungi by genus, hydrophobicity, and exploration type.

Because soil $\delta^{15}N$ increases with increasing depth, the depth at which taxa obtain their N should also correlate with $\delta^{15}N$ values. Some general patterns in depth distributions of fungal activity have emerged from studies using morphological or genetic characteristics to assess fungal identities of hyphae or mycorrhizas in the soil profile (Landeweert *et al.*, 2003; Rosling *et al.*, 2003; Scattolin *et al.*, 2008). A rigorous comparison between exploration depth and $\delta^{15}N$ has not yet been carried out, although Agerer *et al.* (2012) reported that $\delta^{15}N$ of *Ramaria* correlated with the observed depth of hyphal exploration. In two Swedish studies, hyphae harvested from in-growth bags increased by 1.6‰ from 5 to 10 cm depth (Boström *et al.*, 2007), increased by 3.5‰ from 5 to 15 cm, and increased by 4.6‰ from 5 to 25 cm depth (Wallander *et al.*, 2004). Similarly, ectomycorrhizal root tips increased by 5‰ from the uppermost mor layer to 0–5 cm in the mineral soil (Högberg *et al.*, 1996).

High $\delta^{15}N$ values pose particular difficulties in interpretation, as they are generally higher than any concurrently measured bulk soil pool that could serve as a source. In comparing patterns in $\delta^{15}N$ of soil horizons and the colonization by horizon of different ectomycorrhizal taxa (Lindahl *et al.*, 2007) with the $\delta^{15}N$ of those taxa collected from a nearby site (Taylor *et al.*, 2003), ectomycorrhizal fungi appear to be 5–9‰ enriched in ^{15}N relative to the soil horizons from which they assimilate N (Table 6). The limited data from this study confirm that ectomycorrhizal fungi are active

Table 5 $\delta^{15}N$ for common ectomycorrhizal fungi

Genus	Mean (‰)	n	Hydrophobicity[1]	Exploration type[1]
Laccaria	0.5 ± 0.6	15	Hydrophilic	Short
Inocybe	2.3 ± 0.4	24	Hydrophilic	Short
Hebeloma	2.7 ± 1.1	7	Hydrophobic	Short/medium-fringe
Amanita	3.1 ± 0.5	35	Hydrophilic	Medium-smooth
Russula	3.2 ± 0.3	83	Hydrophilic	Contact/short/ medium-smooth
Lactarius	4.2 ± 0.3	54	Hydrophilic	Contact/ medium-smooth
Dermocybe	4.9 ± 1.4	11	Hydrophobic	Medium-fringe
Boletus	5.8 ± 1.0	17	Hydrophobic	Long
Cortinarius	6.8 ± 0.3	100	Hydrophobic	Medium-fringe
Paxillus	7.1 ± 0.7	7	Hydrophobic	Long
Leccinum	8.1 ± 0.7	19	Hydrophobic	Long
Suillus	8.2 ± 0.7	17	Hydrophobic	Long
Tricholoma	9.3 ± 0.6	35	Hydrophobic	Medium-fringe
Hydnellum	9.4 ± 1.0	6	Hydrophobic	Medium-mat
Tuber	15.1 ± 0.6	9	Hydrophilic	Short
Hebeloma	*c.* 21	2	Hydrophobic	Short/ medium-fringe[2]

Values are ± SE. Nitrogen represents the number of distinct species × site combinations. Global averages are derived from Mayor *et al.* (2009) except *Tuber* values which are from Hobbie *et al.* (2001).
[1]Fungal exploration type and presence of hydrophilic or hydrophobic ectomycorrhizas after Agerer (2006).
[2]Ammonophilic taxa, data from Kohzu *et al.* (1999), classifications from Imamura & Yumoto (2008).

deeper in the soil profile than saprotrophic fungi, with a 2.8‰ difference in $\delta^{15}N$ between the potential N sources for ectomycorrhizal fungi (−1.1‰) and saprotrophic fungi (−3.9‰), with two ericoid mycorrhizal taxa intermediate at −1.9‰. With overall averages for Stadsskogen fungi at 5.8‰ for ectomycorrhizal fungi and 0.8‰ for saprotrophic fungi, this translates into a 6.9‰ enrichment in ^{15}N for ectomycorrhizal fungi and a 4.7‰ enrichment in ^{15}N for saprotrophic fungi relative to the soil horizons where these fungi are present.

Many taxa with high $\delta^{15}N$ values also possess strong proteolytic ability (Lilleskov *et al.*, 2002), so high $\delta^{15}N$ may be a marker of organic N uptake (e.g. *Cortinarius*). In addition, two *Hebeloma* species collected by Kohzu *et al.* (1999) in Okinawa and Japan are classified as ammonophilic fungi (Imamura & Yumoto, 2008), with fruiting often indicating the presence of dead animals or rodent burrows. Thus, the very high values for these two taxa (*c.* 20‰) presumably indicate assimilation of ^{15}N-enriched ammonium after ammonia volatilization. Relative to other ectomycorrhizal taxa, $\delta^{15}N$ values for *Tuber* are also very high. However, *Tuber* spp. primarily colonize the mineral soil (Baier *et al.*, 2006; Buée *et al.*, 2007; Scattolin *et al.*, 2008), where the high $\delta^{15}N$ typical of deep mineral soil horizons (Hobbie & Ouimette, 2009) therefore probably contributes to the high $\delta^{15}N$ value of *Tuber*.

2. Patterns within fungi

Isotopic patterns in different fungal components may provide some insight into mechanisms creating isotopic differences among fungi. Taylor *et al.* (1997) reported that protein and amino acids were *c.* 10‰ enriched in ^{15}N relative to chitin in fungi and also suggested that the higher ^{15}N abundance and %N in caps relative to stipes were related to more protein and amino acids in caps than in stipes. This ^{15}N enrichment probably accounts for other reported patterns within fungi, including ^{15}N enrichment of fungal tissue in ectomycorrhizas relative to extraradical hyphae (Hobbie & Colpaert, 2003) and ^{15}N enrichment in sporocarps relative to extraradical hyphae (Kohzu *et al.*, 2000; Wallander *et al.*, 2004; Boström *et al.*, 2007; Table 7).

V. Biochemical basis for the influence of fungi on $\delta^{15}N$ patterns in plant–soil systems

Kinetic isotopic effects associated with movement of ammonia or amino groups within mycorrhizal fungi and the subsequent transfer to host plants of ammonia or amino acids appear to deplete host

Table 6 Estimated $\delta^{15}N$ signature (±SE) of source nitrogen (N) for different taxa, based on colonization patterns of taxa in different soil horizons and the $\delta^{15}N$ values of those horizons (Lindahl *et al.*, 2007)

Taxa	Source $\delta^{15}N$ (‰)	Sporocarp $\delta^{15}N$ (‰)	Taxa	Source $\delta^{15}N$ (‰)
Ectomycorrhizal			Ericoid mycorrhizal	
Cortinarius spp. (*n* = 5)	−2.2 ± 0.5	6.5	*Capronia* sp.	−2.1
C. armeniacus	−2.5	5.4	*Rhizoscyphus ericae*	−1.6
C. armeratus	−0.6		Saprotrophic	
Dermocybe sp.	−3.7		*Galerina atkinsoniana*	−3.5
D. semisanguinea	−2.5	6.4	*Lophodermium*	−4.2
Hydnellum aurantiacum	1.6	10.6	*L. pinastri*	−3.2
Piloderma sp.	2.4		*Marasmius androsaceus*	−4.6
P. reticulatum	−0.9		*Mycena* sp.	−4.0
Rozites caperatus	1.0	6.6	Averages	
Sebacina sp.	−0.5		Ectomycorrhizal	−1.1 ± 0.5
Thelephorales sp.	0.5		Ericoid mycorrhizal	−1.85 ± 0.25
Phialocephala fortinii	−0.6		Saprotrophic	−3.9 ± 0.2

Data are given in Supporting Information Table S3.

Table 7 ^{15}N and %N difference (designated ε) in culture studies and field studies in ectomycorrhizal fungi and plants

Tissues compared	ε ([15]N (‰))	ε (%N)	References
Culture studies			
Rhizomorphs – shoots	3–5[1], 0–3[2]	–	Högberg *et al.* (1999)
Sporocarps – mycelia	2.7[3]	–	Kohzu *et al.* (2000)
Field studies			
Sporocarps – mycelia	2.8[4]	2.0%	Wallander *et al.* (2004)
Sporocarps – mycelia	0.3, 6.8	–	Clemmensen *et al.* (2006)
Sporocarps – mycelia	0.9, 2.5[5]	–	Boström *et al.* (2007)
Caps – stipes (±SE)	2.1 ± 0.8	2.0 ± 1.0	Taylor *et al.* (1997)
Caps – stipes (ho)[6]	3.2	1.9	Hobbie *et al.* (2012)
Caps – stipes (hi)[7]	1.4	1.5	Hobbie *et al.* (2012)
Gills – stipes[8]	*c.* 6	–	Zeller *et al.* (2007)

[1]Ammonium nutrition; *Pinus sylvestris* with *Suillus bovinus*.
[2]Nitrate nutrition.
[3]Culture study with *Suillus variegatus* and *Pinus densiflora*.
[4]Mycelia from 0 to 10, 10 to 20, and 20 to 30 cm in spruce and mixed species stands.
[5]Sporocarps for seven ectomycorrhizal taxa averaged 6.3‰; mycelia from in-growth bags averaged 3.8 ± 0.5 at 5 cm and 5.4 ± 1.5‰ at 10 cm (± SE). Stand is *Picea abies*.
[6]Hydrophobic ectomycorrhizas.
[7]Hydrophilic ectomycorrhizas.
[8]*Tricholoma sciodes*.

plants in ^{15}N relative to fungal symbionts. This process would be analogous to that presumably causing ^{15}N enrichment of up to 10‰ in N_2-fixing root nodules relative to soybean (*Glycine max*)
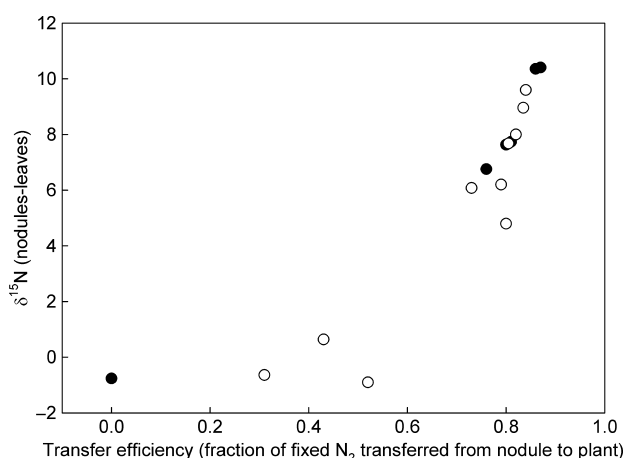
Fig. 2 Enrichment in ^{15}N of root nodules relative to leaves vs the transfer efficiency of nitrogen (N) from root nodules to the rest of the plant. Data are from studies in soybean (*Glycine max*) by Shearer *et al.* (1980) (closed symbols) and Kohl *et al.* (1983) (open symbols).

leaves (Shearer *et al.*, 1980; Kohl *et al.*, 1983; Fig. 2), in which ammonia is the transfer compound. Such processes would also enrich mycorrhizal fungi in ^{15}N relative to saprotrophic fungi, even if source N were similar for these two fungal types. Ammonia is a suspected transfer compound in arbuscular mycorrhizal fungi (Bago *et al.*, 2001), whereas glutamine, glutamate, alanine, and ammonia have all been invoked as potential transfer compounds in ectomycorrhizal fungi (Smith & Smith, 1990; Chalot *et al.*, 2006; Dietz *et al.*, 2011). In the hypothesized glutamine–glutamate shuttle mechanism for N transfer between ectomycorrhizal fungi and plants, the amido group of glutamine may be the source N assimilated by ectomycorrhizal plants (Smith & Smith, 1990), and is the source for N in *N*-acetylglucosamine (Zalkin & Smith, 2006), the monomer of chitin. Thus, ^{15}N depletion of chitin relative to co-occurring protein parallels the ^{15}N depletion of compounds that are subsequently transferred to host plants. The ^{15}N depletion of chitin appears to be a general phenomenon accompanying chitin biosynthesis, as chitin in arthropods, marine invertebrates, or fungi is depleted in ^{15}N relative to muscle, total biomass, or protein by 7–12‰ (Schimmelman & DeNiro, 1986; Macko *et al.*, 1989; Taylor *et al.*, 1997; Webb *et al.*, 1998). Recent work estimated that the ^{15}N enrichment of protein relative to chitin averaged 15‰ for hydrophobic ectomycorrhizal fungi, 10‰ for saprotrophic fungi, and only 7‰ for hydrophilic ectomycorrhizal fungi (Hobbie *et al.*, 2012), with variable isotopic partitioning within a closed system (e.g. Eqn 6) as a plausible mechanism to create these patterns.

VI. Patterns of δ^{15}N in plant and fungal culture studies

To better understand how mycorrhizal fungi influence plant δ^{15}N, several studies have grown plants in symbiosis with arbuscular mycorrhizal or ectomycorrhizal fungi with different N forms and reported the resulting isotopic patterns. The few studies with arbuscular mycorrhizal plants have been difficult to interpret, with no clear indication that arbuscular mycorrhizal fungi are sequestering a ^{15}N-enriched pool or passing ^{15}N-depleted N to host plants (Azcón-G-Aguilar *et al.*, 1998; Wheeler *et al.*, 2000). However, fractionation on uptake cannot be ruled out in these studies, and it is difficult to quantify N retention by arbuscular mycorrhizal fungi. Studies with ectomycorrhizal plants have most commonly used *Pinus sylvestris*. Relative to nonmycorrhizal plants, foliar δ^{15}N declined 0.5–4.6‰ with mycorrhizal colonization, with smaller declines under nitrate supply than under ammonium or ammonium nitrate supply (Table 8). Many ectomycorrhizal fungi appear to assimilate nitrate more slowly than ammonium (Finlay *et al.*, 1992; Keller, 1996), with some transfer of unreduced nitrate to host roots (Ek *et al.*, 1994; Hobbie *et al.*, 2008). The transfer of ^{15}N-depleted N from fungi to plants coupled with N retention by fungi leads to ^{15}N-depleted plants and ^{15}N-enriched fungi. The ^{15}N patterns can therefore provide insight into N partitioning between mycorrhizal fungi and host plants, and also appear to correlate highly with fungal biomass (Fig. 3).

Only two isotopic studies have cultured mycorrhizal plants with organic N forms. In ectomycorrhizal *Betula nana* and ericoid

Table 8 ^{15}N depletion in foliage of mycorrhizal pine vs nonmycorrhizal pine, calculated as $\delta^{15}N_{nonmycorrhizal} - \delta^{15}N_{mycorrhizal}$ in per mil (‰)

| Species/symbiont | Nitrogen form | | | References |
	NH$_4$NO$_3$	NH$_4^+$	NO$_3^-$	
Pinus halepensis[1]	–	2.0	–	Bardin *et al.* (1977)
Pinus densiflora[2]	–	1.7–4.6	–	Kohzu *et al.* (2000)
Pinus sylvestris				
Suillus luteus	0.5[3], 2.8[4]	–	–	Hobbie & Colpaert (2003)
Thelephora terrestris	0.9[3], 1.9[4]	–	–	Hobbie & Colpaert (2003)
Suillus bovinus	–	3.5[3], 1.7[4]	0.5[3], 1.3[4]	Hobbie *et al.* (2008)
Laccaria laccata	–	2.3[3], 3.0[4]	2.2[3], 1.4[4]	Hobbie *et al.* (2008)

[1]Unknown ectomycorrhizal symbionts.
[2]Symbiont, *Suillus variegatus*.
[3]Low nitrogen (N) supply rate.
[4]High N supply rate.
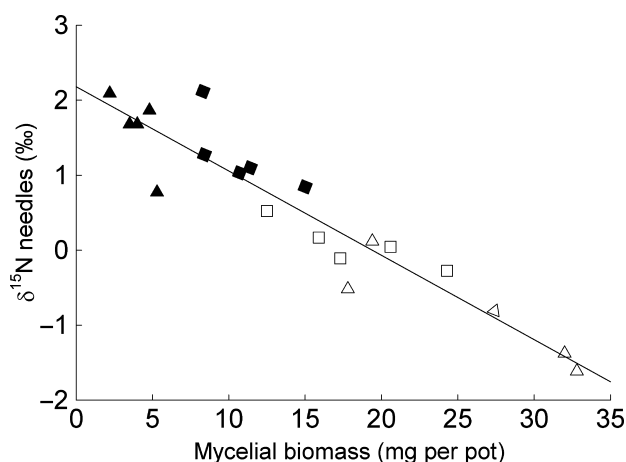
Fig. 3 Fungal biomass correlates strongly with foliar δ^{15}N in mycorrhizal pine ($r^2 = 0.88$; $P < 0.001$). Nitrogen (N) as ammonium nitrate was supplied exponentially at rates of 3% d^{-1} (low N; open symbols) or 5% d^{-1} (high N; closed symbols) to *Pinus sylvestris* cultured with either *Thelephora terrestris* (squares) or *Suillus luteus* (triangles). Nitrogen was the limiting nutrient in both treatments. Data are from Hobbie & Colpaert (2003).

mycorrhizal *Vaccinium vitis-idaea*, only a small proportion of supplied glutamic acid and glycine was assimilated, with ^{15}N fractionation on uptake of at least 5‰ for glutamine, glutamic acid, and glycine (Emmerton *et al.*, 2001a). Similarly, in ectomycorrhizal *Eucalyptus* supplied with glutamine, glutathione, or bovine serine albumin (BSA), < 30% of supplied N was assimilated (estimated graphically; Schmidt *et al.*, 2006). Fractionation for BSA appeared negligible, whereas *Eucalyptus* was actually enriched in ^{15}N relative to the supplied glutathione. To assess whether internal ^{15}N redistributions in the plant–mycorrhizal system differ,

further culture studies with organic N compounds are needed where fungal N retention is quantified and where all supplied N is assimilated.

VII. Mycoheterotrophic and parasitic plants

Mycoheterotrophic plants lack chlorophyll and depend on specific mycorrhizal fungi for supplies of C and N (Leake, 1994). Many of the 400 species are orchids, but some are monotropoid and pyroloid plants (Gebauer & Meyer, 2003; Zimmer *et al.*, 2007). Fully mycoheterotrophic plants were c. 12‰ higher in ^{15}N than autotrophic reference plants (Zimmer *et al.*, 2007), and mycoheterotrophic plants were c. 3–4‰ higher in ^{15}N than their associated ectomycorrhizal fungi (Trudell *et al.*, 2003). Based on consistent ^{15}N enrichment in protein and amino acids relative to chitin in other studies, Trudell *et al.* (2003) suggested that the high δ^{15}N of mycoheterotrophic plants relative to possible fungal symbionts reflected preferential incorporation of protein-derived N vs chitin-derived N. Mycoheterotrophic plants may acquire N by digestion of fungal structures (Smith & Read, 2008), which presumably differs sufficiently from the controlled exchange between mycorrhizal fungi and autotrophic plants for different isotopic patterns to arise. An isotopic mass balance between the 3 and 4‰ enrichment in ^{15}N together with a ^{15}N enrichment of protein relative to chitin of perhaps 10‰ suggests that fungal N is 30–40% chitin. More recently, δ^{15}N patterns in mycoheterotrophic plants associated with either arbuscular mycorrhizal fungi or saprotrophic fungi have been investigated, with mycoheterotrophic plants associated with arbuscular mycorrhizal fungi similar in δ^{15}N to their fungal hosts (Merckx *et al.*, 2010), whereas mycoheterotrophic plants associated with saprotrophic fungi were c. 3‰ depleted in ^{15}N relative to their fungal hosts (Martos *et al.*, 2009; Ogura-Tsujita *et al.*, 2009).

Partial mycoheterotrophy in photosynthetic orchids and pyroloid plants has also been investigated (Gebauer & Meyer, 2003; Hynson *et al.*, 2009). The degree of dependence on heterotrophy for N and C acquisition in partial mycoheterotrophs can be estimated using an isotopic mixing-model analysis with co-existing autotrophs and full mycoheterotrophs as isotopic endmembers. Variation in the δ^{15}N and δ^{13}C among autotrophs, mycoheterotrophs, and partial mycoheterotrophs may accordingly reveal information not only about the sources of N and C, but about the C cost for N uptake in partial mycoheterotrophs.

Parasitic plants that tap into the xylem or phloem of other plants for their N supply provide an interesting isotopic contrast to mycoheterotrophic plants. Autotrophic plant hosts lack the ^{15}N-depleted chitin that can strongly influence internal partitioning of ^{15}N in fungi and, as a consequence, parasitic plants appear similar in δ^{15}N to their plant hosts (within 1‰). Such isotopic measurements were used to determine that *Santalum acuminatum* primarily obtained N from co-occurring N$_2$-fixing legumes in Australian heathlands (Tennakoon *et al.*, 1997) and to determine that *Geocaulon lividum* tapped into the canopy dominants in boreal Alaska (Hobbie *et al.*, 2009).

VIII. Patterns of foliar δ^{15}N in autotrophic plants

1. Global patterns of foliar δ^{15}N in non-N$_2$-fixing autotrophic plants

Researchers have published several compilations of data on foliar δ^{15}N across biomes since 1997. In one survey, foliage from tropical forests averaged 6.5‰ higher than foliage from temperate forests, and tropical soils averaged 8‰ higher than temperate soils (Martinelli *et al.*, 1999). The authors attributed this to a greater availability of N in the tropical systems (which are often limited by phosphorus rather than by N), and hence, larger losses of N through processes that discriminate against ^{15}N. The latter suggestion has been supported by modeling of denitrification in montane tropical rainforests (Houlton *et al.*, 2006). Across a wide range of ecosystems, site-averaged foliar δ^{15}N was negatively correlated with mean annual precipitation, with ecosystems distant from the equator and with high rainfall having particularly low site-averaged foliar δ^{15}N (Handley *et al.*, 1999). The available evidence suggests that plant δ^{15}N broadly reflects soil δ^{15}N, so low δ^{15}N values should reflect the low importance of loss mechanisms of ^{15}N-depleted N$_2$, N$_2$O, and nitrate from soil.

The most complete compilation of data on foliar N isotopes (including 11 000 plants world-wide) was reported by Craine *et al.* (2009). For non-N$_2$-fixing plants, foliar δ^{15}N decreased as precipitation increased (for mean annual temperature > −0.5° C). Importantly, the type of mycorrhiza formed by plants influenced δ^{15}N, with foliar δ^{15}N decreasing in the order nonmycorrhizal (mean ± SE, 0.9 ± 0.2‰) > arbuscular mycorrhizal (−1.1 ± 0.1‰) > ectomycorrhizal (−2.3 ± 0.2‰) > ericoid mycorrhizal plants (−5.0 ± 0.2‰). These values were normalized to a standard value for temperature (13.2°C), precipitation (751 mm yr^{-1}), and N concentration (1.58%). In the database regression of foliar δ^{15}N, 56% of variance could be explained. Of that variance, precipitation explained 14%, temperature and derived variables an additional 14%, mycorrhizal association 29%, and foliar N concentration 44%. Overall, nonmycorrhizal plants were enriched in ^{15}N relative to all mycorrhizal types, suggesting either that the source N for nonmycorrhizal plants was enriched in ^{15}N relative to source N for mycorrhizal plants, or that fractionation during creation of transfer compounds by mycorrhizal fungi resulted in mycorrhizal plants receiving a ^{15}N-depleted N pool. The importance of foliar N concentration as an explanatory variable might reflect a dichotomy between N dynamics in systems with plants of deciduous vs evergreen foliage, but could also reflect correlations among N availability, foliar N concentrations, belowground allocation, relative C allocation to mycorrhizal fungi, and retention of ^{15}N-enriched N belowground (Ågren & Bosatta, 1996; Hobbie, 2006).

These global patterns overestimate isotopic differences among plants of different mycorrhizal type when they co-occur. This is particularly apparent when comparing ericoid mycorrhizal plants against ectomycorrhizal and arbuscular mycorrhizal plants, where site-specific differences are 2‰ less than overall average differences, as shown in Table 9. This pattern presumably arises because N dynamics causing high δ^{15}N in ectomycorrhizal and arbuscular

Table 9 Plant δ^{15}N of differing mycorrhizal types were compared at sites where they co-occur (with paired *t*-tests)

Mycorrhizal types compared	Site-averaged ^{15}N difference (‰)	P	n	Overall ^{15}N difference (‰)
AM – ECM	0.7	0.215	18	1.2
AM – ERM	1.8	0.002	14	3.9
Non – AM	2.3	0.001	21	2.0
ECM – ERM	0.7	0.140	10	2.7
Non – ECM	3.9	0.005	9	3.2
Non – ERM	4.6	0.002	7	5.9

Data are from the Craine *et al.* (2009) database. The number of sites where the specified mycorrhizal types co-occur is given by *n*. Climate-adjusted overall δ^{15}N averages as reported in Craine *et al.* (2009) are also given. AM, arbuscular mycorrhizal; ECM, ectomycorrhizal; ERM, ericoid mycorrhizal; Non, nonmycorrhizal.

mycorrhizal plants (generally, high rates of N cycling) tend to exclude ericoid mycorrhizal plants.

2. Site-specific foliar ^{15}N patterns and the influence of N availability

Many site-specific studies have reported that ectomycorrhizal and ericoid mycorrhizal plants in Arctic, alpine or boreal regions were significantly depleted in ^{15}N relative to co-occurring arbuscular mycorrhizal plants (Schulze *et al.*, 1994; Michelsen *et al.*, 1996, 1998; Hobbie *et al.*, 2005). The very high biomass of ectomycorrhizal fungi that may accompany the N-limited conditions prevalent in these regions could potentially sequester sufficient ^{15}N-enriched N to deplete in ^{15}N the remaining pool of N available for plant transfer. Alternatively, the extensive enzymatic capabilities of ericoid mycorrhizal and ectomycorrhizal fungi may allow their host plants to access fresh, litter-derived N, whereas arbuscular mycorrhizal fungi (and plants) must rely on deeper N that has been enriched in ^{15}N during microbial processing. Comparing tracer ^{15}N labeling studies to natural abundance δ^{15}N patterns to distinguish among N sources from different depths could assess these two competing hypotheses.

Numerous studies have linked changes in foliar δ^{15}N across sites or across time to changes in N availability. Declines over time in foliar δ^{15}N and %N in herbarium specimens have been attributed to increasing N limitation caused by the *c.* 100 ppm rise in atmospheric CO$_2$ over the last 150 yr (Peñuelas & Estiarte, 1997; McLauchlan *et al.*, 2010). Declines (averaging 1‰ in 27 species) in foliar δ^{15}N in free-air CO$_2$ enrichment (FACE) studies were attributed by the authors to either interactions with mycorrhizal fungi or increased assimilation of nitrate in roots (Bassirirad *et al.*, 2003). In a *Pinus strobus* chronosequence in northeastern North America, Compton *et al.* (2007) used an N isotope mass balance approach to conclude that N cycling became tighter during 100 yr of stand development, during which foliar δ^{15}N declined. Declines of 1‰ in δ^{15}N of tree rings and lake sediments also indicated decreased N availability over the last 75 yr of forest development in the northeastern USA (McLauchlan *et al.*, 2007). Such analyses should be restricted to heartwood, as comparing data from old

heartwood vs young sapwood could be easily confounded by differences in metabolic activity.

Long-term records avoid some of the issues with interpreting retrospective analyses such as tree rings or sediment cores. In a study of a boreal pine forest, the $\delta^{15}N$ of needles collected over a period of 35 yr fell by *c.* 3‰ in the control plots (Högberg *et al.*, 2011); in heavily N-fertilized plots the $\delta^{15}N$ of needles increased, but declined after the treatment ended. Strong correlations among the $\delta^{15}N$ of needles, DNA sequences of ectomycorrhizal fungi and a phospholipid fatty acid biomarker attributed to ectomycorrhizal fungi confirmed the role of ectomycorrhizal fungi in determining the $\delta^{15}N$ of the plants. In Hietz *et al.* (2011), increased $\delta^{15}N$ in trees in tropical forests in Panama (leaves) and Thailand (wood) over a 40- to 80-yr period was attributed to anthropogenic N deposition increasing N losses from these ecosystems. After a ploughed agricultural field was planted with ectomycorrhizal pine trees in South Carolina, a 15‰ difference developed over 40 yr between the upper organic layer, which derives from recent aboveground plant litter, and the mineral soil at 35–60 cm depth (Billings & Richter, 2006). The ^{15}N enrichment at depth was attributed to accumulation of ^{15}N-enriched N derived from microbes, including ectomycorrhizal fungi.

3. Using plant $\delta^{15}N$ to estimate carbon flux to mycorrhizal fungi

Because N and C cycling are closely coupled, it is possible to use stoichiometric relationships to mathematically link them. Such stoichiometry is particularly important in mycorrhizal fungi, where the potential N gain from exploring soil must be balanced against both the C cost and the N cost (for chitin and protein) of building the necessary tissues to extend into previously unexploited regions. Under some conditions, plant $\delta^{15}N$ values in mycorrhizal systems may reflect the balance of N between the plant and fungal components of the system (Fig. 1 and Eqns 4 and 5), with the mycorrhizal transfer ratio T_r (the proportion of N taken up by fungi that is subsequently transferred to plant hosts) as the controlling variable. In ectomycorrhizal culture, C allocation to fungi correlates strongly and negatively with plant $\delta^{15}N$ values (Fig. 3). This C allocation can be mathematically expressed as a function of T_r, the plant N supply from mycorrhizal fungi (N_p), the C/N of fungal biomass, and the efficiency (e) with which plant-supplied C is turned into fungal biomass (Hobbie & Hobbie, 2008).

$$C_{fungal} = (1/T_r - 1) \times N_p \times C/N \times e \qquad \text{Eqn 10}$$

The mycorrhizal transfer ratio reflects the C cost to acquire N by mycorrhizal fungi, and is accordingly the key variable determining both C_{fungal} and plant $\delta^{15}N$. This is illustrated in Fig. 4, which expresses the relationship between T_r, plant $\delta^{15}N$, and the plant C cost to acquire a unit of mycorrhizally derived N (C_{fungal}/N_p). The C cost of N acquisition increases as T_r declines, such as under conditions of low N availability in many ectomycorrhizal forests. Hobbie & Hobbie (2006) used equations similar to Eqn 10 and system N isotope patterns to estimate that 8–17% of plant productivity was allocated to mycorrhizal symbionts in a tundra
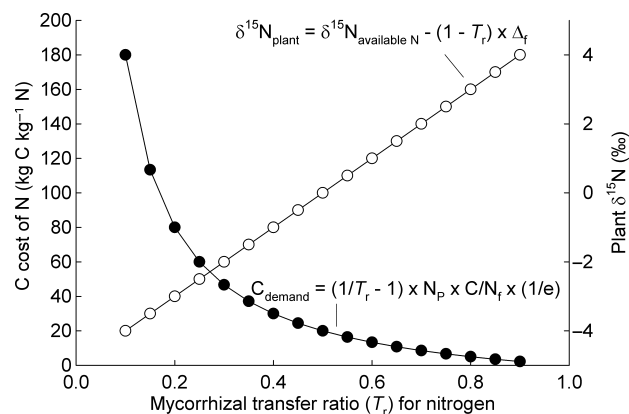

Fig. 4 Carbon (C) cost for nitrogen (N) supply from mycorrhizal fungi to plants (C_{fungal}/N_p) increases as the mycorrhizal transfer ratio (T_r) for N decreases, according to Eqn 10. Fungal C : N is set to 10 and microbial efficiency (e) is set to 0.5. T_r is the proportion of N taken up by fungi that is transferred to host plants. All plant N is assumed to be supplied by mycorrhizal fungi. Variation in plant $\delta^{15}N$ as a function of T_r is also shown, with ^{15}N fractionation during creation of N transfer compounds by mycorrhizal fungi (Δ_f) set at 10‰.

shrub ecosystem. Related equations were used to estimate the fraction of plant N supplied by mycorrhizal fungi.

IX. Controls over plant $\delta^{15}N$

Numerous explanations have been invoked to explain N isotope patterns in plants. One difficulty is that several processes can plausibly account for a given set of isotopic patterns, and it is tempting to explain a specific isotopic pattern by claiming that an unmeasured process is the likely control. Here, we discuss some of the most plausible explanations for patterns of plant $\delta^{15}N$.

1. Rooting depth

Because soils commonly increase in $\delta^{15}N$ with depth, differences in $\delta^{15}N$ are sometimes attributed to differences in rooting depth across species or, less commonly, within a species and across sites. For example, Kohzu *et al.* (2003) correlated rooting depth in different species of mire plants against foliar $\delta^{15}N$, and Hobbie *et al.* (2009) attributed very low $\delta^{15}N$ in *Picea mariana* in Alaska to its association with permafrost, which would restrict foraging to the uppermost litter and soil layers. Unfortunately, little information exists on rooting distributions with depth for most species. An additional difficulty is that rooting distributions are unlikely to correspond closely with uptake depths (as shown for N and other elements by Göransson *et al.*, 2006, 2008), particularly for elements like N whose concentration often declines swiftly with depth.

2. Source differences

Uptake of different forms of N could affect plant $\delta^{15}N$. Ammonium and nitrate are the N forms commonly measured, although a few studies have measured forms of organic N, such as dissolved organic N (Takebayashi *et al.*, 2010), hydrolyzed amino acids

(Bol *et al.*, 2004) or amino sugars (Yano *et al.*, 2009). Because nitrification and denitrification both discriminate against ^{15}N, the relative importance of these two processes controls whether nitrate is higher or lower in $\delta^{15}N$ than ammonium.

Several studies have compared plant $\delta^{15}N$ against measures of nitrate vs ammonium use. In Swedish oak woodlands (Falkengren-Grerup *et al.*, 2004) and in alpine tundra (Miller & Bowman, 2002), greater relative nitrate uptake correlated negatively with foliar $\delta^{15}N$ in herbaceous plants, suggesting $\delta^{15}N_{nitrate} < \delta^{15}N_{ammonium}$, whereas German temperate grass-lands had the reverse pattern, leading Kahmen *et al.* (2008) to conclude that $\delta^{15}N_{nitrate} > \delta^{15}N_{ammonium}$. Denitrification is presumably higher where $\delta^{15}N_{nitrate} > \delta^{15}N_{ammonium}$.

The influence of mycorrhizal fungi on the sources of N available to plants and on the isotopic signature of the N derived from those sources that are ultimately transferred to plants makes estimating the importance of ammonium, nitrate, and organic N as sources very difficult. At sufficiently high concentrations, fractionation against ^{15}N on uptake is an additional factor that is difficult to quantify under field conditions. One possible solution to assess the $\delta^{15}N$ of plant-available N is to use buried cellulose filters as integrators of microbially available N (Hendricks *et al.*, 1997). The cellulose serves as a relatively labile C source for colonizing microbes, which then assimilate available N from the soil. In a ^{15}N labeling study, this approach correlated better with plant $\delta^{15}N$ than using either inorganic N extracted from resin bags or buried bag incubations (Hendricks *et al.*, 2004). Solid samples like cellulose filters are also easier to process than aqueous samples such as ammonium and nitrate, and an integrated signal of available N (including organic N) is assessed, rather than only inorganic N.

3. Fractionation on uptake

Substantial fractionation against ^{15}N on uptake and assimilation is possible if N concentrations are high relative to uptake rates (Handley & Raven, 1992; Fogel & Cifuentes, 1993; Emmerton *et al.*, 2001b). This can be an issue in culture studies, where applied N concentrations are generally higher than in field situations. Therefore, unless N supply is carefully matched to uptake rates, $\delta^{15}N$ patterns in culture have uncertain relevance to field situations, where N supply rates appear generally low. Fractionation on uptake depends on the medium, with diffusion rates and fractionation higher in liquid culture than in agar culture (reviewed in Hobbie & Hobbie, 2008), and also depends on the form of N (generally, diffusion of nitrate > ammonium > organic N).

Fractionation on uptake is generally discounted as a controlling factor for plant $\delta^{15}N$ in field studies, under the assumption that N concentrations at the site of uptake are too low for fractionation to be expressed. However, phosphorus limitation may encourage fractionation against ^{15}N on assimilation (Högberg *et al.*, 1999), as seen in mangroves (McKee *et al.*, 2002) and bogs (Clarkson *et al.*, 2005). Sites with high water tables may also facilitate fractionation on uptake (Kohzu *et al.*, 2003). Field conditions that encourage fractionation against ^{15}N during uptake (such as higher inorganic N concentrations) also favor losses of ^{15}N-depleted N through

ammonia volatilization, nitrate leaching or denitrification, thereby increasing the $\delta^{15}N$ of the remaining N.

4. Mycorrhizal fungi

Mycorrhizal fungi can influence plant $\delta^{15}N$ in several ways. By increasing plant access to recalcitrant and slowly diffusible forms of N, they may alter the average $\delta^{15}N$ of the available N sources. This could include ^{15}N-depleted sources in surficial litter and ^{15}N-enriched sources at greater depths. Their increased surface area and uptake capacities at low external concentrations may also decrease the extent of fractionation on uptake. Finally, as previously discussed, biochemical reactions within fungi may partition N into ^{15}N-enriched and ^{15}N-depleted pools, with plants apparently receiving N primarily from the latter pool.

X. Conclusions and research needs

From the analyses presented in this review, we propose that interactions among plants, mycorrhizal fungi, and soil drive many ecosystem $\delta^{15}N$ patterns, with the type of mycorrhizal association correlating strongly with values in bulk soil, soil profiles, and foliage. By contrast, climatic factors explained little of the observed variance once mycorrhizal associations were accounted for. Although including mycorrhizal fungi in large-scale databases of soil $\delta^{15}N$ increased the fraction of variance explained, overall ability to predict bulk soil $\delta^{15}N$ from climate and mycorrhizal association remained rather poor, indicating that large-scale patterns presumably reflect factors not captured in current statistical models. For foliar $\delta^{15}N$, the large fraction of variance explained by N concentrations should be investigated further. High N availability can increase foliar %N while increasing losses of ^{15}N-depleted N and raising the $\delta^{15}N$ of available N. In addition, high N availability could diminish sequestration of ^{15}N-enriched N in fungal biomass and accordingly diminish the ^{15}N fraction-ation mediated by mycorrhizal fungi between available N and plants.

Colonization by ectomycorrhizal and ericoid mycorrhizal fungi increases plant access to N forms that are poorly available to nonmycorrhizal plants. This N may be ^{15}N-depleted (e.g. litter N) or ^{15}N-enriched (e.g. mineral horizons) relative to that available to nonmycorrhizal plants, but determining the relative contribution from different soil N pools to plant N budgets is not yet generally possible from natural abundance measurements. Low $\delta^{15}N$ in ericoid mycorrhizal plants relative to co-occurring ectomycorrhizal and arbuscular mycorrhizal plants probably arises from ericoid mycorrhizal fungi mainly acquiring N from ^{15}N-depleted litter layers. Consequently, N acquired by ericoid mycorrhizal fungi and plants may be of shallower and more recent origin than N acquired by other plant types.

^{15}N fractionation during creation of transfer compounds by mycorrhizal fungi leads to retention of ^{15}N-enriched N and transfer of ^{15}N-depleted N to the plant symbiont. This ^{15}N fractionation probably occurs in all autotrophic mycorrhizal plants and makes it difficult to directly link plant $\delta^{15}N$ to soil N sources. Transfer compounds may be ammonia or amino acids; ^{15}N fractionation

during amino acid synthesis, ammonia formation from urea, or aminotransferase reactions such as formation of N-acetylglucosamine are possible. Ectomycorrhizal fungi vary widely in their enzymatic capabilities; fungal $\delta^{15}N$ corresponds to the N source (e.g. $\delta^{15}N_{inorganic} < \delta^{15}N_{organic}$, usually), the depth of N acquisition ($\delta^{15}N_{shallow} < \delta^{15}N_{deep}$), and the mycorrhizal transfer ratio, although more work is needed on these factors. Sporocarp $\delta^{15}N$ probably reflects closed-system rather than open-system isotopic fractionation. Partitioning of N between ^{15}N-enriched fungal protein and ^{15}N-depleted fungal chitin appears to be another key control over plant, fungal, and soil $\delta^{15}N$ patterns.

Ecosystem-scale modeling of ^{15}N distributions over time at natural abundance using well-characterized systems (Hobbie *et al.*, 1999; Billings & Richter, 2006) can help to constrain the range of possible scenarios that fit the available data. As one example, the Non-Equilibrium Stable Isotope Simulator (NESIS) model can be used to take output from an element-based model and then predict isotopic signatures for the pools and fluxes in that model (Rastetter *et al.*, 2005).

In another approach, ^{15}N labeling can be followed for multiple years to further constrain the range of possible solutions (Currie & Nadelhoffer, 1999). This approach was used to determine that N from mineral soil was an important N source for trees (Currie *et al.*, 2004). When combined with natural abundance $\delta^{15}N$ data, ^{15}N labeling could further help to distinguish between competing hypotheses. A further approach is to combine isotopes on multiple elements. For example, ^{15}N, ^{18}O and ^{17}O can be measured on nitrate or nitrous oxide to understand the sources and cycling of these important N forms (Pérez *et al.*, 2000; Costa *et al.*, 2011), or parallel measurements on ^{13}C and ^{15}N in soil profiles can be used to trace the coupled cycling of C and N during soil profile development. For example, losses of ^{13}C-depleted methane or ^{15}N-depleted nitrous oxide as major fluxes will disproportionately increase the $\delta^{13}C$ or $\delta^{15}N$ values at specific soil depths favoring those two different processes. Further culture studies on ectomycorrhizal plants other than *Pinus* are needed, particularly of angiosperms, given the potential differences between angiosperms and gymnosperms in reliance on mycorrhizal fungi (Comas & Eissenstat, 2004). Culture studies on arbuscular mycorrhizal plants in which fractionation during mycorrhizal transfer of N from fungi to plants can be assessed are also desirable. Given the probable importance of organic forms of N to plant-available N (Inselsbacher & Näsholm, 2012), both culture studies and field studies should focus on exploring how organic N use in plants and mycorrhizal fungi influences isotopic patterns.

Acknowledgements

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Table S1 Climate, mycorrhizal type of dominant vegetation, and bulk soil δ^{15}N data at 48 sites

Table S2 Climate, mycorrhizal type of dominant vegetation, and ^{15}N enrichment between surface litter and deep soil at 86 sites

Table S3 Data on δ^{15}N by horizon of soil pools and fungal colonization of ectomycorrhizal, ericoid mycorrhizal, and saprotrophic taxa at Jädraås, Sweden

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