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Nitrogen amendments reduce the growth of extramatrical ectomycorrhizal mycelium

Abstract The effect of three different nitrogen sources on the growth of external ectomycorrhizal mycelium was studied in Perspex micorocosms. Nonsterile peat was used as substrate. Five different fungal isolates growing in symbiosis with pine seedlings were investigated: two isolates of *Paxillus involutus*, one of *Suillus bovinus* and two unidentified ectomycorrhizal fungi isolated from ectomycorrhizal root tips. Three different nitrogen sources were used: ammonium as $(NH_4)_2SO_4$, nitrate as $NaNO_3$ and a complete nutrient solution (Ingestad 1979), and three different nitrogen concentrations, 1, 2 or 4 mg N/g dry wt. of peat. The mycelial growth of all fungi was found to be negatively affected by the nitrogen amendments, although the sensitivity to nitrogen varied between the isolates. One of the unidentified isolates was extremely sensitive and growth was completely inhibited by all nitrogen treatments. In contrast, the growth of one of the *P. involutus* isolates was only slightly reduced by the nitrogen amendments. The different nitrogen sources all reduced growth, and since no significant difference was found between the nitrogen sources or between the different nitrogen concentrations the results were pooled to give one value that summarized the effect of nitrogen on mycelial growth. Thus, the mycelial growth of one of the two *P. involutus* isolates was reduced to approximately 80% of the growth in the control, the other *P. involutus* and one of the unidentified fungi, vgk 2 89.10, were reduced to 40–50% of the control growth, *S. bovinus* to 30% of the control and the most sensitive fungus, the unidentified isolate vg 1 87.10, was reduced to 3% of the growth in the control treatment. In all experiments, the shoot to root ratio generally increased, mainly as a result of increased shoot growth.

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Introduction

Most forest trees in coniferous temperate forests live in symbiosis with ectomycorrhizal fungi and are dependent on the activity of these fungi for their nutrient uptake. The extramatrical mycelium of the ectomycorrhizal fungi radiates from the ectomycorrhizal root tips, effectively colonizing the soil substrate. Nutrients are explored, absorbed and translocated to the host plant. The extramatricial mycelia also connect different host plants, and interplant translocation of carbon (Read et al. 1985) and nitrogen (Arnebrant et al. 1993) has been demonstrated. Nitrogen is the nutrient limiting tree growth in these ecosystems, and most ectomycorrhizal fungi are probably adapted to low inorgnaic nitrogen levels in the soil. The potential of many ectomycorrhizal fungi to mobilize nitrogen from proteins, demonstrated by Abuzinadah and Read (e.g. 1986, 1989a, b) and Finlay et al. (1992) is thus of major importance in these ecosystems, as discussed by Read (1991).

These forests are fertilized in order to improve tree growth. In Sweden, 150–300 kg N ha^{-1} as NH_4NO_3 is the most used fertilizer regime. It is well known that nitrogen fertilization affects ectomycorrhizal fungi. An initial reduction in the number of ectomycorrhizal root tips has often been reported, on both planted bait seedlings (Arnebrant and Söderström 1992) and the standing trees (Menge et al. 1977; Tétreault et al. 1978; Alexander and Fairley 1983). This reduction in numbers is no longer apparent after some growing seasons (Menge et al. 1977; Laiho et al. 1987; Arnebrant and Söderström 1992), but a changed ectomycorrhizal community structure appears to be more long-lived, as indicated by the different ectomycorrhizal types colonizing bait seedlings planted in fertilization experiments 13 years after fertilization (Arnebrant and Söderström 1992). Both the number of ectomycorrhizal species and the produc-

tion of fruitbodies have also been reported to decrease after fertilization (Hora 1959; Menge and Grand 1978; Ohenoja 1978; Wästerlund 1982). Some ectomycorrhizal species, however, may actually increase production of fruitbodies as a result of fertilization, for example *Paxillus involutus* (Hora 1959; Ohenoja 1978; Laiho 1979; Shubin 1988) and *Lactarius rufus* (Ohenoja 1978).

The effect of inorganic nitrogen on the extramatrical mycelium has so far received little attention. In laboratory experiments using a semihydroponic cultivation system, decreased production of fungal biomass, estimated as ergosterol, was reported in both roots (Wallander and Nylund 1991, 1992) and extramatrical mycelium for three different ectomycorrhizal fungi (Wallander and Nylund 1992) as a result of increased nitrogen levels continuously distributed in the nutrient solution. A reduction in the amount of mycelium is a possible explanation for the reduced amount of ectomycorrhizal root tips found after fertilization.

In the present study, the effects of inorganic nitrogen on the production and growth of external mycelium of some ectomycorrhizal fungi grown in symbiosis with a host plant was investigated. Transparent Perspex microcosms with nonsterile peat as substrate were used. The production of extramatrical mycelium was estimated as the area colonized by the fungus. The use of transparent microcosms also made it possible to estimate the growth rate of the different fungi in addition to the total area covered by mycelium. Nitrogen was added at one occasion to simulate fertilization.

Materials and methods

Ectomycorrhizal seedlings were obtained using the method described by Duddridge (1986) as modified by Finlay (1989). Five different ectomycorrhizal fungal isolates were used: two isolates of *Paxillus involutus* (Batsch) Fr., nos. 87.017 and 374; one isolate of *Suillus bovinus* (L.:Fr) O. Kuntze; and two unidentified fungi isolated from ectomycorrhizal root tips of bait seedlings planted in a poor pine forest in southern Sweden. *Pinus contorta* (Dougl. ex Loud) was used as host plant for *Paxillus involutus* and *Pinus sylvestris* L. for the other fungi.

Experimental design

The ectomycorrhizal seedlings were planted in small Perspex microcosms (12 cm × 12 cm) prior to the nitrogen experiment. In these small microcosms, untreated peat was used as substrate. When the mycelium covered all of the peat surface, the small microcosms were transfered to larger ones (20 cm × 20 cm) and peat treated with different nitrogen amendments was added to cover the area around the small chambers. Five replicates were used, and two experiments were performed for one of the unidentified isolates, vgk 2 89.10 and for *Paxillus involutus* 87.017.

Throughout the synthesis period and during the experiment, the microcosms were kept in propagators inside growth cabinets at approximately 150 μmol m^{-2} s^{-1} PAR, with a 18 h/6 h 18° C/15° C day/night cycle for 2–4 months depending on the growth rate of the fungus. The growth of the mycelial front was continuously recorded, and the experiment was finished when the fastest growing mycelium (most often the control treatment) reached the margin of the microcosms.

The total area covered by mycelium at harvest was obtained through copying the Perspex lid of the microcosms onto paper and the proper area was cut out, weighted and transformed to area. The maximal area that could be covered by mycelium was 256 cm^2 [(20 cm × 20 cm) − (12 cm × 12 cm)].

At harvest, the dry weights of the seedlings (shoots and roots) were obtained by drying at 85° C for 12 h. The nitrogen content in the shoots was measured using the Kjeldahl technique.

The pH values were measured both prior to and after the experiments: 5 g of wet peat was mixed with 25 ml distilled water and shaken for approximately 1 h on a rotary shaker.

Nitrogen treatments

Nonsterile peat with a pH(H_2O) of 4.0 (1:5 w/w) was used as substrate. Nitrogen was added either as $(NH_4)_2SO_4$, $NaNO_3$ or as a complete nutrient solution (Ingestad 1979) where both NH_4^+ and NO_3^- were used as nitrogen sources and the N:K:P ratio was 100:65:13. Three different nitrogen levels were used, 1, 2 and 4 mg N/g dry wt. peat. Since all nitrogen treatments at least initially decreased the pH of the peat, so-called pH controls were prepared in which 0.18 M H_2SO_4 was added to the peat in order to obtain pH values corresponding to those of the nitrogen-treated peat.

Statistical analyses

Analyses of variance (ANOVA) were used to analyse the effect of the different nitrogen treatments on mycelial growth as well as the different plant variables. Duncan's Multiple Range test was used to evaluate differences between individual nitrogen treatments. In order to summarize the results, and since in most cases no difference was found either within or between the different nitrogen sources, the values of all nitrogen treatments were pooled and treated as one, and ANOVA was again used to evaluate the total effect of nitrogen.

Results

Effects on mycelial growth

The area covered by mycelium showed a tendency to be reduced for all fungi in all nitrogen treatments, although in most cases this reduction was not significant for the individual nitrogen treatments (Table 1). No evident dose-response relationship was seen (Table 1) and since no significant difference was shown between the different nitrogen concentrations within each treatment, the values obtained for each nitrogen source were pooled. Furthermore, since no significant difference between the different nitrogen sources was noted, the values obtained in all the nitrogen treatments were pooled to obtain one value showing the overall effect of nitrogen on mycelial growth. The overall effect of inorganic nitrogen on all fungi was a significant reduction of mycelial growth as shown in Table 1. Although the mycelial growth of all fungi was reduced, the nitrogen tolerance differed very much between the different fungi. The most sensitive fungus was one of the unidentified isolates, vg 1 87.10, the growth of which was almost completely inhibited even at the lowest nitrogen concentrations (Table 1). For this fungus, even the mycelium originally existing in the small microcosms at the

Table 1 Area (cm^2) covered by mycelium at harvest of the different ectomycorrhizal fungi in the different nitrogen treatments. Nitrogen was added in three different levels, 1, 2 and 4 mg N/g dry wt. peat. The maximal area was 256 cm^2. *Pinus contorta* was used as host plant for *Paxillus involutus* and *Pinus sylvestris* for the other fungi. Values followed by different letters within a column indicate significant differences according to Duncan's Multiple Range test ($P<0.05$), $n=5$. Percentages of control values are shown within parentheses. The bottom line includes values showing the effect of nitrogen on the area covered by mycelium. Again, percentages of control values are shown within parentheses. (*ND* Not determined)

	Fungus	*Paxillus involutus* 87.017		*Paxillus involutus* 374	vgk 2 89.10		vg 1 87.10	*Suillus bovinus*
	Experiment	I	II		I	II		
Control		234a (100)	116ab (100)	145a (100)	206a (100)	121a (100)	163a (100)	96a (100)
pH control		214ab (92)	180a (155)	124a (86)	123abc (60)	80ab (66)	146a (90)	81a (84)
$NaNO_3$	1	164ab (70)	93ab (80)	44a (30)	140abc (68)	66ab (55)	2.8b (1.7)	12a (13)
	2	214ab (92)	112ab (97)	117a (81)	135abc (66)	70ab (58)	8.7b (5.3)	26a (27)
	4	172ab (74)	ND	73a (50)	100abc (49)	ND	ND	ND
$(NH_4)_2SO_4$	1	182ab (78)	102ab (88)	26a (18)	187ab (91)	35ab (29)	0.6b (0.37)	42.5a (45)
	2	156b (67)	68ab (59)	49a (34)	96abc (47)	13b (11)	8.5b (5.2)	32.6a (34)
	4	172ab (74)	ND	76a (52)	36c (17)	ND	ND	ND
Complete nutrient treatment	1	211ab (90)	64b (55)	ND	138abc (67)	ND	ND	ND
	2	209ab (89)	46b (40)	ND	82bc (40)	ND	ND	ND
	4	174ab (74)	ND	ND	50c (24)	ND	ND	ND
F-ratio		2.175	3.952	2.088	3.236	2.937	66.70	2.387
Significance level		0.0379	0.0019	0.0820	0.0033	0.0331	0.0000	0.0682
Nitrogen effect		188* (80)	88 (76)	64* (44)	107* (52)	46** (38)	5.1*** (3.1)	28** (29)

* $P<0.05$, ** $P<0.001$, *** $P<0.001$

start of the experiment vanished after the amendment of the nitrogen-treated peat. The most tolerant fungus was one of the *Paxillus involutus* isolates, 87.017, where the area covered by mycelium was reduced to approximately 80% of the control value by the nitrogen treatments (Table 1). The other three fungal isolates showed an intermediate response to the nitrogen treatments, *Paxillus involutus* 374 was reduced to approximately 45% of the control in the nitrogen treatments, the unidentified isolate vkg 2 89.10 to 40–50% and *S. bovinus* to 30% of the growth in the control treatments (Table 1).

Generally the mycelial growth rate was negatively affected by the nitrogen treatments and appeared to be the main reason for the reduced growth (Table 2). There was a very good correlation between total area covered by mycelium at harvest and growth rate. For two of the fungi, *Paxillus involutus* 87.017 and one of the unidentified fungi, vgk 2 89.10, two different experiments were performed. Although the relative effect of nitrogen showed similar trends in both experiments, the total area covered by mycelium (Table 1) and the growth rate were very different (Table 2).

I also obtained results demonstrating that nitrogen regulated mycelial growth. The seedlings infected with the unidentified isolate vg 1 87.10 were transferred to microcosms with untreated peat as substrate after the nitrogen treatments were terminated, and as long as there were still mycorrhizal root tips the growth resumed; after a few weeks the peat was covered with mycelium.

All nitrogen treatments initially decreased the pH of the peat, but at the time of harvest the difference in pH compared to the control value was not as pronounced

Table 2 Growth rate (mm day^{-1}) of the different ectomycorrhizal fungi. Nitrogen treatments as described in Table 1. *Pinus contorta* was used as host plant of *Paxillus involutus,* and *Pinus sylvestris* of the other fungi. To calculate the growth rate only microcosms where growth occurred were included. Values followed by different letters within a column indicate significant differences according to Duncan's Multiple Range test ($P<0.05$), $n=5$. Percentages of control values are shown within parentheses. (*ND* Not determined; *ng* no growth in any of the microcosms)

	Fungus	*Paxillus involutus* 87.017		*Paxillus involutus* 374	vgk 2 89.10		vg 1 87.10	*Suillus bovinus*
	Experiment	I	II		I	II		
Control		4.92a (100)	1.73a (10)	1.48a (100)	2.70ab (100)	1.12a (100)	2.54a (100)	1.77a (100)
pH control		4.10a (83)	2.47b (143)	1.90a (129)	1.48a (55)	0.93a (83)	2.74a (108)	1.28a (72)
$NaNO_3$	1	3.92a (80)	1.77ab (102)	1.20a (81)	3.02ab (112)	1.17a (104)	NG	0.65a (35)
	2	3.94a (80)	1.93ab (112)	2.28a (154)	2.28ab (84)	1.31a (117)	1.0 (39)	1.32a (76)
	4	3.90a (79)	ND	1.57a (106)	2.55ab (94)	ND	ND	ND
$(NH_4)_2SO_4$	1	3.92a (80)	1.80ab (104)	1.47a (99)	3.38b (125)	0.948a (85)	NG	1.23a (70)
	2	4.14a (84)	2.04ab (118)	1.23a (84)	1.50a (56)	1.03a (92)	0.58 (23)	0.86a (49)
	4	3.48a (71)	ND	1.28a (86)	1.31a (49)	ND	ND	ND
Complete nutrient treatment	1	4.70a (96)	1.70a (98)	ND	3.02b (112)	ND	ND	ND
	2	3.92a (80)	1.67a (97)	ND	1.93ab (71)	ND	ND	ND
	4	3.78a (77)	ND	ND	1.98ab (73)	ND	ND	ND
F-ratio		0.987	2.912	0.901	3.226	0.702	2.105	1.261
Significance level		0.4686	0.0258	0.5225	0.0045	0.6280	0.1779	0.3156

or was even nonexistent. An addition of 1 mg N as any of the nitrogen sources initially decreased the pH to 3.6. At harvest, the pH of the 1-mg treatments had increased 0.2–0.4 units. All of the 2-mg N treatments initially reduced the pH to 3.4–3.5 and at harvest these values had also increased 0.2–0.4 units. The highest nitrogen amendments (4 mg) all reduced the initial pH to 3.4, and at harvest the values had increased by 0.1–0.2 units. The pH value of the pH controls was initially 3.4 and at harvest it was 3.6, and thus most often corresponded to the pH values of the highest nitrogen concentrations. Although the mycelial growth of two of the fungi *S. bovinus* and the unidentified isolate vgk 2 89.10, was reduced in the pH controls, this reduction was never as pronounced as the reduction in the nitrogen treatments.

Effects on seedlings

The dry weight of the shoots (Table 3) generally showed a tendency to increase as a result of the nitrogen treatments, with shoots grown in the highest levels of nitrogen being the largest. Treatments with one fungus, however, did not show this trend; with the unidentified isolate vgk 2 89.10 the highest shoot weight was usually recorded at the medium level of nitrogen amendments (experiment I) in the ammonium and nitrate treatments. Furthermore, the plants grown in the full nutrient solution treatment usually had higher shoot weights than those grown on either of the nitrogen sources alone, indicating that nitrogen was no longer growth limiting in the treatments with only nitrogen. The most pronounced increase in shoot growth was in the experiment with the unidentified isolate vg 1 87.10, where the shoot weight increased by approximately 300% in the treatment with 2 mg nitrogen as $(NH_4)_2SO_4$. The nitrogen treatments did not, however, significantly affect the weight of the roots (Table 4), which resulted in an increased shoot to root ratio of the seedlings in the nitrogen treatments (Table 5).

The concentration of nitrogen in the shoots was not significantly affected by the nitrogen treatments (Table

Table 3 Shoot dry weight (mg) of host seedlings at harvest. Nitrogen treatments as described in Table 1. *Pinus contorta* was the host plant of *Paxillus involutus* 87.017, and *Pinus sylvestris* of the other fungi. Values followed by different letters within a column indicate significant differences according to Duncan's Multiple Range test ($P<0.05$), $n=5$. Percentages of control values are shown within parentheses. (*ND* Not determined)

	Fungus	*Paxillus involutus* 87.017		*Paxillus involutus* 374	vgk 2 89.10		vg 1 87.10	*Suillus bovinus*
	Experiment	I	II		I	II		
Control		38.16a (100)	23.82a (100)	36.66ab (100)	65.08abc (100)	56.50a (100)	38.00a (100)	39.50a (100)
pH control		43.70a (115)	27.68a (116)	30.08a (82)	37.10a (57)	44.16a (78)	42.14a (111)	47.92ab (121)
$NaNO_3$	1	47.12a (123)	35.60a (149)	38.50ab (105)	62.08abc (95)	62.08a (110)	73.24a (193)	62.70ab (159)
	2	57.92a (152)	30.44a (128)	73.48b (200)	68.88abc (106)	69.22a (123)	72.86a (192)	57.92ab (147)
	4	63.00ab (165)	ND	54.04ab (147)	50.14ab (77)	ND	ND	ND
$(NH_4)_2SO_4$	1	39.56a (104)	34.72a (146)	37.84ab (103)	63.10abc (97)	40.54a (72)	72.32a (190)	68.20ab (173)
	2	50.26a (132)	37.94a (159)	58.96ab (161)	78.26bc (120)	51.60a (91)	116.1b (306)	74.50b (189)
	4	53.36a (140)	ND	70.58b (193)	41.86a (64)	ND	ND	ND
Complete nutrient treatment	1	46.26a (121)	40.20a (169)	ND	55.16abc (85)	ND	ND	ND
	2	62.42ab (164)	26.72a (112)	ND	86.92c (134)	ND	ND	ND
	4	92.14b (241)	ND	ND	82.62bc (127)	ND	ND	ND
F-ratio		3.488	1.205	3.195	3.258	2.263	6.723	2.985
Sigificance level		0.0019	0.3240	0.0111	0.0032	0.0805	0.0002	0.0311

6), nor was the total nitrogen content in the shoots. No consistent relationship was found between nitrogen concentrations in shoots and mycelial growth.

Discussion

A negative effect of all the different nitrogen treatments on the external mycelial growth was demonstrated, similar to results previously reported by Wallander and Nylund (1992). Although the mycelial growth of all the fungi was reduced by the nitrogen treatments, the different fungal isolates were obviously differentially tolerant. The most sensitive fungus in my study was one of the unidentified isolates, vg 1 87.10. This fungus was isolated from a root tip of a bait seedling planted at Kroksbo, a forest fertilization experimental site, and represented an ectomycorrhizal type common to control and urea-treated plots, but significantly reduced in the ammonium nitrate-treated plots (Arnebrant and Söderström 1992). The least sensitive fungus was *Paxillus involutus* isolate 87.017, the growth of which was only slightly inhibited by the nitrogen amendments. The other isolate of *Paxillus involutus* (isolate no. 374) was less tolerant and these results (as many others) show that the variability between different strains can be as pronounced as the differences between species. It is well known from fruitbody studies that *Paxillus involutus* is one of only a few mycorrhizal fungi that may increase their fruitbody production as a result of forest fertilization (Hora 1959; Ohenoja 1978; Laiho 1979; Wästerlund 1982; Shubin 1988). The other two fungi, *S. bovinus* and the unidentified vgk 2 89.10 were more strongly affected than *Paxillus involutus* 87.017 but less than vg 1 87.10. Wallander and Nylund (1992) found that the growth of three fungi, *Laccaria bicolor, Hebeloma crustuliniforme* and *Suillus bovinus,* was substantially reduced. In their study, *S. bovinus* was the most sensitive fungus and *L. bicolor* the most tolerant.

A regulatory role of nitrogen on extramatrical mycelial growth is indicated by the fact that the repressive effect of nitrogen on mycelial growth was reversible. The external mycelial growth of vg 1 87.10 resumed as

Table 4 Root dry weight (mg) of host seedlings at harvest. Nitrogen treatments as described in Table 1. *Pinus contorta* was the host plant of *Paxillus involutus,* and *P. sylvestris* of the other fungi. Values followed by different letters within a column indicate significant differences according to Duncan's Multiple Range test ($P<0.05$), $n=5$. Percentages of control values are shown within parentheses. (*ND* Not determined)

	Fungus	*Paxillus involutus* 87.017		*Paxillus involutus* 374	vgk 2 89.10		vg 1 87.10	*Suillus bovinus*
	Experiment	I	II		I	II		
Control		35.26ab (100)	19.60a (100)	45.06a (100)	43.56a (100)	57.92a (100)	ND	35.40a (100)
pH control		34.02ab (96)	25.22a (129)	34.94a (78)	24.10ab (55)	42.80ab (74)	ND	43.92a (124)
$NaNO_3$	1	36.18ab (103)	19.58a (100)	25.38a (56)	38.42ab (88)	52.46ab (91)	ND	42.88a (121)
	2	31.08ab (88)	24.84a (127)	40.00a (89)	41.54ab (95)	46.74ab (81)	ND	38.82a (110)
	4	41.64a (118)	ND	38.78a (86)	22.50ab (52)	ND	ND	ND
$(NH_4)_2SO_4$	1	32.80ab (93)	18.82a (96)	29.64a (66)	28.28ab (65)	27.44b (47)	ND	46.90a (132)
	2	38.28ab (109)	17.42a (89)	35.84a (80)	40.14ab (92)	33.90ab (59)	ND	44.32a (125)
	4	23.94b (68)	ND	49.58a (110)	18.78b (43)	ND	ND	ND
Complete nutrient treatment	1	32.82ab (93)	20.12a (103)	ND	33.86ab (78)	ND	ND	ND
	2	35.14ab (97)	21.92a (112)	ND	38.66ab (89)	ND	ND	ND
	4	40.64ab (115)	ND	ND	43.24a (99)	ND	ND	ND
F-ratio		1.248	1.360	1.527	2.463	3.286		0.7110
Significance level		0.2889	0.2470	0.1937	0.0195	0.0212		0.6208

soon as the plants were transferred to peat without extra nitrogen. These results are in line with those obtained in the experiments performed by Wallander and Nylund (1992), who found that production of fungal biomass increased after the nitrogen concentration of the nutrient medium was changed from "high" to "low". These results thus seem to offer an explanation for observations made in many field experiments, i.e. that the ectomycorrhizal infection level decreases temporarily as an effect of nitrogen fertilization. Mycelial growth appears to be generally reduced but some fungi are more adversely affected than others, and might even become extinct while more tolerant varieties are favoured. When the concentraiton of inorganic nitrogen decreases, mycelial growth of surviving fungi resumes. In this way, a single nitrogen addition could affect the community structure of ectomycorrhizal fungi for a long period of time, as indicated by the results of Arnebrant and Söderström (1992), who found an altered composition of ectomycorrhizal fungi colonizing the root systems of bait seedlings planted in a fertilizer experiment 13 years after the fertilizer was applied, although no differences in total colonization level were shown. Such long-term effects have also been demonstrated for saprophytic microfungi (Arnebrant et al. 1990). In contrast, in soils continuously supplied with nutrients either as fertilizers or as pollutants, mycelial growth is likely to remain reduced. This was indicated by results of Arnebrant and Söderström (1992), where bait seedlings planted in a field experiment designed for optimal tree growth still had a significantly reduced total infection level as well as a changed composition of ectomycorrhizal fungi colonizing the root systems after 15 years of treatment. Although differences in species composition have thus clearly been demonstrated, the long-term effect on the ecocystem is not known. Since different ectomycorrhizal species have different properties, exemplified by the ability to use proteins as a nitrogen source (Abuzinadah and Read 1986, 1989a, b; Finlay et al. 1992), a change in the community structure might affect the nutrient uptake of the trees. This has been discussed by Jansen and Dighton (1990), who concluded that one possible effect of nitrogen saturation is a change in the ectomycorrhizal flora to one consisting

Table 5 Shoot/root ratios of the host plants at harvest. Nitrogen treatments as described in Table 1. *Pinus contorta* was the host plant of *Paxillus involutus,* and *Pinus sylvestris* of the other fungi. Values followed by different letters indicate significant differences according to Duncan's Multiple Range test ($P<0.05$), $n=5$. Percentage of the control values are shown within parentheses. (*ND* Not determined)

	Fungus	*Paxillus involutus* 87.017		*Paxillus involutus* 374	vgk 2 89.10		vg 1 87.10	*Suillus bovinus*
	Experiment	I	II		I	II		
Control		1.095a (100)	1.326ab (100)	0.909a (100)	1.610a (100)	0.975a (100)	ND	1.122a (100)
pH control		1.394ab (127)	1.154a (87)	1.040ab (114)	1.607a (100)	1.031ab (106)	ND	1.118a (100)
$NaNO_3$	1	1.280a (117)	1.774ab (134)	1.473ab (162)	1.621a (101)	1.180ab (121)	ND	1.570a (140)
	2	1.932abc (176)	1.456ab (110)	1.836c (202)	1.871a (116)	1.584ab (162)	ND	1.554a (139)
	4	1.453abc (133)	ND	1.391ab (153)	2.263a (141)	ND	ND	ND
$(NH_4)_2SO_4$	1	1.235a (113)	1.724ab (130)	1.310ab (144)	2.302a (143)	1.588ab (163)	ND	1.460a (130)
	2	1.339a (122)	2.002b (151)	1.601bc (176)	1.953a (121)	1.648b (169)	ND	1.629a (145)
	4	2.261bc (206)	ND	1.493ab (164)	2.315a (144)	ND	ND	ND
Complete nutrient treatment	1	1.404ab (128)	1.820ab (137)	ND	1.675a (104)	ND	ND	ND
	2	1.918abc (175)	1.888ab (142)	ND	2.188a (136)	ND	ND	ND
	4	2.323c (212)	ND	ND	2.195a (136)	ND	ND	ND
F-ratio		3.798	2.730	3.112	1.958	3.749		2.889
Significance level		0.0010	0.0184	0.0128	0.0622	0.0119		0.0352

of more r-selected species, or so-called early-stage fungi.

Not surprisingly, the main reason for the decreased mycelial growth was found to be a reduced growth rate. Interestingly the results also show that the same isolate in two different experiments can have very different growth rates. However, the relative effect of the nitrogen treatment was similar. The most probable explanation for these differences is that the peat used in the different experiments originated from different batches.

Ammonium and nitrate, as well as the complete nutrient solution, affected mycelial growth negatively, and no differences in response to these different sources were observed. The reason for the reduced mycelial growth is not known. Direct effects on the fungus or indirect effects mediated via changes in the host plant are both possible, but the design of my experiments did not allow any firm conclusions to be drawn. Indirect effects caused by changes carbon allocation has, however, previously been thoroughly dicussed (Nylund 1988; Wallander and Nylund 1991, 1992; Wallander 1992). In contrast to the results obtained by Wallander and Nylund (1992), who reported a negative correlation between needle concentration of nitrogen and extramatrical mycelial biomass production in *Hebeloma crustuliniforme* (although the relationship was not significant for *Laccaria bicolor*), I found no consistent relationship between the nitrogen concentration of the shoots and production of external mycelium. With the most affected fungi, vg 1 87.10, I found a significantly negative correlation ($r=-0.55$) between shoot dry weight and area colonized by mycelium. This finding is similar to the results of Wallander and Nylund (1992), who concluded that excess nitrogen resulted in lower production of fungal biomass and higher production of shoot biomass. In contrast, I found a significant positive correlation with two of the other fungi between mycelial area and shoot dry weight ($r=0.35$ for *Paxillus involutus* 374 and $r=0.27$ and $r=0.49$ in the two experiments using vgk 2 89.10). The differences between my experiments and those performed by Wallander and Nylund (1992) may be a result of the different experimental conditions used.

Table 6 N concentration ($\mu g\ g^{-1}$ dry wt.) of shoots at harvest. Nitrogen treatments as described in Table 1. *Pinus contorta* was the host plant of *Paxillus involutus,* and *Pinus sylvestris* of the other fungi. Values followed by different letters within each column indicate significant differences according to Duncan's Multiple Range test ($P<0.05$), $n=5$. Percentages of control values are shown within parentheses. (*ND* Not determined)

	Fungus	*Paxillus involutus* 87.017		*Paxillus involutus* 374	vgk 2 89.10		vg 1 87.10	*Suillus bovinus*
	Experiment	I	II		I	II		
Control		23803a (100)	15315a (100)	17437a (100)	20468a (100)	23896a (100)	19196a (100)	23730a (100)
pH control		25723a (108)	17458ab (114)	19538ab (112)	23543a (115)	26596a (111)	16643a (87)	25372ab (107)
$NaNO_3$	1	23230a (98)	17068ab (111)	20018ab (115)	22600a (110)	27205a (114)	13739a (72)	21671ab (91)
	2	18297a (77)	17241ab (113)	19110ab (110)	21503a (105)	27960a (117)	13751a (72)	22163ab (93)
	4	21968a (92)	ND	22244ab (128)	22876a (112)	ND	ND	ND
$(NH_4)_2SO_4$	1	23862a (100)	18544ab (121)	20491ab (118)	22095a (108)	26453a (111)	18127a (94)	23506a (99)
	2	25643a (108)	19529b (128)	22772b (131)	21794a (106)	28305a 118)	14243a (74)	25679a (108)
	4	24029a (101)	ND	21372ab (123)	21594a (106)	ND	ND	ND
Complete nutrient treatment	1	23569a (99)	18232ab (119)	ND	25581a (125)	ND	ND	ND
	2	20834a (88)	18972b (124)	ND	21897a (107)	ND	ND	ND
	4	20228a (85)	ND	ND	22993a (112)	ND	ND	ND
F-ratio		0.503	2.332	1.924	1.447	1.032	1.579	2.026
Significance level		0.8788	0.0395	0.0981	0.1922	0.4213	0.1915	0.1111

Since both microbial biomass and microbial activity have been shown to decrease as a result of forest fertilization (Bååth et al. 1981; Söderström et al. 1983; Nohrstedt et al. 1989), a direct effect on ectomycorrhizal fungi cannot be excluded. The effect on the unidentified fungus vg 1 87.10 was immmediate; the mycelium did not start to colonize the new substrate and even the originally present mycelium vanished. An effect mediated by changes in the plant could not possibly be that rapid. Furthermore, the similar effects obtained with all the different nitrogen sources, and the fact that no clear dose-response effect was found (except for vgk 2 89.10), might also indicate that the negative effect on mycelial growth was a direct one, since it is known that nitrogen amendments of substrates with a high content of organic material can result in the production of toxic substances (Fog 1988). The negative effect on fungal biomass production obtained in the experiments by Wallander and Nylund (1991, 1992) could not, however, be explained by production of toxic substances, since they used expanded clay as substrate.

To summarize, it was demonstrated that the extension of extramatrical ectomycorrhizal mycelium of some different fungi is reduced as an effect of nitrogen amendments. These results are in close agreement with those obtained by Wallander and Nylund (1992), although we used completely different experimental systems. The fungi in my experiments responded in two distinctly different ways to the nitrogen treatment. Although the mycelial growth of all five isolates was reduced, four of them were moderately affected, an effect that could possible be explained by a reduced amount of carbon allocated from the host. The very pronounced and immediate effect on one of the fungi, vg 1 87.10 might indicate a direct effect on this fungus. Since explanations for neither the reduced mycelial production nor the differences in nitrogen tolerance have been established, further investigations are required.

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