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Summary

• A field study was carried out to evaluate the influence of N fertilization on the growth of the external mycelium of ectomycorrhizal (EM) fungi in a Norway spruce forest in SW Sweden.

• Nylon mesh bags filled with sand were buried in the soil for 6–18 months and the ingrowth of mycelium was used as an estimate of EM mycelial growth. Root-isolated, trenched plots were used to estimate background growth of saprotrophic fungi.

• Mycelial growth of EM fungi in N-treated plots was reduced to *c.* 50% of that in nonfertilized plots. Local addition of apatite stimulated the EM mycelial growth in N-treated plots.

• The negative influence of N on the growth of external EM mycelium observed earlier in laboratory studies was confirmed in the present field study. The growth of EM mycelia was not directly related to N concentration in the soil but rather to the N status of the trees, although other factors induced by the N treatment may also have influenced EM mycelial growth.

Key words: ectomycorrhiza, fungi, extramatrical mycelium, nitrogen fertilization, forest soil, nitrate, apatite.

Introduction

Boreal and temperate forests are generally limited by the availability of nitrogen (Tamm, 1991). Ectomycorrhizal (EM) fungi have adapted to these conditions and are efficient in their uptake and subsequent transport of N to tree roots (Smith & Read, 1997). Elevated N levels have a negative impact on the growth of many species of EM fungi; shown both in the field and in laboratory studies, and reviewed by Wallenda & Kottke (1998).

Total number and biomass of EM fruit bodies have been found to decrease in several field studies due to N fertilization of forests (Menge & Grand, 1978; Ohenoja, 1978; Wiklund *et al.*, 1995) or the deposition of airborne N compounds (Termorshuizen & Schaffers, 1991). Some EM species (e.g. *Paxillus involutus* and *Lactarius rufus*) may on the other hand increase their production of fruit bodies in forest soils to which N has been added (Laiho, 1970; Ohenoja, 1978). EM fruit body production does, however, not necessarily reflect the activity of EM root tips and EM extramatrical mycelium (Gardes & Bruns, 1996; Jonsson *et al.*, 2000; Dahlberg, 2001).

EM colonization of root tips tends to be influenced less by the addition of N than fruit body formation (Menge & Grand, 1978; Ritter, 1990; Brandrud, 1995). In some cases the EM colonization of root tips has been reported to be reduced shortly after N fertilization (Menge *et al.*, 1977; Tétrault *et al.*, 1978; Arnebrant & Söderström, 1994). However, recent studies suggest that the main effect of N addition is a shift in the EM fungal community, favoring N-tolerant species, while the frequency of root tips colonized by mycorrhizal fungi remains high following N addition (Kårén & Nylund, 1997; Jonsson *et al.*, 2000; Taylor *et al.*, 2000).

The external mycelium is important in increasing the surface area available for uptake and therefore a reduction in the amount of EM mycelia may reduce the uptake capacity for elements other than N (Read, 1992; Wallenda *et al.*, 2000). The production of extramatrical mycelium by EM fungi is

often reduced in response to N addition in laboratory studies (Wallander & Nylund, 1992; Arnebrant, 1994).

It is difficult to estimate the growth of EM mycelia in the field, because existing methods do not separate fungal mycelia produced by EM fungi from those produced by other groups of fungi. These problems can be avoided by the method recently described by Wallander *et al.* (2001), using fungal ingrowth bags filled with sand. These are buried in forest soil for about 6 months or more and the nylon mesh allows fungal hyphae, but not roots, to enter. Analysis of carbon isotopes revealed that these mesh bags were colonized by mycorrhizal but not saprotrophic fungi (Wallander *et al.*, 2001).

When nitrogen supply exceeds the requirement for the growth of forest trees, other mineral nutrients, for example phosphorus or potassium, may become limiting (Aber *et al.*, 1989). Phosphorus deficiency results in increased allocation of carbon from shoots to roots by trees, while the opposite is true for K (Eriksson, 1995). Laboratory experiments have also shown that the production of extramatrical EM mycelia increased considerably under severe P starvation (Wallander & Nylund, 1992; Ekblad *et al.*, 1995) but decreased under K limitation (Ekblad *et al.*, 1995). Thus, the phosphorus status of forest trees may also influence the production of extramatrical mycelia in the field.

The objective of the present study was to investigate the influence of N fertilization on EM mycelial production under field conditions. We especially wanted to examine whether the decrease in mycelial production by EM fungi earlier found in laboratory studies after N fertilization would also be observed under field conditions. Additionally, we wanted to ascertain whether local amendment using a P containing mineral (apatite) would stimulate EM mycelial production and how this was related to N fertilization of the forest.

Materials and Methods

Site description

The field study was conducted at the Skogaby Experimental Forest in SW Sweden. This former heathland (dominated by *Calluna vulgaris L.*) was planted with Norway spruce (*Picea abies*) in 1966, replacing a first generation of Scots pine planted in 1913. The site is located at an altitude of 95–115 m above sea level, where a maritime climate prevails with a mean precipitation of 1100 mm yr^{-1} and an annual mean temperature of 7.5°C. It has a 200-d vegetation period from the beginning of April to the beginning of November. The soil type is a poorly developed Haplic podzol with a silt loam texture down to 30 cm. The humus layer had an average thickness of 7 cm when examined in 1987 (Bergholm *et al.*, 1994; Nilsson & Wiklund, 1995).

Atmospheric deposition of nitrogen at Skogaby is around 15 kg N ha^{-1} yr^{-1}. In the nitrogen-fertilized plots 100 kg N and 114 kg S ha^{-1} yr^{-1} were added as ammonium sulfate, divided into three applications per year, mainly during June and July. From the start of the treatment (1988) to the first year of our study (1998) 1000 kg N ha^{-1} was added to the N plots. During 1998, fertiliser was applied on May 22nd, June 25th and July 20th; and during 1999 June 3rd, June 21st and July 12th. Four replicates of each N-fertilized and nonfertilized plot were established 1987 and each plot had an area of approximately 2000 m^2 (Bergholm *et al.*, 1994).

Experimental design

To estimate the production of external EM mycelium in the field we followed the method described by Wallander *et al.* (2001). Fungal ingrowth bags (50 × 50 × 10 mm in size) were made of nylon mesh (50 µm mesh size). The bags were filled with 60 g acid-washed sea sand (0.36–2.0 mm, 99.6% SiO_2, Ahlsell, Sweden), or sand mixed with apatite (Kemira, 50–250 µm; to a final apatite concentration of 1% by weight), and sealed.

In order to estimate the background production of mycelium by saprotrophic fungi we established root-isolated, trenched, plots. Here, in the absence of active roots, we assumed EM fungi to have no significant ability to produce mycelia. Plastic tubes (16 cm in diameter, 30 cm in length) were forced into the soil to a depth of 25 cm. Three tubes were inserted into each of the four nonfertilized and N-treated plots at Skogaby by the end of March 1998. The mesh bags were buried at the interface between the organic horizon and the mineral soil, at *c.* 5 cm depth on two occasions, April 5th 1998 and April 14th 1999 (Table 1), one bag inside, and one in the vicinity but outside, each root-isolated plot.

Harvest of fungal ingrowth mesh bags and soil sampling

The mesh bags buried in the spring of 1998 were collected after 6 months, 12 months and 18 months and the mesh bags buried in the spring of 1999 were harvested after 6 months (Table 1). Soil samples were taken from the humus layer in the vicinity outside as well as inside trenched plots in N-treated and in nonfertilized plots in April and October 1999 (Table 1). The samples were stored at −20°C and later used for the analysis of phospholipid fatty acid (PLFA) content. Similar samples were also taken on April 14th 1999 to determine the N content of the humus inside and outside root-isolated plots in N-treated and nonfertilized plots.

Fungal biomass

After harvest of the mesh bags the sand and the sand amended with apatite were observed under a dissecting microscope and the fungal colonization was estimated visually. The degree of colonization was divided into five classes: 0, no mycelia present; 1, sparse mycelia present; 2, mycelia present but no

Table 1 Schedule of the field study to determine the production and content of EM mycelia in N-treated (N) and nonfertilized (C) plots showing mesh bag burial and harvest times and humus soil sampling times and number of replicates. Root-isolated plots were created by inserting plastic tubes into the soil by the end of March 1998. Fertilizer (ammonium sulfate) was applied on May 22nd, June 25th and July 20th 1998, and June 3rd, June 21st and July 12th 1999; 33 kg N ha^{-1} on each occasion

	1998		1999		
	April 5th	October 20th	April 14th	October 13th	October 26th
In-growth mesh bags	1st burial occasion sand + sand w. apatite		2nd burial, sand + sand w. apatite	4 + 4	
C plots	12 + 12		4 + 4		
C plots, root-isolated	12 + 12		4 + 4		
N treated plots	12 + 12		4 + 4		
N treated plots, root-isolated	12 + 12		4 + 4		
Harvest		1st	2nd	3rd	4th
C plots		4 + 4	4 + 4	4 + 4	4 + 4
C plots, root-isolated		4 + 4			
N treated plots		4 + 4	4 + 4	4 + 4	4 + 4
N treated plots, root-isolated		4 + 4			
Duration		6 months	12 months	18 months	6 months
Soil samples					
C plots			4	4	4
C plots, root-isolated			4		
N treated plots			4	4	4
N treated plots, root-isolated			4		

aggregation of the sand particles; 3, plentiful mycelia present and some aggregation of the sand particles; 4, plentiful mycelia present and sand particles aggregated to a large extent.

The sand from the mesh bags was carefully mixed and 10 g of sand were used for the analysis of the PLFA content. The humus samples were treated in the same way as the sand. The samples were stored at −20°C until analyzed. The method of lipid extraction followed that of Frostegård *et al.* (1991). Extracted lipids were fractionated into neutral lipids, glycolipids and polar lipids on silicic acid columns (100–200 mesh, Unisil, Clarkson, Chromatography Products Inc., PA, USA) by successive eluation with chloroform, acetone and methanol. The methanol fraction (containing the phospholipids) was subjected to mild alkaline methanolysis to transform the fatty acids into free methyl esters. These were analyzed on a gas chromatograph with a flame ionisation detector and a 50-cm HP5 capillary column, according the method of Frostegård *et al.* (1993). The PLFA 18 : 2ω6, 9 was used as an indicator of fungal biomass (Frostegård & Bååth, 1996).

To estimate the amount of EM mycelia produced in the in-growth mesh bags and the amount of EM biomass in the humus we subtracted values of fungal biomass obtained inside root-isolated, trenched plots from the values obtained outside. Values from October 1998 (6 months after trenching) were used as estimates of background saprotrophic mycelial production in the mesh bags, because EM mycelium and roots in several cases had entered the tubes on sampling occasions during 1999. For that same reason values from April 1999 (12 months after trenching) were used to estimate the saprotrophic background in the soil samples. In order to calculate the amount of EM biomass in humus samples we assumed that the biological marker for fungi (PLFA 18 : 2ω6,9) that represented the biomass of EM origin, in root-isolated, trenched plots had degraded by 12 months. This is probably a minimum value because all EM mycelia may not have degraded. A conversion factor of 2 μmol of the PLFA 18 : 2ω6,9 g^{-1} fungal biomass (Olsson, 1998) was used to calculate EM biomass from PLFA values.

C : N ratio and carbon isotopic composition of the mycelia

When mycelia were abundantly present in the mesh bags, water was added to a subsample of the sand to make a slurry. Mycelia and rhizomorphs present in the sand floated to the surface and could be collected on a nylon mesh and dried at 70°C for 24 h. The C (both ^{12}C and ^{13}C) and N contents of the mycelia were determined at the Department of Forest Ecology, SLU, Umeå, Sweden, using an online, continuous flow CN analyzer coupled to an isotope mass spectrometer. Results regarding the carbon isotopic composition are expressed in the standard notation ($\delta^{13}C$) in parts per thousand relative to the international standard, Vienna Pee Dee Belemnite (Högberg *et al.*, 1999).

N content in humus samples

Vessels containing 5 g (f. wt) humus and 50 ml 0.2 M $CaCl_2$ were shaken for 1 h on a rotary shaker. The extract was filtered and analyzed for NH_4^+ and NO_3^- by flow-injection analysis

(Falkengren-Grerup *et al.*, 1998). In order to express the N content in relation to the organic matter content, the loss of ignition was estimated by heating soil samples to 600°C for 4 h.

Statistics

The effects of N fertilization on EM colonization of mesh bags, EM biomass in humus samples and on N concentration of the humus and EM mycelia were tested with the Students *t*-test or analysis of the variance (ANOVA). Values from the visual estimations of fungal colonisation were tested statistically with the nonparametric Mann–Whitney *U*-test.

Results

Visual estimation revealed that mesh bags buried in nonfertilized plots were well colonized with mycelia after 6, 12 and 18 months with a mean degree of colonization varying from 3.0 to 3.5 (using the 0–4 classification scale) (Fig. 1a). Significantly less mycelial colonization was observed in N-treated plots at all times (mean degree of colonization between 0.0 and 0.5).

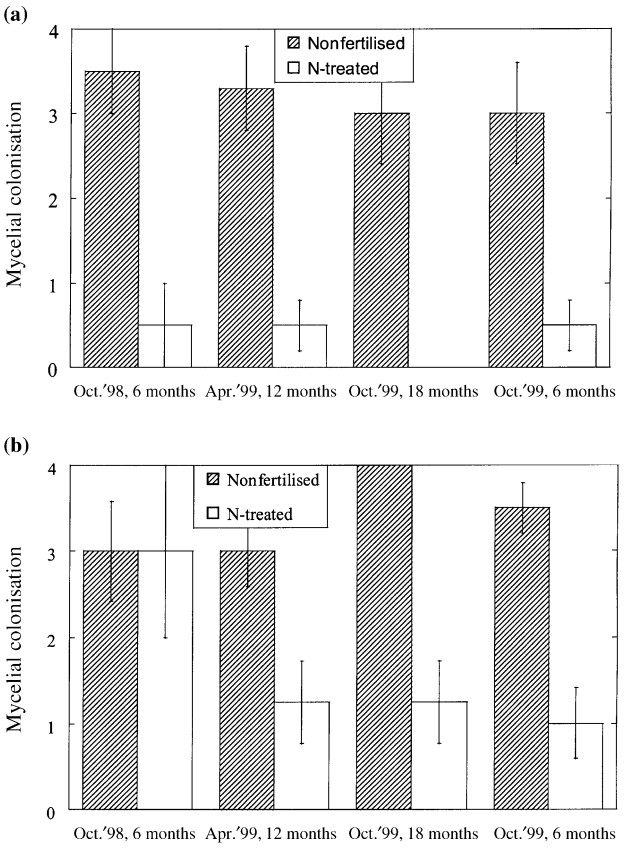

Fig. 1 Fungal colonization of (a) sand and (b) sand amended with apatite (1%) in mesh bags in nonfertilized and N-treated plots based on visual estimation. Bars indicate ± SE, $n = 4$. Mann–Whitney *U*-test: N-treatment (a); $P < 0.001$, apatite amendment N-treated plots (a,b); $P < 0.01$, apatite amendment nonfertilized plots (a,b); (ns).

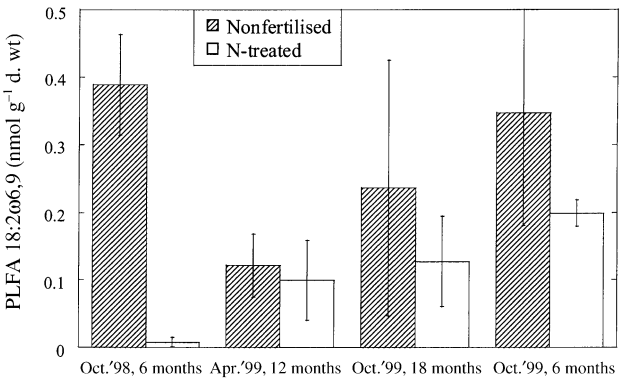

Fig. 2 EM mycelial colonization of sand in mesh bags in nonfertilized and N-treated plots based on the analysis of PLFA 18 : 2ω6,9. Bars indicate ± SE, $n = 4$. (ANOVA: N-treatment; $P = 0.02$, time; ns, N-treatment * time; ns).

Results of the phospholipid fatty acid 18 : 2ω6,9 of EM origin confirmed a significant reduction in the colonization of mesh bags in N-treated compared with nonfertilized plots (Fig. 2) ($P = 0.02$). The mean production of EM mycelia in N-treated plots was on average only 50% of that in the nonfertilized plots. N fertilization appeared to have a greater negative influence on EM mycelial production during 1998 than during 1999. However, the differences between the seasons were not statistically significant and the visual estimation did not reveal any interseasonal differences.

Visual estimation of mesh bags buried in root-isolated, trenched, plots revealed no colonization by fungal mycelia in N-fertilized plots (mean degree of colonisation 0.0 ± 0.0 on all harvesting occasions) and almost no colonization in nonfertilized plots (mean degree of colonization 0.5 ± 0.5 after 6 months and 0.0 ± 0.0 after 12 months). The PLFA 18 : 2ω6,9 was also low in mesh bags collected from trenched plots (0.071 ± 0.017 nmol g^{-1} d. wt in nonfertilised plots and 0.065 ± 0.009 nmol g^{-1} d. wt in N-treated plots after 6 months 1998). However, after 18 months roots and mycelium had entered some of the root-isolated plots, especially the nonfertilized plots where the PLFA 18 : 2ω6,9 contents increased to 0.281 ± 0.032 nmol g^{-1} d. wt (mesh bags buried in 1998) and to 0.471 ± 0.050 nmol g^{-1} d. wt (mesh bags buried in 1999). In N-treated plots PLFA 18 : 2ω6,9 increased 18 months after trenching to 0.220 ± 0.012 nmol g^{-1} d. wt (1998) and 0.217 ± 0.107 nmol g^{-1} d. wt (1999).

The visual estimates of fungal colonization of apatite-amended mesh bags revealed stimulated EM mycelial production by local additions of a P-containing mineral in N-fertilized plots ($P < 0.01$) (Fig. 1a,b). Local addition of apatite had no effect on fungal colonization in nonfertilized plots (Fig. 1a,b).

The proportion of PLFA 18 : 2ω6,9 to total PLFAs that could be attributed to saprophytic fungi in the humus samples

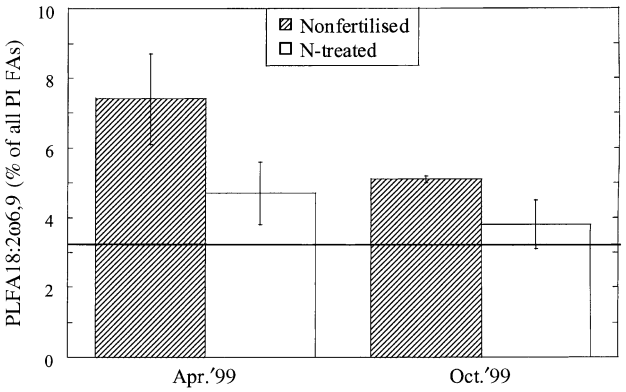

Fig. 3 Fungal biomass in soil samples from nonfertilised and N-treated plots expressed as the phospholipid fatty acid 18 : 2ω6,9 (% of total PLFAs). The horizontal line indicates the background due to saprotrophic fungi obtained in root-isolated plots and the biomass of EM fungi is found above the line. Bars indicate ± SE, $n = 4$ (April 1999), $n = 8$ (October 1999). (ANOVA for EM fungi: N-treatment; $P = 0.06$, time; $P = 0.02$, N-treatment * time; ns).

Table 2 C : N ratio of EM mycelia and rhizomorphs collected from ingrowth mesh bags in nitrogen treated (N) and nonfertilized (C) plots

	C : N ratio	*n*
C plots	20.1 ± 0.8	4
N treated plots	14.8 ± 0.3	2
t-test	$P = 0.013$	

inside the root isolated plots was 3.3 ± 0.02 in nonfertilised plots and 3.2 ± 0.24% PLFA 18 : 2ω6,9 (of total PLFAs) in N-treated plots (Fig. 3). The proportion of PLFA 18 : 2ω6,9 to total PLFAs that could be attributed to EM fungi (additional to the background) in nonfertilized plots in April 1999 was 4.1% (18 : 2ω6,9 of total PLFAs) and in October 1999 1.8% (18 : 2ω6,9 of total PLFAs) (Fig. 3). The EM biomass in humus samples in N-treated plots decreased, although not significantly ($P = 0.06$), to about 35% of that in nonfertilized plots (Fig. 3). Converted to biomass, EM biomass in the humus samples corresponded to *c.* 800 kg ha^{-1} in nonfertilized plots and 300 kg ha^{-1} in N-treated plots in April 1999, and to about 370 kg ha^{-1} and 120 kg ha^{-1}, respectively, in October 1999.

The nitrogen content of mycelia collected from in-growth mesh bags increased in N-treated plots and the C : N ratio was 20.1 ± 0.8 in nonfertilized plots and 14.8 ± 0.3 in N-treated plots (Table 2). The carbon isotopic ($\delta^{13}C$) value was −26.3 ± 0.4 ($n = 4$) in nonfertilized plots and −25.9 ± 0.0 ($n = 2$) in N-treated plots.

The amount of ammonium extracted by 0.2 M $CaCl_2$ was significantly higher in N-treated plots (57.2 μg g^{-1} organic matter, OM) than in nonfertilized plots (4.6 μg g^{-1} OM) when determined in April 1999 ($P < 0.001$) (Table 3). Nitrate concentrations were generally low, but were higher in N-treated plots (1.7 μg g^{-1} OM) than in nonfertilized plots (0.2 μg g^{-1} OM) ($P < 0.001$). Trenching increased the ammonium concentrations in N-treated plots (to 93.3 μg g^{-1} OM) ($P < 0.01$) and in nonfertilized plots (to 68.7 μg g^{-1} OM) ($P < 0.001$). Nitrate levels were unaffected by trenching in N-treated plots (1.5 μg g^{-1} OM), but increased (to 0.8 μg g^{-1} OM) after trenching in nonfertilized plots ($P < 0.001$).

Discussion

Almost all fungal mycelia colonizing the ingrowth mesh bags in this study were of ectomycorrhizal (EM) origin, when calculated as the difference between the fungal biomass in mesh bags collected outside, and those collected inside, the root-isolated, trenched plots. Here, only negligible amounts of fungal mycelia were produced. Furthermore, the carbon isotopic ($\delta^{13}C$) value in mycelia collected from mesh bags confirmed its EM origin, because the values correspond to values found in fruit bodies of ectomycorrhizal fungi in similar forests (Hobbie *et al.*, 1999; Högberg *et al.*, 1999; Wallander *et al.*, 2001).

The present study clearly demonstrates that nitrogen fertilization of a spruce forest caused a significant decrease in the production of external EM mycelium. This decrease may either be an effect of reduced production of external mycelia by individual species or an effect of a changed EM community induced by the N treatment, favoring species that produce lower amounts of external mycelia. The reduction in amounts of EM mycelia produced in N-treated plots, about 50% of the production in nonfertilized plots, was of the same magnitude as that previously found in laboratory studies. In an experiment in a semihydroponics system Wallander & Nylund (1992) found a decrease in the external mycelial biomass

Table 3 NH_4^+ and NO_3^- extracted by 0.2 M $CaCl_2$ from soil samples collected from the organic horizon in nitrogen treated (N) and nonfertilized (C) plots in April 1999. Soil samples were collected both inside and outside root-isolated plots 12 months after trenching of roots. Means ± SE ($n = 12$)

	NH_4^+ (μg g^{-1} OM)	NO_3^- (μg g^{-1} OM)
C plots	4.6 ± 0.4	0.2 ± 0.0
N treated plots	57.2 ± 12.3	1.7 ± 0.2
t-test (treatment)	$P < 0.001$	$P < 0.001$
C plots, root-isolated	68.7 ± 15.1	0.8 ± 0.1
t-test (root isolation)	$P < 0.001$	$P < 0.001$
N treated plots, root-isolated	93.3 ± 10.0	1.5 ± 0.2
t-test (root isolation)	$P < 0.01$	ns

of *Suillus bovinus* to 20% and of *Laccaria bicolor* to 35% with N in excess (100–200 mg N l^{-1}) of that in control (1–10 mg N l^{-1}) 8 wk after N addition started. Arnebrant (1994) found that growth of the mycelium of *S. bovinus* was reduced to 30% and that of *Paxillus involutus* to about 80% of that in control when N was added to the peat substrate at concentrations of 1–4 mg N g^{-1} d. wt.

In our study we found a tendency to a decrease in the amount of EM mycelia in N-treated plots according to phospholipid fatty acid analysis of soil samples. An indirect indication of reduced amounts of fungal mycelium in response to N addition at Skogaby has also been found by Lindberg *et al.* (2001), who noticed a reduction in the abundance of fungivorous collembolans and mites in N-treated compared with nonfertilized plots.

Interestingly, high soil ammonium levels *per se* did not appear to cause any decrease in EM mycelial growth, because EM mycelia and roots entered many of the root-isolated plots after 18 months of trenching (Wallander *et al.*, 2001). This colonization occurred although the inorganic N concentration in these trenched plots was higher than the concentration found in N-treated plots (Table 3), where the growth of EM mycelia was severely inhibited. Thus, it is probably not the N concentration in soil but rather the N status of the trees that regulates growth of EM mycelia. Other factors induced by the N treatment may also have influenced growth of EM mycelia and composition of the EM community, as discussed thoroughly by Kårén & Nylund (1997). From some other studies, designed to evaluate the effect of local patches of high N on mycelial growth in forest soils, conclusions on the importance of tree nutrient status may be drawn. Stober *et al.* (2000) found that hyphal length and density was stimulated by local additions of N in a nitrogen-deficient forest soil, but not in a nitrogen-sufficient site. Moreover, Brandes *et al.* (1998) found increased EM hyphal density when nitrogen and phosphorus were added to mycelial compartments containing sand in a laboratory system with low N availability. On the other hand, Read (1991) found no increase in biomass of EM mycelium in patches with added inorganic N in forest humus in a laboratory system, probably because the humus was originally rather rich in N.

The reduction in EM mycelial production found in our study may, in part, be an effect of reduced growth of mycorrhizal fine roots. Kårén & Nylund (1997) found that the fungal biomass in EM root tips decreased, although not significantly, from 150 kg ha^{-1} in nonfertilized plots to 110 kg ha^{-1} in N-treated plots at Skogaby during 1992 and 1993. However, this was not due to a lower colonization rate by EM fungi, but to reduced biomass of fine roots following the additions of nitrogen. In any case, the more pronounced reduction in EM mycelia found in this study suggests that the influence of N on the production of external EM mycelia is probably much greater than the effect on mycorrhizal short roots (Kårén & Nylund, 1997). This has also been found in laboratory studies (Wallander & Nylund, 1992).

The influence of N fertilization on EM fruit bodies at Skogaby was very rapid and vigorous (Wiklund *et al.*, 1995), as the production of EM sporocarps decreased from 6 kg ha^{-1} y^{-1} (mean values 1989–93) in nonfertilized plots to 0 kg ha^{-1} y^{-1} in N-treated plots. Fruit body production may be a good early indicator of the effects of N on EM fungi, but the sporocarps represent only a small fraction of the EM biomass compared with fine roots and external mycelia (Wallander *et al.*, 2001). In conclusion, under field conditions, N fertilization affects the production of EM fruit bodies drastically; the production of EM mycelia is also severely affected, while the fungal biomass of EM root tips seems to be affected to a lesser extent.

Tree growth was initially stimulated by N fertilization at Skogaby, but after about 8 yr of nitrogen treatment tree growth in these plots started to decline compared with nonfertilized plots. At this time, tree growth was considered to be limited first by P and thereafter probably by K or Mg (Nilsson *et al.*, 2001). Furthermore, Rosengren-Brinck & Nihlgård (1995) found an accumulation of N in old needles in N-treated plots, which they interpreted as an indication of N saturation. Our results support, to some extent, the idea that the limiting factor for tree growth has shifted from N to P, because local addition of apatite stimulated the growth of EM mycelia in N-treated plots (Fig. 1a,b). Hagerberg *et al.* (2003) found a similar increase in colonisation by EM mycelia of apatite-amended mesh bags in a forest with poor P status, while this was not the case in forests with a good P status.

A considerable variation in NO_3 leaching from N-treated plots at Skogaby has been reported for the 2 yr of the present study; 20 mg N l^{-1} in the runoff during 1998 and 2 mg N l^{-1} during 1999 (Bergholm in: Högberg *et al.*, 2001). This coincided with a variation in the production of EM mycelia, although time showed no significant effect in our study. The considerable loss of nitrate from the N-treated plots during 1998 could thus be the result of poor growth of the EM mycelia during this year. Although other factors may be important, the role of EM mycelium in preventing nitrate leaching should also be considered in future studies.

Needle concentrations of Mg and Ca have decreased in N-treated plots to 55–75% that in nonfertilized plots at Skogaby, and are negatively correlated to nitrate leaching (Nilsson *et al.*, 2001). Although this decrease in Mg and Ca concentrations in N-treated plots is probably associated with nitrate leaching, the EM mycelium may also be important for the uptake of Mg and Ca, as suggested by Jentschke *et al.* (2000) and Blum *et al.* (2002).

The technique of using in-growth mesh bags enables determination of nutrient content in naturally occurring forest soil EM mycelia. We found a C : N ratio of about 20 in EM mycelia in nonfertilized plots at Skogaby, and similar C : N ratios are also reported from other forests in south Sweden

(Wallander *et al.*, 2003). The C : N ratio of EM mycelia in ingrowth mesh bags decreased to 15 in N-treated plots at Skogaby. Despite the increase in EM mycelial N content after N fertilization, the EM mycelia contained less nitrogen based on area in N-treated (3.0 kg N ha^{-1}) than in nonfertilized plots (3.8 kg N ha^{-1}), due to the decease in EM mycelial biomass.

In conclusion, for the first time it has been shown that N fertilization of a spruce forest has a negative influence on the mycelial growth of EM fungi in the field. This reduction was not directly related to N concentration in the soil. The N status of the trees is one possible explanation of reduced production of mycelia by EM fungi, but other factors may also be of importance. We found that local amendment with a P-containing mineral may stimulate the growth of EM mycelia in soils in N-treated forests, which have probably moved from being N-limited to P-limited as a result of the N fertilization. Our continued studies on EM mycelia in the field will include investigations of the potential of EM mycelia to retain nitrogen in forests exposed to increased input of N through deposition.

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