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Relationships between fungal uptake of ammonium, fungal growth and nitrogen availability in ectomycorrhizal *Pinus sylvestris* seedlings

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Abstract Nitrogen deposition and intentional forest fertilisation with nitrogen are known to affect the species composition of ectomycorrhizal fungal communities. To learn more about the mechanisms responsible for these effects, the relations between fungal growth, nitrogen uptake and nitrogen availability were studied in ectomycorrhizal fungi in axenic cultures and in symbiosis with pine seedlings. Effects of different levels of inorganic nitrogen (NH_4) on the mycelial growth of four isolates of *Paxillus involutus* and two isolates of *Suillus bovinus* were assessed. With pine seedlings, fungal uptake of ^{15}N-labelled NH_4 was studied in short-term incubation experiments (72 h) in microcosms and in long-term incubation experiments (3 months) in pot cultures. For *P. involutus* growing in symbiosis with pine seedlings, isolates with higher NH_4 uptake were affected more negatively at high levels of nitrogen availability than isolates with lower uptake. More NH_4 was allocated to shoots of seedlings colonised by a high-uptake isolate, indicating transfer of a larger fraction of assimilated NH_4 to the host than with isolates showing lower NH_4 uptake rates. Thus low rates of N uptake and N transfer to the host may enable EM fungi avoid stress induced by elevated levels of nitrogen. Seedlings colonised by *S. bovinus* transferred a larger fraction of the ^{15}N label to the shoots than seedlings colonised by *P. involutus.* Seedling shoot growth probably constituted a greater carbon sink in pot cultures than in microcosms, since the mycelial growth of *P. involutus* was more sensitive to high NH_4 in pots. There was no homology in mycelial growth rate between pure culture and growth in symbiosis, but N uptake in pure culture corresponded to that during growth in symbiosis. No relationship was found between deposition of antropogenic nitrogen at the sites of origin of the *P. involutus* isolates and their mycelial growth or uptake of inorganic nitrogen.

Key words Ammonium · Ergosterol · Extramatrical mycelium · *Paxillus* · *Suillus*

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Introduction

Boreal forest ecosystems are characterised by a high degree of nutrient immobilisation in organic residues, with nitrogen being the predominant growth-limiting nutrient (Tamm 1991). It has long been known that the soil volume exploited by trees is substantially increased by ectomycorrhizal (EM) mycelium radiating out from the mycorrhizal roots (Read et al. 1985; Harley 1989). Frank (1894) first suggested the potential of EM fungi to mobilise organic nutrients and this has recently been recognised (cf. Abuzinadah and Read 1986; Smith and Read 1997; Näsholm et al. 1998). However, the relative contribution of organic and inorganic nitrogen sources utilised by EM fungi remains to be examined. The importance of inorganic nitrogen can be expected to increase in forests exposed to high levels of antropogenic deposition of nitrogen, where the availability of soil nitrogen is increasing. It should be kept in mind that EM fungi are adapted to a have evolved in forest soils characterised by a low availability of nitrogen (Arnolds 1988 Alexander 1983).

Antropogenic nitrogen input to forest ecosystems has been reported to change the composition of ectomycorrhizal species (Arnebrant and Söderström 1994; Kårén and Nylund 1997), reduce amounts of EM root tips (Alexander and Fairley 1983; Arnebrant and Söderström 1994; Kårén and Nylund 1997; Menge and Grand 1977), decrease amounts of EM fruit bodies, and reduce the diversity of EM species (Arnolds 1991). Observed changes in EM fruit-body production probably reflect alterations in the fitness of EM species as a re-

sult of changes in soil nitrogen. For example, the production of sporocarps of *P. involutus* is commonly enhanced in areas receiving large inputs of N (Hora 1959; Ohenoja 1978; Shubin 1988), whereas sporocarps of most *Cortinarius* species decline (Brandrud 1995).

Availability of inorganic nitrogen and phosphorus has been shown to be important factors in regulating mycelial growth in symbiotic associations (Wallander and Nylund 1992; Ekblad et al. 1995). Wallander and Nylund (1992) demonstrated that growth of extramatrical mycelium of *Laccaria bicolor* in symbiosis with *Pinus sylvestris* seedlings was inhibited when the nitrogen concentration was increased from 50 to 200 mg l^{-1} in the nutrient solution added. Interestingly, mycelial growth resumed as soon as the nitrogen level was reduced to the lower level. Similar results were obtained by Arnebrant (1994), who grew *P. sylvestris* seedlings in microcosms colonised by five different isolates of ectomycorrhizal fungi. Wallander (1995) argued that such reduced mycelial growth resulted from a change in the pattern of carbon allocation in the fungi, with more carbon being allocated to nitrogen assimilation at the expense of fungal growth. Under such conditions, available carbon would be used as energy and to form carbon skeletons for the assimilation of ammonium to amino acids. This could explain why fungal growth rapidly resumed once N had been reduced to low levels. EM fungi with high nitrogen uptake rates would then require more carbon for nitrogen assimilation. Colpaert et al. (1992) demonstrated that EM fungi retained nitrogen in the extramatrical mycelium, which induced N deficiency symptoms in the associated seedlings.

In the present study, four isolates of *P. involutus* were collected in forests from regions differing in antropogenic N deposition. This species was chosen since it occurs in forests with low as well as high N deposition. Since the mycelial growth of *S. bovinus* is reported to be more sensitive to elevated N availability than *P. involutus* (Arnebrant 1994), two isolates of this fungus were included in some experiments. The objectives of the study were to assess intraspecific differences in (1) mycelial growth and (2) uptake of nitrogen by *P. involutus* and *S. bovinus* isolates growing in axenic cultures and in symbiosis with pine seedlings at different levels of NH_4 availability. Further, we studied partitioning of nitrogen between shoot and root in the colonised seedlings. We tested the prediction that elevated levels of nitrogen availability have a more inhibiting effect on fungal growth of EM isolates characterised by (a) low nitrogen uptake than those with a high capacity for nitrogen uptake or (b) low transfer of assimilated nitrogen to the host than those with a high capacity for N transfer to the host.

The effect of nitrogen on mycelial growth was also studied in axenic cultures and compared to results obtained in symbiosis, in order to test the screening of the higher number of EM isolates possible in axenic cultures. Corresponding results would allow axenic selection of isolates with a broad spectrum of nitrogen uptake rates which could then be tested in symbiotic association.

Material and methods

Plant and fungal material

Ectomycorrhizal *P. sylvestris* seedlings were obtained by growing the fungus and 4-week-old seedlings in Petri dishes filled with peat and vermiculite soaked in Modified Melin Norkrans medium, according to the method described by Duddridge (1986) as modified by Finlay (1989). After 6 weeks, the seedlings colonised by the introduced EM fungi were used in experiments 1 and 2 described below. Four isolates of *P. involutus* and two of *S. bovinus* were used (Table 1), all originating from sporocarp collections. Three of the *P. involutus* isolates and one *S. bovinus* isolate were collected in *P. sylvestris* forests in northern Sweden with low loads of antropogenic N deposition. Two *P. involutus* isolates were from areas in southern Sweden with a high antropogenic-N load, and one isolate was from a coal waste tip in Scotland with an N deposition load of 8 kg/ha. One *S. bovinus* isolate came from the culture collection at the department of Animal and Plant Science, University of Sheffield, UK.

Experiment 1

EM seedlings colonised by four isolates of *P. involutus* were planted in small perspex microcosms (12 cm × 12 cm) prior to the nitrogen treatments, with untreated (unsterile) sphagnum peat ($pH_{(H_2O)}$ 4.0) as substrate. Once the mycelium covered all of the peat surface (3 weeks), the microcosms were transferred to larger ones (20 cm × 20 cm) with a latex barrier 3 cm from the bottom to facilitate nitrogen uptake experiments. Peat amended with 0, 1 or 4 mg N g^{-1} (dry wt.) of $(NH_4)_2SO_4$ was added to cover the area around the small chambers. Seven replicate microcosms for each isolate were used for each treatment. Unamended peat was added to the area below the barrier in all microcosms.

Throughout the synthesis period and during the experiment, the microcosms were kept in propagators inside growth cabinets at approximately 300 μmol m^{-2} s^{-1} PAR, with a 18 h/6 h 18 °C/15 °C day/night cycle. Growth of the mycelial front, which was recorded weekly, continued until it had reached the barrier at the lower end of the microcosm. Initial colonisation was delayed to various degrees, as a result of which some fungi reached the edge of the perspex window after 3 weeks, while other isolates needed up to 8 weeks. Therefore the increase in area covered between 7 and 14 days was used as an estimate of mycelial growth rate during the exponential growth phase for all isolates. To estimate the area covered by mycelium at the different sampling occasions, the perspex lid of the microcosms was photocopied and the image of the mycelium cut out, weighed and transformed into area.

To study the fungal uptake of NH_4, 3 ml of a 200 mg N l^{-1} solution of $^{15}(NH_4)_2SO_4$ (99 atom% excess) was supplied to the partitioned area of peat where only fungal mycelium was present. No diffusion of ^{15}N was possible across the latex barrier. The perspex microcosms were incubated for 72 h under the light conditions of the previous treatment, to allow for uptake and translocation of the labelled N. At harvest, the seedlings were divided into EM root tips, residual roots, and shoots. Each fraction was lyophilised, the dry weights measured and the total ^{15}N content determined. Available NH_4 in the peat at harvest was determined by extracting the peat with 1 M KCl for 2 h on a rotary shaker. NH_4 was analysed by flow injection analysis at the Department of Plant Ecology, Lund University.

Experiment 2

Ectomycorrhizal seedlings (3–4 replicates) colonised by four *P. involutus* isolates, two *S. bovinus* isolates or non-mycorrhizal see-

Table 1 Origin of fungal isolates used in the study (*Pi Paxillus involutus, Sb Suillus bovinus*)

Designation of EM isolates	Location and coordinates	Description of forest	Approximate N deposition (kg ha^{-1})
Pi 1	Månskogstjärn, northern Sweden 65°35′N, 18°40E	100-year-old *Pinus sylvestris* forest	1
Pi 2	Billingen, southern Sweden 58°22′N, 13°45′E	75- to 125-year-old *Picea abies* dominated forest; ground layer dominated by *Vaccinium myrtillus*	5–10
Pi 3	Gårdsjön, southern Sweden 58°04′N, 12°01′E	80-year-old *Picea abies* forest; ground layer dominated by *V. myrtillus*	15–20
Pi 4	Midlothian, Scotland (isolate 87.017 culture collection Lund University)	15- to 30-year-old *Betula pendula* forest	8
Sb 1	Månskogstjärn northern Sweden 65°35′N, 18°40E	100-year-old *Pinus sylvestris* forest	1
Sb 2	Sheffield culture collection isolate no. 85.022 (096)	not known	not known

dlings were planted in 5 × 5 × 5 cm plastic pots. The substrate was a peat/quartz sand mixture 1:3 (v/v). To estimate nitrogen uptake, two wells of labelled N were placed in each pot consisting of scintillation-vial lids filled with the same substrate as the pot but with 1 ml of an $^{15}NH_4SO_4$ solution (100 mg N l^{-1}) (95% atom excess) added. The lids were covered with a nylon mesh (100 μm) to allow access for fungi but not roots. To reduce diffusion of ^{15}N into the surrounding substrate, an air-gap 2–3 mm wide was maintained between the nylon mesh and the labelled solution. Non-mycorrhizal seedlings were included in the experiment to estimate the amount of ^{15}N that leaving the wells by diffusion or through translocation by indigenous fungi in the substrate and thereby becoming available to the roots. All pots were placed in boxes with a capillary mat in the bottom to transport nutrient solution from a 6-l reservoir located 5 cm below the seedlings.

Nutrients and water were added to the reservoir every 2 weeks in amounts corresponding to seedling demands. To optimise uptake of nitrogen from the wells, a solution meeting the requirements for all nutrients except nitrogen was added to all seedling reservoirs. Approximate concentrations of P and K were 7.5 mg P l^{-1} and 25 mg K l^{-1} (for details on other elements, see Nylund and Wallander 1989). In the nitrogen treatment, nitrogen was added as $(NH_4)_2SO_4$ at a concentration of 100 mg N l^{-1}. The seedlings were harvested after 3 months, the root and shoots washed under tap water, frozen at –20 °C and lyophilised. Dry weights were recorded and the ^{15}Ncontent of the root and shoots was determined.

Experiment 3

All EM isolates were grown on 20 ml MMN agar medium with various concentrations of N and glucose added with 5 replicates for each treatment. The agar surface was covered with a sheet of autoclaved cellophane (the cellophane contained 30 mg N g^{-1} and each sheet weighed approximately 100 mg). Nitrogen was supplied as $(NH_4)_2SO_4$ at 0.284 g l^{-1} or 0.852 g l^{-1}. Glucose was added as the sole carbon source at 3 g l^{-1} or 6 g l^{-1}. The fungal cultures were grown for 30 days and then each colony was measured dried at 80 °C and weighed. The nitrogen concentration of the fungal mycelium was determined by Kjelldahl analysis at the Department of Plant Ecology, University of Lund.

Analyses

The ^{15}N content of the seedlings was determined using an Automated Nitrogen Carbon Analyser-Mass Spectrometer (Europa Scientific Ltd) at the Swedish University of Agricultural Sciences, Uppsala. Ergosterol, as an indicator of EM fungal biomass in roots, was determined as described by Nylund and Wallander (1992) and modified by Ek et al. (1995).

Results

In experiment 1 the influence of nitrogen on fungal growth, and on uptake and translocation of ^{15}N labelled ammonium by EM isolates growing in symbiosis with pine seedlings was examined.

There was no significant effect of N addition on mycelial growth rate of any of the *P. involutus* isolates, although isolates Pi 1, Pi 2 and Pi 3 showed an apparent reduction and Pi 4 an increase in mycelial growth rate in response to N addition (Table 2). Nitrogen addition had no significant influence on the total biomass of the seedlings, except for those colonised by Pi 4. The latter, which were treated with 4 mg N g peat^{-1}, weighed on

Table 2 Shoot:root ratio of pine seedlings and fungal growth of *Paxillus involutus* isolates growing in symbiosis with pine seedlings in microcosms at different N availability (experiment 1). Different letters within each fungal isolate indicate statistically different values (ANOVA with LSD, $P < 0.05$, to separate the means) (*n.s.* not significant)

Fungal isolate	N treatment	Shoot:root ratio	Growth of mycelium (cm^2 day^{-1})
Pi 1	0 mg	1.2 b	13.2 cd
	1 mg	1.8 ab	12.6 cd
	4 mg	2.1 a	10.9 d
Pi 2	0 mg	1.6 ab	15.1 bc
	1 mg	2.0 a	12.9 cd
	4 mg	2.0 a	11.7 cd
Pi 3	0 mg	1.4 b	20.2 a
	1 mg	1.6 ab	18.7 a
	4 mg	2.1 a	17.8 ab
Pi 4	0 mg	1.05 b	11.7 cd
	1 mg	1.5 b	14.6 bc
	4 mg	2.5 a	15.0 bc
Anova	Fungus	n.s.	0.000
P-value	N treatment	0.000	n.s.
	F*N	n.s.	n.s.

average 113 mg compared with other seedlings weights of 66–90 mg (data not shown). On the other hand, higher added N resulted in an increased shoot:root ratio for Pi 1-, Pi 3- and Pi 4-colonised seedlings; the shoot:root ratio of Pi 2- colonised seedlings was not influenced by nitrogen (Table 2).

The fungal uptake of ^{15}N-labelled ammonium was low in experiment 1, probably because of the short incubation time. Less than 1.7% of the total N in root tips was labelled. At zero N availability (0 mg N l^{-1}), the concentration of ^{15}N in mycorrhizal root tips was higher for Pi 2 and Pi 3 than Pi 1 and Pi 4 (Fig. 1). At high N availability, the ^{15}N concentration in the root tips was generally much lower with no differences between the isolates (data not shown). No ^{15}N was detected in the residual root system or in shoots of seedlings in the high-N or low-N treatments and, thus, no conclusions on partitioning of nitrogen from fungal uptake between shoot and root could be drawn. N concentration in shoots, roots and root tips increased in response to N addition (Table 3). At low N availability, the N concentration was lower in root tips colonised by Pi 1 than by other isolates. At harvest, peat from the control treatment contained 0.12 ± 0.01 mg N g^{-1} (as KCl extractable NH_4), the 1 mg N treatment contained 0.87 ± 0.1 mg N g^{-1} and the 4 mg N treatment contained 2.8 ± 0.2 mg N g^{-1}.

Experiment 2 was performed to study the effect of nitrogen addition on the fungal growth of EM-colonised seedlings grown in pots. Pot-grown seedlings grow much better than seedlings in microcosms; thus the shoots constitute a much greater sink for carbon than the less actively growing shoots in experiment 1. Furthermore, the EM fungi had more time (3 months) to take up and allocate the added ^{15}N-labelled nitrogen.

For seedlings growing in pots, high N availability significantly reduced the concentration of ergosterol in the roots colonised by Pi 2 (by 71%) and Pi 3 (by 65%), but the ergosterol concentration in Pi 1- and Pi 4-colonised seedlings was not significantly affected. In the high N treatment, the concentration of ergosterol in roots of *S. bovinus* was reduced significantly by 93% in Sb 1 but was not significantly affected in Sb 2 (Fig. 2). Seedling biomass and shoot:root ratio were not influenced by fungal colonisation but were positively influenced by nitrogen addition (Table 4). In the low N treatment in experiment 2, *S. bovinus*-colonised seedlings were smaller but had higher shoot:root ratios than *P. involutus*-colonised and non-mycorrhizal seedlings (Table 4).

The longer ^{15}N uptake period in this experiment resulted in an ^{15}N labelling of 13–17% of the total N in the seedlings in the low N treatment. Isolate Pi 2 took up significantly more ^{15}N than isolate Pi 1 (Fig. 3). The fraction of the labelled nitrogen present in shoots was higher in Pi 2-colonised seedlings (shoot:root ratio 1.6) than in seedlings colonised by Pi 3 (ratio 1.2), Pi 1 (ra-

Table 3 N concentration in root tips, roots and shoots of seedlings colonised by four different *P. involutus* isolates grown with low or high N addition. Different letters within each column indicate statistically different values (ANOVA/LSD, $P < 0.05$)

Fungal isolate	N treatment	Shoot N concentration (%)	Root N concentration (%)	Root tip N concentration (%)
Pi 1	0 mg	1.55 e	1.02 b	1.1 c
Pi 1	4 mg	2.1 abc	1.76 a	2.3 ab
Pi 2	0 mg	1.9 cde	1.16 b	2.2 b
Pi 2	4 mg	2.3 a	1.82 a	2.6 a
Pi 3	0 mg	1.7 de	1.20 b	2.3 ab
Pi 3	4 mg	2.0 bcd	1.7 a	2.3 ab
Pi 4	0 mg	1.7 d	0.96 b	2.1 b
Pi 4	4 mg	2.3 ab	1.91 a	2.3 ab
Anova	Fungus	0.045	n.s	0.012
	N treatment	0.000	0.000	0.003
	F*N	n.s	n.s	n.s

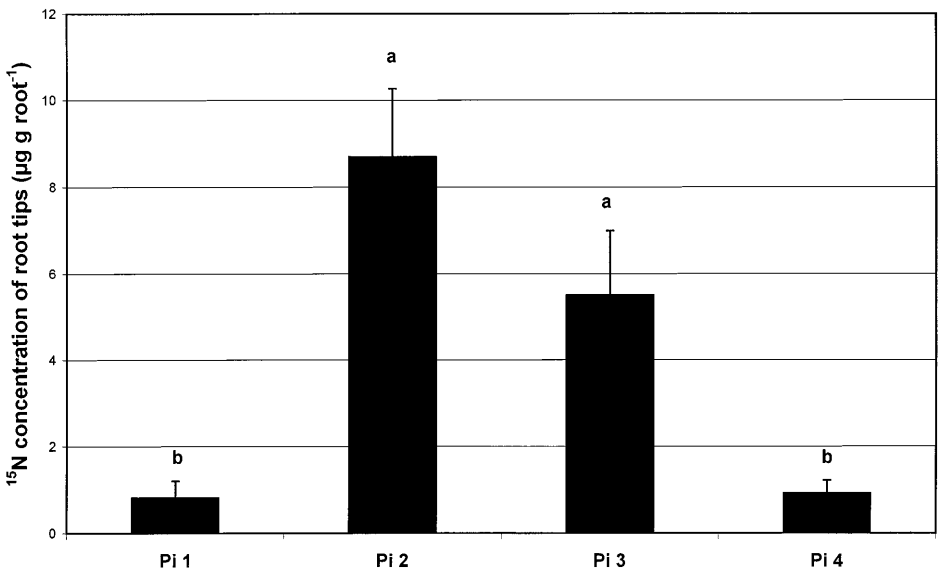

Fig. 1 Concentration of ^{15}N in root tips of *Pinus sylvestris* (pine) seedlings colonised by different *Paxillus involutus* isolates in the low N treatment (0 mg N l^{-1}) of experiment 1. The ^{15}N label originated from ammonium, which was available to the fungal mycelium alone. Different letters indicate statistically different values using ANOVA and LSD, $P < 0.05$, to separate the means; *bars* standard errors

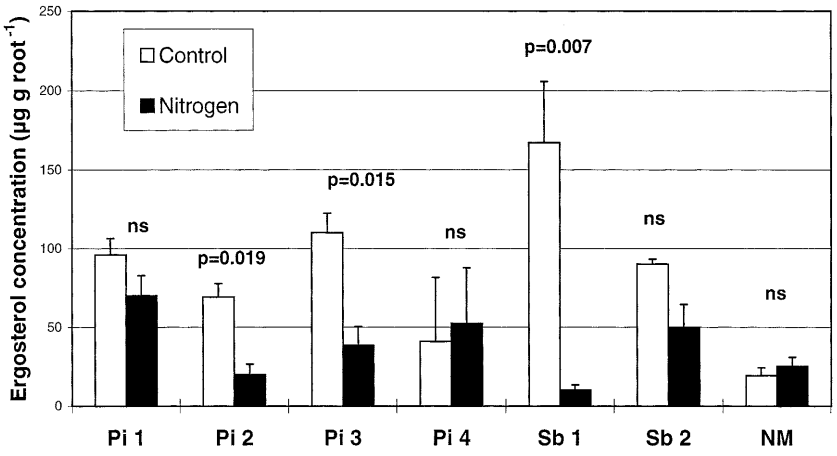

Fig. 2 Concentration of ergosterol in roots of non-mycorrhizal pine seedlings or pine seedlings colonised by different isolates of *P. involutus or Suillus bovinus* grown with or without N addition (experiment 2). A significant effect (ANOVA/LSD) of N addition on ergosterol concentration within each fungal treatment is indicated in the Fig. with a *P* value (*ns* no significant effect of the N addition)

tio 0.9) or Pi 4 (ratio 1.0) (Fig. 4). Seedlings colonised by the *S. bovinus* isolates had a larger fraction of labelled nitrogen in the shoots than seedlings colonised by *P. involutus* (Fig. 4), which is in line with the higher shoot:root ratio of *S. bovinus*- than *P. involutus*-colonised seedlings (Table 4). In the high N treatment, only 0.4–1% of the nitrogen in the seedlings was labelled, and uptake by the EM fungi was not measurable. Non-mycorrhizal seedlings took up much less ^{15}N from the wells than mycorrhizal seedlings in the low N treatment (Fig. 3). Fungal colonisation did not influence root N concentration, while shoot N concentration was higher in *S. bovinus*- than in *P. involutus*-colonised or non-mycorrhizal seedlings in the low N treatment (Table 5).

In experiment 3, the same EM isolates were grown in axenic cultures to compare the result obtained when the fungus is growing with or without the host plant.

The EM isolates varied both interspecifically and intraspecifically in mycelial growth. In the axenic cultures, the growth of some isolates (Pi 1, Pi 4) was hampered by high N availability where glucose was added at low concentrations. Isolates receiving large amounts of glucose grew at similar rates regardless of the N level (Table 6).

The concentration of N in the mycelium of *P. involutus* isolates varied between 5.1 and 9.0%. Mycelial N concentration dropped at low glucose availability in isolates Pi 1 and Pi 4. The nitrogen concentration was generally higher in *S. bovinus* isolates (9.1–10.5%) than in *P. involutus* isolates (Table 6).

Discussion

The differences in concentration of ^{15}N in the seedlings in our study could be due to differences in either the rate of N uptake by the EM isolates or in the amount of EM mycelium colonising the ^{15}N sources. Assuming different rates of N uptake, the differences in fungal growth, N uptake and allocation of labelled nitrogen in seedlings colonised by the different *P. involutus* isolates could be interpreted as follows: Pi 2, and to some extent Pi 3, used a larger portion of the available carbon

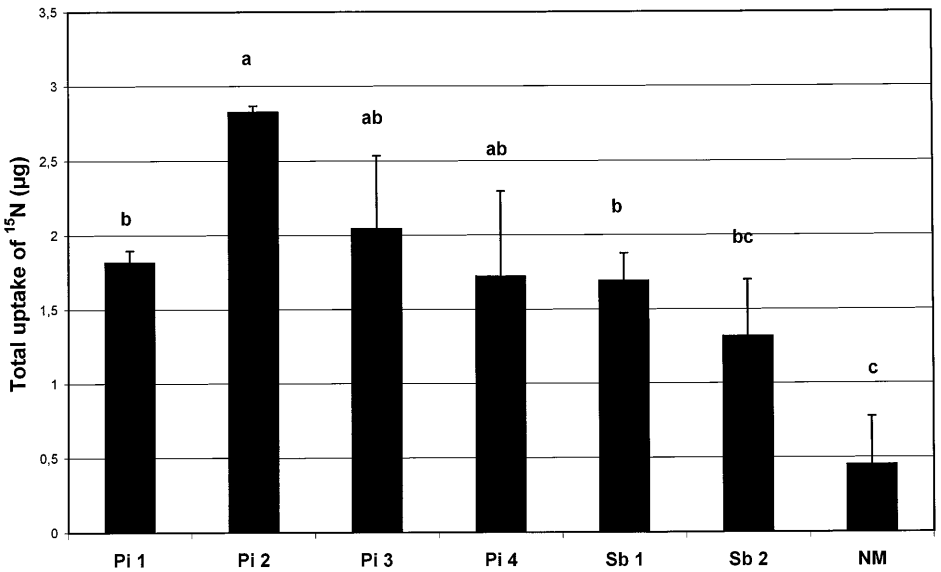

Fig. 3 Total content of ^{15}N in non-mycorrhizal seedlings or seedlings colonised by different *P. involutus* or *S. bovinus* isolates. The ^{15}N label originated from ammonium, which was available to the fungal mycelium alone. Different letters indicate statistically different values using ANOVA and LSD, $P < 0.05$, to separate the means; *bars* standard errors

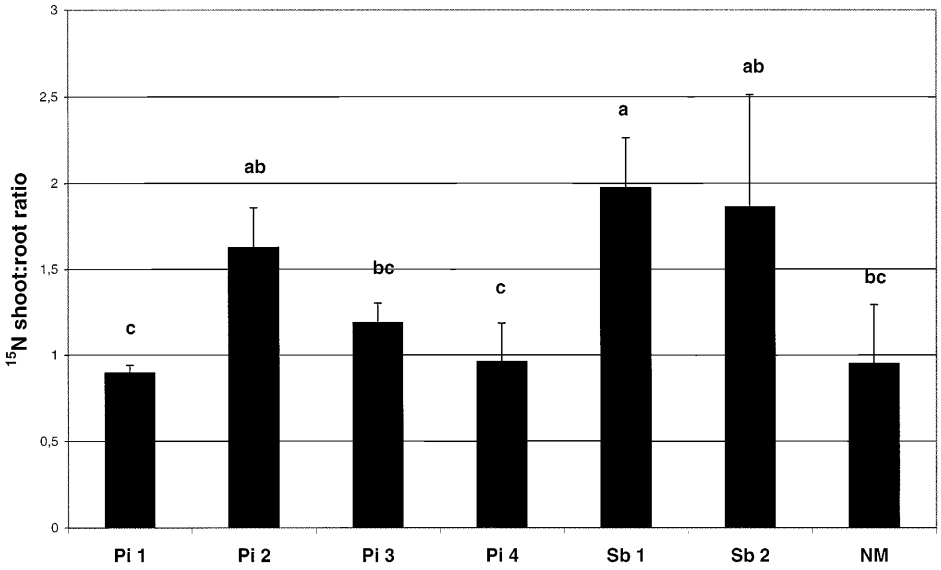

Fig. 4 Relation between ^{15}N label accumulating in the shoot and in the root of non-mycorrhizal seedlings or seedlings colonised by different *P. involutus* or *S. bovinus* isolates. Different letters indicate statistically different values using ANOVA and LSD, $P<0.05$, to separate the means; *bars* standard errors

Table 4 Effects of nitrogen and colonisation by different *P. involutus* and *S. bovinus* isolates on growth parameters of pine seedlings (experiment 2). Values are means 3–4 replicate seedlings in each treatment. Different letters within each column indicate statistically different values (ANOVA/LSD, $P<0.05$) (*NM* non-mycorrhizal seedlings)

Fungus	N addition (mg l^{-1})	Total d wt (g)	Shoot:root ratio
Pi 1	0	0.23 cd	0.77 d
Pi 1	100	0.39 c	2.6 a
Pi 2	0	0.26 cd	0.98 cd
Pi 2	100	0.48 b	2.0 ab
Pi 3	0	0.21 cd	1.0 cd
Pi 3	100	0.69 a	2.5 a
Pi 4	0	0.19 cd	0.7 d
Pi 4	100	0.53 ab	2.4 a
Sb 1	0	0.14 d	1.6 bc
Sb 1	100	0.46 b	2.8 a
Sb 2	0	0.11 d	1.7 bc
Sb 2	100	0.46 ab	1.8 bc
NM	0	0.24 cd	0.85 cd
NM	100	0.35 c	2.2 a
Anova	Fungus	n.s.	n.s.
P-value	N-treatment	0.000	0.000
	F*N	n.s.	0.021

Table 5 Effects of nitrogen and colonisation by different *P. involutus* and *S. bovinus* isolates on N concentration in shoots and roots of pine seedlings (experiment 2). Different letters within each column indicate statistically different values (ANOVA/LSD, $P<0.05$) (*NM* non-mycorrhizal seedlings)

Fungal isolate	N addition (mg N l^{-1})	Root N concentration (%)	Shoot N concentration (%)
Pi 1	0	0.53 b	0.63 c
Pi 1	100	2.1 a	2.4 a
Pi 2	0	0.55 b	0.79 c
Pi 2	100	2.2 a	2.5 a
Pi 3	0	0.59 b	0.65 c
Pi 3	100	2.3 a	2.4 a
Pi 4	0	0.54 b	0.7 c
Pi 4	100	2.5 a	2.7 a
Sb 1	0	0.88 b	1.7 b
Sb 1	100	2.5 a	2.6 a
Sb 2	0	0.7 b	1.7 b
Sb 2	100	2.3 a	2.6 a
NM	0	0.59 b	0.7 c
NM	100	2.6 a	2.5 a
ANOVA	Fungus	n.s.	0.000
	N treatment	0.000	0.000
	F*N	n.s.	0.021

in the assimilation of inorganic nitrogen and this carbon was reallocated to the host to a greater than in seedlings colonised by Pi 1 or Pi 4. Pi 1 and Pi 4 were then able to use a larger portion of the available carbon for fungal growth. Such results support the hypothesis that the enhanced assimilation of inorganic nitrogen reduces the amount of energy and carbon skeletons available for fungal growth (Wallander 1995). Further support for this view was found by Wallenda et al. (1996), who analysed the concentration of fungus-specific carbohydrates in mycorrhizal roots exposed to different amounts of nitrogen.

The indication that those isolates of EM fungi which transfer a larger fraction of the assimilated nitrogen to the host than other isolates also produce less biomass at elevated N levels, is further supported by the difference between *P. involutus* and *S. bovinus*. In terms of fungal growth, *S. bovinus* is regarded as being more sensitive than *P. involutus* to elevated N levels (Hora 1959; Ohenoja 1978; Arnebrant 1994), although in the present study only one of the *S. bovinus* isolates was significantly influenced by nitrogen. The larger fraction of the assimilated nitrogen allocated to shoots than to roots of seedlings colonised by *S. bovinus* than *P. involutus* may indicate that *S. bovinus* transfers a larger fraction of assimilated nitrogen to the host than *P. involutus*. Fungal uptake and transfer of nitrogen to the host seemed to have regulated the growth of the EM fungi in this

Table 6 Radial growth, biomass and N concentration in *P. involutus* and *S. bovinus* mycelium grown axenically with different glucose and ammonium additions. Different letters within each isolate indicate statistically different values (ANOVA/LSD, $P<0.05$)

Fungal isolate	Glucose addition (g l^{-1})	N addition (mg N l^{-1})	Lateral growth (mm)	Biomass (mg)	N concentration (%)
Pi 1	3	50	60 a	5 b	5.8 b
Pi 1	3	150	38 b	5 b	5.9 b
Pi 1	6	50	67 a	14 a	7.4 a
Pi 1	6	150	61 a	15 a	7.9 a
Anova	Glucose		0.000	0.000	0.000
(*P*-value)	Nitrogen		0.000	n.s	n.s
	G*N		0.005	n.s	n.s
Pi 2	3	50	65 b	8.6 b	7.6 a
Pi 2	3	150	66 ab	8.5 b	8.0 a
Pi 2	6	50	68 a	15 a	8.1 a
Pi 2	6	150	67 ab	14 a	8.0 a
Anova	Glucose		0.02	0.000	n.s
	Nitrogen		n.s	n.s	n.s
	G*N		n.s	n.s	n.s
Pi 3	3	50	65 ab	8.1 b	9.0 a
Pi 3	3	150	61 b	8.1 b	8.9 a
Pi 3	6	50	60 b	14.8 a	7.5 b
Pi 3	6	150	69 a	15.1 a	9.1 a
Anova	Glucose		n.s	0.000	0.014
	Nitrogen		n.s	n.s	0.041
	G*N		0.008	n.s	0.024
Pi 4	3	50	53 b	7 b	5.4 b
Pi 4	3	150	23 c	4.4 c	5.3 b
Pi 4	6	50	65 a	15.5 a	6.4 a
Pi 4	6	150	63 ab	15.1 a	5.8 ab
Anova	Glucose		0.000	0.000	0.006
	Nitrogen		0.001	0.002	n.s
	G*N		0.003	0.017	n.s
Sb 1	3	50	26 a	4.5 b	9.5 a
Sb 1	3	150	25 a	4.5 b	9.5 a
Sb 1	6	50	32 a	7.3 a	9.5 a
Sb 1	6	150	25 a	7.4 a	9.5 a
Anova	Glucose		n.s	0.000	n.s.
	Nitrogen		n.s	n.s	n.s
	G*N		n.s	n.s	n.s
Sb 2	3	50	16 b	7 b	10a
Sb 2	3	150	17 b	7 b	9.1 b
Sb 2	6	50	20 a	10a	10.5 a
Sb 2	6	150	17 b	12 a	10.4 a
Anova	Glucose		n.s.	0.000	0.002
	Nitrogen		0.024	n.s	n.s
	G*N		n.s	n.s	n.s

study. However, there may be other more direct effects of nitrogen on EM fungi. For instance, one EM isolate studied by Arnebrant (1994) seemed inefficient in taking up nitrogen and transferring it to the host, but mycelial growth was still severely inhibited at elevated levels of nitrogen availability.

The lower shoot:root ratio of seedlings growing without N addition and colonised by Pi 1 and Pi 4 than by the other isolates suggests that a reduction in allocation of nitrogen from fungus to host results in an N deficiency in the seedlings. Colpaert et al. (1992) stressed that the development of mycorrhizal mycelium is a nitrogen-consuming process that can be linked to reduced nitrogen concentrations in mycorrhizal seedlings. In their study, *Scleroderma citrinum* appeared to retain more nitrogen in the fungal mycelium as seedling growth and shoot N concentration were reduced, whereas the nitrogen content of the fungal mycelium was higher in this plant-fungus combination than in seedlings colonised by *Thelephora terrestris* or *S. bovinus.* Conjeaud et al. (1996) demonstrated that *Hebeloma cylindrosporum* reduced the growth and shoot N concentration of pine seedlings, and suggested that the fungus retains substantial quantities of nitrogen in the mycelium for its own growth.

The amount of carbon allocated to the fungus is of vital importance in determining its response to N addition. It was clearly shown in the pure culture experiment of the present study that uptake of NH_4 increased in response to glucose addition in some of the isolates. The amount of carbon available to the fungus will vary depending on both the amount of carbon assimilated by the plant and the activity of carbon-utilising sinks in the plant other than the EM fungi. The growth of EM

fungi demands significant amounts of carbon (Smith and Read 1997), and extensive fungal growth can reduce growth of the host plants (Nylund and Wallander 1989; Dosskey et al. 1990; Colpaert et al. 1992). At low rates of nitrogen addition to small seedlings, shoot growth will become a weak sink for carbon and, consequently, more carbon can be allocated below ground to support root growth and associated EM fungi (Ericsson 1995; Wallander 1995). Shoot growth responded more positively to N addition in pot-grown seedlings than the seedlings in the microcosms. Thus, it seemed that the shift in carbon allocation from fungal growth to shoot growth at higher rates of N addition was more pronounced in pot-grown seedlings. This could explain why fungal growth was inhibited more strongly in pots than in microcosms for some *P. involutus* isolates (Pi 2, Pi 3).

We found no agreement when comparing the influence of nitrogen on mycelial growth of EM isolates in axenic and symbiotic cultures. It may, thus, be difficult to draw any ecologically meaningful conclusions based on the experiment with axenic cultures. The growth of Pi 1 and Pi 4 were reduced in response to N addition when growing axenically at low glucose availability. This response diminished at higher glucose availability. It is interesting to note that these two isolates were least effected by N addition when growing in symbiosis. The reduced growth in axenic cultures may be a result of autolysis. Alexander (1983) found that *P. involutus* rapidly autolysed and released ammonium to the growth medium when the carbon source was exhausted. The N concentration of isolates Pi 1 and Pi 4 dropped at lower glucose availability, which may indicate ammonium release by autolysis. This was never the case for *S. bovinus* in our study or *Lactarius rufus* in that by Alexander (1983). On the other hand, there was good agreement between the nitrogen concentration in axenically grown mycelium and the uptake of nitrogen by the fungus in symbiosis. Isolates Pi 1 and Pi 4 took up less ^{15}N-labelled ammonium when grown in symbiosis, and the fungal tissue of these isolates had lower nitrogen concentrations when grown axenically. Similarly, isolate Pi 2 took up more $^{15}NH_4$ in symbiosis and had a higher nitrogen concentration in axenically grown mycelium than other isolates.

There was no relationship between the ability of the isolates to grow at high N availability and the amount of deposited anthropogenic nitrogen at the collection site. The most sensitive isolate (Pi 2) was collected from southern Sweden, with a nitrogen deposition load of 5–10 kg ha^{-1} yr^{-1}, whereas the most tolerant isolate (Pi 1) was from northern Sweden, where the load is much lower (1 kg N ha^{-1} yr^{-1}). Similarly, Leski et al. (1995) found no relation between aluminium tolerance (in terms of fungal growth) and the concentration of aluminium in the soil from which *S. luteus* isolates had been collected. On the other hand, isolates of *P. tinctorius* collected at coal-mine tailings with high concentrations of Al were found to grow better in soils with high concentrations of Al than isolates from forest soils with lower Al levels (Egerton-Warburton and Griffin 1995). Thus, certain chemical characteristics of a site may not necessarily be of primary selective value for the physiological properties of an EM population. However, as the generation time of EM fungi in boreal forest may be longer than a forest generation (Dahlberg and Stenlid 1995), the elapsed time with enhanced nitrogen may not yet have been manifested in changed properties of EM populations.

The additions of ammonium in our study were higher than those fungi would be subjected to in a natural field situation. The KCl- extractable NH_4 was 140 μg N g^{2-1} peat in the untreated peat used in the microcosm experiment but 2800 μg g^{2-1} in the highest N addition. By comparison, Persson and Wirén (1995) reported that net mineralisation rates of humus from spruce forests in southern Sweden averaged 1000 μg N g^{-1} humus during 100 days under laboratory conditions. Setälä et al. (1997) found that KCl-extractable NH_4 increased from 0.002 μg g^{-1} in the humus of control plots to 76 μg g^{-1} in that of N-fertilised forest plots in Finland. The biomass of EM fungi growing in symbiosis with pine seedlings was severely reduced in seedlings grown in the fertilised forest soil compared with seedlings grown in soil collected from control plots. In soil water in the field, concentrations of inorganic nitrogen rarely exceed 1000 μg^{-1} (Nohrstedt et al 1996), owing to rapid immobilisation by roots and microorganisms (Jonasson et al. 1996). However, the inorganic N concentration in percolation water has occasionally been found to reach 10 000 μg l^{-1} in beech forest soil in southern Sweden (Falkengren-Grerup 1994).

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