New Phytologist

Research

Carbon–nitrogen relations of ectomycorrhizal mycelium across a natural nitrogen supply gradient in boreal forest

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Received: *31 May 2021*
Accepted: *17 August 2021*

New Phytologist (2021) **232:** 1839–1848
doi: 10.1111/nph.17701

Key words: Betsele N supply gradient, boreal forest, carbon limitation, ectomycorrhizal fungi, extramatrical ectomycorrhizal mycelium, natural abundance of ^{13}C and ^{15}N, nitrogen limitation, stable isotope mixing model analysis.

Summary

- The supply of carbon (C) from tree photosynthesis to ectomycorrhizal (ECM) fungi is known to decrease with increasing plant nitrogen (N) supply, but how this affects fungal nutrition and growth remains to be clarified.
- We placed mesh-bags with quartz sand, with or without an organic N (^{15}N-, ^{13}C-labeled) source, in the soil along a natural N supply gradient in boreal forest, to measure growth and use of N and C by ECM extramatrical mycelia.
- Mycelial C : N declined with increasing N supply. Addition of N increased mycelial growth at the low-N end of the gradient. We found an inverse relationship between uptake of added N and C; the use of added N was high when ambient N was low, whereas use of added C was high when C from photosynthesis was low.
- We propose that growth of ECM fungi is N-limited when soil N is scarce and tree belowground C allocation to ECM fungi is high, but is C-limited when N supply is high and tree belowground C allocation is low. This suggests that ECM fungi have a major role in soil N retention in nutrient-poor, but less so in nutrient-rich boreal forests.

Introduction

Many ecosystems dominated by ectomycorrhizal (ECM) trees are characterized by low nitrogen (N) supply and low plant production (Smith & Read, 2008). Numerous studies in ECM boreal forests, in particular, have demonstrated increased plant productivity after N addition (Tamm, 1991; Högberg *et al.*, 2017). However, the physiological responses of ECM fungi to variations in N supply have not yet been fully explored under intact plant–soil conditions in the field (Högberg & Read, 2006; Smith & Read, 2008).

While plant growth is often N-limited, soil microorganisms are commonly considered to be carbon (C)-limited (e.g., Kaye & Hart, 1997; Nazir *et al.*, 2010). The C-limitation also applies to ECM fungi because of their dependence of photosynthates supplied by the tree hosts (Högberg & Högberg, 2002; Zak *et al.*, 2019). Any reductions in this supply may induce C deficiency in these fungi and shifts in biomass, species richness and community composition (Högberg *et al.*, 2001; Ekblad *et al.*, 2013; Lilleskov *et al.*, 2019). The extent to which an N limitation also occurs among soil microorganisms (Schimel & Weintraub, 2003), including ECM fungi, is poorly studied (Hart & Stark, 1997; Ekblad & Nordgren, 2002; Allen & Schlesinger, 2004; Schimel & Bennett, 2004; Camenzind *et al.*, 2020). Thus, the important question remains if the ECM fungi just act as indifferent elongations of the tree root systems, or if their own growth is, like the growth of the trees, also hampered by the exceptionally low N supply in most boreal forests.

Tracer studies report strong microbial N retention in N-poor forest ecosystems (Perakis & Hedin, 2001; Kaiser *et al.*, 2011; Blaško *et al.*, 2013), but is not evident in more nutrient-rich systems (Högberg *et al.*, 2006; Corre *et al.*, 2007). In a large-scale $^{13}CO_2$, ^{15}N-labeling experiment in a boreal *Pinus sylvestris* forest it was shown that ECM fungi exchanged more N per C from the trees after N addition (Näsholm *et al.*, 2013), which indicates that N additions might not only release the N limitation of the trees in forests, but also that of the ECM fungi. This, and the facts that tree belowground C allocation and nutrient supply vary seasonally and spatially (Högberg *et al.*, 2010, 2017) suggests that the ECM symbiosis may be more dynamic and responsive to variations in N supply than formerly believed (Nilsson *et al.*, 2005; Högberg *et al.*, 2011; Lilleskov *et al.*, 2019).

We have previously reported declining abundance of biomarkers of ECM fungi in soil with increasing availability of N across a 90-m-long N supply gradient (e.g., Högberg *et al.*, 2003, 2007; Nilsson *et al.*, 2005; Högberg, 2006). This gradient represents much of the variability in the boreal forest landscape in terms of variations in plant productivity and for example soil pH and C :

N ratio, N forms, N supply rates, and microbial community composition (Högberg *et al.*, 2017). We proposed that the decline in ECM fungi was driven by decreasing tree belowground C allocation to ECM fungi and other soil microorganisms in response to increasing N supply in accordance with plant C allocation theory (Nilsson *et al.*, 2005; Högberg *et al.*, 2007, 2014, 2017; Yarwood *et al.*, 2009).

Here, we focus on the C–N relations and the physiology of the extramatrical ECM mycelium in soil under varying supplies of C and N. We hypothesize that growth of ECM mycelium is stimulated by N addition when tree belowground C allocation of photosynthates is relatively high in response to low N availability, whereas the mycelium is C-limited when tree belowground C allocation is low at high soil N.

Nutrient limitations of organisms is demonstrated by increased growth after addition of the limiting substrate in the absence of competitors (Kaye & Hart, 1997). We quantify natural and induced responses of ECM mycelial production to shifting N supply in boreal forest by combining isotope mixing model analysis with an established fungal ingrowth mesh-bag methodology *in situ,* in which competing roots and saprobic fungi are effectively excluded (Wallander *et al.*, 2001, 2013; Hagenbo *et al.*, 2018). We measure the response of ECM fungal growth to N by comparing the mycelial ingrowth into bags with just quartz sand and bags with extra C and N of known isotopic signatures added. We test if (1) the production of ECM mycelium in N poor forest is N-limited and (2) whether this condition changes and gradually shifts toward C limitation when the natural N supply increases and the tree belowground C allocation decreases.

Materials and Methods

Study site

The site is located northwest of Betsele in northern Sweden (64°39′ N, 18°30′ E, 235 m above sea level). We used a 90-m-long transect (Giesler *et al.*, 1998; Högberg *et al.*, 2017, 2020), through a *c.* 130-yr-old forest, previously used as a model for landscape-scale variations in N supply and plant productivity in Fennoscandian boreal forests (Smith & Read, 2008) (Table 1; Supporting Information Table S1). In this study, we extended the transect length by 10 m at the N-poor end to ensure good representation of N-poor systems; this location is denoted −10 m. Soils are podzols and are classified as Haplic Podzols (FAO, 1988). The mor layer (O horizon) is *c.* 0.05 m thick. The N-poor end is dominated by *Pinus sylvestris* L. trees and ericaceaous dwarf shrubs, then short herbs appear under the canopy of *Picea abies* (L.) H. Karst, but are replaced by tall herbs at the N-rich end. Three forest types (based on the composition of field-layer plants) have been defined along the gradient, a dwarf-shrub (DS) forest type between −10 and 40 m, a short-herb (SH) forest type between 50 and 80 m, and a tall-herb (TH) forest type at 90 m. The field layer shifts from ericoid mycorrhizal plants, to plants with arbuscular mycorrhiza (AM) in the direction from low to high N supply, along with a large decrease in soil fungi and a concomitant increase in bacterial abundance (Table 1). On average, the ground is covered by snow from late October to early May. Mean annual temperature and precipitation are 1.0°C and 570 mm, respectively. The vegetation period lasts for *c.* 5 months from beginning of May through September (Swedish University of Agricultural Sciences, Svartberget Research Station, data from 1990 to 2017).

Table 1 Characteristics of the mor-layer soil along the local natural nitrogen (N) gradient at Betsele in northern Sweden.

Parameter	DS	SH	TH
pH_{H20}	4.0	4.6	5.3
C : N ratio	38.1	22.9	14.9
NH_4-N (μg g^{-1} OM)	4.6	5.2	15.9
NO_3-N (μg g^{-1} OM)	0.9	0.7	3.4
GNMR (kg N ha^{-1} d^{-1})	0.3	1.1	4.1
Immediate $^{15}NH_4^+$ retention (%)	79.0	35.0	23.0
Microbial C : N	11.7	6.9	4.8
Ratio fungi : bacteria	0.44	0.18	0.02

OM, soil organic matter.
Forest types: dwarf-shrub (DS) understorey between 0 and 40 m, short-herb (SH) understorey between 50 and 80 m and a tall-herb (TH) community (at 90 m distance on transect). Soil pH, ammonium nitrogen (NH_4-N), nitrate nitrogen (NO_3-N), and carbon : nitrogen (C : N) data are from Högberg *et al*. (2007), gross N mineralization rate (GNMR) and immediate NH_4^+ retention are from Högberg *et al*. (2006), and microbial data are from Högberg *et al*. (2003).

Quantification of ECM mycelial biomass and nutrient limitation in the field

We followed the so called mesh-bag ingrowth method, which selects for ingrowth of ECM fungal mycelia over that of roots and saprobic fungi (Wallander *et al.*, 2001, 2013; Nilsson & Wallander, 2003; Hagenbo *et al.*, 2018). We used mesh-bags (nylon mesh, 50 μm mesh size, 50 mm × 50 mm × 10 mm in size) each containing 40 g acid-washed quartz sand (0.36–2.0 mm, 99.6% silicon dioxide (SiO_2); Ahlsell, Sweden) as a control treatment. To quantify putative limitations of C and N to the growth of fungi colonizing the mesh-bags, the sand in a separate treatment was amended with an organic N source, the urea analogue methylurea (IUPAC nomenclature, $C_2H_6N_2O$, Merck CAS 598-50-5; Merck, Darmstadt, Germany) corresponding to 0.014% N and 0.012% C by weight, respectively. We choose this substrate because it is not as quickly mineralized in the soil (Praveen-Kumar & Brumme, 1995), and has lower N, but higher C content as compared to urea. This structural analogue to urea act simultaneously as substrate and an inhibitor of urease, which slows down the catalytic activities considerably (Zerner, 1991; Smith *et al.*, 1993; Qin & Cabral, 2002). The methylurea had a distinct isotopic signature. Thus, we used the natural abundances of ^{13}C and ^{15}N and stable isotope mixing model analysis to delineate ECM fungal use of C from tree photosynthates, soil-derived N, and added substrate C and N. Furthermore, ECM fungi show signatures of stable isotope ^{13}C and ^{15}N distinct from other fungi (e.g. Högberg *et al.*, 1999; Henn & Chapela, 2001; Hobbie *et al.*,

2001; Taylor *et al.*, 2003; Chen *et al.*, 2019). Details of the isotope mixing model analysis are shown later in the section on isotope analysis.

Experimental design

On 8 October 2003, five controls and five N-amended bags were laid out at each of eight positions along the gradient (Fig. 1). To avoid some of the spatial autocorrelation among ECM fungi, bags were buried at *c.* 1 m distance from each other (Pickles *et al.*, 2010), perpendicular to the N supply gradient. This was repeated at −10, 0, 30, 40, 50, 60, 80 and 90 m distance on the gradient. To minimize disturbance of the humus layer we made a 5 cm deep cut using a knife. Disposable gloves were used when bags were carefully placed horizontally at the boundary between the humus layer and the mineral soil. The mesh-bags were incubated for two growing seasons to ensure appropriate biomass for subsequent analyses (Wallander *et al.*, 2013). This prolonged incubation time has recently been shown to greatly reduce the contribution of saprobic fungi observed the first months after the installation of mesh-bags (Hagenbo *et al.*, 2018).

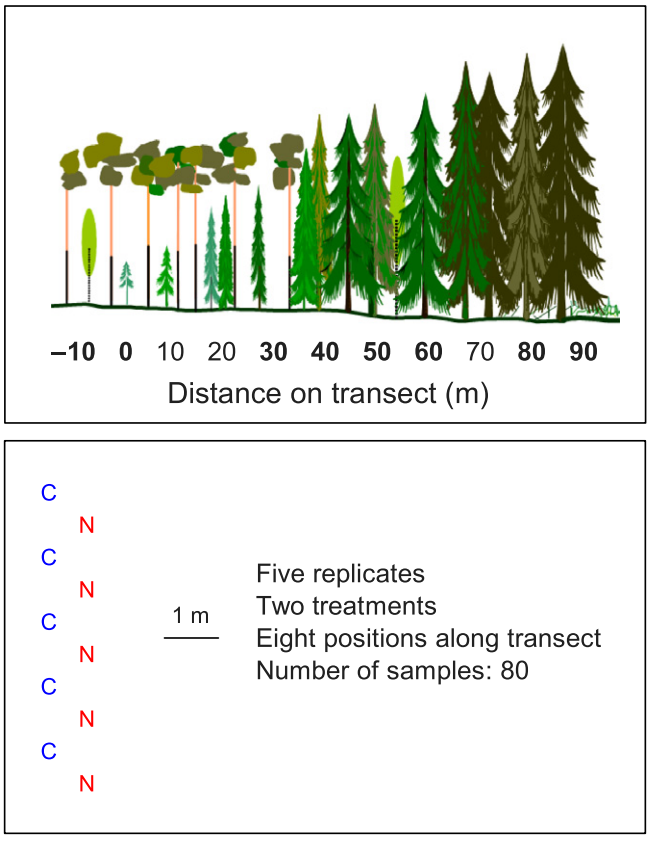

Fig. 1 Layout of the ectomycorrhizal fungal ingrowth experiment at Betsele, northern Sweden. Upper panel: Schematic illustration of the shifting vegetation and distances across the local nitrogen (N) supply gradient. Pine (*Pinus sylvestris* L.) with ericaceous dwarf shrub (DS) understorey between −10 and 40 m was progressively replaced by spruce (*Picea abies* (L.) H. Karst), first with a short-herb (SH) understorey between 50 and 80 m and then with a tall-herb (TH) understorey at 90 m. Lower panel: Mesh-bags were placed perpendicular to the transect at eight positions along the transect as shown in the graph. Treatments are control bags with sand only (blue), and methylurea labeled sand (red). Bar, 1 m.

Harvest of mycelia

The mesh-bags were collected on 17 October 2005 (outdoor temperature 7.5°C) and stored in a refrigerator overnight at 4°C. The bags were opened and a visual estimation of the degree of fungal colonization of the sand was made under a dissecting microscope (Nilsson *et al.*, 2005). The degree of colonization was divided into four classes: 0, no mycelia present; 1, sparse mycelia present; 2, mycelia present, but no aggregation of the sand particles; 3, plenty of mycelia present and some aggregation of the sand particles; 4, plenty of mycelia present and sand particles aggregated to a large extent. For extraction of mycelium water was added to 10 g field-moist sand the mixture was stirred 10 times using a glass rod, and the mixture of floating aggregated mycelia and water was decanted through a funnel equipped with a nylon mesh filter (Wallander *et al.*, 2004). The mycelium was then washed using 3 × 30 ml portions of water. The collected mycelia were observed under a dissecting microscope to ensure effective washing. The mycelium was frozen at −20°C and subsequently lyophilized. This was followed by another round of inspection under the microscope and cleaning out of contaminating particles using forceps. Mycelial biomass was quantified by weighing the dried mycelium (microbalance model XP6; Mettler Toledo, Greifensee, Switzerland). Subsamples of dried mycelia were used to determine the C and N content and isotopic composition (see later). Unless otherwise stated, the mycelial data were corrected to a fungal C content of 45% (Taylor *et al.*, 2003; Wallander *et al.*, 2013; Chen *et al.*, 2019).

Stable isotopic analyses

The C%, $\delta^{13}C$, N%, and $\delta^{15}N$ of fungal mycelium were analysed (Werner *et al.*, 1999) on an isotope ratio mass spectrometer (model DeltaV; Thermo Fisher Scientific, Bremen, Germany) interfaced to an element analyser (model Flash EA 2000; Thermo Fisher Scientific). We report data in ‰, using the δ notation to report deviations from the natural abundance as defined by the Vienna Peedee belemnite (V-PDB) or atmospheric N_2 standards:

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

where $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$. Working standards were wheat and maize flours calibrated against the reference standards, as follows: for N%, we used atropine and NIST 1515 and for $\delta^{15}N$, IAEA-600, IAEA-N-2, USGS40 and USGS41. For C%, we used cyclohexanone, nicotinamide, and sucrose, and for $\delta^{13}C$, IAEA-600, IAEA-CH-6, USGS40. Measurement uncertainty was 0.14‰ for ^{13}C and 0.02‰ for ^{15}N.

By applying isotope mixing model analysis on fungal C and N data from the control and N addition treatments, it was possible to calculate the fungal content of endogenous C and N originating from methylurea as well as that of exogenous C and N, i.e. C

from photosynthate and N from surrounding soil. For these calculations we used the means of control, 'c', and N amended samples, 'm', per distance along the N supply gradient, and the natural abundance of ^{13}C (−49.66 ± 0.08‰) and ^{15}N (−0.07 ± 0.07‰) of methylurea. The C : N of pure methylurea is 0.857. We assumed that fungal biomass C (or N) in substrate amended sand consists of endogenous C (or N) and exogenous C (or N):

$$C_{fungal}(\text{or } N_{fungal}) = C_{endo}(\text{or } N_{endo}) + C_{exo}(\text{or } N_{exo}) \quad \text{Eqn 2}$$

The percentage contributions of endogenous, 'endo', or exogenous, 'exo', C (or N) to total fungal C (or N) were calculated:

$$C_{endo}(\text{or } N_{endo})\% = 100 \times [\delta^{13}C_c(\text{or } \delta^{15}N_c) - \delta^{13}C_m(\text{or } \delta^{15}N_m)/$$

$$\delta^{13}C_{MeU}(\text{or } \delta^{15}N_{MeU}) - \delta^{13}C_c(\text{or } \delta^{15}N_c)] \quad \text{Eqn 3}$$

where 'c' is control treatment, 'm' the methylurea treatment, and 'MeU' stands for pure methylurea.

Thus,

$$C_{exo}(\text{or } N_{exo})\% = 100\% - C_{endo}(\text{or } N_{endo})\% \quad \text{Eqn 4}$$

Statistics

We used the SigmaStat 4.0 software (Systat Software Inc., San José, CA, USA) for all statistics. Data are presented as means ± 1 SE. For comparison between treatments we applied the pairwise *t*-test using population means (N) of up to five replicates (n) for each distance. When a normality test (Shapiro–Wilk) failed, we used the nonparametric Wilcoxon signed rank test. The Pearson correlation coefficient is referred to when data are normally distributed; when the normality test failed, the Spearman rank correlation coefficient is reported. For isotopic mixing model analysis mean values for each position along the transect were used. Significant differences refer to the $P < 0.05$ level.

Results

The two estimates of ECM fungal colonization (visual observation and fungal dry mass per 10 g field moist sand) correlated well ($R^2 = 0.631$, $P < 0.001$, $N = 16$) (Fig. 2a). In the DS forest type (−10 to 40 m), the ECM fungal dry mass was 85.4% higher (paired *t*-test $P = 0.033$, $N = 4$) in the N-amended samples (2.393 ± 0.235 mg) than in the controls (1.291 ± 0.252 mg) (Fig. 2b). This difference was apparent already at the visual observation (Fig. 2c). In the SH forest type (50–80 m) there was no difference in ECM biomass between the treatments, and the same was true in the tall herb forest (90 m), where mycelial dry mass was very low in both treatments. Thus, the control treatment showed a hump shaped pattern, with the highest ECM mycelial colonization in the middle of the N supply gradient.

The ECM mycelial N concentration in control mesh-bags reflected the changes in soil N, i.e. ambient N (Table 1), and

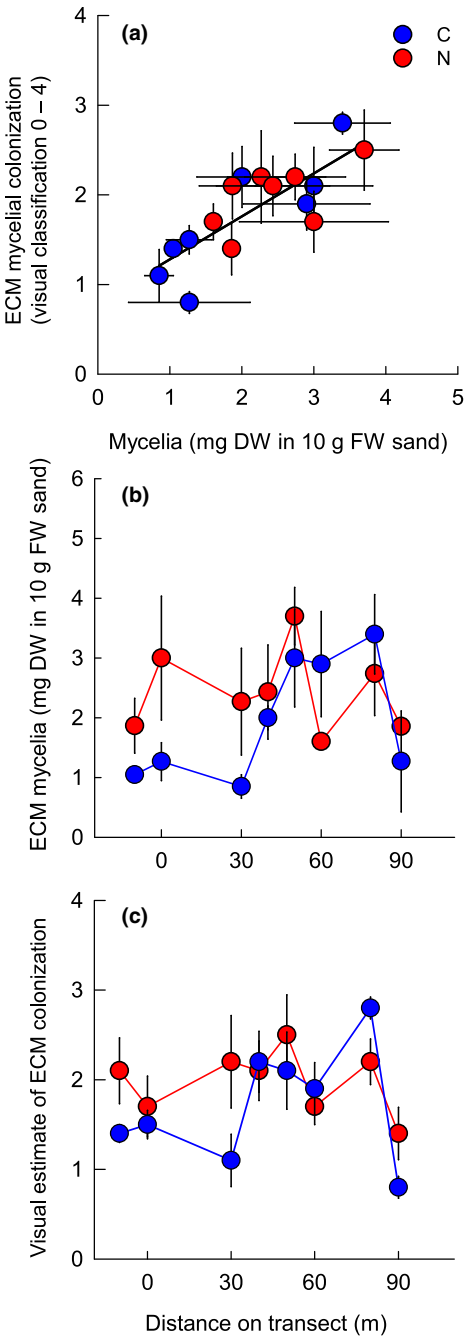

Fig. 2 Relationship between visual observation of colonization degree and biomass of ectomycorrhizal (ECM) mycelia and rhizomorphs along the natural nitrogen (N) supply and productivity gradient at Betsele in northern Sweden. Treatments are controls (blue circles) and organic N amendment (red circles) (a), ECM mycelial biomass (b), and visual estimate of ECM colonization (c). Values are means ± 1 SE, n = 2–5.

increased from 1.3 in the nutrient poor to 4.5% in the nutrient rich end and averaged 2.2 ± 0.4% (Fig. 3a). In the N-amended samples the N concentration varied between 4.7 and 9.8% (mean 6.8 ± 0.7%). Thus, the C : N ratio in control samples declined from 37.6 to 10.1 and averaged 23.6 ± 2.9, whereas in the N-amended samples it was rather stable *c.* 7.6 ± 0.7. (Fig. 3b). Throughout the gradient both the mycelial N concentration and

the C : N ratio differed between the control and the N treatment (paired *t*-test $P < 0.001$, $N = 8$, both variables).

The natural abundance of ^{13}C of ECM mycelia in control mesh-bags increased from −28.5 in the nutrient poor to −26.7 at 80 m and then declined again to −28.6‰ at 90 m in the nutrient-rich end (Fig. 3c), whereas at the same time the $\delta^{15}N$ declined from 5.5 to 0.2‰ (Fig. 3d). The $\delta^{13}C$ and $\delta^{15}N$ averaged −27.5 ± 0.3‰ and 2.8 ± 0.7‰, respectively. The $\delta^{13}C$ of mycelia in N-amended samples varied between −33.6 and −28.3‰ across the gradient (mean −30.5 ± 0.6‰), whereas the ^{15}N abundance was rather constant *c.* 0.3 ± 0.1‰. Both $\delta^{13}C$ and $\delta^{15}N$ differed significantly between treatments (*t*-test $P = 0.008$, $N = 8$ and $P = 0.018$, $N = 6$, respectively). The mycelial $\delta^{13}C$ and $\delta^{15}N$ did not correlate in any of the treatments (Fig. 3e). However, the $\delta^{15}N$ correlated positively to C : N ratio ($R^2 = 0.728$, $P = 0.031$, $N = 6$) in the control treatment (Fig. 3f).

Isotope mixing modelling allowed calculations of ECM mycelial content of endogenous C and N (originating from methylurea) and exogenous C and N sources (fungal C and soil N translocated into mesh-bags). The C : N ratio of the endogenous substrate, the methylurea, was 1.3 ± 0.2. Between 70 to

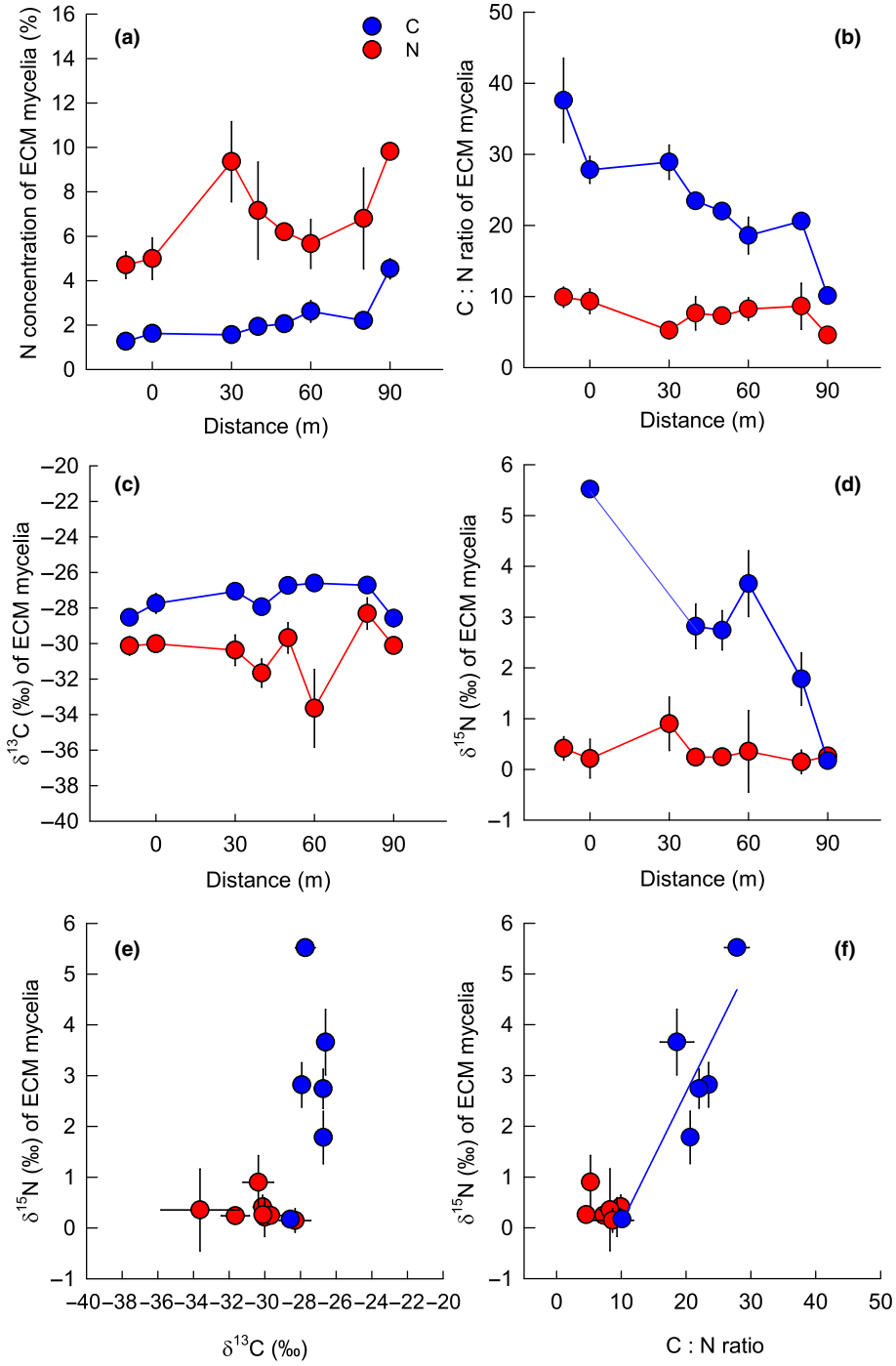

Fig. 3 Carbon (C) and nitrogen (N) relations of ectomycorrhizal (ECM) mycelia and rhizomorphs collected from ingrowth mesh-bags incubated *in situ* along a natural N supply gradient in northern Sweden. Control bags (blue circles) and bags labelled with methylurea (red circles). (a) N content, (b) C : N ratio, (c) $\delta^{13}C$, (d) $\delta^{15}N$, (e) $\delta^{13}C$ vs $\delta^{15}N$, and (f) C : N ratio vs $\delta^{15}N$. Values are means ± 1 SE, n = 1–5.

93% of fungal C originated from exogenous sources, i.e. most likely from plant photosynthate. There was a shift from high exogenous C and endogenic N use (Fig. 4a,d) towards more of endogenous C and exogenous N use as natural N supply increased from the N-poor DS forest type through the intermediate SH forest type to the richer end of the gradient (Fig. 4b,c). The amount of mycelial C correlated with the exogenous C, i.e. imported C ($R = 0.99$, $P = 0.000$, $N = 8$) (Fig. 5a) and endogenous N ($R = 0.90$, $P < 0.05$, $N = 5$, not shown). Thus, assimilation of endogenous N correlated strongly to exogenous C ($R = 0.89$, $P = 0.042$, $N = 5$) (Fig 5b). Mycelial N correlated with exogenous N ($R = 0.98$, $P = 0.004$, $N = 5$) (Fig. 5c). The assimilation of endogenous C did not correlate with any of these variables ($P > 0.05$, $N = 5$).

Discussion

We can draw three conclusions. First, the combined use of control and N-amended fungal ingrowth mesh-bags and isotope mixing model analysis is novel and demonstrates a new window of opportunities to study the nutrition of ECM fungi *in situ*. Second, and most important, we show that the growth of ECM fungi can be limited by the supply of soil N under extremely N-poor conditions. This must aggravate the N limitation experienced by ECM trees under such conditions. Third, the use of C by ECM fungi from an organic N source can be detected and quantified.

Our study focusses on the growth of ECM mycelium in defined substrates along a natural gradient of soil N supply. The patterns in fungal C–N relations we found, thus, relate both to larger-scale natural variations in tree–microbe–soil interactions along the gradient and to the local patches in the N-amended and non-N-amended control mesh-bags. Hence, we tested whether extramatrical mycelia of ECM fungi are C-limited and/or N-limited in environments where plant roots were excluded and competing saprobes (i.e. saprotrophic fungi) were greatly reduced (Hagenbo *et al.*, 2018). The ingrowth of ECM mycelium to the mesh-bags is expected to be context-dependent (Hendricks *et al.*, 2006) at the ecosystem level as well as the nutrient patch level. The experimental design of our study enables insight into this complexity.

Studies *in situ* along this gradient have consistently revealed decreases in two indicators of ECM fungi, the phospholipid fatty acid (PLFA) 18:2ω6,9 and the number of ECM root tips per unit area, in the direction of N-richer conditions (Fig. 6). Ergosterol, another indicator of fungal biomass, also decreases in the same way (Fig. 6). We would like to point out that the soil horizon sampled is dominated by ECM fungi (Lindahl *et al.*, 2007), which suggests that the earlier-mentioned patterns in 18:2ω6,9 and ergosterol are likely reflecting a decline in ECM rather than a decline in fungi in general.

Mycorrhizal fungi are known to respond to nutrient-rich patches by increased growth (Bending & Read, 1995a,b; Leake *et al.*, 2001). Here, this corresponds to the N-amended mesh-bags. The control mesh-bags on the contrary represent an extremely poor substrate. Despite this fact, the mycelial C : N ratio showed extremely broad variation and declined from almost 40 in the N poor end to 10–20 in the richer end of the gradient (Fig. 3b).

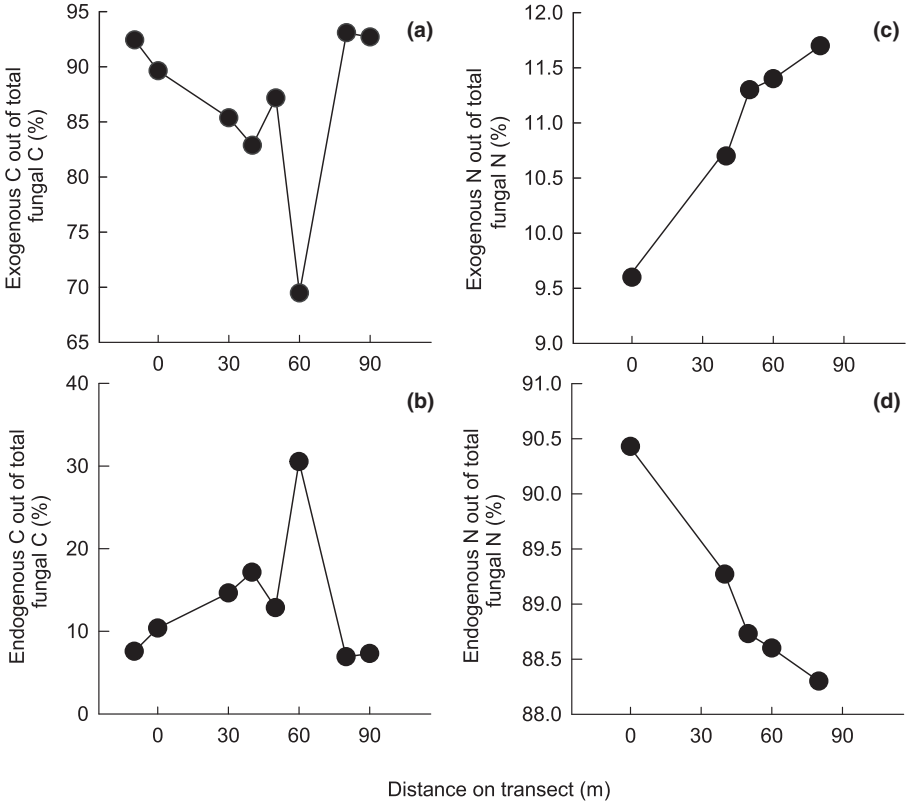

Fig. 4 Outcomes of stable isotope mixing model analysis ectomycorrhizal fungal carbon (C) and nitrogen (N) use along a natural N supply gradient at Betsele in northern Sweden. Percentage exogenous (or endogenous) C and N out of total fungal C and N. (a, c) Exogenous C and N, respectively. (b, d) endogenous C and N. Mycelia were from ingrowth mesh-bags containing sand which was labeled or not labeled with methylurea.

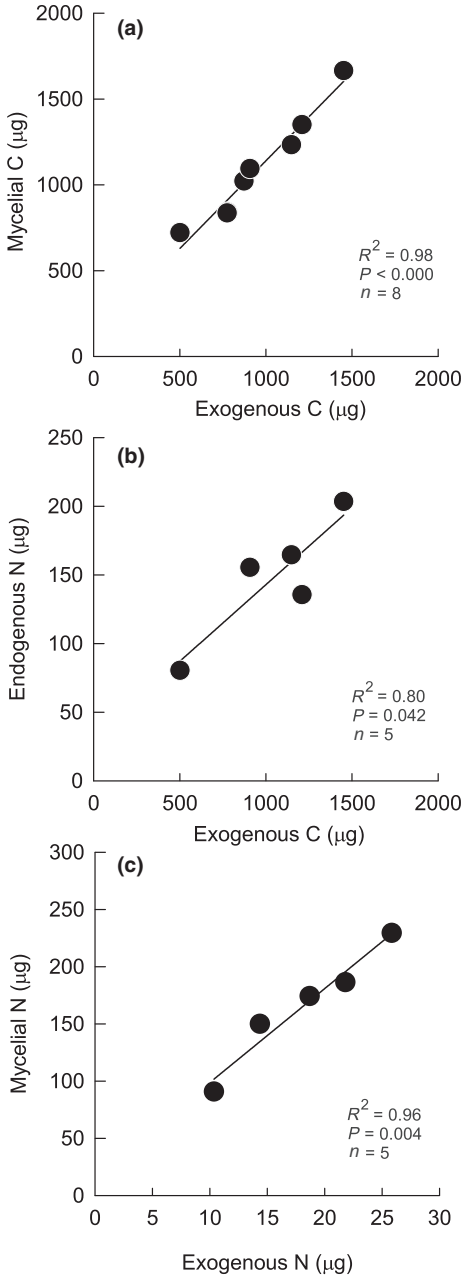

Fig. 5 Outcomes of stable isotope mixing model analysis of ectomycorrhizal fungal carbon (C) and nitrogen (N) use. Relationship between exogenous (imported) C and (a) mycelial C, (b) endogenous N, and between exogenous N and (c) mycelial N.

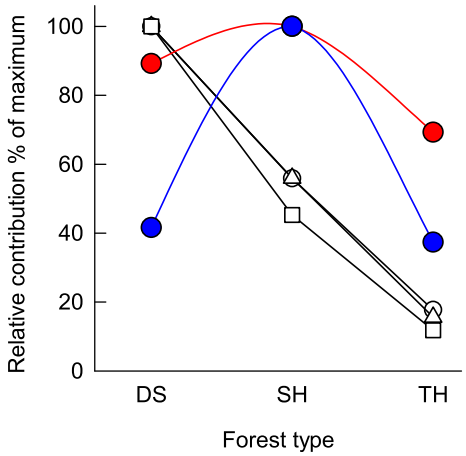

Fig. 6 Summary of relative changes in ectomycorrhizal (ECM) fungal biomass in the dwarf-shrub (DS), short-herb (SH) and tall-herb (TH) forest types along the natural nitrogen (N) supply gradient at Betsele, northern Sweden. The classical estimates of ECM fungal biomass in soil and number of ECM root tips follows the same declining trend with increasing nutrient status, whereas the ECM production in mesh-bags filled with sand peaks at intermediate N supply. Production of ECM extramatrical mycelia out of maximum in control (blue circles) and methylurea labelled mesh-bags (red circles). Data on phosholipid fatty acid 18:2ω6,9 (unfilled circle) and ergosterol (unfilled triangle) biomarkers are from Högberg (2006). Number of ECM root tips per cm^2 (unfilled square) are from Hoffland *et al*. (2003).

Water leaching through the mor-layer may contribute some N to this poor substrate, but as compared to the surrounding soil, the control mesh-bags represent poor spots. Indeed, isotopic mixing-model analysis reveals that between 88.3 and 90.4% of the N in ECM mycelium in N-amended mesh-bags comes from the urea. While including both treatments, the C : N of ECM fungi in this study compares with the wide range found by Zhang & Elser (2017).

A previous study using mesh-bags with (non-N-amended) sand at Betsele found a similar hump shape, with highest ECM mycelial growth in the middle of the gradient, i.e. not at the N-poor end (Nilsson *et al.*, 2005). Thus, this unexpected response has now been found in two studies using mesh-bags along the gradient at Betsele. In both these studies, standing biomass of ECM was clearly highest at the N-poor end according to the PLFA biomarkers. A similar pattern was also observed at the site Varjisån, another natural N supply gradient in boreal forest (Nilsson *et al.*, 2005).

Our findings support the idea of an N-limitation to growth in ECM mycelia at the N-poor end of the gradient. First, we observe an increase in ECM growth in response to N-amendment (in mesh-bags) at the N-poor end (Fig. 2). Second, the mycelium in the bags at this end has a very high C : N ratio (Fig. 3b), i.e. very little N relative to C has been imported from the surrounding soil and mycelium. We interpret this as evidence of both an extremely low availability of N in the mesh-bag substrate and as a low availability of N in the surrounding soil matrix. Thirdly, at this end of the gradient the mycelium in the control bags has a high $\delta^{15}N$ (Fig. 3d), which indicates a low efficiency of transfer of N from the soil through ECM fungi to the plant hosts (Hobbie & Högberg, 2012).

Our finding of an N-limitation to ECM mycelial growth supports the hypothesis of ECM fungi as important immobilizers of N in nutrient-poor boreal forests as demonstrated experimentally by Näsholm *et al.* (1998, 2013), Hasselquist *et al.* (2016) and Högberg *et al.* (2017). At the N-poor end, the immediate retention of $^{15}NH_4^+$ added to the soil is 80%, which compares with 20% at the N-rich end (Table 1). Toljander *et al.* (2006) report from this particular gradient a group of ECM fungal species with a clear preference for the N-poor end, but *Cortinarius* spp.

attributed decomposing and N scavenging activities (Lindahl & Tunlid, 2015), were not in this group. Apparently, N-scavenging by these and other ECM species does not relieve the N limitation of ECM mycelial growth at the N-poor end. The hump shaped pattern of ECM growth found here (Fig. 6) is in line with a study of AM fungi by Treseder & Allen (2002), in which it was concluded that responses of mycorrhizal fungi to local N-amendment (in this study N-amended mesh-bags) varied with the N status of the studied ecosystems.

The signatures of ^{13}C were within the range for ECM fungi and well below the −26 to −21‰ typical of saprobic fungi (Högberg *et al.*, 1999; Henn & Chapela, 2001; Taylor *et al.*, 2003; Hendricks *et al.*, 2006; Chen *et al.*, 2019). We thus confirm that the method applied targeted mycorrhizal fungi. This was further supported by the comparably higher $\delta^{15}N$ values than those expected for saprobes (Taylor *et al.*, 1997, 2003; Chen *et al.*, 2019) (Fig. 3d).

Tree foliage $\delta^{13}C$ collected along the gradient increases towards the N-rich end (Högberg *et al.*, 2005), which fits with the general increase in $\delta^{13}C$ in mycelium in control bags seen here. However, between 80 and 90 m this increase is disrupted as the $\delta^{13}C$ of the mycelium is lower at 90 m (Fig. 3c). We would like to stress that the samples from 90 m contain very little mycelium (Fig. 2), as is expected in a N-rich soil with very little ECM biomarkers and ECM root-tips (Fig. 6).

In the TH forest type, it is possible that the mycelium in non-N-amended mesh-bags contains AM mycelium supported by the rich local flora of AM herbs. AM spores were visible in the mycelium in mesh-bags and PLFA/NLFA (neutral lipid fatty acid) biomarkers of AM were abundant at this location both in mesh-bags and in the soil (Nilsson *et al.*, 2005; Högberg *et al.*, 2007). The comparatively low $\delta^{13}C$ of mycelium at 90 m may be attributed to photosynthate C coming from field-layer AM plants with a low $\delta^{13}C$ growing in the shade of the tall trees (Högberg *et al.*, 2005). Shade plants photosynthesis operates at a higher ratio of internal over external CO_2 concentration, which lowers the $\delta^{13}C$ (Brooks *et al.*, 1997). Moreover, AM spores contain much C in the form of lipids, which commonly are depleted in ^{13}C compared with, e.g. host carbohydrates (DeNiro & Epstein, 1977; Nakano *et al.*, 1999). This may also contribute to the somewhat lower ^{13}C signal. As regard C use, the ECM fungi generally show higher C demands than the AM fungi (Smith & Read, 2008). Hence, intensified colonization of mesh-bags by the latter when tree belowground allocation to C limited ECM fungi is low, might explain the sharp increase in fungal use of exogenous C out of total fungal C and the highest exogenous N use of total fungal N use (Fig. 4a,c). Thus, we propose that the apparent increase in use of exogenous C at this end of the gradient does not reflect the response of ECM fungi.

The consistently lower $\delta^{13}C$ of mycelium in N-amended mesh-bags shows that throughout the gradient, the mycelium assimilated C from the added organic substrate. Isotopic mixing-model analysis suggests that C from methylurea contributes between 7 and 30% of the C in the mycelium in the amended mesh-bags (Fig. 4b). The contribution of urea N to the mycelial biomass was very high (*c.* 90%) (Fig. 4d). We, thus, demonstrate fungal uptake of both C and N from an organic N source.

As was hypothesized, the ECM fungal use of photosynthates and biomass C declined in concert when N supply increased (Fig. 5a). The strong and positive relationship between fungal use of photosynthate and endogenic N (Fig. 5b) lends further support to the idea that ECM fungi become N-limited under high host C supply in response to N poor soil conditions. Not surprisingly therefore, there was a very strong relationship between mycelial biomass N and exogenous N use (Fig. 5c).

Fungi, including ECM fungi, are able to take up and metabolize urea directly (e.g. Mobley & Hinsinger, 1989; Morel *et al.*, 2008), but we cannot exclude the possibility of syntrophy among microbes, where several organisms combine their metabolic capabilities to catabolize substrates, or other interactions (Warmink *et al.*, 2009; Kaiser *et al.*, 2015; Deveau *et al.*, 2018). Perhaps so called mycorrhiza helper bacteria (MHB) are involved (Garbaye, 1994; Frey-Klett *et al.*, 2007). Curiously, in a urease model system, methylurea was found to be catalysed not only to ammonia and CO_2, but also to methylamine in equimolar amounts (Barrios & Lippard, 2001). This one-C–one-N compound can be used as the sole C and N source by many species among methylotrophs, out of which the *Methylocella* (Chen *et al.*, 2010) is referred to as MHB of ECM fungi as are the extremely nutritionally versatile *Pseudomonas* and *Burkholderia* species (Madigan & Martinko, 2006; Frey-Klett *et al.*, 2007).

The earlier-mentioned mechanisms are hypothetical and suggests that more detailed studies should be done. However, we can conclude that ECM fungal use of C and N from the amended organic substrate varied with the natural variations in photosynthate C supply and soil N supply to these fungi. When the rate of photosynthate supply was high in the N poor end of the gradient the fungal supply of N was limiting, whereas as the rate of photosynthate supply to ECM declined while N increased in the N-rich end, the supply of photosynthate C was limiting (Fig. 6). This was supported by higher ECM mycelial biomass in N-amended mesh-bags than in controls in the DS forest type at the nutrient poor end, and the high records of mycelial biomass in the SH forest type under intermediate N supply. A more detailed picture of the interactions could be obtained by using several levels of N and C amendments and by combining molecular and isotopic methods in the analysis of the mycelium.

Acknowledgements

This work was supported by the research councils VR (20145-356) and FORMAS (20160-1658) to MNH and FORMAS (2004-0216) to LON. Authors declare there are no conflicts of interests.

Author contributions

MNH and L-ON planned the research, and performed field and laboratory work. HW suggested the use of methylurea as N additive. MNH analysed the data. MNH and PH wrote the manuscript. All authors commented on the manuscript.

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Data availability

The data that support the findings in this study are available in the text or are presented in the figures.

References

Allen AS, Schlesinger WH. 2004. Nutrient limitations to soil microbial biomass and activity in loblolly pine forests. *Soil Biology and Biochemistry* **36**: 581–589.

Barrios AM, Lippard SJ. 2001. Decomposition of alkyl-substituted urea molecules at a Hydroxide-Bridged Dinickel Center. *Inorganic Chemistry* **40**: 1250–1255.

Bending GD, Read DJ. 1995a. The structure and function of the vegetative mycelium of ectomycorrhizal plants: V. The foraging behaviour of ectomycorrhizal mycelium and the translocation of nutrients from exploited organic matter. *New Phytologist* **130**: 401–409.

Bending GD, Read DJ. 1995b. The structure and function of the vegetative mycelium of ectomycorrhizal plants: VI. Activities of nutrient mobilizing enzymes in birch litter colonized by *Paxillus involutus* (Fr.) Fr. *New Phytologist* **130**: 411–417.

Blaško R, Högberg P, Holm Bach L, Högberg MN. 2013. Relations among soil microbial community composition, nitrogen turnover, and tree growth in N-loaded and previously N-loaded spruce forest. *Forest Ecology and Management* **302**: 319–328.

Brooks JR, Flanagan LB, Buchmann N, Ehleringer JR. 1997. Carbon isotope composition of boreal plants: functional grouping of life forms. *Oecologia* **110**: 301–311.

Camenzind T, Lehmann A, Ahland J, Rumpel S, Rillig MC. 2020. Trait-based approaches reveal fungal adaptations to nutrient-limiting conditions. *Environmental Microbiology* **22**: 3548–3560.

Chen J, Heikkinen J, Hobbie EA, Rinne-Garmston KT, Penttilä R, Mäkipää R. 2019. Strategies of carbon and nitrogen acquisition by saprotrophic and ectomycorrhizal fungi in Finnish boreal *Picea abies*-dominated forests. *Fungal Biology* **123**: 456–464.

Chen Y, Scanlan J, Song L, Crombie A, Rahman MT, Schäfer H, Murrell JC. 2010. γ-Glutamylmethylamide is an essential intermediate in the metabolism of methylamine by *Methylocella silvestris*. *Applied and Environmental Microbiology* **76**: 4530–4537.

Corre MD, Brumme R, Veldkamp E, Beese FO. 2007. Changes in nitrogen cycling and retention processes in soils under spruce forests along a nitrogen enrichment gradient in Germany. *Global Change Biology* **13**: 1509–1527.

DeNiro M, Epstein S. 1977. Mechanism of carbon isotope fractionation associated with lipid synthesis. *Science* **197**: 261–263.

Deveau A, Bonito G, Uehling J, Paoletti M, Becker M, Bindschedler S, Hacquard S, Hervé V, Labbé J, Lastovetsky OA *et al.* 2018. Bacterial–fungal interactions: ecology, mechanisms and challenges. *FEMS Microbiology Reviews* **42**: 335–352.

Ekblad A, Nordgren A. 2002. Is growth of soil microorganisms in boreal forests limited by carbon or nitrogen availability? *Plant and Soil* **242**: 115–122.

Ekblad A, Wallander H, Godbold DL, Cruz C, Johnson D, Baldrian P, Björk RG, Epron D, Kieliszewska-Rokicka B, Kjøller R *et al.* 2013. The production and turnover of extramatrical mycelium of ectomycorrhizal fungi in forest soils: role in carbon cycling. *Plant and Soil* **366**: 1–27.

FAO. 1988. *Food and Agriculture Organization of the United Nations (FAO). FAO/UNESCO Soil Map of the World. Revised legend. World Resources Report.* Rome, Italy: FAO.

Frey-Klett P, Garbaye J, Tarkka M. 2007. The mycorrhiza helper bacteria revisited. *New Phytologist* **176**: 22–36.

Garbaye J. 1994. Helper bacteria: a new dimension to the mycorrhizal symbiosis. Tansley Review, 76. *New Phytologist* **128**: 197–210.

Giesler R, Högberg M, Högberg P. 1998. Soil chemistry and plants in Fennoscandian boreal forest as exemplified by a local gradient. *Ecology* **79**: 119–137.

Hagenbo A, Kyaschenko J, Clemmensen KE, Lindahl BD, Fransson P. 2018. Fungal community shifts underpin declining mycelial production and turnover across a *Pinus sylvestris* chronosequence. *Journal of Ecology* **106**: 490–501.

Hart CS, Stark MJ. 1997. Nitrogen limitation of the microbial biomass in an old-growth forest soil. *Ecoscience* **4**: 91–98.

Hasselquist NJ, Metcalfe DB, Inselsbacher E, Stangl Z, Oren R, Näsholm T, Högberg P. 2016. Greater carbon allocation to mycorrhizal fungi reduces tree nitrogen uptake in a boreal forest. *Ecology* **97**: 1012–1022.

Hendricks JJ, Mitchell RJ, Kuehn KA, Pecot SD, Sims SE. 2006. Measuring external mycelia production of ectomycorrhizal fungi in the field: the soil matrix matters. *New Phytologist* **171**: 179–186.

Henn MR, Chapela IH. 2001. Ecophysiology of ^{13}C and ^{15}N isotopic fractionation in forest fungi and the roots of the saprophytic-mycorrhizal divide. *Oecologia* **128**: 480–487.

Hobbie EA, Högberg P. 2012. Nitrogen isotopes link mycorrhizal fungi and plants to nitrogen dynamics. *New Phytologist* **196**: 367–382.

Hobbie EA, Weber NS, Trappe JM. 2001. Mycorrhizal vs saprotrophic status of fungi: the isotopic evidence. *New Phytologist* **150**: 601–610.

Hoffland E, Giesler R, Jongmans AG, van Breemen N. 2003. Feldspar tunneling by fungi along natural productivity gradients. *Ecosystems* **6**: 739–746.

Högberg MN. 2006. Discrepancies between ergosterol and the phospholipid fatty acid 18:2ω6,9 as biomarkers for fungi in boreal forest soils. *Soil Biology and Biochemistry* **38**: 3431–3435.

Högberg MN, Bååth E, Nordgren A, Arnebrant K, Högberg P. 2003. Contrasting effects of nitrogen availability on plant carbon supply to mycorrhizal fungi and saprotrophs: a hypothesis based on field observations in boreal forest. *New Phytologist* **160**: 225–238.

Högberg MN, Blaško R, Holm Bach L, Hasselquist NJ, Egnell G, Näsholm T, Högberg P. 2014. The return of an experimentally N-saturated boreal forest to an N-limited state: observations on the soil microbial community structure, biotic N retention capacity and gross N mineralization. *Plant and Soil* **381**: 45–60.

Högberg MN, Briones MJI, Keel SG, Metcalfe DB, Campbell C, Midwood AJ, Thornton B, Hurry V, Linder S, Näsholm T *et al.* 2010. Quantification of effects of season and nitrogen supply on tree below-ground carbon transfer to ectomycorrhizal fungi and other soil organisms in a boreal pine forest. *New Phytologist* **187**: 485–493.

Högberg MN, Högberg P. 2002. Extramatrical ectomycorrhizal mycelium contributes one-third of microbial biomass and produces, together with associated roots, half the extractable dissolved organic carbon in a forest soil. *New Phytologist* **154**: 791–796.

Högberg MN, Högberg P, Myrold DD. 2007. Is microbial community composition in boreal forest soils determined by pH, C-to-N ratio, the trees, or all three? *Oecologia* **150**: 590–601.

Högberg MN, Myrold DD, Giesler R, Högberg P. 2006. Contrasting patterns of soil N-cycling in model ecosystems of Fennoscandian boreal forests. *Oecologia* **147**: 96–107.

Högberg MN, Skyllberg U, Högberg P, Knicker H. 2020. Does ectomycorrhiza have a universal key role in the formation of soil organic matter in boreal forests? *Soil Biology and Biochemistry* **140**: 107635.

Högberg P, Ekblad A, Nordgren A, Plamboeck AH, Ohlsson A, Bhupinderpal-Singh HMN. 2005. Factors determining the ^{13}C abundance of soil–respired CO_2 in boreal forests. In: Flanagan LB, Ehleringer JR, Pataki DE, eds. *Stable isotopes and biosphere–atmosphere interactions: processes and biological controls.* San Diego, CA, USA: Elsevier Academic Press, 47–68.

Högberg P, Johannisson C, Yarwood S, Callesen I, Nasholm T, Myrold DD, Högberg MN. 2011. Recovery of ectomycorrhiza after 'nitrogen saturation' of a conifer forest. *New Phytologist* **189**: 515–525.

Högberg P, Näsholm T, Franklin O, Högberg MN. 2017. Tamm Review: on the nature of the nitrogen limitation to plant growth in Fennoscandian boreal forests. *Forest Ecology and Management* **403**: 161–185.

Högberg P, Nordgren A, Buchmann N, Taylor AFS, Ekblad A, Högberg MN, Nyberg G, Ottosson-Löfvenius M, Read DJ. 2001. Large-scale forest girdling shows that current photosynthesis drives soil respiration. *Nature* **411**: 789–792.

Högberg P, Plamboeck AH, Taylor AFS, Fransson PMA. 1999. Natural ^{13}C abundance reveals trophic status of fungi and host-origin of carbon in mycorrhizal fungi in mixed forests. *Proceedings of the National Academy of Sciences, USA* **96**: 8534–8539.

Högberg P, Read DJ. 2006. Towards a more plant physiological perspective on soil ecology. *Trends in Ecology and Evolution* **21**: 548–554.

Kaiser C, Franklin O, Richter A, Dieckmann U. 2015. Social dynamics within decomposer communities lead to nitrogen retention and organic matter build-up in soils. *Nature Communications* **6**: 1860.

Kaiser C, Fuchslueger L, Koranda M, Gorfer M, Stange CF, Kitzler B, Rasche F, Strauss J, Sessitsch A, Zechmeister-Boltenstern S *et al.* 2011. Plants control the seasonal dynamics of microbial N cycling in a beech forest soil by belowground C allocation. *Ecology* **92**: 1036–1051.

Kaye JP, Hart SC. 1997. Competition for nitrogen between plants and soil microorganisms. *Trends in Ecology and Evolution* **12**: 139–143.

Leake JR, Donnelly DP, Saunders EM, Boddy L, Read DJ. 2001. Rates and quantities of carbon flux to ectomycorrhizal mycelium following ^{14}C pulse labeling of *Pinus sylvestris* seedlings: effects of litter patches and interaction with a wood-decomposer fungus. *Tree Physiology* **21**: 71–82.

Lilleskov EA, Kuyper TW, Bidartondo MI, Hobbie EA. 2019. Atmospheric nitrogen deposition impacts on the structure and function of forest mycorrhizal communities: a review. *Environmental Pollution* **246**: 148–162.

Lindahl BD, Ihrmark K, Boberg J, Trumbore SE, Högberg P, Stenlid J, Finlay RD. 2007. Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. *New Phytologist* **173**: 611–620.

Lindahl BD, Tunlid A. 2015. Ectomycorrhizal fungi – potential organic matter decomposers, yet not saprotrophs. *New Phytologist* **205**: 1443–1447.

Madigan MT, Martinko JM. 2006. *Biology of microorganisms.* Upper Saddle River, NJ, USA: Pearson Prentice Hall.

Mobley HL, Hausinger RP. 1989. Microbial ureases: significance, regulation, and molecular characterization. *Microbiological Reviews* **53**: 85–108.

Morel M, Jacob C, Fitz M, Wipf D, Chalot M, Brun A. 2008. Characterization and regulation of PiDur3, a permease involved in the acquisition of urea by the ectomycorrhizal fungus *Paxillus involutus*. *Fungal Genetics and Biology* **45**: 912–921.

Nakano A, Takahashi K, Kimura M. 1999. The carbon origin of arbuscular mycorrhizal fungi estimated from $\delta^{13}C$ values of individual spores. *Mycorrhiza* **9**: 41–47.

Näsholm T, Ekblad A, Nordin A, Giesler R, Högberg MN, Högberg P. 1998. Boreal forest plants take up organic nitrogen. *Nature* **392**: 914–916.

Näsholm T, Högberg P, Franklin O, Metcalfe D, Keel SG, Campbell C, Hurry V, Linder S, Högberg MN. 2013. Are ectomycorrhizal fungi alleviating or aggravating nitrogen limitation to tree growth in boreal forests? *New Phytologist* **198**: 214–221.

Nazir R, Warmink JA, Boersma H, Van Elsas JD. 2010. Mechanisms that promote bacterial fitness in fungal-affected soil microhabitats. *Fems Microbiology Ecology* **71**: 169–185.

Nilsson L-O, Giesler R, Bååth E, Wallander H. 2005. Growth and biomass of mycorrhizal mycelia in coniferous forests along short natural nutrient gradients. *New Phytologist* **165**: 613–622.

Nilsson LO, Wallander H. 2003. Production of external mycelium by ectomycorrhizal fungi in a Norway spruce forest was reduced in response to nitrogen fertilization. *New Phytologist* **158**: 409–416.

Perakis SS, Hedin LO. 2001. Fluxes and fates of nitrogen in soil of an unpolluted old-growth temperate forest, southern Chile. *Ecology* **82**: 2245–2260.

Pickles BJ, Genney DR, Potts JM, Lennon JJ, Anderson IC, Alexander IJ. 2010. Spatial and temporal ecology of Scots pine ectomycorrhizas. *New Phytologist* **186**: 755–768.

Praveen-Kumar, Brumme R. 1995. Alkylated ureas: mineralization and evaluation as N sources. *Fertilizer Research* **41**: 117–124.

Qin Y, Cabral JMS. 2002. Review. Properties and applications of urease. *Biocatalysis and Biotransformation* **20**: 1–14.

Schimel JP, Bennett J. 2004. Nitrogen mineralization: challenges of a changing paradigm. *Ecology* **85**: 591–602.

Schimel JP, Weintraub MN. 2003. The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. *Soil Biology and Biochemistry* **35**: 549–563.

Smith PT, King AD, Goodman N. 1993. Isolation and characterization of urease from *Aspergillus niger*. *Microbiology* **139**: 957–962.

Smith SE, Read DJ. 2008. *Mycorrhizal symbiosis.* Cambridge, UK: Academic Press.

Tamm CO. 1991. *Nitrogen in terrestrial ecosystems.* Berlin, Germany: Springer-Verlag.

Taylor AFS, Fransson PM, Högberg P, Högberg MN, Plamboeck AH. 2003. Species level patterns in ^{13}C and ^{15}N abundance of ectomycorrhizal and saprotrophic fungal sporocarps. *New Phytologist* **159**: 757–774.

Taylor AFS, Högbom L, Högberg M, Lyon AJE, Näsholm T, Högberg P. 1997. Natural ^{15}N abundance in fruit bodies of ectomycorrhizal fungi from boreal forests. *New Phytologist* **136**: 713–720.

Toljander JF, Eberhardt U, Toljander YK, Paul LR, Taylor AFS. 2006. Species composition of an ectomycorrhizal fungal community along a local nutrient gradient in a boreal forest. *New Phytologist* **170**: 873–883.

Treseder KK, Allen MF. 2002. Direct nitrogen and phosphorus limitation of arbuscular mycorrhizal fungi: a model and field test. *New Phytologist* **155**: 507–515.

Wallander H, Ekblad A, Godbold DL, Johnson D, Bahr A, Baldrian P, Björk RG, Kieliszewska-Rokicka B, Kjøller R, Kraigher H *et al.* 2013. Evaluation of methods to estimate production, biomass and turnover of ectomycorrhizal mycelium in forests soils – a review. *Soil Biology and Biochemistry* **57**: 1034–1047.

Wallander H, Göransson H, Rosengren U. 2004. Production, standing biomass and natural abundance of ^{15}N and ^{13}C in ectomycorrhizal mycelia collected at different soil depths in two forest types. *Oecologia* **139**: 89–97.

Wallander H, Nilsson L-O, Hagerberg D, Bååth E. 2001. Estimation of the biomass and seasonal growth of external mycelium of ectomycorrhizal fungi in the field. *New Phytologist* **151**: 753–760.

Warmink JA, Nazir R, Van Elsas JD. 2009. Universal and species-specific bacterial 'fungiphiles' in the mycospheres of different basidiomycetous fungi. *Environmental Microbiology* **11**: 300–312.

Werner RA, Bruch BA, Brand WA. 1999. ConFlo III – an interface for high precision $\delta^{13}C$ and $\delta^{15}N$ analysis with an extended dynamic range. *Rapid Communications in Mass Spectrometry* **13**: 1237–1241.

Yarwood SA, Myrold DD, Högberg MN. 2009. Termination of belowground C allocation by trees alters soil fungal and bacterial communities in a boreal forest. *Fems Microbiology Ecology* **70**: 151–162.

Zak DR, Pellitier PT, Argiroff WilliamA, Castillo B, James TY, Nave LE, Averill C, Beidler KV, Bhatnagar J, Blesh J *et al.* 2019. Exploring the role of ectomycorrhizal fungi in soil carbon dynamics. *New Phytologist* **223**: 33–39.

Zerner B. 1991. Recent advances in the chemistry of an old enzyme, urease. *Bioorganic Chemistry* **19**: 116–131.

Zhang J, Elser JJ. 2017. Carbon:nitrogen:phosphorus stoichiometry in fungi: a meta-analysis. *Frontiers in Microbiology* **8**: 1281.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Table S1 Soil characteristics.

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