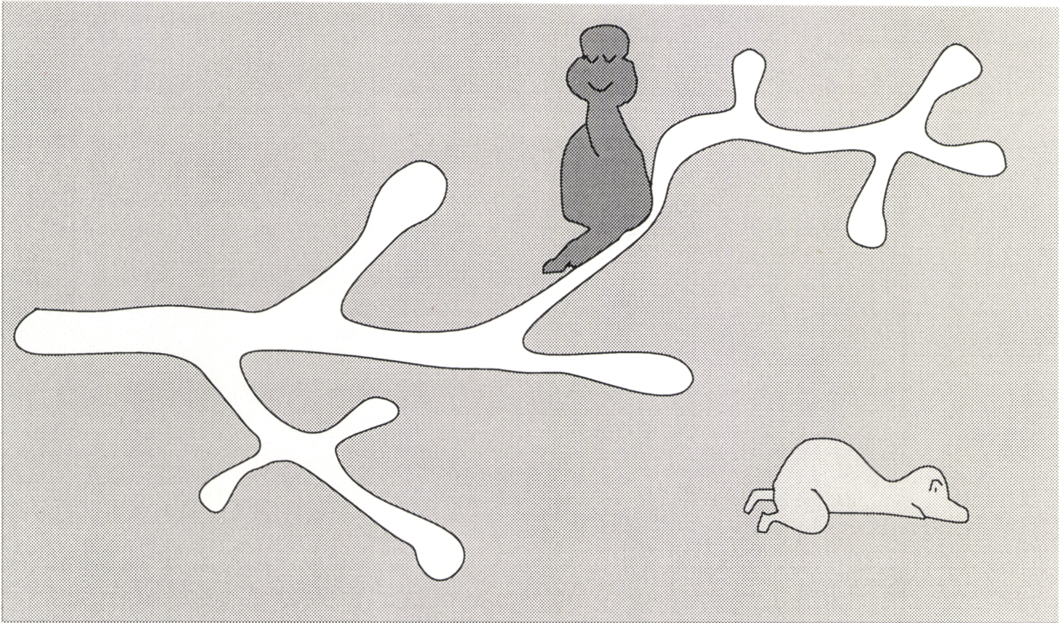

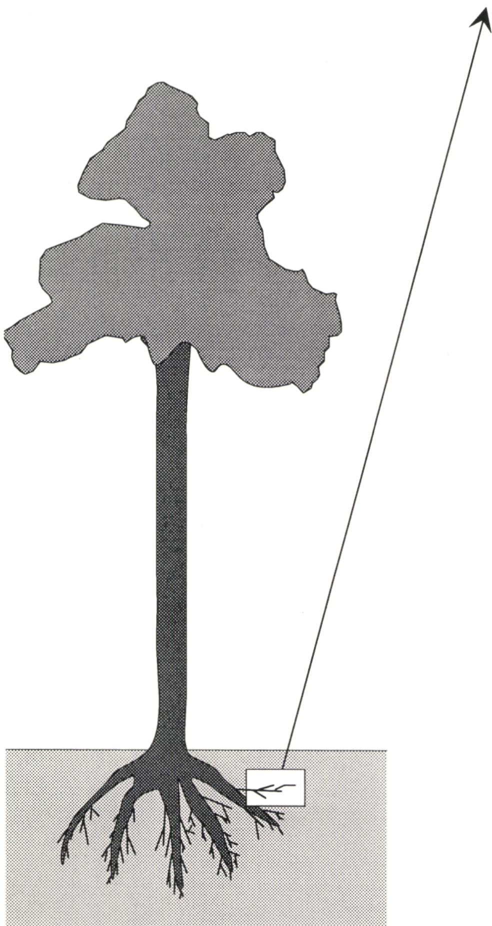

Roots, Mycorrhizas and Rhizosphere Microbes

NorFa – Workshop
in Hyytiälä, Finland
20.–22.9.1994

METSÄNTUTKIMUSLAITOS
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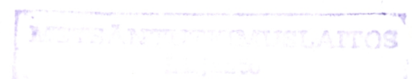

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Foreword

This volume contains the extended abstracts of papers and posters presented in the Nordic Workshop "The role of roots, mycorrhizas and rhizosphere microbes in carbon cycling in forest soil" , which took place 20-22 September 1994 in Hyytiälä Forest Station. The workshop was organised with the economic support kindly provided by NorFa.

The aim of the workshop was to bring together Nordic scientists working with the dynamics of belowground organisms in carbon cycling, to increase communication and understanding between researchers working in different disciplines and on different organisational levels (from cell to ecosystem), and to exchange information on new methods and research results dealing with the belowground processes connected with roots in forest soil.

We would like to thank the authors of papers and posters and all participants for contributing to a stimulating and successful meeting.

Vantaa 16th December 1994

On behalf of the organizers

Heljä-Sisko Helmisaari Aino Smolander

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Effects of nitrogen amendments on the growth and dispersal of some different ectomycorrhizal fungi grown in symbiosis with a host plant

Kristina Arnebrant

Introduction

It has often been reported that ectomycorrhizal fungi are negatively affected by nitrogen fertilization. Generally these studies have been focussed on the effects found on fruitbodies (Menge and Grand 1978, Ohenoja 1978, Wästerlund 1982) and ectomycorrhizal root tips (Menge et al. 1977, Alexander and Fairley 1983, Arnebrant and Söderström 1992). The effects of N amendments on the mycelial part of the symbioses have thus far received much less interest. In many aspects, however, the extramatrical mycelium is the essential part of the symbiosis, since it, for an individual host plant, will substantially increase the surface area in contact with the substrate, while for the individual fungus it is a prerequisite for dispersal and thus for the fitness of the fungi. Furthermore, at the plant community level the mycelium is of importance since it connects different plants with each other, thereby creating the possibility of translocating carbon (Read et al. 1985, Finlay and Read 1986) and nitrogen (Arnebrant et al. 1993) from one host to another. Thus, possible

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negative effects of nitrogen on the mycelial growth are of importance both to the host plant as well as to the fungus.

In laboratory experiments, using a semi-hydroponic cultivation system, a decreased production of fungal biomass, estimated as ergosterol, has been reported both in roots (Wallander and Nylund 1991, 1992) as well as extramatrical mycelium for three different ectomycorrhizal fungi (Wallander and Nylund 1992) as a result of increased nitrogen levels continously distributed in the nutrient solution.

In the present study the effects of inorganic nitrogen on the production growth and dispersal of some ectomycorrhizal fungi grown in symbiosis with a host plant was studied.

Material and methods

Transparent perspex microcosms with non-sterile peat as substrate were used in the experiments where the production of extramatrical mycelium was studied. 1, 2 or 4 mg of N g^{-1} dry weight peat as $(NH_4)_2SO_4$, $NaNO_3$ or as a complete nutrient solution were used. Five different fungal isolates, grown in symbiosis with pine seedlings, were investigated.

The effect of different N treatments on dispersal and competition between pairs of ectomycorrhizal fungi was investigated in pot experiments. Four pine seedlings, two colonized by one fungus and two with the other,were diagonally planted in a pot. In the center of the pot, one bait-seedling, at the start of the experiment uncolonized, was planted. The pots were continously watered with different nitrogen solutions, 50 and 100 ppm N solutions of KNO_3, $(NH_4)_2SO_4$ and a complete nutrient solution were used.

Results

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 treatment (Table 1). However, no significant difference was shown neither between the different N-concentrations within each nitrogen treatment nor between the different nitrogen sources. Thus, the values obtained in all nitrogen treatments were pooled to obtain one value which showed the overall effect of nitrogen on mycelial growth. The overall effect of inorganic nitrogen on all the fungi was a significant reduction of the mycelial growth as

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^{-1} dry weight peat. The maximum area was 256 cm^2. *Pinus contorta* was used as host plant for *Paxillus involutus* and *P. sylvestris* to 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. On the bottom line the table also includes values showing the effect of **nitrogen** on the area covered by mycelium. Percentages of control values are shown within brackets (from Arnebrant 1994).

	Fungus	*P. inv.* 87.02		*P. inv.* 374	vgk 2 89.10		vg 1 87.10	*S. bov.*
	Experiment	I	II		I	II		
Control		234^{a} (100)	116^{ab} (100)	145^{a} (100)	206^{a} (100)	121^{a} (100)	163^{a} (100)	96^{a} (100)
pH-control		214^{ab} (92)	180^{a} (155)	124^{a} (86)	123^{abc} (60)	80^{ab} (66)	146^{a} (90)	81^{a} (84)
$NaNO_3$	1	164^{ab} (70)	93^{ab} (80)	44^{a} (30)	140^{abc} (68)	66^{ab} (55)	2.8^{b} (1.7)	12^{a} (13)
	2	214^{ab} (92)	112^{ab} (97)	117^{a} (81)	135^{abc} (66)	70^{ab} (58)	8.7^{b} (5.3)	26^{a} (27)
	4	172^{ab} (74)	n.d[1]	73^{a} (50)	100^{abc} (49)	n.d	n.d	n.d
$(NH_4)_2SO_4$	1	182^{ab} (78)	102^{ab} (88)	26^{a} (18)	187^{ab} (91)	35^{ab} (29)	0.6^{b} (0.37)	43^{a} (45)
	2	156^{b} (67)	68^{ab} (59)	49^{a} (34)	96^{abc} (47)	13^{b} (11)	8.5^{b} (5.2)	33^{a} (34)
	4	172^{ab} (74)	n.d	76^{a} (52)	36^{c} (17)	n.d	n.d	n.d
Complete nutrient solution	1	211^{ab} (90)	64^{b} (55)	n.d	138^{abc} (67)	n.d	n.d	n.d
	2	209^{ab} (89)	46^{b} (40)	n.d	82^{bc} (40)	n.d	n.d	n.d
	4	174^{ab} (74)	n.d	n.d	50^{c} (24)	n.d	n.d	n.d
F-ratio		2.175	3.952	2.088	3.236	2.937	66.697	2.387
Sign. level		0.038	0.002	0.082	0.003	0.033	0.000	0.068
nitrogen effect		188* (80)	88* (76)	64* (44)	107* (52)	46** (38)	5.1*** (3.1)	28** (29)

shown in Table 1. The mycelial growth of all fungi was found to be reduced by the nitrogen amendments, although the sensitivity to N varied between the isolates. One unidentified fungus was extremely sensitive and its growth was completely inhibited by all the N treatments. In contrast, the least sensitive fungus was one of the *Paxillus involutus* isolates which growth decreased by approximately 20 %. The mycelial growth of the other three isolates was reduced by 50-70 %. In the experiments studying effects of nitrogen treatments on colonization potential a significant reduction of ectomycorrhizal root tips was shown for all nitrogen treatments within one experiment including *Hebeloma crustuliniforme* and *Piloderma croceum* (Table 2). The distribution of the two fungal species on the bait seedling was, however, shown to be different depending on the nitrogen treatment (Table 2). In contrast, in another experiment that included *P. involutus* and *Suillus bovinus* all N treatments negatively affected *S. bovinus* (Table 3).

Discussion

It was clearly demonstrated that nitrogen can affect the competitive interactions between different ectomycorrhizal fungi. In the experiment that included *H. crustuliniforme* and *P. croceum*, it was indicated that different nitrogen sources can have different effects on the competitive success of different fungi. In this case, although both fungi were negatively affected of all nitrogen treatments, *P. croceum* appeared to be relatively more sensitive to ammonium than to nitrate compared to *H. crustuliniforme* and thus lost in competitive success. The opposite was indicated in the treatment with a complete nutrient solution where the percentage of ectomycorrhizal root tips colonized with *P. croceum* tended to increase compared to all other treatments, and thus the relative success of this fungus was increased compared to *H. crustuliniforme*.

In contrast, all nitrogen treatments including the complete nutrient solution, all affected the relative competitive success of *Suillus bovinus* and *Paxillus involutus* in the same way. Although the total percentage of ectomycorrhizal root tips only were significantly reduced in the nitrate compared to the ammonium treatment, all significantly reduced the relative success of *S. bovinus*. Thus, not only the relative success of *P. involutus* increased in all nitrogen treatments, but also the total colonization level by this fungus tended to increase in the ammonium treatment. In contrast

Table 2. Data showing the colonization pattern of the bait-seedling from the pot experiment including *Hebeloma crustuliniforme* and *Piloderma croceum*. The pots were treated with 50 or 100 ppm N solutions of KNO_3 or $(NH_4)_2SO_4$ or a 50 ppm N of a complete nutrient mixture, and incubated for four months. One-way ANOVA was performed on either log- or arcsine square root-transformed values (percentages). Duncan's multiple range test $p<0.05$, N=5.

Treatment	total no. of root tips	total no. of ectomyc. root tips	percentage ectomyc. root tips	total no. of *P. croceum*	percentage *P. croceum* of total ectom.
Control	436[a]	204[a]	48.1[a]	15.2[a]	7.88[a]
KNO_3					
50	855[b]	189[a]	24.2[b]	13.4[ab]	7.56[a]
100	1097[bc]	133[ab]	12.7[bc]	6.2[ab]	6.71[a]
$(NH_4)_2SO_4$					
50	766[b]	184[a]	24.6[b]	3.2[ab]	2.58[a]
100	994[b]	67[b]	7.5[c]	2.6[b]	2.04[a]
Complete nutrient solution					
50	1746[c]	103[ab]	6.8[c]	16.5[a]	17.80[a]
F-ratio	14.42	6.229	14.19	2.708	1.971
p (sign. level)	< 0.001	< 0.05	< 0.001	< 0.05	0.121

Table 3. Data showing the colonization pattern of the bait-seedling from the pot experiment including *Paxillus involutus* and *Suillus bovinus*. The pots were treated with 50 ppm solutions of KNO_3, $(NH_4)_2SO_4$ or a complete nutrient mixture, and incubated for 6 months. One-way ANOVA was performed on either log- or arcsine square root-transformed (percentages) values. Duncan's multiple range test $p<0.05$, N=5.

Treatment	total no. of root tips	total no. ectom. root tips	percentage ectom. root tips	total no. *S. bovinus*	percentage *S. bovinus* of total ectom.
Control	496[ab]	178[a]	36.0[ab]	93.6[a]	56.3[a]
KNO_3					
50	1087[bc]	113[a]	10.9[a]	9.2[b]	10.5[b]
$(NH_4)_2SO_4$					
50	343[a]	178[a]	46.8[b]	21.6[b]	13.1[b]
Complete nutrient solution					
50	1155[c]	260[a]	22.7[ab]	25.8[b]	7.3[b]
F-ratio	11.785	0.909	2.844	4.622	10.937
p (sign. level)	< 0.001	0.459	0.071	< 0.05	< 0.001

to the fungi used in the other pot experiment, both the fungi in this were included in the study concerning effects of different nitrogen sources on mycelial growth (Arnebrant, in press), and the differences in competitive ability could possibly be explained by the differences in nitrogen sensitivity. *P. involutus* was in that study shown to be the least affected fungus, although it's growth was significantly reduced with approximately 20 %. The mycelial growth of *S. bovinus* (although another isolate than was used in this study) was reduced with approximately 70 % compared to the growth in the control. This fungus was also the most sensitive one of the three used by Wallander and Nylund (1992). The fruitbody production by *S. bovinus* has been shown to decrease in ammonium nitrate fertilized plots (Wästerlund 1982), while *P. involutus* is known to be one of those few that actually increases it's fruitbody production as an effect of ammonium treatment (Hora 1959, Laiho 1979, Shubin 1988).

The explanation to the reduced mycelial growth caused by increased nitrogen level is not evident but has been substantially discussed by Nylund (1988) and Wallander (1994). What's even more intriguing to discuss is why different ectomycorrhizal fungi are differently sensitive to nitrogen. What mechanism makes *P. involutus* more successful than for example *S. bovinus* in nitrogen treated soils?

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Effects of collembolan grazing on the ectomycorrhizal symbiosis

Kristina Arnebrant[1], Hans Ek[2], Maria Sjögren[3] and Bengt Söderström[1]

Introduction

Little is known about effects of microarthropods on the function of the ectomycorrhizal symbiosis (Moore 1988). There are, however, a few studies focusing on the relationship between arbuscular mycorrhiza and their grazers and the effects on the host plant. Kaiser and Lussenhop (1991) demonstrated that collembolans can interfere with the establishment of arbuscular mycorrhiza. Warnock et al. (1982) found that the positive effect of arbuscular mycorrhizal colonization on the growth of leek disappeared in the presence of collembolans. This was explained as an effect of the collembolans grazing on the external hyphae. Finlay (1985) studied interactions between the collembola *Onychiurus ambulans* and arbuscular mycorrhizal *Allium porrum* and observed that at low grazing intensities, mycorrhizal plant phosphorus uptake was stimulated, but at higher grazing pressure P uptake was reduced. Similar non-linear effects of collembolans on plant growth and P uptake have also been shown in arbuscular mycorrhizal *Geranium robertianum* (Harris and Boerner 1990).

The Collembola-ectomycorrhiza interaction involves a host plant which may be indirectly affected by the grazing activities. In our study we have therefore tried to evaluate the effects of grazing

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not only on the two main components directly affected, the ecto-mycorrhizal fungus and the Collembola, but also on the plant.

Material and methods

A microcosm system consisting of transparent polystyrene chambers 245mm x 245mm x 25mm (so called screening plates) was used. In these systems three *Pinus contorta* seedlings were planted, one colonized by the ectomycorrhizal fungus *Paxillus involutus* planted in the center and on each side of this, two at the start of the experiment noncolonized seedlings. A sandy soil, with a pH (H_2O) of 4.6 was used as substrate. The soil was microwaved prior to the experiment to obtain a sterile soil. To the microcosms 12 small cups (1.5 cm in diameter), each containing soil with 100 µg N as $^{15}NH_4Cl$, covered with a net that allowed the fungal hyphae to penetrate but neither Collembola nor plant roots, were added. The microcosms were incubated for 88 days in growth cabinets.

There were four different treatments, mycorrhizal or non-mycorrhizal, with 0 or 50 collembolans of the species *Onychiurus armatus* added per microcosm. Ten microcosms of each treatment were used.

In a pilot experiment the effect of different densities of *O. armatus* on mycelial growth was studied. In this experiment we added 0, 20, 75 or 200 animals per microcosm.

Mycelial growth was measured continously. At harvest we measured plant biomass (as dry weight), ^{15}N-uptake, fungal biomass (as ergosterol) on roots and in the soil, microbial biomass (as ATP content) in the soil and numbers of Collembola and nematodes.

Results and discussion

The mycelial growth rate was significantly stimulated by the presence of a low density (50) of Collembola, the fungus grew 60 % faster in the grazed treatment, 13.6 cm^2 day^{-1} compared to 8.4 cm^2 day^{-1} in the control. The colonization rate of the side plants was also higher in the microcosms with animals (data not shown). The production of extramatrical mycelium was increased in the grazed treatment, while the amount of fungal biomass found in the roots was unaffected (Table 1). Thus, in the grazed microcosms 62 % of the *P. involutus* grew as extramatrical mycelium compared to 42 % in the nongrazed. Furthermore,

Table 1. Biomass of *Paxillus involutus* in plant roots and soil. A conversion factor of 9.6 µg ergosterol mg^{-1} fungal dry weight was used to calculate biomass from ergosterol values. The amount of ergosterol found in soil from non-mycorrhizal microcosms was subtracted from the corresponding mycorrhizal treatment (modified from Ek et al., 1994).

Grazing	Amount of *P. involutus* in root (mg)	Amount of *P. involutus* in soil (mg)
+	12.8[a]	20.4[a]
-	13.2[a]	11.5[b]

Table 2. Number of Collembola at start and at harvest of the two experiments (modified from Ek et al., 1994).

	Mycorrhiza	No. of collembola at start	No. of collembola at harvest	Percentage increase
Main	+	50	63	25
	-	50	57	14
Pilot	+	20	126	630
	+	75	459	610
	+	200	384	190
	-	75	332	440

Table 3. Seedling biomass and proportion of the plant allocated to the shoot (modified from Ek et al., 1994).

Mycorrhiza	Grazing	Plant biomass (mg)	Biomass allocated to shoot
+	+	394[a]	41%[a]
+	-	344[a]	36%[a]
-	+	156[b]	30%[b]
-	-	175[b]	24%[b]

Table 4. Amount of ^{15}N taken up by the plants. In total 1.2 mg of ^{15}N, available only to the fungus, was added to each microcosm (from Ek et al., 1994).

Mycorrhiza	Grazing	Mid-plant (µg ^{15}N)	Side-plant (µg ^{15}N)
+	+	232[a]	215[a]
+	-	132[b]	177[a]
-	+	5.6[c]	12[b]
-	-	4.3[c]	12[b]

the proportion of fungal biomass of the total plant and fungal biomass increased in the grazed system to 5.3 % compared to being 3.7 % in the ungrazed. The Collembola thus induced a change in the carbon allocation pattern and increased the amount allocated to the soil. In the pilot experiment the collembolan population increased dramatically during the experiment, and even the lowest density was higher than in the main experiment (Table 2). The mycelial growth was reduced in all treatments with collembolans and was only 66 % of the growth rate in the control in the treatments with the highest density of *O. armatus*.

The plant biomass was significantly higher in the mycorrhizal treatment, while grazing had no apparent effect (Table 3). Furthermore, the proportion of biomass allocated to the shoot was significantly increased by the mycorrhizal treatment. The nitrogen content (both ^{14}N and ^{15}N) was consistently higher in mycorrhizal compared to non-mycorrhizal plants. There was no effect of *O. armatus* on the total nitrogen content but the uptake of ^{15}N was significantly higher in the mycorrhizal and grazed treatment, thus the mycorrhizal midplants in the grazed microcosms had 76 % higher ^{15}N content than the corresponding plants with mycorrhiza but without grazing pressure (Table 4). There was, however, no linear relationship neither between the amount of ergosterol in the substrate and total nitrogen content in the plants nor between fungal biomass in the cups and ^{15}N content in the plants. In the first case the grazed microcosms had higher amount of fungal biomass but no difference in total N content in the host plant was found, while in the second there was no difference in fungal biomass in the cups but significantly higher amount of ^{15}N in the plants.

We thus found a similar effect of collembolan grazing as has been reported for arbuscular mycorrhizal systems, i.e. that a low density increase growth and nutrient uptake, while higher densities reduce growth. There are a few possible mechanisms for the stimulatory effect. The first is that the fungus reacts with overcompensatory growth at a low grazing pressure. The increased amount of fungal biomass in combination with that younger hyphae might be more efficient in nutrient uptake explains the increased nutrient uptake. A prerequisite for this explanation is that the collembolans are grazing directly of the ectomycorrhizal fungus. We have, however, no such evidence. In a third experiment we collected the Collembola and analyzed their content of ^{15}N. No difference was found between animals from mycorrhizal or nonmycorrhizal microcosms but since the biomass of the collem-

bolans was low the measurement was close to the detection limit. Furthermore, in the pilot experiment the population of *O. armatus* increased up to sixfold, and the Collembola were seen to be gathered at the top of the chambers were a band of green algae grew. In the main experiment where we had taken precautions to avoid algal growth there was only a slight increase of the collembolan population and there was no difference between the mycorrhizal and nonmycorrhizal treatments. Thus, *P. involutus* does not appear to be a good food source for *O. armatus*. However, the reduced mycelial growth as an effect of higher densities of *O. armatus* indicate a direct grazing effect.

Another explanation involves selective grazing by the collembolans on other microorganisms, in this way reducing the competition. This hypothesis has been discussed by Newell (1984a and b) and Parkinson et al. (1979). However, we could not find any clear evidence of this either since the ATP content was not reduced and in a later experiment, where we studied the microfugal community structure, no obvious differences were noted between treatments (data not shown).

A third explanation could be that the Collembola makes nutrients more easy available to the fungus.

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Fine root dynamics in Canadian boreal forest stands at different successional stages after fire

Leena Finér[1] *and Christian Messier*[2]

Background

Vegetation types vary in southern boreal forests in the Northern Clay Belt of Québec and Ontario in relation to the surficial deposits and successional stages. Clay deposits are typical on lowlands where early successional stands are characterized by an abundance of deciduous species, primarily trembling aspen (*Populus tremuloides*) and paper birch (*Betula papyrifera*), which are replaced later in succession by the coniferous species white spruce (*Picea glauca*), balsam fir (*Abies balsamea*) and eastern red cedar (*Thuja occidentalis*) (Bergeron and Dubuc 1989). The growth and structure of the forests are controlled by three disturbances: fire, spruce budworm (*Choristoneura fumiferana*) and man. The fire cycle is around 100 years, and the forests in the area seldom reach the climax stage of succession. Spruce budworm outbreaks occur at regular intervals. The forest is vulnerable to attack in the late successional stages dominated by white spruce and balsam fir. Conifers grow in mixed stands, and deaths caused by spruce budworm create gaps. The human impact has been minor so far.

The above-ground plant succession is relatively well documented in forests of the Clay Belt region, but hardly anything is known about the below-ground dynamics. The aim of the study

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was to investigate how the fine root distribution and production of trees and ground vegetation vary in forest stands at different successional stages after fire. This was one of the many projects being carried out in the boreal forests of the area, and was conducted by the forest research group of the University of Québec in Montréal.

Material and methods

Study sites

The material was collected from three southern boreal forest stands at different successional stages after fire around lake Duparquet in northeastern Québec, Canada. The youngest stand, which had developed since the last fire 48 years ago, was dominated by paper birch and trembling aspen. The ground layer was dominated by herbs and mountain maple (*Acer spicatum*). Trembling aspen, white spruce and balsam fir were the main tree species in the mid successional stand, which had been burnt 122 years ago. The ground layer was formed by herbs, grasses and shrubs; the mountain maple and American yew (*Taxus canadensis*) especially were important species. The late successional, 232-year-old stand was characterized by eastern red cedar, balsam fir and aspen. The ground layer was dominated by mountain maple and American yew. The thickness of the organic layer had increased as a result of ageing of the stand from 5.3 cm to 8.0 cm.

Sampling

The study was carried out in each of the tree stands on three replicates of 10 x 10 m plots for each of the two treatments: 1) gap 2) control. Fine and small roots (Ø $\leq$ 10 mm) were sampled on the control plots by the core method at the beginning of June 1993. In the laboratory the roots were separated from eight soil cores per plot (area 38 cm^2) and divided into diameter classes. The small roots (Ø 2-10 mm) were further separated by species. The length and biomass of fine roots were measured.

The fine root production was measured by the ingrowth bag method on both the control plots and gaps. Six 30 cm long bags, filled with rootfree clay from the site, were installed on each plot in August 1992. The ingrowth bags were dug up in September 1993.

Table 1. Total fine and small root (Ø $\leq$ 10 mm) biomass, length and biomass production in stands at different successional stages after fire. Standard deviation in parentheses.

Site	Root biomass g/m^2	Root length m/m^2	Root biomass production $g/m^2/yr$
48 yrs	1056 (289)	12857 (3667)	130 (75)
122 yrs	827 (193)	8200 (1107)	61 (36)
232 yrs	952 (170)	7181 (404)	47 (18)
	F-value 0.80 p-value 0.49	F-value 5.55 p-value 0.04	F-value 2.43 p-value 0.17

Preliminary results and discussion

Root biomass

The youngest of our stands had not yet reached its maximum above-ground biomass, whereas the 122- and 232-year-old stands had passed the stage of maximum biomass, partly due to spruce budworm outbreaks in the late 1980's. The fine and small root biomass did not vary between the sites (Table 1). The proportion of fine and small root biomass out of the total biomass correspondingly increased with the aging of the stand. However, total root length was longer in the youngest stand than in the older stands, which could indicate a better nutrient uptake ability in the early successional stage compared to later stages.

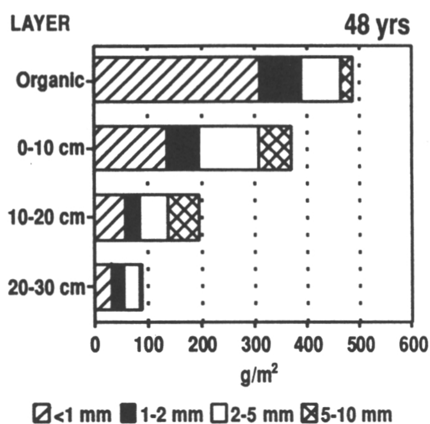
As in many studied forest ecosystems, the root systems were superficial in all stands (Fig. 1) (Persson 1980a, 1983, Gale and Grigal 1987). The results did not indicate any changes in total rooting depth along with succession.

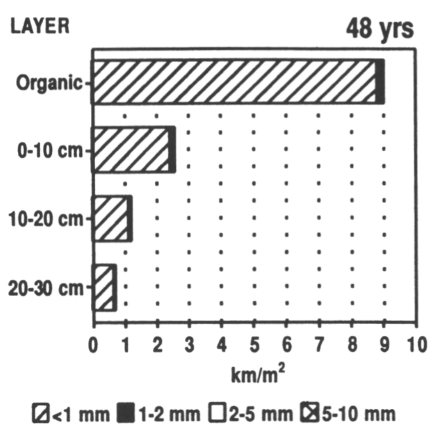
The roots of tree species accounted for 80, 66 and 67 % of the small root biomass, respectively (Fig. 2). The tree density did not vary between the sites, which may explain why the proportion did not correlate with stand age as has been reported in some other stands (Persson1979, Vogt et al. 1983). Aspen dominated the early and mid successional stands, but was replaced by cedar in the old stand. The abundance of birch decreased along with the increase in the abundance of spruce and fir. American yew became more dominant than the other shrubs at mid succession.

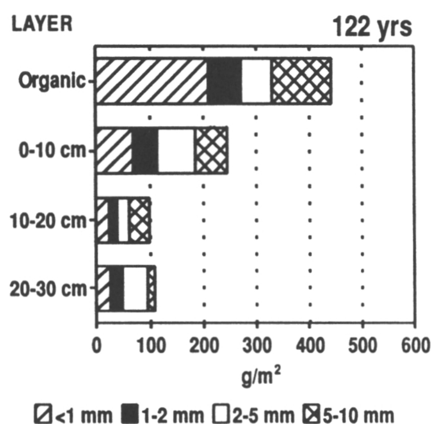
Root production

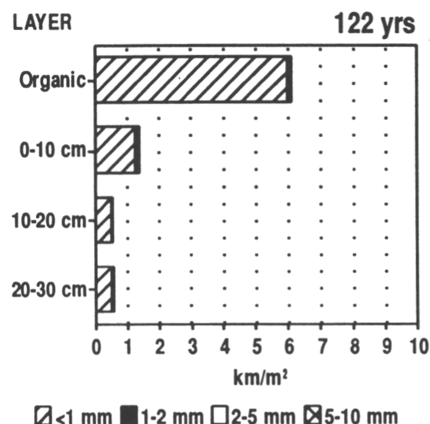
The fine root production was studied with the ingrowth bag method, which is known to give results comparable to the

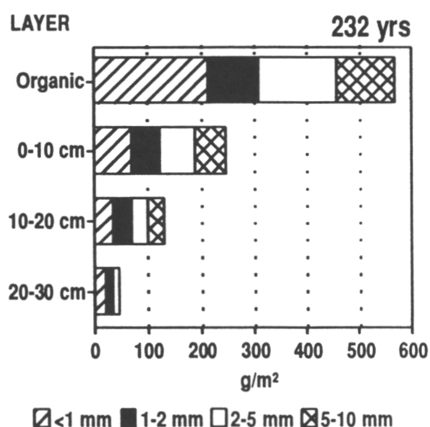
Biomass — *Length*











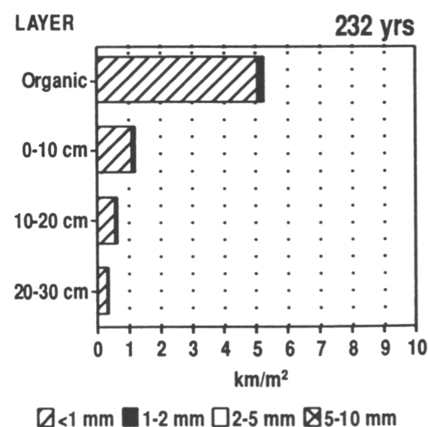


Figure 1. Fine and small root (Ø ≤ 10 mm) biomass and length in different soil layers and diameter classes in stands at different successional stages after fire.

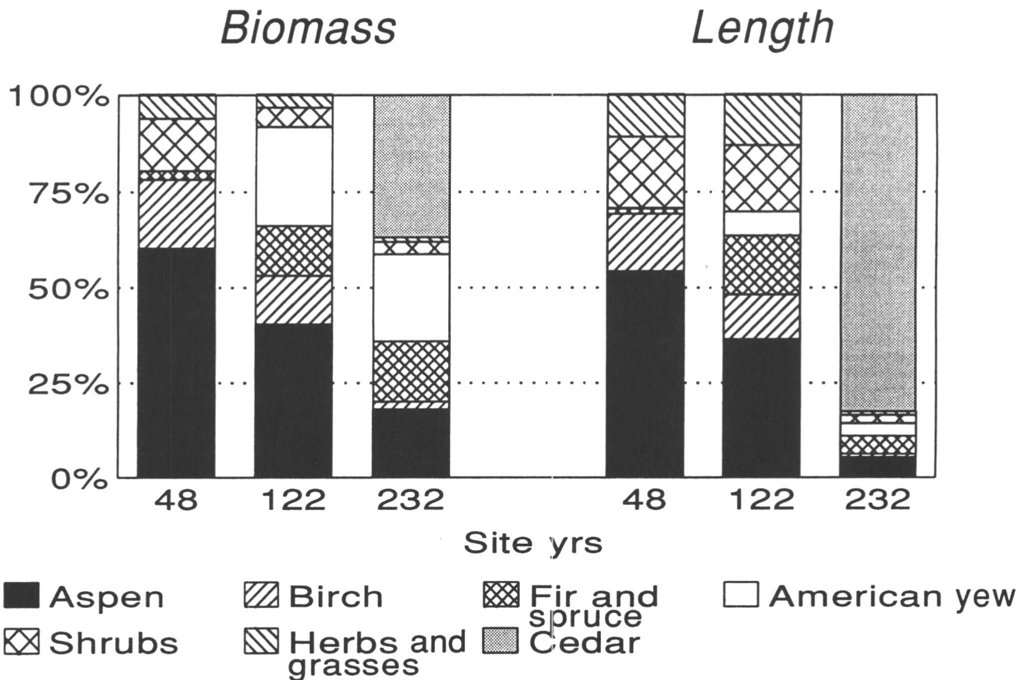


Figure 2. Distribution of small root (Ø 2-10 mm) biomass and length between different plant species in stands at different successional stages after fire.

sequential coring method if the time elapsed since installation of the bags to sampling is long (Persson 1979, 1980b). The results clearly indicated that the time was too short for the roots to have grown into the bags, since the total root biomass (Ø < 5 mm) found in the bags was only 6-14 % of that in the cores (Table 1). The results did not show any statistically significant differences in fine root production between the sites. In absolute figures, however, the production seemed to decrease along with the age of the stand.

The effect of gaps

The effect of the small gaps in the stand was studied by the ingrowth bag method in artificially created gaps to simulate those gaps caused by spruce budworm attacks. The formation of an above-ground gap did not create any below-ground gaps (Tables 1 and 2). Fine root production was similar in the center, at the edge and in the intact forest outside the gap. The short study period or the small size of the gaps may have affected the results. The results from some previous studies are in accordance with ours (Vogt et al. 1993), but some are contradictory (Wilczynski and Pickett 1993).

Table 2. Total small root (≤ 5 mm) biomass production in the center, at the edge and in the forest outside the gap in stands at different successional stages after fire. Standard deviation in parentheses.

	Root biomass production g/m^2/yr				
Site	Center	Edge gap	Edge forest	F-value	p-value
48 yrs	76 (13)	76 (24)	78 (24)	0.01	0.99
122 yrs	35 (11)	37 (10)	51 (13)	1.56	0.29
232 yrs	36 (40)	73 (24)	64 (5)	1.47	0.30

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Fine-root biomass and turnover in Norway spruce and Scots pine stands

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Introduction

The production, turnover and decomposition of roots are major processes in the carbon and nutrient dynamics of forest ecosystems. Fine root production represents a large and relatively unknown portion of the production/decomposition carbon balance (Aber et al. 1985). Fine roots are in constant flux, with death and replacement taking place simultaneously. Even though the proportion of fine roots and mycorrhizas of the total tree biomass is not large, their growth and maintenance require a major part of total net primary production. Therefore their role in the carbon cycle is more important than may be expected on the basis of instantaneous biomass measurements. Vogt et al. (1982) estimated that although only about 1 % of the biomass of *Abies amabilis* stands was in mycorrhizal fungi, about 15 % of the net primary production was allocated into these fungi. For total fine root production, allocation values between 60-73 % were reported by Ågren et al. (1980) and Fogel and Hunt (1983). The majority of organic input to the decomposition process results from fine-root production (78-84 % of total tree return, Fogel and Hunt 1983).

The objective of this research was to determine Norway spruce and Scots pine fine-root biomass and turnover in different conditions. The fine-root studies are a part of several on-going studies dealing with carbon and nutrient cycling in forests.

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Methods

Fine-root and mycorrhiza turnover (seasonal growth and death), and microbial biomass and activities (Smolander et al., this volume) relevant to carbon and nutrient cycles are studied in several forest stands in Finland. Norway spruce fine root biomass and turnover is studied during 1993-1995 related to soil moisture in a hydrological catena close to Hyytiälä field station in southern Finland. The root research is a part of the project "Carbon in boreal coniferous forest soil" carried out by the University of Helsinki, Dept. of Forest Ecology, and the Finnish Forest Research Institute. Norway spruce fine root biomass and turnover is also studied during 1993-1995 in stands that have received different long-term nutrient applications. Scots pine fine root biomass and turnover was studied in 1985-88 related to stand age in eastern Finland (Helmisaari 1994, Makkonen, this volume), and is studied in 1993-1995.

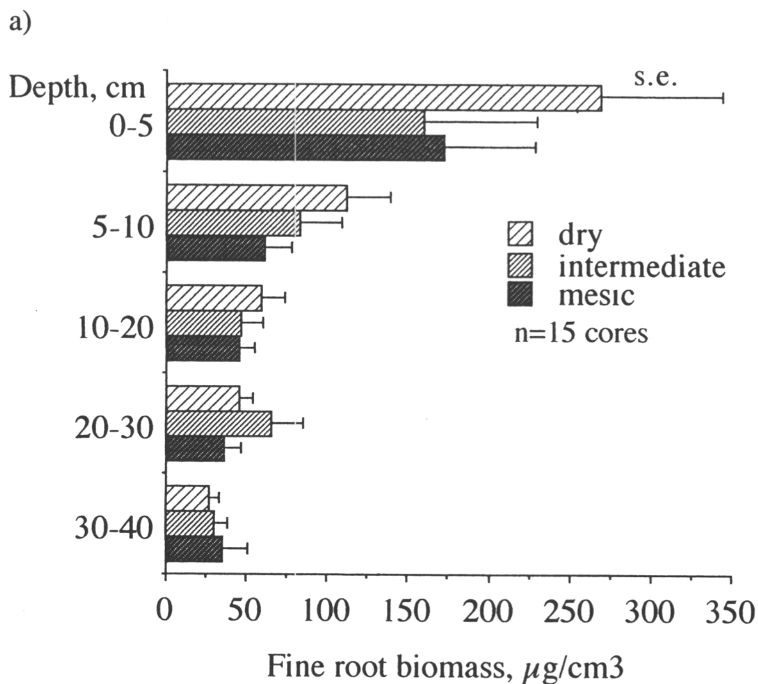
Fine-root turnover is studied with root ingrowth cores. Ingrowth core is a nylon net mesh filled with sorted and rootless mineral soil, and inserted into the hole in soil made by a cylindrical soil corer. The total amount of living and dead fine roots was studied with cylindrical soil core sampling to the depth of 40 cm mineral soil at installing the ingrowth cores.

Roots which had grown into the ingrowth and soil cores were sorted out through dry sorting and washing. Roots were sorted for tree species and field layer and for living and dead. The dry weights of roots were measured for different root diameter classes. The changes in the amounts of living and dead roots within a certain time gives an estimate of the root turnover. The increase in the amount of dead roots gives an estimation of the production of root litter. The changes in the number of mycorrhizal root tips was studied using subsamples of the samples for estimating the amounts of roots.

Results and discussion

Soil temperature and moisture affect fine-root growth. Results from the study about Norway spruce fine root turnover related to soil moisture indicate that during the first year after installation more fine roots and their mycorrhiza had grown into the ingrowth cores in the dry site. Ratio dead/living roots and dead/living mycorrhiza increased with depth in soil (Fig. 1). Fine root ingrowth was low during the first year after placing the ingrowth

a)



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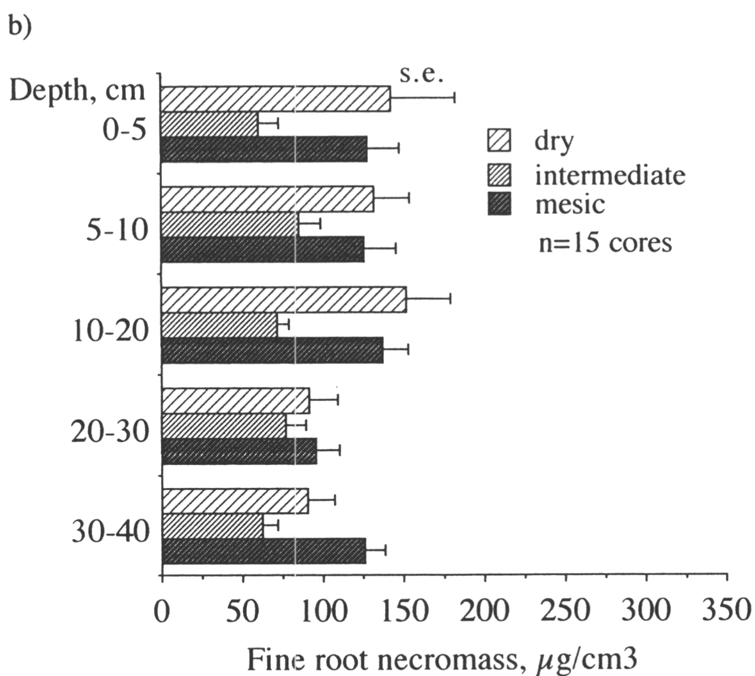

Figure 1a. Conifer fine-root (d<1 mm) biomass and b) necromass in the ingrowth cores in three Norway spruce stands in September 1993, one year after placing the ingrowth cores in soil.

cores, but is expected to increase considerably during the second year. Therefore, realistic estimates of fine roots and mycorrhiza as carbon sinks are not expected before monthly samplings in 1994.

Changes in nutrient availability may affect carbon allocation into different parts of the tree. The results from the study concerning Norway spruce fine-root biomass related to long-term lime and nitrogen applications in eastern Finland show that there were less living and more dead Norway spruce fine roots on the limed plot compared with the N-fertilized or control plots (Fig. 2). These results are in agreement with results by Lehto (1994).

There is a large variation in ratio of aboveground to belowground biomass and production in different forest communities of the same species, age being one of the determining factors. Results concerning the Scots pine fine root biomass related to stand age show that the stand belowground / aboveground biomass ratio decreased with tree age. In the sapling stand, 61 % of belowground biomass were fine roots. Similar values for pole stage and mature stands were 36 and 16 %, respectively. Of the total tree biomass, 16, 8 and 2 % were fine roots in sapling, pole stage and mature stands, respectively. Despite great differences in the aboveground biomass, the amount of living fine roots was only

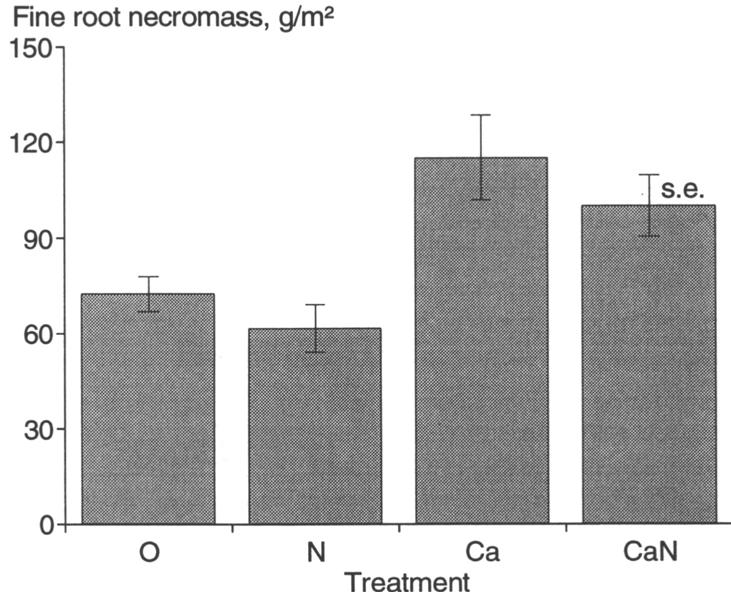

Figure 2. Norway spruce fine-root (d< 2 mm) necromass in the humus layer in May 1993 in plots (25 samples per plot) given lime 6 000 kg/ha and nitrogen 858 kg/ha separately and in combination during a period of 30 years.

slightly greater in the mature stand compared with the sapling stand. However, almost three times more nitrogen had to be taken-up for biomass production in the mature stand than in the sapling stand (Helmisaari 1994). These results confirm the finding of Nikinmaa (1993), according to which root to shoot ratio depends inversely on the nutrient uptake per unit mass of fine roots.

The results presented here were mostly first year fine-root biomass results from different studies. Results on fine-root growth dynamics from intensive sampling of root ingrowth cores are under preparation. Fine-root biomass varies considerably during the growing season (Makkonen, this volume). Seasonal variation should be considered when interpreting biomass results from different experiments based on sampling in single occasions.

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Cellular changes and accelerated senescence of conifer mycorrhizas related to high nitrogen availability

Toini Holopainen[1], Sari Janhunen[1] and Helvi Heinonen-Tanski[2]

Introduction

Wide forest areas are nowadays exposed to elevated nitrogen deposition (Nihlgård 1985). Depending on soil characteristics and the concentration of other nutrients, there exists an optimum level of N for tree growth and also for mycorrhizal development. However, some studies have indicated that the N optimum for mycorrhizas is rather low and serious decline of the fungal partner may occur before the optimum for tree growth has been achieved (Alexander and Fairley 1983, Laiho et al. 1987, Holopainen and Heinonen-Tanski 1993). In mycorrhizal roots the nitrogen and carbon metabolisms are closely related and increased carbon demand for synthesized nitrogen compounds may partly explain the limited growth of fungi (Plassard et al. 1991, Wallander and Nylund 1991). In this presentation we summarize some of the most important observations on Scots pine mycorrhizas exposed to high nitrogen availability, concentrating on ultrastructural methods.

Materials and methods

Experimental nitrogen exposures of mycorrhizal Scots pine seedlings: Scots pine seedlings were grown in natural forest

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humus receiving the mycorrhizal inoculum from the growth medium. In the first experiment (Holopainen and Heinonen-Tanski 1993) the seedlings received three different nitrogen sources, $NaNO_3$, NH_4-acetate and ureaformaldehyde each in four levels corresponding to 0, 50, 150 and 300 kg/ha N. In the second experiment (Kottke et al. 1994) two ammonium sources, NH_4Cl and $(NH_4)_2SO_4$, at total doses of 50 and 100 kg/ha N were used.

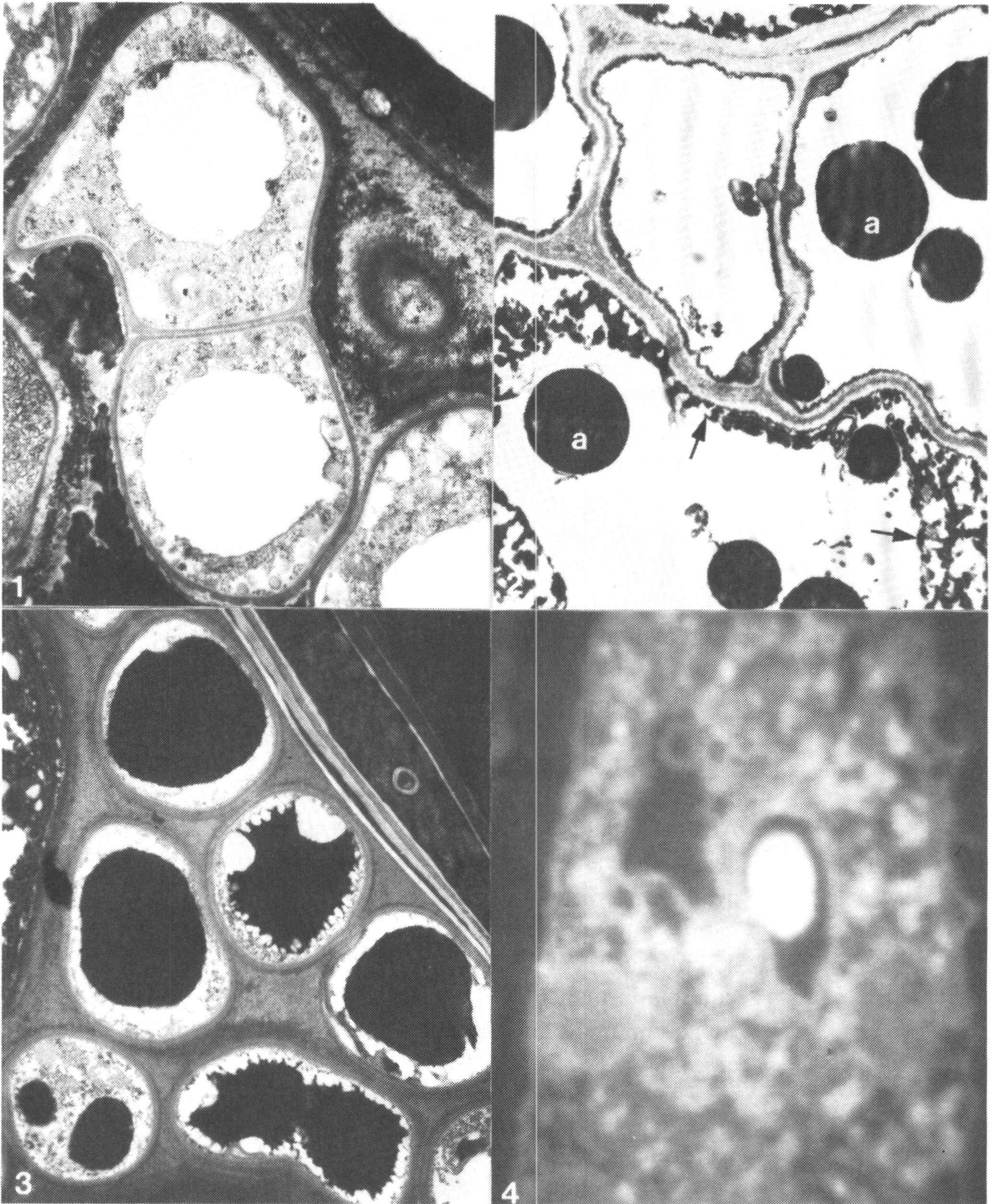
Mycorrhizas in industrial environment: Samples of several mycorrhizal types of Scots pine were collected from the environment of a pulp mill near the city of Kuopio. During the study period, the annual SO_2 emissions were about 10 000 t/y, NO_x emissions 400 t/y and NH_4 emissions 16 t/y. Elevated nitrogen concentrations of the humus layer have been measured in this environment (Holopainen et al. 1994).

Pure cultures of mycorrhizal fungi: Experiments for comparative purposes were carried out with pure cultures of *Cenococcum geophilum* and *Paxillus involutus*. The fungi were grown on Petri dishes on MMN medium where the levels of ammoniumchloride (NH_4Cl) added were 62.5, 125, 250 (optimum), 500 and 750 mg/L.

Methods: The different mycorrhizal types were identified and counted under a dissecting microscope (Holopainen and Heinonen-Tanski 1993). For electron microscopy mycorrhizas and pieces of pure cultures were fixed in glutaraldehyde and osmiumtetroxide and thin sections stained on grids with uranylacetate and lead citrate (Holopainen and Heinonen-Tanski 1993). Electron energy loss spectroscopy (EELS/ESI) was applied at the University of Tübingen, Germany (Kottke et al. 1994).

Results and discussion

Our results on shoot and root growth and mycorrhiza development in relation to nitrogen availability in experimental exposures were in agreement with earlier observations (Alexander and Fairley 1983, Laiho et al. 1987, Gagnon et al. 1988, Högberg 1989). In our experiments nitrate inhibited more clearly the mycorrhiza development than ammonia (Holopainen and Heinonen-Tanski 1993). At the cellular level all the used nitrogen sources induced a similar developing pattern of changes (Figs. 1 and 2). In the first phase, development of dark vacuolar accumulations accompanied with a decline of glycogen granules and lipid bodies, were evident. A dark staining and gradual disintegration of fungal cytoplasm were observed after exposure to higher nitro-



Figures 1-4. Electron micrographs of Scots pine mycorrhizas. 1) Normal structure of *C. geophilum* in sheath (low nitrogen availability). 2) Fungal cells of *C. geophilum* in sheath showing cytoplasm disintegration (arrows) and large electron dense vacuolar accumulations (a) (NH_4-N, 300 kg/ha). 3) Electron dense vacuolar bodies in a brown dichotomous mycorrhiza in an industrial environment (pulp mill, 600 m N). 4) EELS/ESI-image showing nitrogen deposition (light area) in a vacuolar body in *C. geophilum* under high nitrogen availability.

gen levels (Fig. 2). Intracellular penetrations of fungi were often observed in senescing host cells (Holopainen and Heinonen-Tanski 1993). Very similar cellular changes and limited infection levels were observed in mycorrhizas collected from the industrial environment (Fig. 3) (Holopainen et al. 1994).

The EELS/ESI-analysis revealed that the vacuolar bodies in *C. geophilum* contained high levels of nitrogen (Fig. 4) accompanied with accumulation of phosphorus (Kottke et al. 1994). This observation suggests that vacuolar bodies may consist of proteinaceous material (Turnau et al. 1994) or stored aminoacids (Plassard et al. 1991), although other elements seem to deposit into the vacuoles together with stored nitrogen, as well (Turnau et al. 1994).

The structural observations from the pure cultures of *C. geophilum* and *P. involutus* agreed very well to the observations from mycorrhizas. This indicates that mycorrhizal state is not necessary for the formation of vacuolar storage bodies and accelerated senescence of fungi, which suggests that nitrogen has also direct degenerating effects on mycorrhizal fungi.

Conclusions

Under high nitrogen availability mycorrhizal fungi, both in symbiosis and pure cultures, can store nitrogen as nitrogen-rich vacuolar deposits. Sooner or later, depending on species, the storage capacity is saturated, leading to toxic effects of excess nitrogen. At ultrastructural level this is observable as accelerated senescence and degeneration of fungal cytoplasm. Since the carbon availability was not a limiting factor in pure cultures, these observations suggest that mycorrhizal fungi can be directly affected by soil nitrogen, possibly leading to declined infection level and root vitality. Further studies are needed to identify the stored nitrogen-rich compounds and mechanisms involved in cytoplasmic degeneration.

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The effects of soil moisture and temperature on carbon allocation of Scots pine seedlings

Hannu Ilvesniemi, Jyrki Haataja, Markus Huttunen and Eeva Korpilahti

Introduction

The growth and the partition of carbohydrates to different plant organs of the seedlings is a dynamic function of light, temperature, moisture and nutritional conditions of the site, and the allocation pattern has an effect on the growth of trees (Linder and Rook 1984, Axelsson and Axelsson 1986, Mäkelä 1988, Nikinmaa 1993). The effects of these environmental factors can be seen e.g. on the root to shoot ratio of plants. The aim of this study was to analyze the effects of soil moisture and temperature on the growth and growth allocation of Scots pine seedlings.

Material and methods

The experiment was carried out in the field laboratory of the Hyytiälä experimental station (61° 48' N, 24°19'E). 112 two year old seedlings were grown in 8 liter draining pots, the temperature and moisture of which were adjusted. There were two temperature and two moisture treatments and the treatments were named as moist-warm (MW), moist-cold (MC), dry-warm (DW) and dry-cold (DC). The experiment consisted of two growing seasons. 56 of the seedlings were over wintered outdoors, and during this period all the seedlings, also those of the dry treatment

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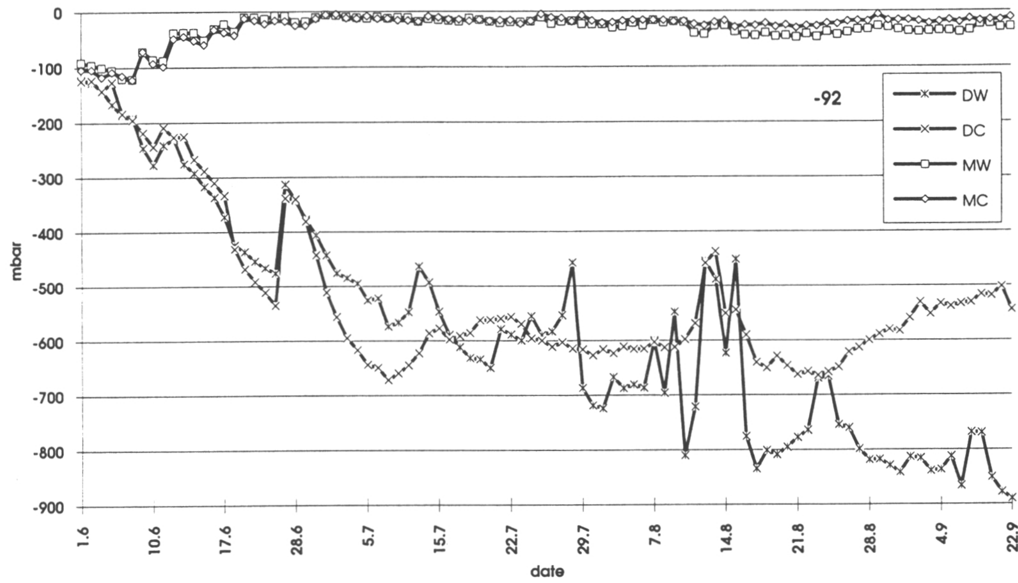
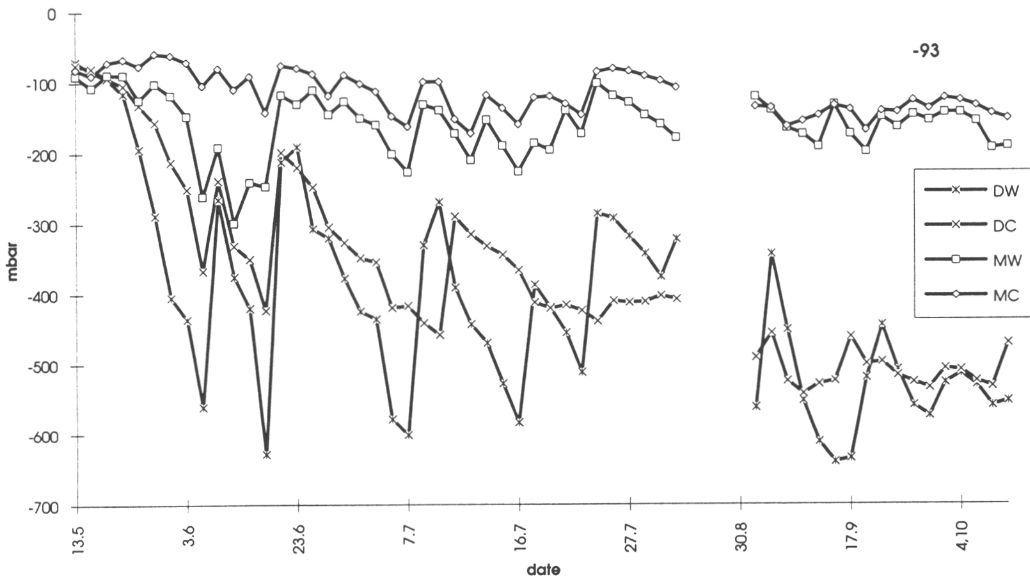




Figures 1a,b. The soil water potential of the growth media in different treatments during the growth period 1992 (a) and 1993 (b).

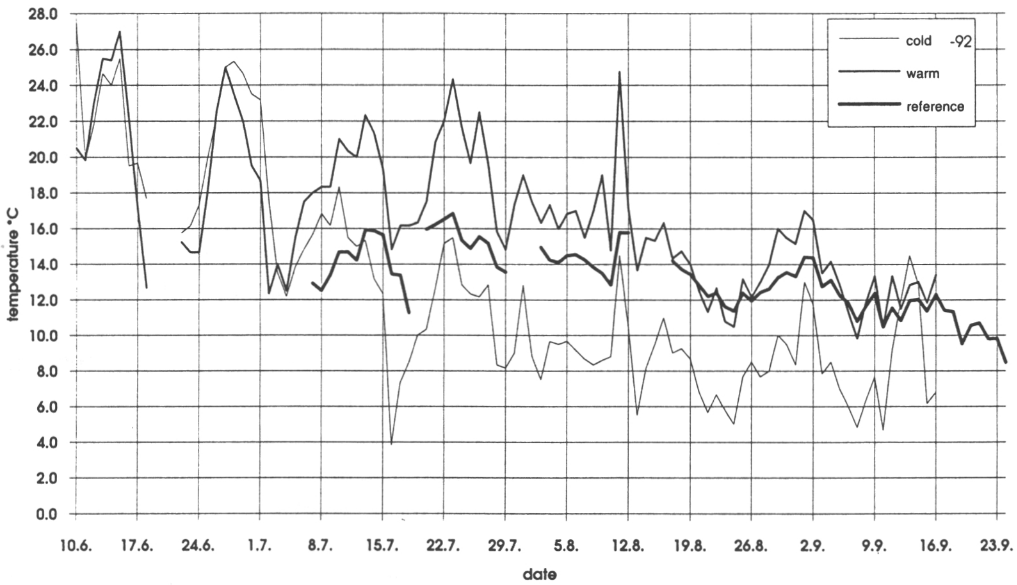
were watered. The growth media was homogenized and sieved (< 0.5 cm) forest soil consisting of eluvial and upper part of illuvial layers mixed together. The same amount of soil (7.85 kg) was weighed to all pots to obtain soil bulk density of 1.2 kg dm^{-3}.

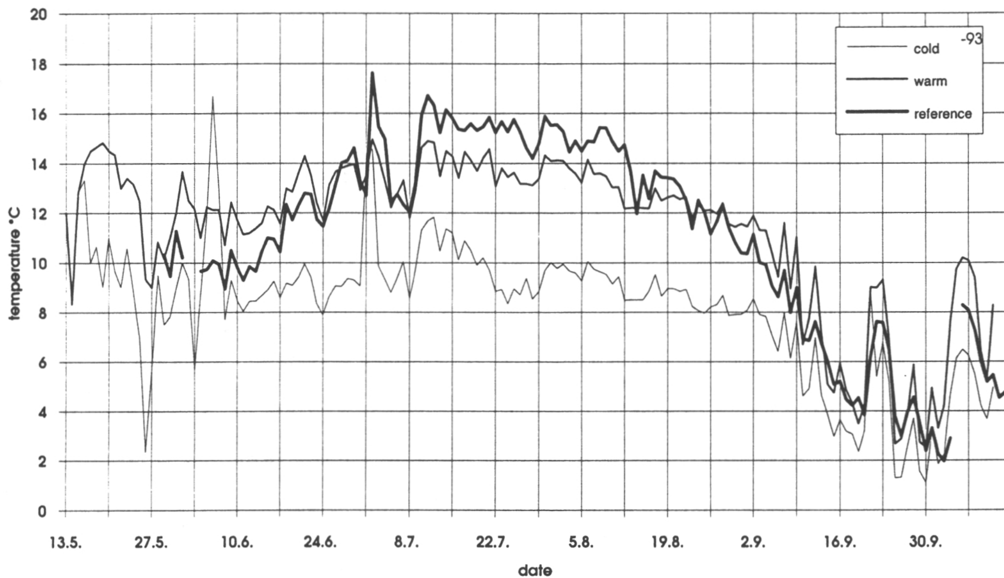
When the seedlings were planted (1.6. 1992) all seedlings were watered to the -10 kPa soil water potential. During the following month the soil water potential was allowed to decrease to -60 kPa. Some water was given to these seedlings during the dry-

ing period to avoid strong moisture gradient along the depth of the pot. Meanwhile the seedlings growing in moist treatment were watered with successive water additions so that the treatment conditions from -1 to -2 kPa was reached (Fig. 1a). In the beginning of the growth period 1993 the soil water potential in all pots was around -10 kPa which level was kept over the growth period for the moist treatment. The treatment level of the dry soil -40-50 kPa was achieved in the beginning of June (Fig. 1b).

The temperature treatments were arranged so that a reference electrode was installed to the depth of 7 cm in soil in an open spot, resembling the soil temperatures of a clear-cut area. In 1992 the warm treatment was adjusted to be 4 to 1 °C higher than the reference temperature the difference decreasing along the decrease in soil temperature. The cold treatment was around 5 °C colder than the soil temperature in an open area. Due to the breakdown of the thermostat, the temperatures in treatments were higher and the difference between treatments was smaller as planned in the beginning of the period (Fig. 2a). The effective temperature sum in soil (+5 °C threshold value) between 10.6 and 14.9 was 1133 d.d. in warm treatment and the corresponding value in cold soil was 721 d.d. During the growth period 1993 the temperature of the warm soil treatment followed that of the reference soil and the cold treatment was 4 °C colder than that (Fig. 2b). The effective temperature sum between 13.5 and 11.10 was 977 d.d. in warm soil and 525 d.d. in cold soil.

In summer 1992 eight and in 1993 ten measurement periods of gas exchange were performed covering evenly the whole growth period. At the same occasion one or two seedlings from each treatment was randomly chosen to the measurement. In 1992 six gas exchange measurement cuvettes were available so that at every other measurement period two seedlings from two treatment combinations was measured at the same time. In 1993 only four cuvettes were used, and one seedling from each treatment was measured at time. The measurement period was 5-6 days except the last four measurements which lasted for 10 days. The amount of transpiration and net photosynthesis, as well as the photosynthetically active radiation and air temperature was measured. The cuvette was closed during the measurement period (around one minute), otherwise the cuvettes were open. The technical details of the measurement system has been presented in Hari et. al. 1990. Each of the cuvettes was measured with the interval of 22 minutes in 1992 and 16 minutes in 1993 day and night.





Figures 2a,b. The temperature of the growth media during the growth periods 1992 (a) and 1993 (b). The thick line corresponds to the reference value, and the thin lines are those measured from two different temperature treatments.

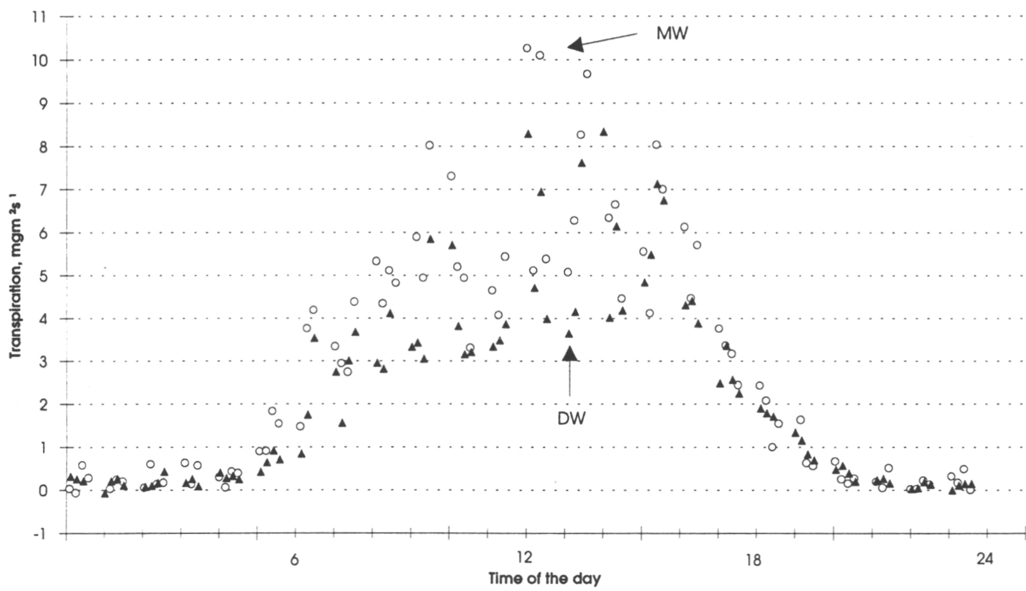
The growth and growth allocation was studied by measuring the increase in dry-weight and by using ^{14}C labeled carbon dioxide as a tracer. In the beginning of the experiment, before the potting, the fresh weight of the seedlings and the volume of the roots was measured as follows: After gently rinsing the roots the fresh weight of the seedlings was recorded. The root system was then immersed in a water filled container of known weight, placed on a weight-recorder. The weight of the container was then reweighed to determine the weight (volume) of water displaced

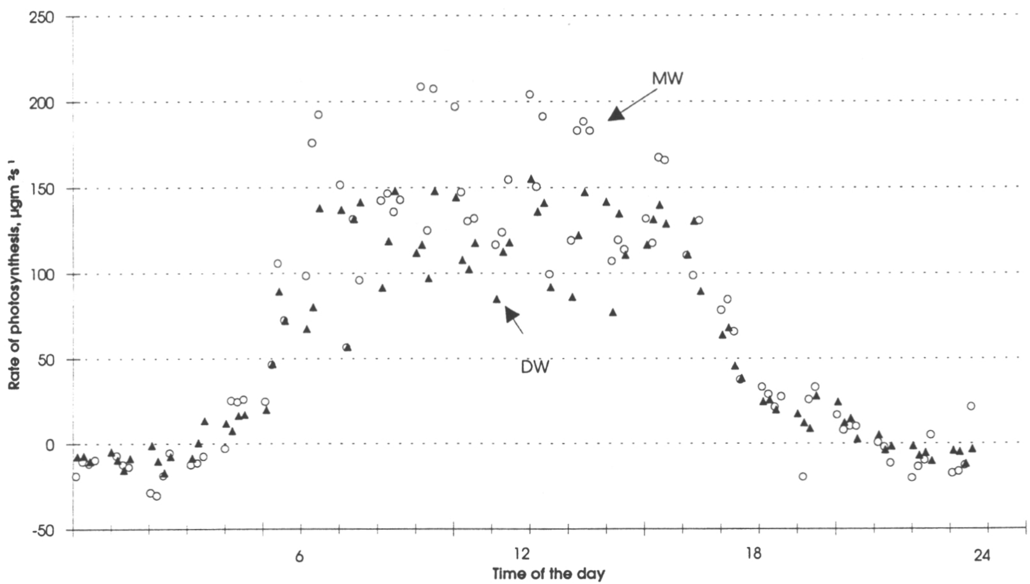
equivalent to the volume of the roots. The volume dry weight of the roots was determined of an independent material following the same procedure to determine root volume. The dry-weight of the experimental seedlings root biomass was then calculated using the recorded water displacement weight (vol) and volume dry weight value. Shoot dry weight was calculated by subtraction and corresponding correction for moisture. By subtracting the dry weight in the beginning of the period from the dry-weight at harvesting, the increase in dry weight could be calculated.

At the end of the treatment period the seedlings measured were lifted, washed free from sand, and separated into stem, needles and roots. The needles were further divided by age and the roots to into fine (diameter < 1 mm) and coarse (diameter > 1 mm) roots. Sample needles were selected and the length and the width of the needles were measured for the surface-area determinations. A coefficient to transform dry-weight values for needle surface area was obtained. The different plant parts were then oven dried at 105 °C for 24 h after which their dry weights were determined. The tissue N concentrations were analyzed with the CSN-analyser. For the analyses of other elements the samples were digested in the mixture of HNO_3 and $HClO_4$ by heating the samples stepwise up to 250 °C. The element concentrations were measured by the plasma emission spectrometry (ICP).

For the tracer addition, seedlings in photosynthesis measurement were enclosed to the measurement cuvette and the tracer brought into the cuvette as $NaH^{14}CO_3$ from which $^{14}CO_2$ was released by addition of H_2SO_4. In 1992 the seedlings were enclosed to the cuvette for 20 minutes, and in 1993 for one hour. The tracer levels used in the successive years were 0.03 and 0.09 µCi. $^{14}CO_2$ respiration of the roots was measured after this for the rest of the measurement period. The evolved $^{14}CO_2$ was trapped by sucking air from the pot through Lumasorb II solution. The disintegration of the ^{14}C was determined from the trap solution using a scintillation counter.

For the determination of ^{14}C assimilated into the biomass, sub samples were prepared from the different plant parts after the seedlings were lifted and dried. The samples were oxidized in a sample oxidizer (Junitek) and the released carbon was trapped in a solution, the activity of which was determined. From these measurements the total activities of different plant parts were calculated.





Figures 3a,b: The transpiration (a) and photosynthesis (b) rates of the seedlings grown on MW or DW conditions on 1.7. 1993. The lower water potential in dry soil has decreased both the transpiration and photosynthesis rates.

Results

Photosynthesis and transpiration

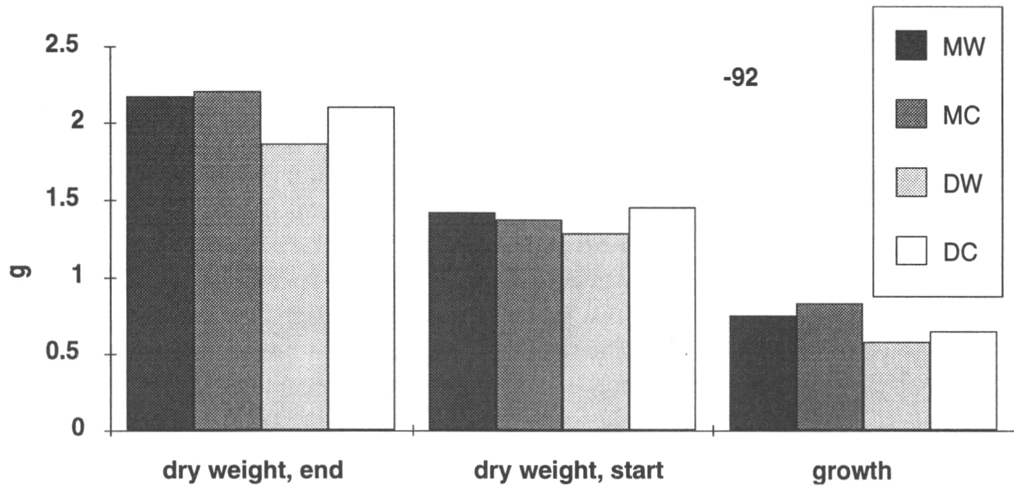
The results of the photosynthesis and transpiration measurements are still to be analyzed. For the purpose of an example some periods of measurement were calculated. Results from the first of July 1993 are presented here. During the day air temperature increased from 10 °C to 23 °C, and the water content in the air

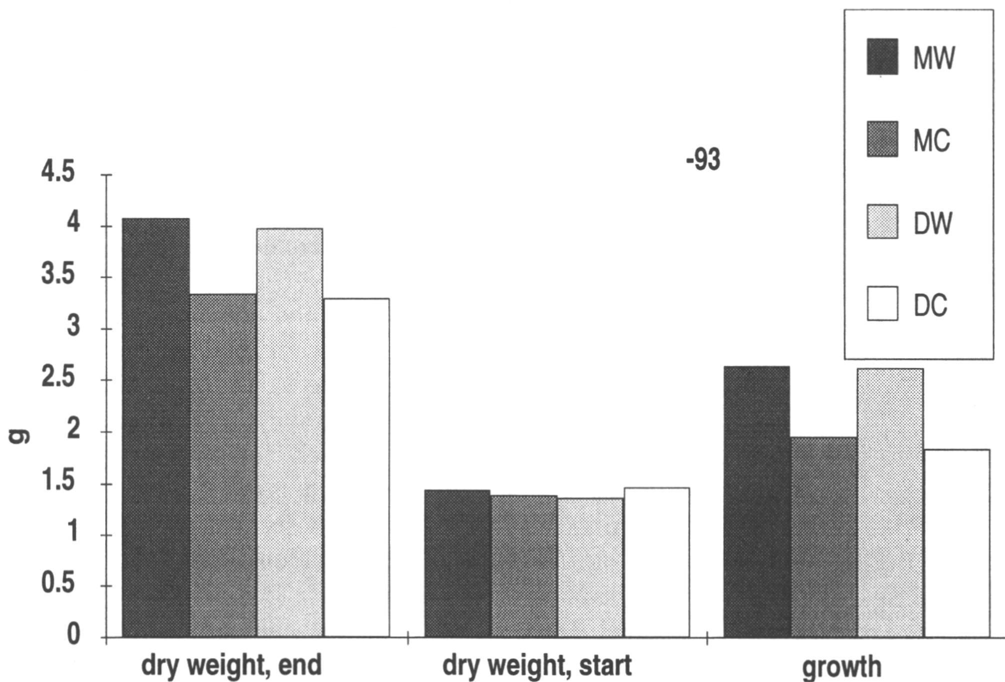
was between 7 and 8 gm^{-3} . The soil water potential in dry treatments was -35 kPa (DC) and-42 (DW), and -12 kPa in moist treatments. For the seedlings growing in dry soil, the maximum transpiration rates were 8 $mgm^{-2}s^{-1}$ (DC) and 7 $mgm^{-2}s^{-1}$ (DW). In moist soil the maximal transpiration rates on that day were 10 $mgm^{-2}s^{-1}$. It can be seen that the dry soil decreased the maximal transpiration rate, but especially in moist soil the temperature did not have any effect on transpiration rate (Fig. 3a). The photosynthetically active radiation varied occasionally between 200-1100 $\mu molm^{-2}s^{-1}$. The rate of photosynthesis followed closely the intensity of the radiation. In both moist treatments the maximum rates of photosynthesis were 200 $\mu gm^{-2}s^{-1}$. In DW treatment the maximum was 150 $\mu gm^{-2}s^{-1}$ and in DC treatment 130 $\mu gm^{-2}s^{-1}$ (Fig. 3b). As the transpiration, also the photosynthesis rate was affected by the soil water potential.

Growth

In the first growing season, the soil water potential had greater effect on soil growth than soil temperature, seedlings in moist soil growing more than those in dry soil (Fig. 4). In 1993 both the seedlings as well as roots separately, were growing faster throughout the growing period in warm soil. The soil water potential did not seem to have an effect on growth. The growth rate of the seedlings growing in DW soil was remarkably high. The difference to other treatments was largest in the growth of fine roots. This response on growth rate is not in good agreement with the results seen on the photosynthesis and transpiration rates on the example day. Any good explanation for this is not available before the photosynthesis measurements have been analyzed in more detail. The explanation for the smaller effect of temperature on the first growing season can be in the fact, that for the first month there was no temperature difference between the treatments and the temperatures were also remarkable high during that period (Fig. 2a, 4a, b). In the beginning of the experiment the seedlings were not of equal size and the effect of the original biomass can be seen especially in the material collected during 1992. This had also effect on the differences detected between different treatments.

The growth of the needles was highest in the DW treatment, and that of fine roots in MW soil. In 1993 the amount of dead needles was highest in seedlings grown in cold soil, only some of the needles formed in 1991 still alive.





Figures 4a, b. The weights of the seedlings presented as a treatment mean of the samples collected over the growing season, (a) 1992, (b) 1993. The dry-weight in the beginning of the experiment was calculated from the fresh-weight by a coefficient obtained from an independent material.

Biomass allocation

In the beginning of the experiment the root to shoot ratio was 2/5 and in the next growth period the mean for the whole material was 2/3. This can be an acclimation reaction to the lower nutrient supply from the forest soil compared to the conditions in nursery. The root to shoot ratio showed similar trend as the growth, the ratio being highest in the seedlings grown on moist soil. The highest root to shoot ratio was found in MW treatment (Fig. 5).

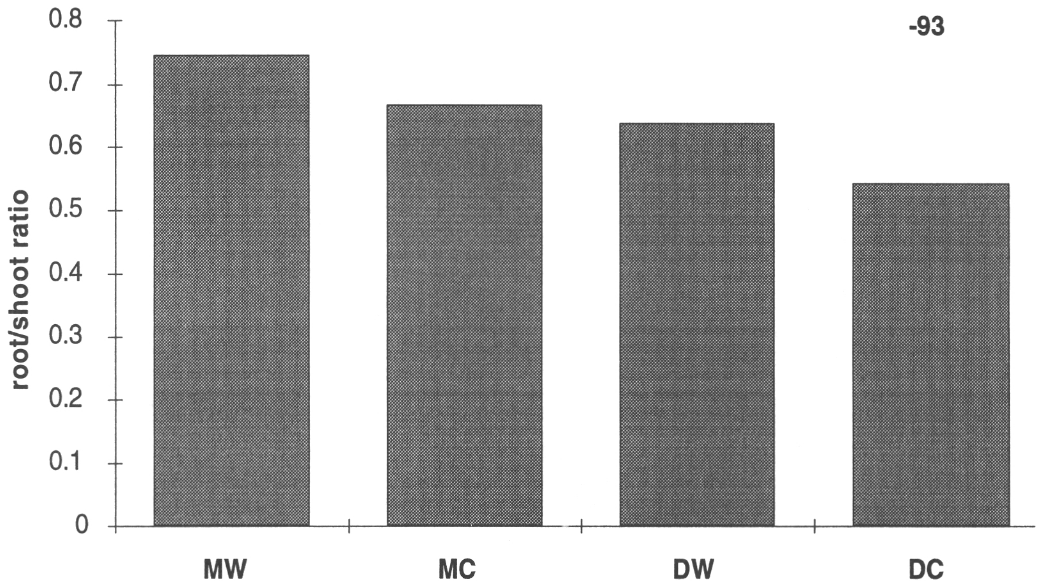


Figure 5. The dry-weight based root to shoot ratio in 1993 presented as a treatment mean over the whole growth period.

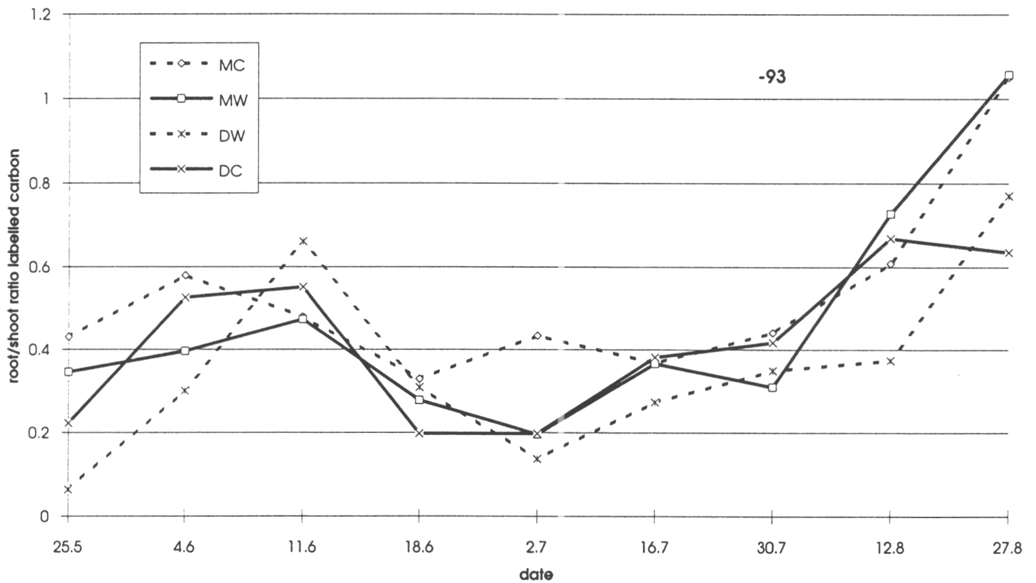


Figure 6. The root to shoot ratios in treatments at different harvesting periods over the growth period 1993 measured as ^{14}C bound to biomass.

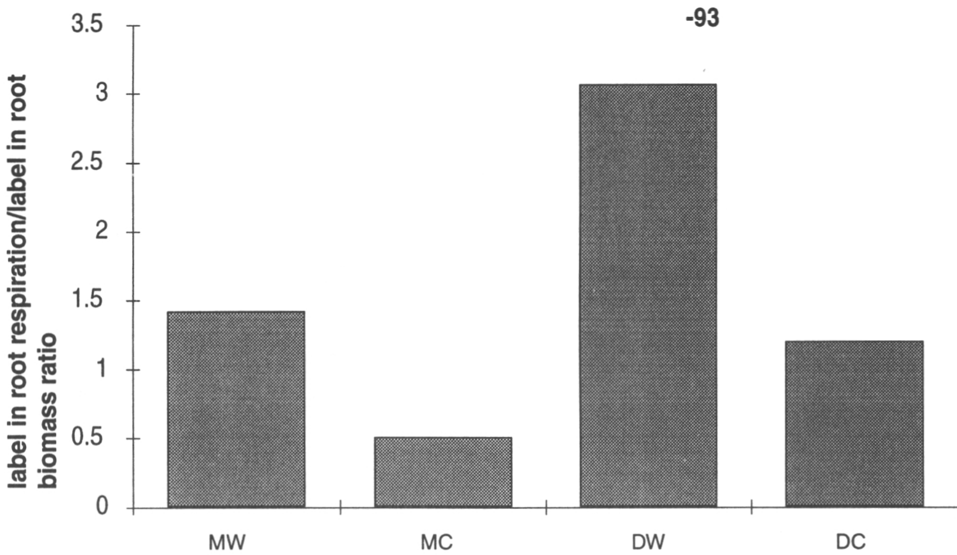


Figure 7. The ratio between $^{14}CO_2$ released from the pot as respiration and ^{14}C bound to the root biomass.

The treatments did not seem to have dramatic effect on the seasonal ^{14}C-allocation pattern of the seedlings during the growth period. In general, an early spring peak on proportional root growth was followed by an increase in the allocation to the needles in late June and July. In August, when the new needles were not growing any more, but were producing effectively carbohydrates, the proportion of the carbon allocated to the roots increased again (Fig. 6).

Root respiration

In the rate of respiration from the pot, remarkable differences were found between different treatments. The respiration expressed as a ratio to the ^{14}C bound into the root biomass was highest in DW treatment and smallest in MC treatment (Fig. 7) The increase in soil water deficit and also higher soil temperature seemed to increase respiration. The respiration rate here consists of root respiration and microbial respiration in rhizosphere. All the labeled ^{14}C released has anyhow been synthesized, transported and allocated by the seedling, because no ^{14}C was given directly to the soil.

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Unbalanced nutrient status and mycorrhizal roots of Scots pine

Sari Janhunen[1], Anneli Ylimartimo[2] and Toini Holopainen[1]

Introduction

Recently there has been a wide interest in effects of excessive nitrogen on mycorrhizal roots, and the majority of the reports indicate negative effects on mycorrhiza development (Alexander and Fairley 1983, Wallander and Nylund 1991, Holopainen and Heinonen-Tanski 1993). However, research on the effects of excess nitrogen on tree nutrition in relation to other nutrients, and mycorrhiza development, is still limited. The aim of this study was to assess the effects of unbalanced nutrient status involving excessive N, and deficient K, Mg and Ca concentrations, alone and in combination, on the development of mycorrhizal roots of Scots pine.

Materials and methods

Scots pine (*Pinus sylvestris L.*) seedlings in this factorial experiment, with 16 nutrient combinations, were 1.5-yr-old. The seedling material and growing methods are described in more details in Ylimartimo et al. (1994). Nutrients were given along with the irrigation water once a week during the growing season. The mass ratios of N, K, P, Ca and Mg in the optimal nutrient solution were 100:45:14:8:8. The aim of the treatments was to induce

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deficient (0), normal (1), or excess (2) foliar N, K, Mg and Ca concentrations as follows:

Nutrient	Concentration		
N	-	1	2
K	0	1	-
Mg	0	1	-
Ca	0	1	-

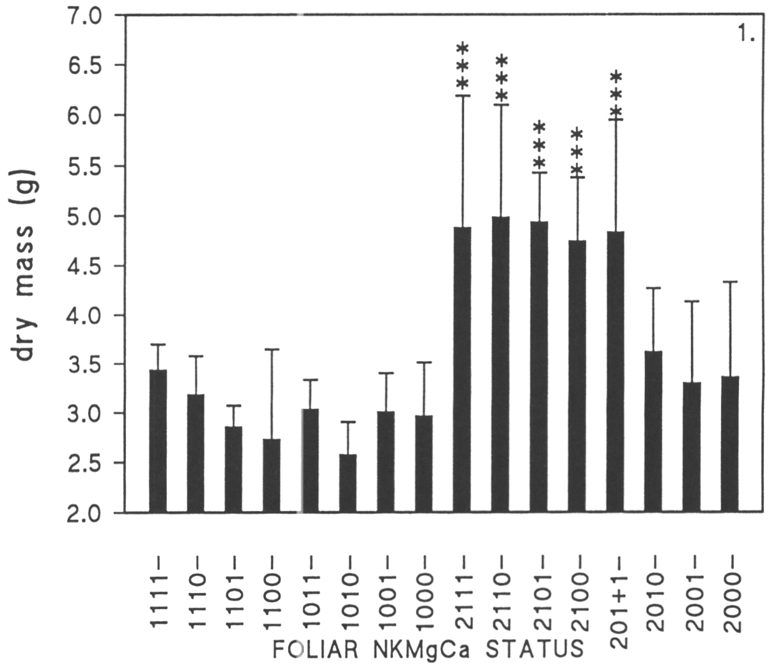
Combined needle and root samples were taken from each treatment for nutrient analysis at the end of September 1992. Nitrogen was analysed by the micro Kjeldahl method. Concentrations of P, K, Ca, Mg and B in the needles were analyzed by ICP and AAS (Williams 1984), Ca, Mg and K of the roots were determined by AAS and P by molybdenum blue method (Allen 1989).

The mycorrhizal short root tips were identified after staining in Ponceau S (Daughtridge et al. 1986), and the total number of mycorrhizas and short roots / 1 m was determined. The calculation was made from the total samples of about 100 cm, consisting of 1-5 cm long root pieces collected randomly throughout the root system.

Results and discussion

The dry masses of roots were highest in the seedlings with excessive N and mostly with optimum K nutrition (Fig.1). In these seedlings the N and K concentrations in the needles (Ylimartimo et al. 1994) and in the roots were higher than in the seedlings with optimum N and deficient K nutrition. Nitrogen also affected the number of mycorrhizal short roots. Approximately 57% of root tips in the seedlings with optimum N treatment were mycorrhizal (living mycorrhizas), but in excessive N treatments only 23%, respectively. This difference was very significant ($p<0.001$). With optimum N treatment only 6% of the root tips were non-mycorrhizal, but in excessive N treatments even 26% (difference was significant, $p<0.001$), respectively. At the same time, the total number of short roots was significantly ($p<0.001$) lower in the seedlings with excessive N treatment (Fig. 2). The reason for lower mycorrhization with excess N is possibly the statistically significant decrease in the numbers of monopodal

Figure 1. The dry mass (means ± S.D.) of Scots pine roots according to foliar NKMgCa status, i.e. status of N, K, Mg, and Ca concentration levels in current needles: 0, deficient; 1, normal; 2, excess level (e.g., 2101 indicates a combination of excess N, normal K and Ca, and deficient Mg concentrations. One level marked with + is considered a borderline case). Stars indicate means significantly ($p<0.001$) greater than in one or more of the other means marked without stars (according to Tukey's Multiple Range Test).



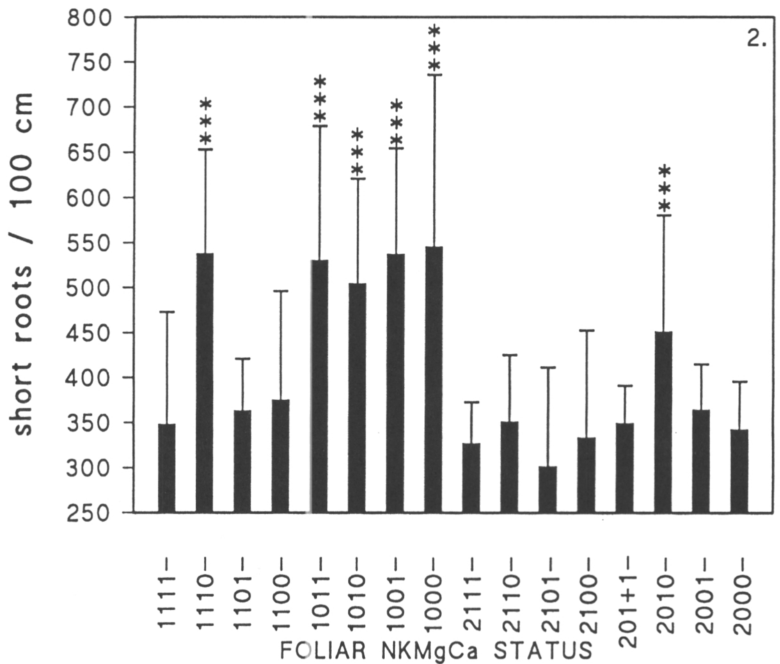


Figure 2. The total number of short roots / 100 cm (including both the mycorrhizal and non-mycorrhizal short roots, means ± S.D.) according to foliar NKMgCa status. Stars indicate means significantly ($p<0.001$) greater than in one or more of the other means marked without stars (according to LSD Multiple Range Test).

($p<0.001$), dichotomously branched ($p<0.001$) and dark stained and black mycorrhizas ($p<0.010$). Deficient K nutrition increased the total number of short roots (Fig. 2.), which appeared to be related to significant ($p<0.01$) increase of old mycorrhizas. The effects of N on mycorrhiza formation are well reported. However, the effects of K are not so well known. The other nutrients

did not have any drastic effects on the development of short roots, though Mg and Ca concentrations in the roots were lower in deficient treatments compared to optimum treatments.

Conclusions

Excessive N reduces very clearly the short root development and the numbers of mycorrhizal short roots, which is in agreement with several earlier observations (Alexander and Fairley 1983, Wallander and Nylund 1991, Holopainen and Heinonen-Tanski 1993). Besides nitrogen, potassium also has a role in controlling the development of mycorrhizas in Scots pine roots. Deficient K nutrition increases the total number of short roots by increasing the proportion of old mycorrhizas.

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Rhizodeposition in arable crops

Bendt Jensen

Introduction

Quantification of the annual input of crop residues to arable soils is a key issue in the study of soil organic matter dynamics. Unlike inputs from above-ground plant parts, quantification of the below-ground C input is much more complicated. Isolation of roots by soil washing underestimates the input to soil of root related C. A considerable portion of the fine roots and root-hairs is lost during the soil washing procedure. Root exudates, leakates, lysates and sloughed-off cells are not accounted for. Most of the C lost during the soil washing procedure is very labile and influences the soil microbial biomass and activity.

In order to quantify and distinguish root-derived C from indigenous soil organic C, $^{14}CO_2$-labelling of plant tops has been used. Most studies under controlled conditions in the laboratory are based on a continuous exposure of plant tops to $^{14}CO_2$. Under field conditions, pulse-labelling with $^{14}CO_2$ is easier to handle compared to continuous labelling, and series of pulses at regular intervals during the growth period have been found to provide a reasonable estimate of the cumulative below-ground C input.

Experiments under field conditions have been based on labelling periods ranging from 15 min to 2 hours, and it has been assumed that the time of labelling during the day was of minor importance for the distribution of assimilated C. However, this assumption is questionable since temperature and light intensity are subject to diurnal and seasonal fluctuations.

This project examines the rhizodeposition from cereal crops under field conditions. Effects of labelling time and light intensity on assimilate distribution in spring barley were also included. Distribution of assimilates in three cruciferous species was exa-

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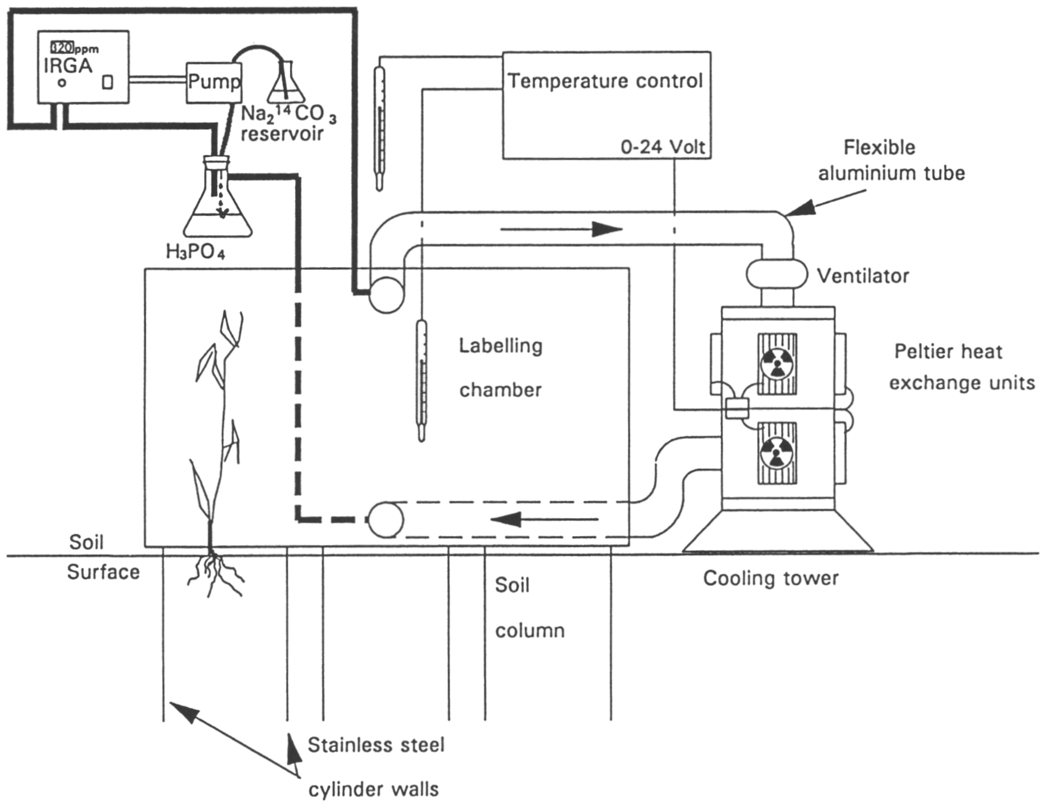


Figure 1. Equipment used in the field for $^{14}CO_2$-generation, temperature control and photosynthesis chamber.

mined under different autumnal conditions (low light and temperature regimes).

Materials and methods

Figure 1 shows the mobile system developed for ^{14}C-pulse labelling plants under field conditions (Jensen 1993). The system allows the rhizosphere respiration (root and microbial respiration) to be determined during and after labelling by placing a seal between above- and below-ground compartments.

The labelling system was used during the growing seasons 1990 and 1991 to determine the rhizodeposition of spring- and winter barley, respectively (Jensen 1993, 1994a). Crops were labelled at four growth stages, each labelling lasting 8 h. The barley plants were grown in stainless steel cylinders (20 cm diam., 50 cm deep) that were pushed into the soil. Decomposition of labelled C left in the root-soil system after labelling was followed for 3 to 5 months in the winter barley experiment.

In two additional experiments the below- and aboveground plant parts were not separated and consequently the rhizosphere respiration was not measured. The effect of labelling time (1½, 4 or 8½ h after onset of light) and of light intensity (75 and 160 W m^{-2}) on the distribution of ^{14}C in spring barley was examined in a green house experiment (Jensen 1994b).

The effect of temperature (5 and 10°C) on assimilate distribution in three cruciferous species (winter rape, turnip and white mustard) was examined in temperature regulated rooms (Jensen 1995). The effects of light intensity (75 and 160 W m^{-2}) and temperature (5 and 10 and 20°C) were examined for white mustard.

Results and discussion

Distribution of labelled C

Table 1 shows the distribution of labelled C in spring barley six days after labelling. During early elongation (29 May), 36.7% of the recovered ^{14}C was translocated below-ground. Equal amounts of ^{14}C are generally translocated below-ground and retained in the shoots at earlier growth stages and thus 43.2% of ^{14}C recovered was translocated below-ground during tillering of winter barley. The proportion of ^{14}C recovered below-ground decreased with plant age.

Rhizosphere respiration accounted for 1.5-8.3% and 2.5-32.5 of the ^{14}C recovered in spring (Table 1) and winter barley, respectively. This corresponds to 20-67% and 18-69%, respectively, of ^{14}C translocated below-ground.

Estimated C input to the soil during the growing season

When estimating the total C input to the soil during the growing season of spring barley, it is assumed that the instantaneous total below-ground C-production (Table 1, lower part) can be applied to periods before and after each labelling date. The growing season was set to 95 days (22 days after sowing to 10 days before maturity). Values from the first, second, third and fourth labelling were taken to represent 45, 20, 15 and 15 days periods, respectively. Furthermore, it was assumed that two times more ^{14}C would have been translocated below-ground if the labelling period had been 24 hours. Consequently, the amount of labelled C retained in the macro-root free soil for more than 6 days was estimated to 95.5 g C m^{-2} yr^{-1}. As macro-root C (30.3 g C m^{-2} yr^{-1}) is

Table 1. Distribution of ^{14}C recovered and estimated C-production (g C m^{-2} $labelling^{-1}$) as measured 6 days after labelling spring barley. Values are given for macro-roots, soil plus micro-roots, rhizosphere respiration, total below-ground and shoot as mean of 3 replicates (standard deviation in parenthesis).

Labelling date	Labelled macro-roots	Whole soil minus macro-roots	Rhizosphere respiration	Total below ground	Shoot
		% of ^{14}C recovered			
29 May	7.6 (2.1)	20.7 (3.0)	8.3 (2.2)	36.7 (3.5)	63.3 (3.5)
21 June	2.0 (0.1)	7.8 (6.5)	4.4 (0.7)	14.3 (6.3)	85.7 (6.3)
4 July	1.0 (0.4)	5.8 (7.1)	1.5 (0.5)	8.3 (6.7)	91.7 (6.7)
18 July	0.7 (0.4)	1.1 (0.6)	3.0 (0.9)	4.7 (0.7)	95.3 (0.7)
		g labelled C m^{-2}			
29 May	0.30 (0.3)	0.87 (2.5)	0.33 (1.9)	1.50 (0.4)	2.55 (1.4)
21 June	0.07 (0.1)	0.31 (1.6)	0.16 (0.1)	0.54 (0.3)	3.13 (4.6)
4 July	0.03 (0.1)	0.17 (1.2)	0.05 (0.1)	0.24 (0.2)	2.71 (4.6)
18 July	0.01 (0.0)	0.01 (0.0)	0.04 (0.2)	0.06 (0.0)	1.15 (3.0)

eventually left in the soil as dead root biomass, this contribution was included too. Rhizosphere respiration (39.4 g C m^{-2} yr^{-1}) made up 23.3% of the total amount of ^{14}C translocated below-ground during the growth period, the total amount of C translocated below-ground being 165.2 g C m^{-2} yr^{-1}.

Figure 2 shows the amount of spring barley macro-root C and the cumulated below-ground labelled C. At maturity (7 August) only 45.0 g C m^{-2} could be isolated in macro-roots. Thus macro-roots isolated at harvest made up only 36% of labelled C retained in the soil for more than 6 days corresponding to 27% of the C translocated below-ground. Total below-ground input correspon-ded to one-third of above-ground C harvested at maturity.

In the winter barley experiment the growing season was set to 120 days that is from 13 March, when the soil temperature exceeded 5° C in a depth of 30 cm, to 10 days before maturity (2 August). The amount of labelled C retained in the macro-root free soil, in macro-root C and in rhizosphere respiration was 96.4, 47.2 and 93.6 g C m^{-2} yr^{-1}, respectively. The total amount of C translocated below-ground was 237.2 g C m^{-2} yr^{-1}, of which 45% was rhizosphere respiration. At maturity (2 August), only 79.7 g C m^{-2} could be isolated in macro-roots.

For both crops, the cumulated amounts of labelled C retained in macro-roots was much smaller than the amounts of macro-root-C isolated at maturity by soil washing.

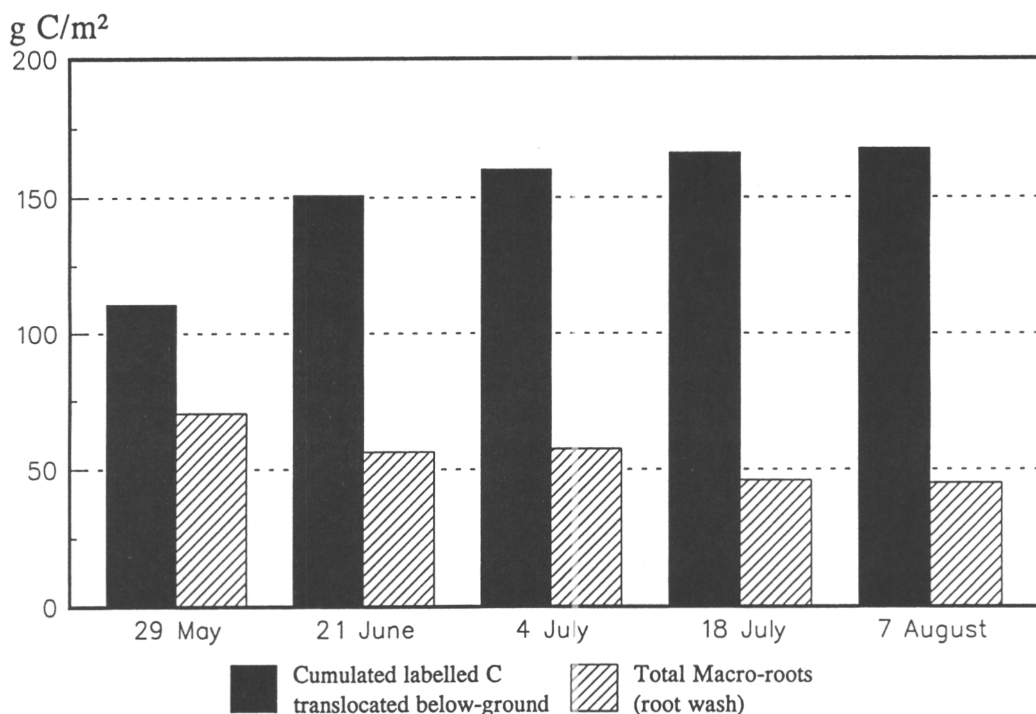


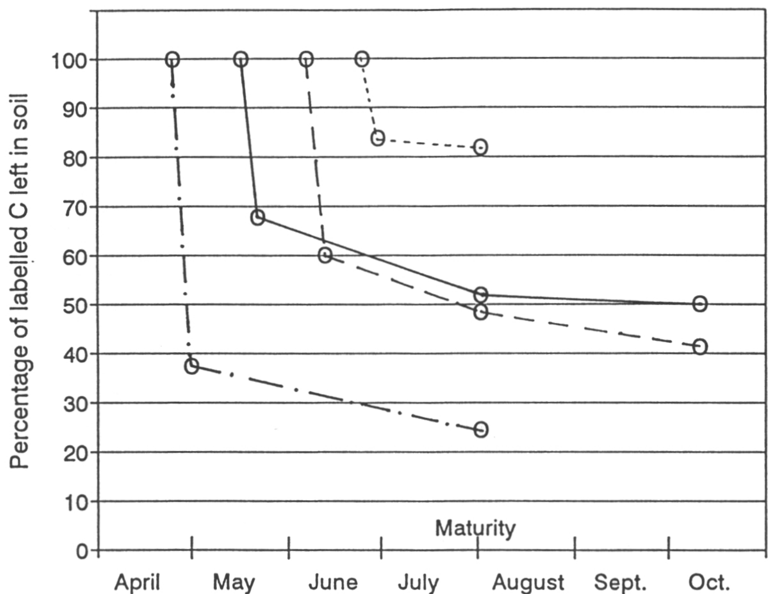
Figure 2. Spring barley experiment. Comparison of macro-root biomass from root wash (g C m^{-2}) and cumulated total amount of C translocated below-ground.

Other studies have estimated the total below-ground input from wheat to 900-2950 kg C ha^{-1} yr^{-1}. Lower values of 475 and 583 kg C ha^{-1} yr^{-1} in wheat and barley, respectively have been reported in experiments, where the $^{14}CO_2$ labelling was carried out between 8.45 and 10.15 am and the plant-soil-systems harvested after 24 h. The ^{14}C movement between above- and below-ground plant parts has been shown to occur mainly between labelling and day 5, and the time of labelling during the day may affect the assimilate distribution when short pulses are applied.

Exudation from roots may be overestimated if roots are not removed from the soil soon after shoot detatchment. Part of the ^{14}C in the soil plus root may therefore be material released from dying and lysing root cells. Thus if the plants were allowed to grow on beyond 6 days after labelling, part of the ^{14}C found in the soil in this study would not have been released to the soil but retranslocated to the shoot later in the growth period. The estimated total input of C into the soil may therefore overestimate the "true" rhizodeposition.

Based on similar assumptions as applied to the below-ground production of spring barley (Table 1), the total shoot production can be calculated to 470.3 g C m^{-2}. At maturity the yield of straw plus grain was 534.9 g C m^{-2} in adjacent reference plots not ex-

Figure 3. Winter barley experiment. The relative amounts of labelled C left in the root-soil system 6 days after each labelling, at maturity and on 11 October. The amount of labelled C translocated below-ground after labelling is set to 100%.



posed to the labelling procedure. Corresponding harvest yield in plots passing the labelling procedure on 18 July was 467.9 g C m^{-2}.

Decomposition of below-ground labelled C (winter barley)

In the winter barley experiment, decomposition of labelled C left in the root-soil system was followed for 3-5 months (Fig. 3). The amount of labelled C left in the root-soil system 6 days after labelling plus labelled C in rhizosphere respiration (root- and microbial respiration) during these 6 days was set to 100%. The decomposition of labelled C following day 6 was slower than in day 1-6. At maturity (2 August), 24-82% of total below-ground translocated C was left. On 11 October the corresponding figures were 41-50%.

Of the labelled C left in the root-soil system 6 days after labelling, 65-98% was still retained at maturity and 69-73% on 11 October. Relating these percentages to the estimated total cumulated C left in soil 6 days after labelling (143.6 g C m^{-2}), about 117 g C m^{-2} was still in soil at plant maturity.

Effect of labelling time, light intensity and temperature

The green house experiment showed, that neither the time of labelling nor the light intensity significantly influenced the ^{14}C distribution four hours after labelling start (Table 2). However,

Table 2. The proportion of ^{14}C recovered below-ground in percent of total ^{14}C recovery. Mean of three pots. Values in each column followed by the same letter are not significantly different at P=0.05 (standard deviation in brackets).

Time after onset of light (h)	Light intensity	Late tillering	Late elongation
1.5	low	^{A} 19.8 (5.2)	^{A} 7.6 (0.9)
4.0	low	^{AB}15.4 (1.5)	^{AB}6.0 (1.2)
4.0	high	^{B}12.1 (3.0)	^{B}5.2 (0.8)
8.5	low	^{AB}16.0 (1.8)	^{AB}6.4 (1.1)
	$LSD_{95\%}$	6.1	1.9

when exposed to low light intensity the percentage of ^{14}C in the soil-root system tended to be higher (15.4% and 6.0% at late tillering and late elongation, respectively) than when exposed to high light intensity (12.1% and 5.2%, respectively). Also, the percentage of ^{14}C in the soil-root system tended to be higher early in the morning (1.5 h after onset of light) than later during the day.

Under field conditions, the light intensity is generally higher in the middle of the day than in the morning. In that respect it is interesting to see, that the distribution of ^{14}C in the morning (low light) is significantly different from the distribution at higher light intensities in the middle of the day. The present results suggest that the total C-input to the soil may be overestimated, when the distribution and the total below-ground C translocation of photosyntate is based on a short pulse-labelling very early in the morning of a predominant sunny day.

The temperature regulated room experiment demonstrated that the temperature effect on assimilate distribution in three cruciferous species was small (Table 3). 7 weeks after planting all three species showed an increasing below-ground -to- shoot ratio of labelled C by increasing temperature, whereas the ratio decreased by temperature 11 weeks after planting. The only significant effect of temperature was found for winter rape 11 weeks after sowing. The effect of temperature may depend on plant development and the effect may be greater in winter rape which is physiologically able to adapt to low temperatures with the purpose of living through the winter.

In a second experiment with white mustard, the effect of temperature and light intensity was examined in more detail. Again there was no significant effect of temperature. As in the green

Table 3. Total below-ground -to- shoot ratio of labelled C determined 6 days after labelling of plants labelled at different temperature and plant age. Mean of three pots. Values in each column followed by the same letter are not significantly different at P=0.05 (standatd deviation in brackets).

		Species		
Weeks after sowing	Temperature (°C)	Winter rape	White mustard	Tyfon
7	5	^{B}0.28 (0.06)	^{A}0.40 (0.11)	^{AB}0.37 (0.15)
7	10	^{AB}0.34 (0.04)	^{A}0.61 (0.26)	^{A}0.51 (0.09)
11	5	^{A}0.38 (0.03)	^{A}0.68 (0.15)	^{AB}0.37 (0.07)
11	10	^{B}0.27 (0.05)	^{A}0.56 (0.02)	^{B}0.32 (0.05)
	$LSD_{95\%}$	0.082	0.293	0.175

house experiment the below-ground -to- shoot ratio of labelled C tended to increase by decreasing light intensity.

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Tomentelloid fungi (Basidiomycetes, Thelephoraceae s. str.) - are they true mycorrhizal fungi?

Urmas Kõljalg

The fungal family Thelephoraceae *sensu strictu* contains well-known (generally accepted) mycorrhizal genera as *Bankera* Coker and Beers ex Pouzar, *Boletopsis* Fayod, *Hydnellum* P. Karst., *Phellodon* P. Karst., *Sarcodon* Quelet ex P. Karst. and *Thelephora* Ehrh.: Fr. There are also six resupinate genera (viz. *Pseudotomentella* Svrcek, *Tomentella* (Pers) Pat., *Tomentellago* Hjortstam & Ryvarden, *Tomentellastrum* (Bourd. and Galz.) Svrcek, *Tomentellina* Höhnel and Litsch. and *Tomentellopsis* Hjortstam) with over 100 species in use (Hjortstam 1974, Hjortstam and Ryvarden 1988, Larsen 1971, 1974, 1981) belonging to this family. The term tomentelloid fungi has been used for all of them. Their fruitbodies occur mainly on the underside of well-decayed wood and inside litter in forest ecosystems. It has been asserted that tomentelloid fungi can form ectomyccorhiza (Miller 1982), and the term *Tomentella*-like has been used for several unidentified ectomycorrhiza (Danielson and Pruden 1989). The basidiospores of *Tomentella crinalis* (Fr.) M. J. Larsen have been used for synthesizing mycorrhiza with *Pinus sylvestris* L. seedlings under sterile conditions (Kõljalg 1992).

Up to present time the tomentelloid fungi have been investigated mainly by taxonomists or so called organism-mycologists. The main reason for this is that the germination of spores and isolation from fruitbody tissue on artificial media as rule have failed. Only known species of these fungi in culture is *T. crinalis*. The

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aqueous suspension of basidiospores of this fungus and one week old seedlings of *P. sylvestris* were introduced simultaneously into 500 ml flasks, containing vermiculite and ground peat moss moistened with modified Melin-Norkrans nutrient solution, so that the spores fell close to and on the radicle. The basidiospores germinated in two flasks of four and mycorrhiza developed which resembled, morphologically as well as anatomically, ectendomycorrhiza. The negatively geotropic fruitbodies, with toothed hymenophore typical of *T. crinalis*, also formed on the substrate (Kõljalg 1992). Several species like *Pseudotomentella tristis* (P. Karst.) M. J. Larsen and *Tomentella punicea* (Alb. and Schw.: Fr.) Schröter can form their fruitbodies under forest litter near tree roots, and these species have rhizomorphs connected with roots (Kõljalg 1992).

Our knowledge is very limited on the ecology of tomentelloid fungi. We cannot answer exhaustively for the questions like: Are tomentelloid fungi mycorrhizal, weakly pathogenic or saprotrophic fungi? What is the task of these fungi in carbon cycling in forest belowground ecosystems?

The sum of authors observations on the ecology of tomentelloid fungi are briefly as follows:

1. The fruitbodies of tomentelloid fungi come out mainly in old forests and usually on the underside of well-decayed logs or inside litter. Both of them are as rule full of living roots of trees. For example the fruitbodies of most species of *Pseudotomentella* are collected from old mixed coniferous forests in Europe, Asia and North-America. The *P. tristis* is almost only species of *Pseudotomentella* which can fruit (we do not know anything about mycelia!) in comparatively young or managed forests.
2. Also, the fruitbodies can develop on recently fallen trees but in this case the mycelia is distributed (if it is possible to follow it) in the litter. It means that nutrients can arrive from other source.
3. Sometimes we can follow mycelia or rhizomorphs emanating from the fruitbodies up to the living roots of trees.
4. We do not know how long the individual mycelia have been growing before fruitbody will develop and what is the size of area the individual mycelia can colonize.

From all what has been said above we can conclude that the fruitbodies of tomentelloid fungi come out first of all in the late successional stages of plant communities (old coniferous, mixed or broad-leaved forests, old wood-meadows) and the mycelia

(when it comes we do not know) colonize mainly litter and well-decayed wood. There are probably different successors of mycorrhizal fungi in different succession stages of forest. If we accept that tomentelloid fungi could be weakly pathogenic or mycorrhizal then they are probably late successors which colonize special substrate - old logs and litter. As we know the well-decayed logs are like seed panks and often there are numerous seedlings growing on these logs. In this case it is possible that tomentelloid fungi have, among others (viz. *Amphinema byssoides* (Fr.) J. Eriksson), weakly pathogenic or mutually beneficial relationships with those seedlings.

Finally, in this stage of knowledge we can just suppose that tomentelloid fungi are true mycorrhizal fungi and call them non-traditional mycorrhizal fungi.

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Ectomycorrhizal fungi and fungicides

Tarja Laatikainen and Helvi Heinonen-Tanski

Introduction

This study deals with the fungicide effects on ectomycorrhizal fungi of coniferous trees in Finland. The effects on the rhizosphere microbial population on plant roots are also studied. Additionally, the capability of ectomycorrhizal fungi to degrade those fungicides is determined.

Microorganisms in the rhizosphere have a marked influence on the growth of plants. Microbial population benefits the plant in many ways: by increasing, recycling and solubilization of mineral nutrients; by synthesizing vitamins, amino acids, auxins and gibberellins, which stimulate plant growth; and by producing antibiotics against potential plant pathogens (Atlas and Bartha 1992). Ectomycorrhizal fungi have the most important role in this association because of their close relationship to plant roots. The allocation and partitioning of carbon provide resources to plants for acclimation to environmental stress (Geiger and Servaites 1991). Ectomycorrhizal fungi take part in that allocation, for example, by transporting carbon between plants.

Pesticides have widely been used in the forests and nurseries of forest trees against fungal diseases, weeds and herbivores. However, only little is known about the effects of those pesticides on the ectomycorrhizal fungi and other microorganisms. Pesticide use has been more common in agriculture fields, especially in industrial countries. Many of these pesticides are persistent in soil and can, therefore, effect seedling growth when those fields will be afforested. This can be a greater problem in Finland than in lower latitudes because of cold climate: the half life of pesticides can be much longer here (Heinonen-Tanski 1989).

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In an earlier study the fungicides have proved to be the most toxic to ectomycorrhizal fungi of Scots pine and Norway spruce (Laatikainen and Heinonen-Tanski 1994). Three herbicides, hexazinone, chlorthiamid and linuron, two fungicides, benomyl and propiconazole, and an insecticide cypermethrin were tested with 56 different mycorrhizal fungi taken from our culture collection. Concentrations were 1 ppm and 10 ppm for fungicides and cypermethrin and 1 ppm for herbicides. The fungicides tested in this study proved to be more toxic to mycorrhizal fungi than herbicides and insecticide cypermethrin. Fungicide propiconazole had the clearest inhibitory effect on mycorrhizal growth.

Material and methods

In the summer 1994 a research was started to find out if fungicides copper oxychloride, propiconazole and chlorothalonil have any effect on ectomycorrhizal fungi of pine seedlings. These fungicides are some of the commonly used fungicides at forest nurseries in Finland.

Two-years-old Scots pine seedlings were grown in sand-peat-filled pots during the summer. After the period of growth the seedlings were treated with one of these fungicides at the same concentrations and the same intervals as recommended at the nurseries of forest trees. The fungicide was given straight to the surface of the pot. There was a control group of seedlings which was not treated at all.

The first sampling was performed two weeks after the first treatment. The seedlings were cut off and the needles were collected and frozen for the later analysis of residues of the pesticides and their major metabolites. The surface soil (0-5 cm) of the pots was separately collected. The samples from the surface soil and the rest of the soil from the pots were dried at room temperature and frozen for the later residue analysis.

The activity of soil microorganisms of the surface soil (0-5 cm) was examined as soil respiratory measurement, and by the active bacteria counts, and measuring active hyphal lengths. Soil respiration was performed as CO_2-measurement where 0.1 M NaOH-solution was used to trap CO_2 (Paul and Clark 1989). The numbers of active bacteria and active hyphal lengths were estimated by direct observation after FDA staining with epifluorescent microscopy.

The toxic effects of fungicides on the ectomycorrhizal fungi will be obtained by ultrastructural observations with electron mi-

croscopy. The roots of the seedlings were cleaned with tap water on sieves and the samples from the short-root tips infected by different type of mycorrhizal fungi were taken and stained for later observation. The short roots of different type of mycorrhizal fungi present will be counted according to five classes (Holopainen and Heinonen-Tanski 1993).

The fungicides and their major metabolites both from needle and soil samples will be analyzed by GLC. The temperature and the rainfall during the test period were measured and recorded by datalogger and computer.

Discussion

Healthy mycorrhizal fungi are important and even necessary for the proper growth of seedlings at the nurseries and for the successful start for development of the seedlings after the plantation (Halonen and Laiho 1993). The inoculation of ectomycorrhizal fungi to the soil of nurseries have proved to produce marked decrease in mortality of host trees (Atlas and Bartha 1992).

Only little attention has been paid on the resistance of mycorrhizal fungi to the pesticides, despite of the extend usage of those pesticides. Some pesticides have been studied earlier (Laiho and Mikola 1964, Marx and Rowan 1981), but the use of the most of those pesticides has been rejected nowadays, except benomyl. Pesticides, specially fungicides which are used for fungal diseases, seem to affect also the ectomycorrhical fungi of the plant protected. Cultivations on petri dishes have shown inhibitory effect of commonly used fungicides on the most of the ectomycorrhizal fungi of coniferous trees tested (Laatikainen and Heinonen-Tanski 1994). In some studies, when some significant pesticide effects on soil microbial populations and activities have been indicated, recovery has generally been rapid (Tu 1978, Tu 1993). Surviving microorganisms had replaced the sensitive species and, thus, maintaining the metabolic integrity of the soil (Tu 1978).

Information of fungal capability to degrade pesticides may help to decide which pesticides would be less harmful when using them at the nurseries of forest trees and in the forests (Glad et al. 1981, Tu 1993). Martin et al. (1991) discovered that rate of microbial degradation of certain fungicides was faster in soil previously treated with these same fungicides than in untreated soils. The microflora, which is able to degrade those fungicides, increases as the treatments are repeated (Martin et al. 1991, Donelly et al. 1994). Both selective inhibitory and stimulatory effects of pesti-

cides due to concentration used on microorganisms have been recorded. For instance, phenoxyacetic acid-containing herbicides had slightly stimulatory effect on the mycorrhizal fungi at low concentrations (Dasilva et al. 1977).

Biodegradation can be a result of cooperation of different microorganisms. For example, combinations of various bacteria strains were shown to degrade the PCBs more effectively (Donelly et al. 1994). Synergist effects between fungi and bacteria in degradation of pesticides have also been observed (Levanon et al. 1994).

At the nurseries of forest trees simultaneous treatments with fungicides and herbicides are common. Furthermore, sometimes one fungicide during the summer and another at the end of the autumn are used with the same seedlings at nurseries of forest trees. There is no information how all these pesticides will affect together ectomycorrhizal fungi of seedlings. Coappearance of different pollutants and their metabolites has shown to reduce biodegradation by microorganisms in the same cases (Burback et al. 1994).

Different fungicides can affect fungi in different ways. Copper oxychloride, one of the fungicides tested in this study, is degraded to Cu^{2+}-ions, which are very persistent is soil. Cu^{2+}-ions are known to accumulate into cells, especially into spores, which causes the coagulation of cell proteins. Cu^{2+} has shown to inhibit some and stimulate the other bacteria to degrade insecticide parathion (Tchelet et al. 1993).

Propiconazole is the sterol biosynthesis inhibitor (SBI) fungicide. In laboratory tests, low dosages of propiconazole stimulated the soil respiration (Elmholt 1992). The stimulation may have been due to the fungicide causing a sub-toxic stress effect, resulting in a diversion of carbon from biosynthesis to maintenance energy requirements. Chlorothalonil is a non-systemic foliar fungicide. Tests with chlorothalonil have shown significant increase in oxygen consumption from the decomposition of organic matter indigenous to the soil. Suppression of invertase and amylase enzymatic activities for one day was also observed. The inhibitory effect disappeared after two days (Tu 1993).

Most of the side-effect studies of pesticides are performed as laboratory experiments and usually at higher temperatures than occurring here in Finland. Furthermore, the results from laboratory side-effect tests can not be extrapolated to the field situation. It has been proved that the side-effects in the field can be provoked at dosages considerably lower and they may last longer than in the laboratory, because the treatments affect the soil microorganisms indirectly (Elmholt 1992). Therefore, field studies

are needed for assessing which pesticides and in which concentrations could safely be used in plant protection.

The information how fungicides affect cell structures of ectomycorrhizal fungi will help to analyze the mechanisms by which the fungal growth and, thereby, the growth of seedling is disturbed by fungicide. That may be important when considering, why so many seedlings will die when planted to forests or old agricultural fields. New information about pesticide effects on microbiological activity and nutrient contents of soil will also be important. This knowledge can also be useful when developing new biotechniques for fungi.

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Variation in the amount of organic carbon in soil within a forest stand: effect of trees and implications for sampling

Jari Liski

Introduction

Soil properties, both physical and chemical, vary considerably even within distances of a few meters (e.g. Ilvesniemi 1991, Järvinen et al. 1993). In forests, besides the geological factors, trees are important causes for the variation (Zinke 1962). In addition to the importance for designing an effective soil sampling, a thorough description of the variation can also provide useful information on the processes that generate the variation.

Materials and methods

For studying the variation in the amount of organic carbon (kg C/m²), a total of 99 soil cores (50 cm deep) were taken from a 4 x 8 m grid in a 130 year old Scots pine (*Pinus sylvestris* L.) stand on a glaciofluvial sand deposit in southern Finland (Fig. 3). One 4 x 4 m half of the grid was placed under tree canopies and the other in a small within-stand opening. In order to study the effect of trees in more detail, 27 additional cores were taken around three trees. Thicknesses of the horizons of the podzolized soil (F/H, E, B) were measured on the cores. Then, the cores were divided into the organic F/H and 0-10 cm (E and top of B horizon), 10-20 cm (middle layer of B horizon) and 20-40 cm (bottom of B horizon and top of C layer) mineral soil layers for analyzing the amount

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of carbon. For 0-40 cm mineral soil layer, the amount was obtained by totalling the amounts in the sublayers.

The number of samples, **n**, needed for given confidence, **d**, in the mean estimate was assessed using the formula $\mathbf{n=(z\,s\,d^{-1})^2}$, where **s** is the standard deviation of the data and **z** the 95 % fractile of Student's t-distribution (Ranta et al. 1989). Semivariograms were used for studying spatial dependence and ordinary block kriging for interpolating the values for 20 x 20 cm blocks at the study site (Isaaks and Srivastava 1989).

Results and discussion

The amount of carbon varied 4-8 fold within the soil layers and the coefficients of variation ranged from 21 % to 41 % (Table 1). On the basis of the observed standard deviations, 8-9 samples result in a mean estimate that differs less than 0.5 kg/m² from the true mean by the probability of 95 % both in the F/H and 0-40 cm layers (Fig. 1). Sample numbers in excess of 10 do not substantially increase the accuracy of estimating the mean.

Table 1. Amount of organic carbon in the different soil layers (kg/m², except % for the CV), n=126.

layer	mean	med	SD	min	max	CV
F/H	1.88	1.77	0.524	1.00	3.60	28.0
0-10 cm	1.41	1.43	0.312	0.668	2.42	22.1
10-20 cm	0.805	0.755	0.252	0.410	1.88	31.3
20-40 cm	0.424	0.384	0.174	0.175	1.49	41.1
0-40 cm	2.64	2.66	0.562	1.43	4.85	21.3

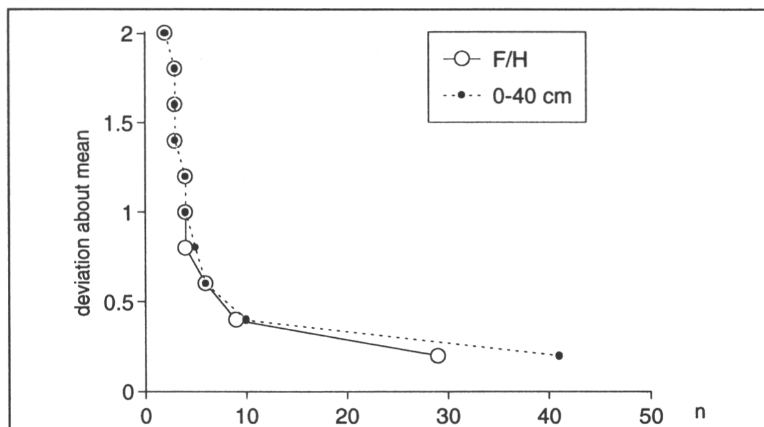


Figure 1. 95 % confidence limits for estimating the mean amount of organic carbon (kg/m²) in the organic F/H layer and 0-40 cm mineral soil layer as a function of number of samples.

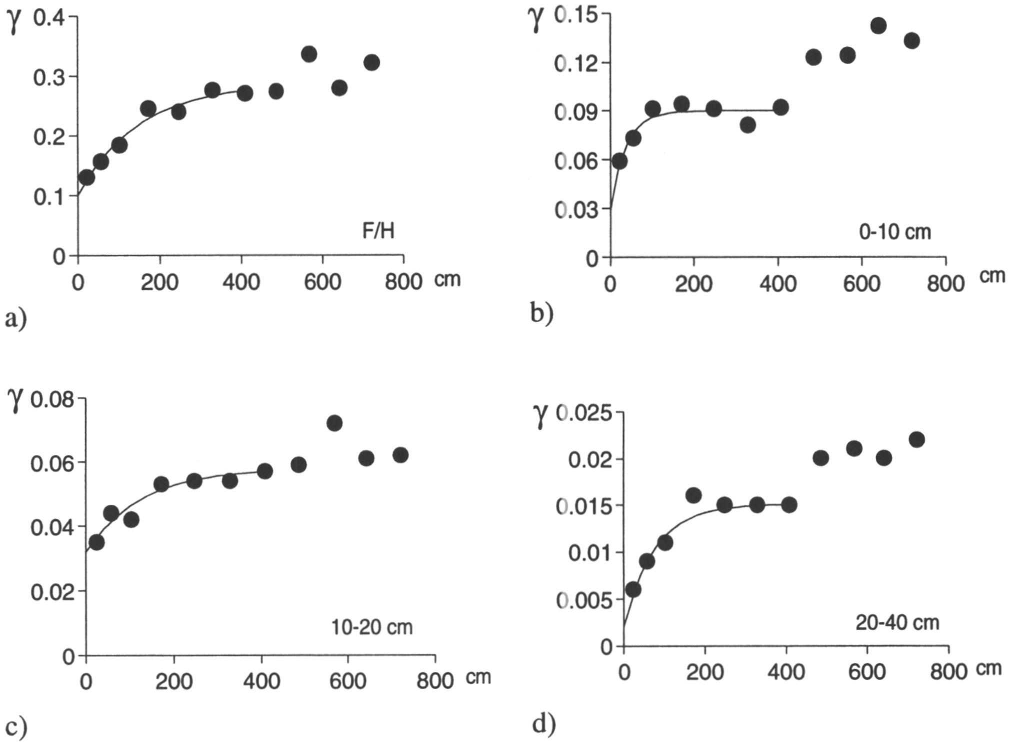


Figure 2. Semivariograms of the amount of organic carbon (kg/m²) in the a) organic F/H layer, b) 0-10 cm, c) 10-20 cm and d) 20-40 cm mineral soil layers. The lines represent models fitted to the semivariances at the distances of less than 4.5 m for the kriging interpolation.

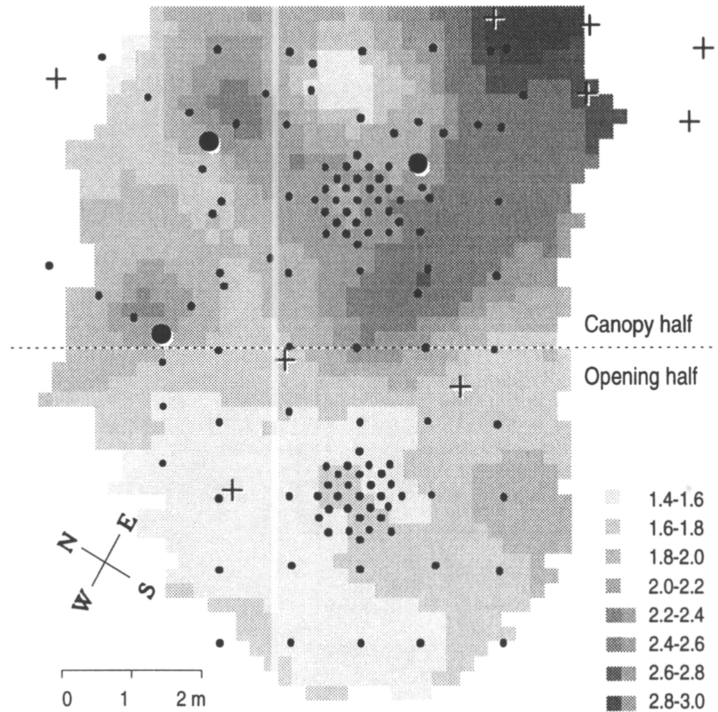
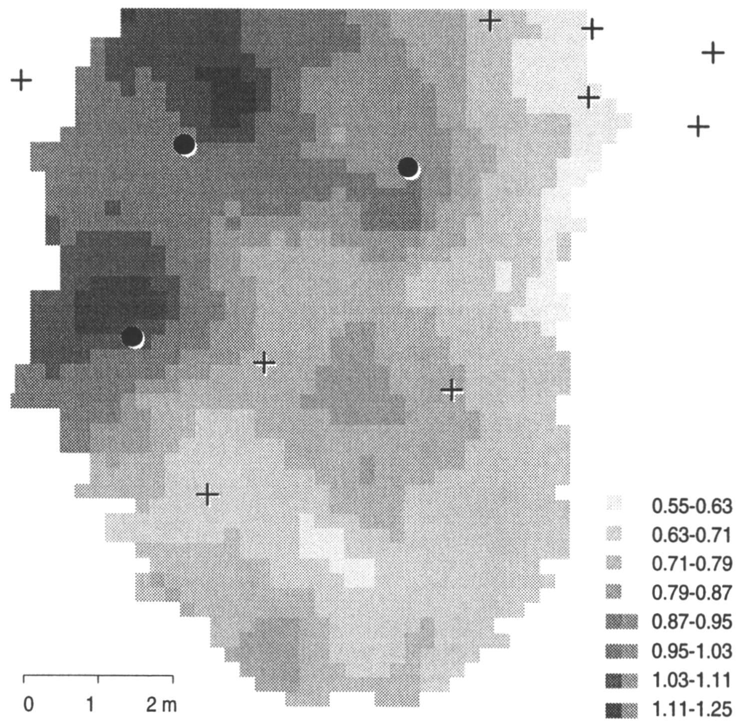
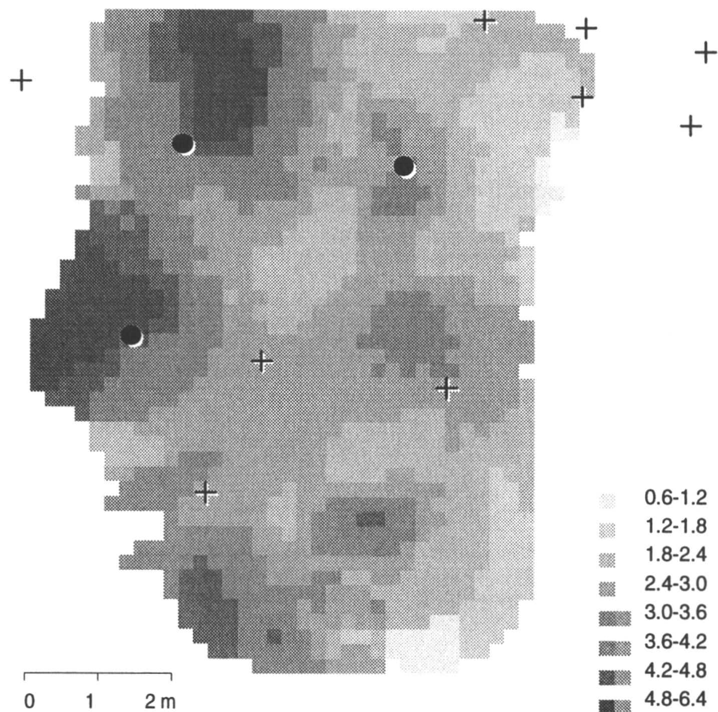


Figure 3. Interpolated map of the amount of carbon (kg/m²) in the organic F/H layer. Sampling points are indicated by the small dots, trees by the large dots and stumps by the crosses. The dashed line indicates the division of the site into the canopy and opening halves.

Figure 4. Interpolated maps of a) the amount of organic carbon (kg/m^2) in the 10-20 cm mineral soil layer and b) the thickness (cm) of the E horizon. Trees are indicated by the dots and stumps by the crosses.





The amount of carbon was spatially dependent at distances of less than 1-4 m and the spatial dependence accounted for 45-86 % of the total variance, depending on the soil layer (Figs. 2a-d). Therefore, to fulfill the criteria of statistical independence, samples should be taken further apart than the range of spatial dependence. Conversely, to utilize the dependence when interpolating (kriging), samples should be taken closer than the range.

The F/H layer was 15 % thicker and contained 33 % more carbon in the canopy half than in the opening. The highest carbon contents tended to occur around the trees (Fig. 3). These differences, due to changes in litter deposition and decomposition rates, had developed over a period of some tens of years which was, on the basis of the stumps, the age of the opening. In the 0-10 cm layer the amount of carbon was patchy and not associated with the trees. On the other hand, the 10-20 cm and 20-40 cm layers contained more carbon near the trees than elsewhere and the largest quantities were found in the immediate vicinities of the stems (Fig. 4a). The spatial patterning of the E horizon thickness was similar (Fig. 4b). The exceptionally thick E horizon near the stems was most probably caused by stemflow and the large amount of carbon in the B horizon below was, in turn, due to the organic compounds transported into the soil by the stemflow. Owing to the podzolic properties, the organic compounds may have remained dissolved in water in the conditions of the E horizon and precipitated first in the B horizon. It seems, that even if the volume of stemflow is not more than 1-2 % of precipitation in Scots pine stands (Päivänen 1966), it still induces remarkable variation in soil properties. This is most probably due to concentrated routes of stemflow into the soil and high carbon concentration, up to 200 mg/l (Gersper and Holowaychuk 1971) compared to an average of 26 mg/l in throughfall (Westman et al. 1994), in the stemflow. According to these results, trees induce heterogeneity in soil properties and clearly observable alterations may develop fairly quickly considering the time scale of soil formation, namely in less than hundred years.

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Decomposition of fine roots of Norway spruce (*Picea abies* (L.) Karst.) and Scots pine (*Pinus sylvestris* L.) in different soils

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Introduction

The most interesting results of root investigations during the last decade are connected with fine-root productivity and turnover. These indicate that the production and replacement of fine roots in boreal forests can form a major part of net primary production (Persson 1983). Therefore, root decomposition is a key process in nutrient, mass and energy dynamics of a coniferous forest. The paper represents a summary of the results of long-term decomposition studies of finest and fine roots in Norway spruce and fine roots in Scots pine, which were carried out with the aim:

(1) to describe the dynamics of the organic matter, ash, nitrogen and energy content during decomposition;
(2) to analyze variability between different sites and incubation depths;
(3) to analyze variability between different conifer species (Norway spruce and Scots pine).

Proceeding from this, the root litter-bag technique was employed.

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Table 1. Characteristics of the permanent plots in Estonia.

A. Root decomposition and stand characteristics

Site	Canopy composition	Age (years)	Mean height (m)	Site quality class	Basal area (m^2)	Remaining amount of roots after five years (% from the initial weight)
Voore 1	10S	40	19	I[a]	48.9	Spruce < 1 mm, 60 %
Voore 2	9S 1B	50	20	I	33.0	Spruce < 2 mm, 54 %
Haanja	9S 1B	45	20	I	43.2	Spruce < 2 mm, 54 %
Väätsa	9S 1B	63	25	I	29.7	—
Vigala	6S 3P	43	18	I	35.6	Spruce < 2 mm, 61 %
Putkaste	9S 1B	64	19	I I	34.0	Spruce < 2 mm, 49 %
Kuusnõmme	5S 5P	73	11	I V - V	11.8	Spruce < 2 mm, 73 %
Tipu	8S 1P 1B	56	22	I	44.3	Spruce < 2 mm, 53 %
Pikasilla	7P 3S	63	11	I I I	26.8	Spruce < 2 mm, 70 % Pine < 2 mm, 73 %
Nõva	10P	143	15	V	10.7	Pine < 2 mm, 69 %

S - *Picea abies*, P - *Pinus sylvestris*, B - *Betula pubescens*

B. Soil characteristics

Site	Soil type	Parent material	Humus form	pH (H_2O) in 0 - 20 cm
Voore 1	Umbric Luvisol	Red-brown till	mull	5.5 - 6.0
Voore 2	Umbric Luvisol	Red-brown till	mull	4.4 - 5.6
Haanja	Dystric Podzoluvisol	Red-brown till	moder	4.3 - 5.6
Väätsa	Umbric Cambisol	Yellow grey till	mull	6.5 - 7.2
Vigala	Dystric Gleysol	Vawed clay	moder-mull	4.4 - 4.9
Putkaste	Gleyic Podzol	Aqueous sand	mor	5.3 - 5.8
Kuusnõmme	Rendzic Leptosol on pebble	Pebble till	mull	7.0 - 8.3
Tipu	Haplo-Gleyic Podzol	Fluvioglacial sand	mor	4.5 - 5.0
Pikasilla	Sombri-Ferric Podzol	Fluvioglacial sand	mor-moder	4.4 - 5.3
Nõva	Sombri-Ferric-Gleyic Podzol	Fluvioglacial sand	mor	5.7 - 6.0

Materials and methods

For Norway spruce the study of the decomposition of the finest (<1 mm in diameter) roots was carried out in a 40-year-old high site quality (I^a) Norway spruce (*Picea abies* (L.) Karst.) stand described in Ivask et al. (1991), and Lõhmus and Ivask (1994) and in Table 1 (Site Voore 1). Fine root (<2 mm in diameter) decomposition studies were conducted on 8 permanent plots in Estonia.

Stand and soil characteristics are given in Table 1 (Sites: Voore 2, Haanja, Väätsa, Vigala, Putkaste, Kuusnõmme, Tipu, Pikasilla). Scots pine decomposition of fine roots (<2 mm in diameter) was studied in Pikasilla and Nõva (Table 1).

The initial material for decomposition was collected for the Norway spruce at high-quality spruce stands: finest roots (<1 mm in diameter) at site Voore 1 and fine roots (<2 mm in diameter) at site Haanja (Table 1); for the Scots pine the fine roots were collected at the low quality pine stand Nõva (Table 1). The methods are described in Lõhmus and Ivask (1994). The mesh size of the root-litter bags was about 0.1 mm, the size of litter bags was 5x5 cm^2 for finest and 8x8 cm^2 for fine roots. Each bag contained 1000 mg of finest or about 500 mg of fine roots. One hundred bags of finest roots were incubated randomly under the forest floor and in subsequent 10 cm soil layers down to the depth of 40 cm in site Voore 1 in July 1986. The litterbags of fine roots were incubated in soil at a depth of 10 cm in July 1989. The bags were collected once or twice a year except for Voore 1 and Voore 2 sites, where the seasonal dynamics was investigated. In all initial and decomposing samples oven-dry weight, ash and energy content (by the macrocalorimeter KL-5) and nitrogen concentration (by the Kjeldahl method) was determined.

Results

1. Finest (<1 mm in diameter) spruce root decomposition

The finest spruce root decomposition dynamics was studied in site Voore 1 (Table 1) and discussed in Lõhmus and Ivask (1994). The initial N, ash and lignin concentrations were 1.29 %, 5.7 % and 34.8 % respectively, caloricity was 18.48 kJ/g. By the multiple comparison of means no significant differences were found between various depths of decomposing samples for the remaining oven-dry and ash-free mass, caloricity and N concentration. The ash-free dry weight of the samples decreased by 14.3 % of the initial dry weight during the first month; the low caloricity of the dry weight loss indicates that the main loss was formed by soluble carbohydrates with a caloricity of 17.0 (Morowitz 1968). After five years the finest roots had lost 40 % of their initial weight, half of it during the first year. Following in time the absolute amount of N in remaining material, the phases of leaching, accumulation and final release (Berg and Staaf 1981) are

observed, the mean nitrogen concentrations varied during the incubation from 1.47 to 1.78 %.

2. Fine (<2 mm in diameter) spruce root decomposition studies

The initial N, ash and lignin concentrations were 0.73 %, 1.8 % and 37.0 % respectively, the caloricity was 20.0 kJ/g. The rate of fine-root decomposition in the Voore forest (Site Voore 2 in Table 1.) is somewhat different from that of the finest roots (Lõhmus and Ivask 1994). During the first month ash-free dry weight decreased by 7.3 % of the initial dry weight, which shows that the amounts of soluble compounds in fine roots are smaller than in the finest root fraction. Due to the lower initial N concentration, 0.73 % in fine roots (for finest roots 1.29 %), the change of the absolute amount of nitrogen over time is different. An accumulation phase can be distinguished, after which a release phase (Berg and Staaf 1981) begins. During the first three years the decay rates of the finest and fine spruce roots in the same soil were similar. In different soils after the first year fine spruce roots had lost 21.0 to 32.7 % of their initial dry weight, after two years the loss was 22.5 to 43.2 %. The remaining dry weight percentages from the initial dry weight after five years are given in Table 1. In all sites the N concentration in five years was higher than the initial concentration and varied from 0.97 to 1.70 % in different sites.

3. Fine (<2 mm in diameter) pine root decomposition studies

The initial N, ash and lignin concentrations were 0.47 %, 1.1 % and 22.7 % respectively, the caloricity was 19.2 kJ/g. After the first year fine pine roots had lost of their initial dry weight at Pikasilla and Nõva sites 19 % and 31 %, respectively; the nitrogen concentration varied in five years from 0.62 to 1.12 %. The remaining dry weight percentages after five years are given in Table 1.

We may conclude that the decay rates of the Norway spruce and Scots pine fine root litter are smaller at the sites where the site quality is lower.

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Seasonal variation of fine-root biomass in *Pinus sylvestris* (L.) stand

Kirsi Makkonen

Introduction

Roots, especially fine roots, have a most important role for the function of forest trees. They take up and transport water and nutrients to the aboveground tree parts.

Although fine roots are so important for the tree, they have seldom been included in ecophysiological studies. One reason for this is the tremendous amount of work and processing time, which root research involves.

The objective of this study was to determine the seasonal variation of fine roots in a 35-year-old Scots pine (*Pinus sylvestris* L.) stand in eastern Finland. This research was a part of the research project "Nutrient dynamics and biomass production of Scots pine" carried out by the Finnish Forest Research Institute and the University of Joensuu. The Society of Forestry in Finland supported my work, for which I am very grateful.

Materials and methods

Experimental stand

The research was carried out in a Scots pine (*Pinus sylvestris* L.) stand at Ilomantsi near Mekrijärvi Research Station (62° 47' N; 30° 58' E; 144m a.s.l.) in eastern Finland.

The research stand is a naturally regenerated 35-year-old pole stage stand (Table 1). The site type is a *Vaccinium*-type, according to the classification of Cajander (Cajander 1949). The field layer

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is dominated by *Vaccinium vitis-idaea* (L.) and *Calluna vulgaris* (L.) Hull. The bottom layer is dominated by *Pleurozium schreberi* (Brid.) Mitt. and some *Cladonia* species.

The soil is an iron podsol, relatively poor in available nutrients. The thicknesses of the soil horizons were humus 2.5 cm, eluvial horizon 5.0 cm, and illuvial horizon 11.0 cm (Helmisaari and Mälkönen 1989).

Table 1. Some characteristics of the experimental stand in 1985.

Age, a	35
Number of trees/ha	2980
Mean diameter, cm	7.4
Mean height, m	6.4
Basal area, m^2/ha	14,0
Stem volume, m^3/ha	11.3
Volume increment, m^3/ha/a	11.3
Plot area, m^2	500

The climate of the research area is continental. Mean annual temperature was 1.0 °C and annual rainfall 699 mm during the study period (1985-1988).

Root sampling

Eleven samplings were carried out during the growing seasons 1985-1988. 20 soils cores per sampling (volumetric samples, core diameter 36 mm) were taken for fine-root biomass determinations. The soil samples were divided into three layers: humus, 0-10 cm mineral soil and 10-30 cm mineral soil. Samples were transported to the laboratory and stored in a deep-freeze.

Laboratory analysis

In the laboratory roots were separated from soil by washing and sorted into Scots pine living roots, other living roots and dead roots. The sorted living roots were separated into three classes by diameter <2 mm, 2-5 mm and >5 mm and dead roots (necromass) <2 mm and >2 mm. Only results from fine roots (<2 mm diameter) will be reported here. The classified roots were dried at 70 °C for 5 days and weighed to determine the oven-dry biomass per area basis.

Results

The variation of Scots pine fine-root biomass

The biomass of Scots pine fine roots varied seasonally in different layers (Fig. 1). Particularly the fine-root biomass in humus varied seasonally and between years. The major part of living fine roots was in the upper mineral soil layer, 53 % more than in humus and 31 % more than in the lower mineral soil layer.

The seasonal variation was statistically significant only in humus layer in 1988: the amount of fine roots was greater in June ($p<0.01$) and in October ($p<0.001$) than in July. Differences between the same months of different years were statistically significant only in July; differences between 1985 and 1988 were rather significant ($p<0.05$), and between 1987 and 1988 significant ($p<0.01$).

In the mineral soil layers the seasonal or between-year variation was not statistically significant.

The variation of other living fine-root biomass

The divided part "other living roots" consisted of dwarf shrubs roots and grass roots. 90 % of these roots were in humus and upper mineral soil layers (Fig.1). Seasonal variation was statistically significant only in 1988; the amount of roots was significant greater ($p<0.01$) in July than in October. There were no significant differences between years.

The variation of necromass

"Necromass" included all dead roots, both dead Scots pine fine roots, dead dwarf shrubs roots and dead grass roots. The major part of dead roots was in humus and the upper mineral soil layer (Fig.1). In the humus layer the seasonal variation was statistically significant in 1987 and 1988; the fine-root necromass differed significantly between June and July 1987 ($p<0.001$), between July and September 1987 ($p<0.01$) and between June and October 1988 ($p<0.01$).

The seasonal variation was significant in the mineral soil only in the upper layer between June and July 1987 ($p<0.01$). The amount of dead roots increased from the beginning to the end of research period. The variation between the same months of different years was significant in humus in July between 1985 and 1988 ($p<0.001$) and between 1987 and 1988 ($p<0.001$).

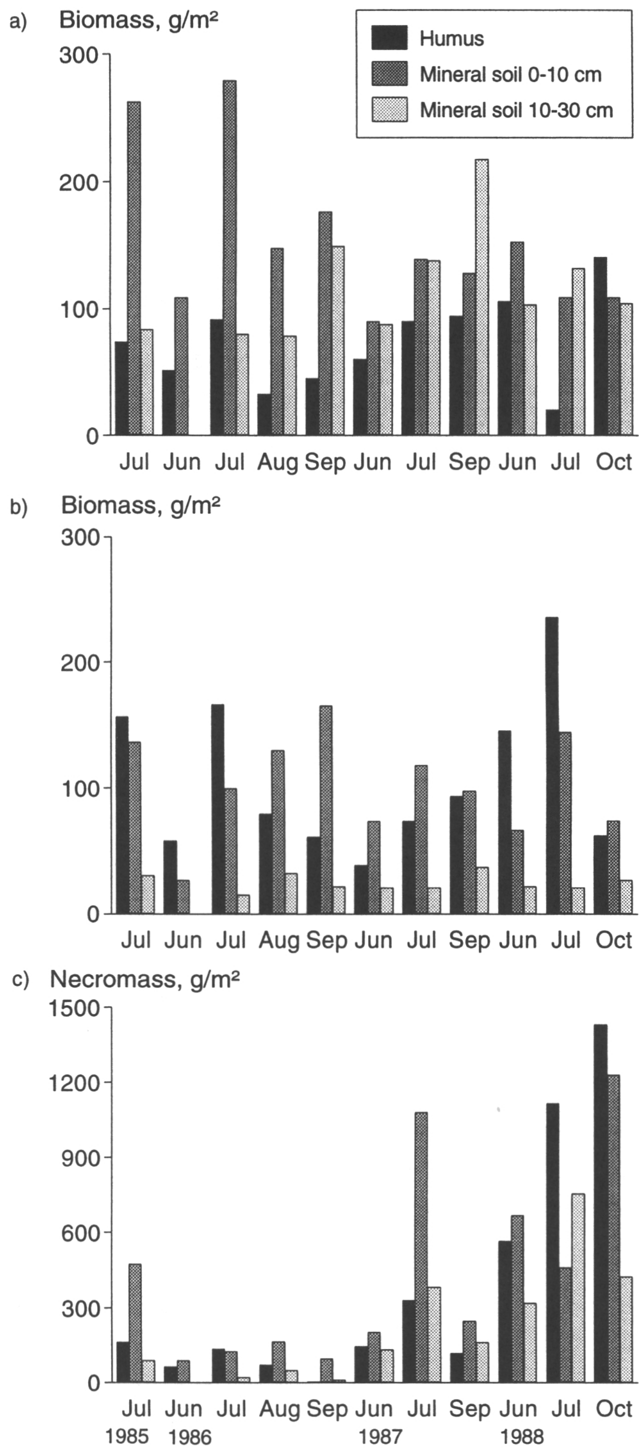

Figure 1. The seasonal variation of a) fine-root biomass of Pinus sylvestris, b) biomass of other fine roots, c) necromass in different soil layers in a Scots pine stand during the research period July 1985 - October 1988.

Discussion

Major part of dwarf shrub roots and grass roots were in the humus layer while Scots pine roots were in mineral soil. Similar results have been reported by Persson (Persson 1978, 1980a, 1983). Differences between species groups depend on the strategies of root growth.

The amount of dead fine roots was larger than the amount of living fine-root biomass. In this study living fine-root biomass was only 55 % of necromass. The fine roots have been shown to replace their weight several times during the growing season (Persson 1978, 1980a, 1980b, 1992).

Fine root growth is a complex process affected by environmental factors. In this study the year 1988 varied from the others. In humus the amount of living Scots pine fine roots decreased in July 1988, but the biomass of dwarf shrubs roots and grass roots did not decrease. The sudden decrease of living Scots pine fine roots may be related to a chance in the environmental factors. The seasonal variation of dwarf shrub roots and grass roots might mostly be related to their seasonal growth dynamics. The biomass of other fine roots decreased and the amount of dead roots increased in October at the end of the growing season. In July 1988 the dying of Scots pine fine roots gave space to a rapid increase in dwarf shrub root and grass root biomass.

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Effects of mycorrhizas on the defensive chemistry in Scots pine seedlings

Anne Nerg, Toini Holopainen and Jarmo K. Holopainen

Introduction

Mycorrhizas improve growth and survival of plants by enhancing nutrient uptake. Improved nutrient uptake allows mycorrhizal plants to allocate more carbon to shoot growth and possibly to antiherbivore defenses in the form of carbon-based defensive compounds. Carbon allocation to antiherbivore defense reduces investments for plant growth, but it may provide protection against herbivores, pathogens and fungal diseases. It is also known, that some ectomycorrhizal fungi themselves can synthesize carbon-based secondary metabolites using carbon partitioned to roots and fungus or induce production of secondary metabolites in roots and thus protecting plants from root pathogens and belowground grazers (Jones and Last 1991). If these metabolites are also transported to shoots, they can influence the aboveground resistance as well.

Plant phenolics are carbon-based metabolites and practically all higher plant phenolics are formed via the shikimic acid pathway from shikimate through the intermediacy of phenylalanine (Harborne 1980). Terpenoids are also carbon-based metabolites and are formed from mevalonic acid via the acetate-mevalonate pathway after the glycolysis of carbohydrates to pyruvic acid and further to acetylCoA (Vickery and Vickery 1981). When conditions are favorable for the plant growth, carbon is allocated to growth processes instead of these secondary metabolites (Bryant et al. 1983). Different environmental stresses may inhibit more growth potential than photosynthetic potential in plants leading to

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increased carbohydrate concentrations. This carbohydrate can be used for secondary metabolite production (Bryant et al. 1983). Some air pollutants, nutrient stresses and water stress have their major impact on photosynthesis and thus may decrease translocated carbon for secondary metabolites (Chapin 1991) and also for fungal and root growth (Andersen and Rygiewicz 1991).

In this study we aimed to find out the effects of mycorrhizal infection on the concentrations of some defensive compounds. The concentrations of total phenolics in *Pinus sylvestris* L. shoots and roots and resin acids in shoots were determined.

Materials and methods

Surface sterilized *Pinus sylvestris* L. seeds were germinated on water agar. After 20 days, the seedlings were transferred to the Petri dishes using a modified version of the sterile Petri dish technique earlier developed by Wong and Fortin (1989). The roots of six-weeks-old seedlings were inoculated with *Cenococcum graniforme* or *Suillus variegatus* and allowed to grow nine weeks until the shoots and roots from individual seedlings were harvested for chemical analyses. The total phenolics were extracted with 80 % acetone from pine shoots and roots and analysed with Folin-Ciocalteu reagent as reported by Julkunen-Tiitto (1985). The absorbances of samples were measured with spectrophoto-meter at 735 nm. Resin acids from pine shoots were extracted following the procedures of Gref and Ericsson (1985). Samples were analysed by gas chromatography-mass spectrometry. The infection level in roots was determined calculating the mycorrhizal and non-mycorrhizal short roots.

Results

The mean infection level in *C. graniforme* roots was 35 % and in *S. variegatus* roots 26 %. According to ultrastructural studies *C. graniforme* formed normal looking mycorrhiza, but the infection with *S. variegatus* was poorer. The root/shoot ratio was significantly ($P<0.05$) higher in the seedlings inoculated with *C. graniforme* than in other treatments. The total phenolic concentration was higher in *S. variegatus* inoculated roots than in *C. graniforme* inoculated roots and on the other hand higher in the shoots of seedlings inoculated with *C. graniforme* than in seedlings inoculated with *S. variegatus* (Fig. 1). Abietic and neoabietic

Figure 1. The total phenolic concentration (mg/g d.wt) in pine roots and shoots in different treatments. C.gr = inoculated with *Cenococcum graniforme*, S.var = inoculated with *Suillus variegatus*. The concentrations in roots or shoots followed by different letters differ significantly at $P<0.05$ according to Duncan's test. The error bars indicate standard deviations.

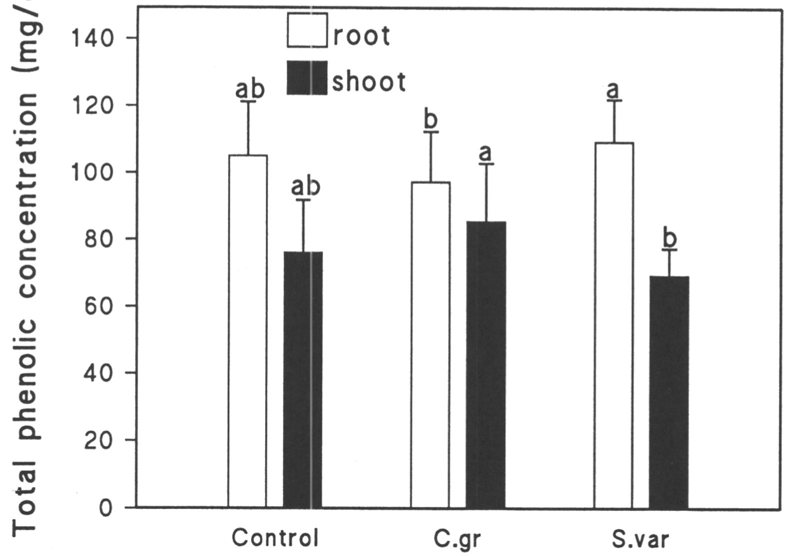
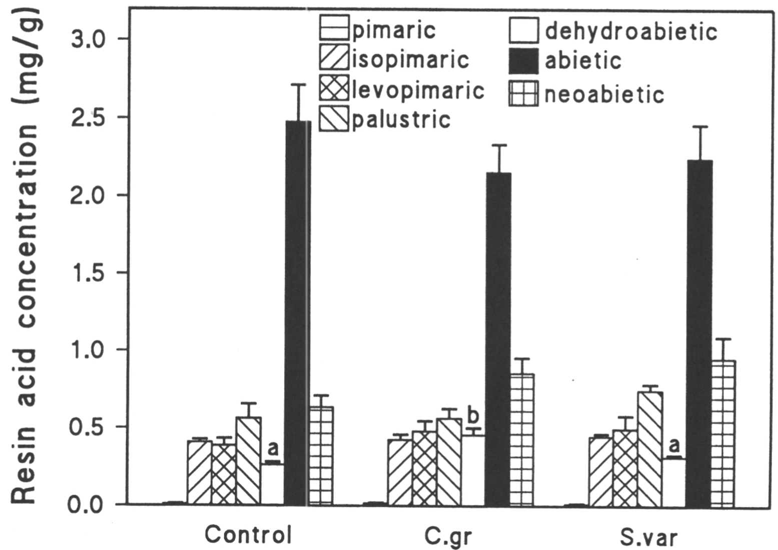


Figure 2. The concentrations of different resin acids (mg/g d.wt) in pine shoots in different treatments. Treatments as in Fig. 1. The concentration of dehydroabietic acid with different letter differs significantly at $P<0.05$ according to Duncan's test. The error bars indicate standard deviations.



acids were the most common resin acids (Fig. 2). The concentration of dehydroabietic acid was significantly higher in shoots of seedlings inoculated with C. *graniforme* than in other treatments (Fig. 2). The total resin acid concentration in the shoots of non-mycorrhizal seedlings was slightly lower than in mycorrhizal seedlings, but there were not significant differences. The proportional quantity of palustric acid was significantly ($P=0.021$) higher in the shoots of seedlings inoculated with *S. variegatus* than in the shoots of seedlings inoculated with *C. graniforme.* Instead in the shoots of non-mycorrhizal seedlings the propor-

tional quantity of abietic acid was significantly (P=0.000) higher than in *C. graniforme* treatment and also significantly (P=0.003) higher than in *S. variegatus* treatment.

Discussion

Results concerning the effects of mycorrhizal infection on the concentration of phenolics in host plant roots are highly varying. Krishna and Bagyaraj (1984) found increase in total phenolics in VA-mycorrhizal *Arachis hypogaea* roots and they suggested that the situation is similar if a pathogenic fungus is invading a host plant. In *Allium porrum* L. and *Ginkgo biloba* L. inoculated with VA-mycorrhiza, the concentrations of cell wall bound phenolics were not changed due to mycorrhizal infection (Codignola et al. 1989). Instead Münzenberger et al. (1990) found a reduction of phenolics in ectomycorrhizas of Norway spruce roots and they suggested that this reduction enables the mycorrhizal fungus to penetrate root tissue. If phenolics are accumulating in roots, they can gradually reduce the plasticity and elasticity of the fungal matrix (Grandmaison et al. 1993). In Scots pine, Bonello et al. (1993) found increased concentrations of some phenolics in the non-mycorrhizal roots after challenging the roots with the root pathogen. Mycorrhizal infection had a dampening effect to the induction of these compounds.

In this study the only difference in total phenolics was between two different mycorrhizal types, both in roots and shoots. The difference between them cannot be explained by higher infection level because the mycorrhizal infection was lower in the roots inoculated with *S. variegatus*. The formation of mycorrhizas also demands part of the assimilated carbon and it may explain lower phenolic concentration in the shoots of *S. variegatus* seedlings. It seems that total phenolics is not sensitive enough revealing the effects of mycorrhizas on plant phenolics. Individual phenolic compounds should be analysed in order to better understand possible mechanisms.

There are only few studies about the effects of mycorrhizas on the biosynthesis of terpenoids. Krupa and Fries (1971) found the accumulation of volatile compounds, primarily terpenes and sesquiterpenes, in the mycorrhizal roots of *Pinus sylvestris* L. The authors proposed that the response of the host to mycorrhizal infection is non-specific and similar to a wound response: increased production of volatile (terpenes etc.) and nonvolatile (phenolics, resin acids etc.) substances. If these compounds are in sufficient

concentrations, they may restrict the growth of mycorrhizal fungi and also inhibit the growth of root pathogens. Cheniclet et al. (1988) have found that fungal infection mainly induces accumulation of volatile terpenes while wounding and insect attacks induce the accumulation of non-volatile terpenes. In this study nonvolatile resin acid concentrations, except dehydroabietic acid, in pine shoots were not changed according to mycorrhizal infection.

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Nitrite reductase activity in the mycorrhizal roots of Scots pine seedlings

Karoliina Niemi[1], Helvi Heinonen-Tanski[2] and Toini Holopainen[3]

Abstract

Nitrite reductase (NiR) activity was shown in roots of Scots pine (*Pinus sylvestris*) seedlings inoculated with three strains of *Cenococcum geophilum* and two strains of *Paxillus involutus*. All mycorrhizal associations could use nitrite (NO^-_2) after both aseptic and non-aseptic cultivation. Nitrogen fertilization increased significantly the NiR activity in *P. involutus* mycorrhizas of aseptic seedlings.

Introduction

Nitrification is low in many acid coniferous forest soils and ammonium (NH_4^+) is the dominant form of mineral N (Adams and Attiwill 1982, Martikainen 1984). The significance of nitrate (NO_3^-) as a N source has, however, increaced due to NO_x pollution and NO_3^- fertilization in forests and nurseries.

Most NO_3^- assimilation in Scots pine (*Pinus sylvestris* L.) may occur in roots (Sarjala et al. 1987, Pietiläinen and Lähdesmäki 1988, Seith et al. 1994). Therefore the mycorrhizal symbiosis formed in roots has a significant effect on NO_3^- nutrition of Scots pine. Nitrate utilization has been observed both in the mycorrhizal (Sarjala 1991) and non-mycorrhizal roots of Scots pine

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(Seith et al. 1994). NO_3^- assimilation presumes the activity of nitrite reductase (NiR) (EC 1.7.7.1 in plants, EC 1.6.6.4 in fungi). Seith et al. (1994) showed NiR to work in the non-mycorrhizal roots of Scots pine. It is, however, quite unclear, how this enzyme works in the mycorrhizal roots of conifer trees.

This study was designed to test the NiR activity in the mycorrhizal roots of Scots pine seedlings and the influence of N fertilization on it.

Materials and methods

Surface sterilized Scots pine seeds, representing a central Finland provenance, were sown into a 1:1 (v:v) sterile vermiculite:peat mixture. The seedlings were grown in a controlled growth room where the day:night temperatures were 26:21 °C and the light:dark cycle was 22:2 h. Seedlings were watered with sterile water when required.

Non-aseptic seeds were sown into peat. The seedlings were grown in a greenhouse in the same temperature and light conditions as the aseptic ones. Seedlings were watered with lake water.

After three weeks the seedlings were inoculated with an agar bloc of mycelium taken from pure cultures of two *Paxillus involutus* (Batch.) Fr. (referred here numbers 12 and 14) and three *Cenococcum geophilum* (Sow.) Ferd. & Winge (36, 38 and 53) strains. All the strains were taken from the culture collection of University of Kuopio.

The seedlings were fertilized twice over the experimental period (6 and 8 weeks after sowing) with $NaNO_3$. Both treatments were calculated to correspond to 50 kg/ha NO_3^- -N. The control seedlings received only water.

NiR activity of pine mycorrhizas was determined 14 weeks after sowing. The enzyme was isolated by a modification of the method of Yoneyama and Sasakawa (1979). The roots were cut into small pieces and ground with a grinding medium composed of potassium phosphate buffer (pH 7.5), cysteine and EDTA. The homogenate was squeezed and the filtrate was centrifuged. The supernatant was used as a crude enzyme extract. The NiR activity measurements were based on the method of Ida and Morita (1973). The enzyme assay mixture contained Tris-HCl buffer, $NaNO_2$, methyl viologen MQ water and the enzyme. All measurements were carried out below +4 °C. The reaction was started by adding sodium dithionite in sodium bicarbonate to the reaction mixture. After 20 min of incubation in the dark at 30 °C, the loss

of NO_2^- was determined by the addition of Griess reagents. Absorbance of the diazo color was read at 540 nm after 20 min.

Results

The infection of mycorrhizal fungi was obvious in each case. All five mycorrhizal associations of Scots pine seedlings could use NO_2^-. NiR activity in unfertilized pines with *P. involutus* mycorrhizas (12 and 14), grown in aseptic conditions, was weak, only about 60 % of that of *C. geophilum* strain 38 (Fig.1). N fertilization (2 x 50 kg NO_3^- -N/ha) improved NiR activity in *P. involutus* mycorrhizas quite clearly.

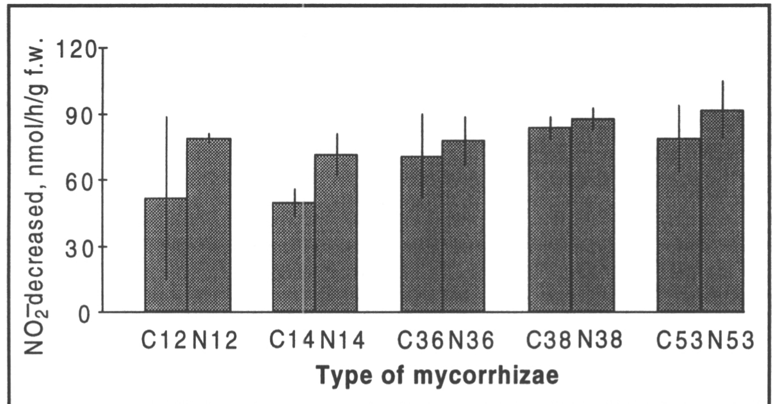

Figure 1. Nitrite reductase activity, NO_2^- decreased nmol/h/g the fresh weight of root mass, in different associations of aseptic Scots pine (*Pinus sylvestris*) mycorrhizas (*Paxillus involutus* strains 12 and 14, *Cenococcum geophilum* strains 36, 38 and 53). C =control (unfertilized), N = fertilized (2 x 50 kg NO_3^- -N/ha). Vertical bars represent SD (n = 3, except C38 n = 2).

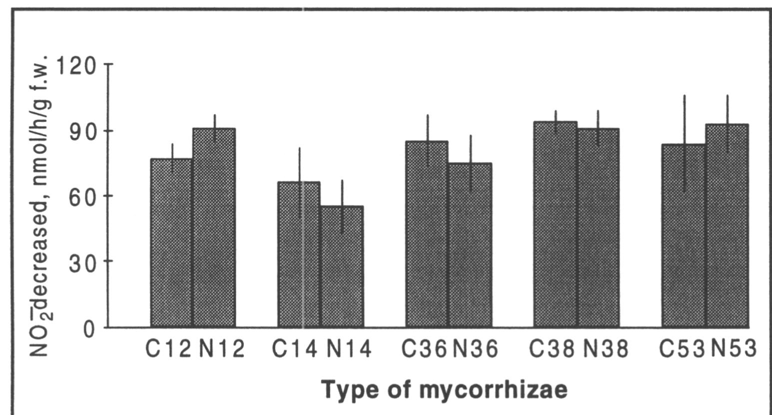

Figure 2. Nitrite reductase activity, NO_2^- decreased nmol/h/g the fresh weight of root mass, in different associations of non-aseptic Scots pine (*Pinus sylvestris*) mycorrhizas (*Paxillus involutus* strains 12 and 14, *Cenococcum geophilum* strains 36, 38 and 53). C = control (unfertilized), N = fertilized (2 x 50 kg NO_3^- -N/ha). Vertical bars represent SD (n = 3).

Unfertilized control mycorrhizas of the strain 14 used NO_2^- least of all non-aseptic controls, about 70 % of the amount that the strain 38 used (Fig. 2). The effects of fertilization were slight, all-together.

Discussion

The fate of NO_2^- formed in NO_3^- reduction is not well known. NO_2^- should be assimilated into some organic form quickly after NO_3^- reduction in the mycorrhizae, because NO_2^- and NH_4^+ are toxic to cells. That is why NiR activity might follow the level of NO_3^- reduction.

In the present study all five mycorrhizal associations of Scots pine seedlings could use NO_2^-. The strains 38 and 53 of *C. geophilum* were isolated from NO_3^- and NH_4^+ rich soil near a pulp mill. In this study the mycorrhizas formed by these strains used NO_2^- the best.

Fertilization (2 x 50 kg/ha NO_3^--N) during the growing period did not affect NiR activity in aseptic *C. geophilum* mycorrhizas. The stimulating effect on *P. involutus* was, however, significant. Seith et al. (1994) showed NO_3 to stimulate NiR in the non-mycorrhizal roots in aseptic conditions. In the present study NiR activity was higher in non-aseptic controls than aseptic ones, which could result from some kind of association between mycorrhizas and microbes in non-aseptic soil. Fertilization did not actually improve the NiR activity of non-aseptic mycorrhizas. The total amount of NO_3^- in the fertilizer was propably too high and even inhibited NiR. This could be seen especially in the strains 14 and 36.

NiR activities of mycorrhizas presented here are much lower than activities (NO_2^- decreased over 10 000 nmol/h/g fresh weight) of pure *Hebeloma cylindrosporum* (Romagn.) mycelium reported by Plassard et al. (1984). The low activities can be partly a result of conditions. The method used here has been developed for the optimal conditions in spinach leaf (Ida and Morita 1973), where NiR activity may be very high. When determining NiR activities the strength of reagents and conditions were not tested and it may be that they were not ideal for NiR of pine mycorrhizas.

The results of the present experiments, however, show clear NiR activity in Scots pine ectomycorrhizas. This supports earlier observations of the ability of some ectomycorrhizal fungi (Ho and Trappe 1980, Sarjala 1990) and plants (Sarjala 1990, Scheromm et. al. 1990) to assimilate NO_3^- and use it as a source of N. A new

approach of studing NO_3^- metabolism of mycorrhizas may be found by developing a method optimal for mycorrhizal NiR. After that the effects of NO_3^- fertilization and pollution on the whole NO_3^- assimilation cycle could be better understood.

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Ectomycorrhiza development: 2-dimensional analysis of the cytoskeleton

Sara Niini, Mika Tarkka and Marjatta Raudaskoski

Cytoskeleton is an essential part of a functioning cell, it plays a major role i.e. in cell division, plant cell wall orientation and in fungal tip growth. The main components of cytoskeleton in eukaryotic cells are microtubules, microfilaments and intermediate filaments. The subunits of microtubules are α- and ß-tubulin, while microfilaments are formed of actin. Intermediate filaments consist of several proteins. Immunological research has shown that microtubules and microfilaments are common structural components in plant and fungal cells, and there is data about the occurrence of intermediate filaments in plant cells. In addition to the main components the cytoskeleton consists of accessory proteins which can be subdivided in assembly controlling, linkage and motor proteins.

The interest in the function of microtubules and microfilaments in mycorrhizal associations originates from the ability to identify the cytoskeletal components by immunological methods both in the fungal and plant cells. Indirect immunofluorescence microscopy has been applied to study the cytoskeletal elements in fungal pure cultures (Runeberg et al. 1986, Raudaskoski et al. 1988, Salo et al. 1989, Niini and Raudaskoski 1993) and also in mycorrhizal associations (Timonen et al. 1993). One dimensional electrophoresis and immunoblotting with specific monoclonal antibodies reveals cytoskeletal proteins in all analyzed ectomycorrhizal fungi (Niini and Raudaskoski 1993) and in ectomycorrhizal associations (Timonen et al. 1993). It is possible to separate *Pinus sylvestris* and *Suillus bovinus* tubulins in 1-D electrophoresis with a sligth difference in molecular weight.

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A good correlation has been shown to exist between the polymerization state of the cytoplasmic microtubules and the growth of the fungi (Niini and Raudaskoski 1993). This supports the idea that intact cytoplasmic microtubules are involved or needed for the extension growth of the fungal apical cells. A change of microtubule pattern from a strand-like to more reticulate in the fungal cells has been observed to be related to the formation of the Hartig net (Timonen et al. 1993). The role of cytoskeleton in the development of ectomycorrhiza is an interesting question as the formation of coralloid mycorrhizas typical to *Pinus sylvestris-Suillus bovinus* symbiosis involves meristematic activity of the root cells, which first leads to the development of dichotomous short roots and later to the coralloid mycorrhizas with numerous root tips. It may be assumed that the plant cell cycle in the short roots is affected by the fungus, especially since no coralloid roots are found in the non-mycorrhizal pine roots.

In order to obtain a more detailed understanding of the changes especially in the cytoskeletal proteins during the ectomycorrhiza development 2-dimensional electrophoresis gels and their immunoblots were prepared and analyzed from main roots and short roots of *Pinus sylvestris* seedlings with or without mycorrhizas. In addition pure cultures and extramatrical mycelium of *Suillus bovinus* were also used. The mycorrhizas were classified in four groups on the basis of their developmental morphology: undivided short root tips with A1) exposed meristem or A2) complete fungal sheath, B) dichotomously branching mycorrhiza and C) coralloid mycorrhiza with three or more tips. Extra care was taken in sample preparation and by using several proteinase inhibitors during the extraction procedure (Åström et al. 1991). Careful solubilization of the proteins ensured the successful immunodetection of cytoskeletal proteins.

The analysis of the total polypeptide patterns indicate that the different developmental stages of ectomycorrhiza share 19 polypeptides that are not present in the seedling or fungus, and thus they could be putative ectomycorrhizins. Relative intensity of the OD readings of the silver stained gels reveal that these ectomycorrhizins can be constitutive during the development of the mycorrhiza or they can be present only in the early phase or late stage. Their amount may also increase through the different stages being most abundant in the coralloid phase.

The immunoblots of ectomycorrhizal extracts with α-tubulin antibody revealed seven isotypes of α-tubulin, four of which are of fungal origin and three of plant origin. Plant α-tubulin could be recognized by its faster migration than that of fungal α-tubu-

lin, which has already been shown in 1-D gels (Timonen et al. 1993). Two strongly expressed α-tubulin isotypes and two weaker signals were detected in the immunoblots of the mycelial extracts of *Suillus*. There was a marked increase in the expression of the weaker fungal α-tubulin signals in the samples from the mycorrhizal roots. In the immunoblots with actin antibody two plant and two fungal actins could be visualized. It seems that in the early mycorrhizal developmental stage the plant actins are strongly dominating species but as the mycorrhiza development proceeds to dichotomous and coralloid stage the fungal actins become visible. The resolution of ß-tubulins in the immunoblots is not as good as that of actin and in the immunoblots of the mycorrhizal samples the plant and fungal ß-tubulins overlap, thus making the quantification difficult. Deducing of the ß-tubulin immunoblots there could be two plant and two fungal ß-tubulins distinguished in the mycorrhizas.

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Ectomycorrhiza: carbohydrates, minerals and hormones

Jan-Erik Nylund

Frank (1885) assumed that (ecto)mycorrhiza received its carbohydrate nourishment from the host, but his ideas took long to be proved; most of his contemporaries believed that the mycobiont took an active part in decomposition. It was only Melins many physiological experiments which showed that Frank, in this as in so many other of his observations, had been (almost) right. Now, when data on the precise share for the fungus of the plant's total carbohydrate are forthcoming at an accelerating pace, there is also increasing evidence for fungal decomposition of organic matter (cf. Abuzinadah and Read 1987), albeit this is of minor, but not marginal importance to the overall energy budget of the mycobiont.

However, Melin's findings and other current physiological work in the '30s made Hatch (1937) propose that carbohydrate supply depends on the host's mineral nutrition status in a paper as hard to access as central in its theory; which in turn led Björkman (1942) to formulate his theory for the role of carbohydrate in mycorrhiza formation: *Mycorrhiza develops only where the host has a "surplus" of carbohydrate available.*

There is much to say about this hypothesis (cf Nylund 1988), which for a long time was not subject to experimental testing to the extent it would have merited. Björkman himself was so convinced of its validity that he never questioned the generalizability of his very limited proof. Yet, even his opponents tacitly acknowledged the central role of carbohydrates in regulating the symbiosis, they only tried to attack the assumed link to N and P nutrition.

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The chief alternative theory, proposing auxin to be the primary regulator rather than N and P, was drawn up by Slankis (summary in Slankis 1973), while Meyer (1962) presented confusing results where mineral nutrition seemed to correlate with ectomycorrhiza just contrary to Björkman's statements.

The story is not very edifying: a poorly supported theory was contested with even less evidence in favour of the counterproposal; it has been told elsewhere (Nylund 1988). This paper will try to disentagle the controversy and set it in an ecological context, based on recent research, mainly in the Uppsala lab. The original research is presented in a series of articles by Nylund and Wallander (Nylund and Wallander 1989, Wallander and Nylund 1991, 1992, Wallander, et al. 1992, 1994, Nylund et al. 1994).

The three interactions

In a long series of hydroponic culture experiments based in Ingestad's well known principles of steady-state nutrition, the following picture emerged:

1. ***Nitrogen*** (superoptimal) suppresses the development of mycorrhiza, and has some influence on the carbohydrate pool.
2. ***Mycorrhiza***. Increases in mycorrhizal biomass reduce the root carbohydrate pool.
3. ***Increased carbohydrate availability*** boosts mycorrhiza development.

Surplus N usually reduces the carbohydrate pool in uninfected seedlings, as does severe starvation. The reason why the N effect on the carbohydrate pool is not so easy to see in mycorrhizal plants is that the depression of the mycorrhiza reduces the demand for carbohydrate counteracting the direct nutrition effect. The mechanisms of nitrogen depression of mycorrhiza will be discussed in detail in a following paper by Håkan Wallander, only the implications will be treated here.

How does this compare with Björkman's statements? Regarding N and P, he showed a pronounced N optimum, and a weaker but nevertheless clear optimum for P; the two factors clearly interacted. He also showed that reduced light and impeded carbon translocation caused mycorrhiza to vanish. Yet, we find no evidence of his claim that *surplus* cabohydrate, i.e. a large pool, would be present in mycorrhizal roots; both we and colleagues have repeatedly found the opposite. Strong mycorrhiza develop-

ment, caused by "virulent" fungi, always reduces the host carbohydrate pool compared with controls and weaker mycorbionts. Available carbohydrate is normally taken care of by the fungus, never allowing a *pool* to build up. If we had methods of monitoring the carbohydrate *flow*, it would most probably be found to increase in proportion to mycorrhiza development. But in my opinion, the surplus-N-and-reduced-P-situation provides enough circumstantial evidence for Björkman's underlying standpoint that carbohydrate sets a limit to mycorrhiza development: N as such depresses mycorrhizal growth, and may tend to reduce carbohydrate availability by requiring carbon skeletons for assimilation, a basic assumption by Björkman, taken over from Hatch. But P starvation reduces the amount of AMP available for making ATP and fuelling all metabolic processes, thereby reducing growth and causing photosynthetic carbon to accumulate even to the point of feedback inhibition of photosynthesis. This more than offsets the N effect, the result being a vigorous development of mycorrhiza. This was empirically found by Stenström et al. (1990) a few years before our work: the success of nursery inoculations required a reduction of P supply compared with standard nursery practices, while attempt at reducing N had no comparable effect.

The elusive auxin

So, we come out in support of Björkman on a more fundamental plane, while rejecting some of his more specific statements. But what about the hormone theory? Even its originator considers carbohydrate supply to be the ultimate regulator, but proposes that this is controlled by auxin action. Based on root culture experiments, Slankis (1973) claimed that (1) IAA of fungal origin causes mycorrhizal morphogenesis *and* (2) induces a flow of carbohydrate from host to fungus, proportional to the IAA supply. This, in turn, (3) was considered to be influenced by the nitrogen availability.

The first statement may well be true. The second may be partially true but oversimplified. The last statement is completely untenable. We have run a series of assays, using the only reliable method available: GC-MS with inner standards. Added nitrogen actually increases the secretion of IAA and the pool in the host, since the hormone is manufactured from tryptophan, which of course becomes more abundant when other amino acids abound. Carbohydrate certainly flows to a plant part to which IAA is ap-

plied, but we could only detect *reduced,* not the predicted *increased* IAA pools in mycorrhizal short roots and root systems. On the other hand, there is increasing evidence that IAA pools are really of no significance at all, turnover being potentially a better indicator, positional effects and hormone-receptor relationships being the important terms in which IAA effects are to be analysed. The monitoring of pools or even production may be trivial and lead nowhere.

We expected much from a series of experiments with IAA hyperproducing mutants. The hypothesis was that the high IAA secretion would lead to high pools in the roots, and an enhanced carbohydrate drain from the host to the fungus. This would be seen as reduced growth of the host and strong growth of the mycobiont. Unfortunately, we found nothing, which most probably was due to the physiology of the mutation, enhancing tryptophan accumulation, not IAA production directly.

So, whatever the interactions between IAA, carbohydrates and mycorrhiza development may be, they are difficult to demonstrate, and far more complex than Slankis thought. Yet, there is circumstantial evidence that IAA does have a role in the symbiosis.

Application of the theory

But why so much noise about a half-century old theory, which in spite of faltering original evidence was found correct? Well, above all since nutritional interactions are central to the understanding of pollution and possible fertilization effects, a red hot subject in Swedish debate today, where the Forest Service and landowners' groups want to remedy the situation by liming, but are doggedly resisted by many researchers, particularly at the Agricultural University. Our studies have reference to one of the fundamental processes which are affected: the carbohydrate cycling in the tree-mycorrhiza system. Whatever the immediate effect of, say pollution, may be: wax layer and stomata damage by acid; ozone poisoning of photosynthesis or nitrogen saturation: effects on the carbohydrate flow to the roots affect the entire rhizosphere-mycorrhiza-root system. Disturbed carbohydrate allocation to the root has several consequences. First, fruit body development is affected. In our work, even a slight increment of nitrogen concentrations in the medium (far less than standard nursery applications) suppressed fructification in the "hungry" *Laccaria bicolor.* This is also seen in the field: fertilization suppresses mushroom and toadstool (but not conch!) formation,

while our recent results (Kårén and Nylund, unpublished) reveal only marginal effects on the species composition of mycorrhiza. The study by Fortin and coworkers (Godbout and Fortin 1992) very nicely illustrated how fruitbodies constitute a direct sink for photosynthetic carbohydrate. Secondly, soil mycelium development is suppressed. This was analysed in detail by us, but extensive research by the Lund team (Söderström 1991) has also demonstrated a suppression varying with species in nitrogen-rich soils. Ultimately, the fungal biomass on the root tips, or mycorrhiza proper, is affected, but even very heavy doses (200 ppm) could not entirely remove mycorrhiza in our culture trays. Similarly, in the field, mycorrhizal roots displayed very thin mantles, and morphotypes became ever more difficult to discern, but no single case of non-mycorrhizal short roots could be detected in the Skogaby trials, where the ammonium sulphate treatments amounted to more than one hundred kg of each element N and S per hectare *a year.*

As we see it, the main culprit is the nitrogen. Acidification may have many negative effects on cuticles and soil cation saturation, but neither lab or field studies demonstrate any notable effects on the mycorrhiza itself. This implies that any policies of forest "life-saving" have to address the nitrogen issue, not only the acidification. This most probably applies to fine root development also: the observed reductions seem almost entirely be a response to the nitrogen load, or in more severe cases also to disturbed photosynthesis, but not to acid as such (direct poisoning in Central Europe is another case not discussed here). And the theory makes no provision for pH effects (tested but not discussed here): whatever there is, is indirect.

So far, our discussion has concerned generalizations. A main point is of course that species and strains are quite different. Some, such as *Laccaria* and *Pisolithus* are very "virulent", excreting much IAA in pure culture, drain the hosts carbohydrate reserves, and take a strong hold on their roots. Some are nitrogen tolerant, other refuse to grow into N fertilized soil even from a well colonized root in low-nutrient medium. pH optima certainly vary, but due to the conditioning effect of roots and soil mycelia on their immediate environment, *in vitro* studies may be of limited value. The fungi also differ in their soil mycelium, some forming hydrophobic strands and mycelial mats, other having a diffuse hydrophilic mycelial structures hard to detect, while some seem to lack a stronger connection with the soil at all. Returning to nitrogen-stressed mycorrhiza, the smooth brown mycorrhiza type into which so many species turn seems to do with little soil mycelium,

perhaps a reaction to the abundancy of the regulatory mineral nutrient in their immediate surroundings.

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The role of roots in carbon cycling in forests

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Available data in literature on forest trees indicate a substantial fine-root production with different seasonal patterns in needle or leaf production. Root growth is sensitive to different climatic factors imposed or strengthened by human activities. Carbon incorporation into the soil by dead roots is an important pathway in the total carbon flow. Factors that lead to growth suspension may or may not be the same as those that result in root shedding or senescence.

Sequential core sampling, ingrowth cores (initially root free cores with sifted mineral soil removed on successive sampling occasions) and minirhizotrons were used in coniferous forest stands in order to investigate how the fine-root turnover rate was influenced by liquid fertilization (IF) with a complete set of essential nutrients with the aim of eliminating water and mineral nutrients as growth-limiting factors. The data reveal a more substantial total fine-root production in the IF treated plots than in the control (C) plots. As regards the increments in fine-root length of the <1 mm diameter fraction, the production proceeded much more rapidly in the IF-plots subjected to "optimum fertilization with irrigation" than in the C plots. It was demonstrated that major environmental problems result from the lack of understanding of the part played by tree roots in the total carbon flow. New pathways of carbon and nutrient cycling require further assessment.

Background

Large diameter roots in forest trees die with the tree itself, while the fine roots have been shown to be in a constant flux, often with

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a high rate of death and renewal (Persson 1979, 1992). Roots < 1 mm in diameter consist of fine ramifications with mycorrhizal root tips that are morphologically very distinctive from the rest of the root system and include both mycorrhizal host and fungal mantle tissues. Comparable with definitions for populations, a birth rate, death rate and average life expectancy can be defined for fine roots (Majdi and Persson, manuscript). The most widely used approach for estimating fine-root production and mortality has been from changes in dry weight in live and dead roots, often less than 1 or 2 mm in diameter (Vogt and Persson 1991). Closely related to the cost-benefit balance of the forest trees are different selective tactics in growth patterns and structures. Although no one would refute the important role played by roots in water and nutrient uptake, species that minimize the investment of energy into these functions seem to be suitable for silvicultural practice.

Fine-root growth dynamics

Growth dynamics of fine roots may differ considerably between different sites, tree species and from one year to another (Santantonio and Hermann 1985, Persson 1980). Most earlier works on the temporal pattern of fine-root growth indicate a considerable variation in the amount of fine-roots during the growing season, as a consequence of a high turnover rate (cf. Persson 1980). These turnover figures constitute the background for the long term fluctuations in the soil organic matter during the life of a forest since the dead root material constitutes one important source of organic input into the soil environment. The main factors that may influence root growth of forest trees are age and type of tree species, carbon economy, nutrient and water supply, other abiotic factors such as soil temperature, soil strength and aeration, and finally chemical toxicity and allelopathy (Persson 1992). Besides local climatic and edaphic factors, silvicultural practice may vary the picture considerably. Site quality may significantly affect the relationship between the amount of fine roots and foliage produced annually (Santantonio et al. 1977). In closed canopy stands, there is a consistent, strong negative relationship in dry matter partitioning between fine roots and stems (Santantonio and Grace 1987). The carbon cost necessary for the uptake of water and nutrients by the fine roots appears to be in balance with the carbon partitioning to the foliage (Santantonio and Grace 1987). The annual turnover of fine-roots in a young and a mature Scots pine stand was shown to be at least be twice

and equal to the average fine-root biomass (Persson 1979). Available data suggest that the fluctuations and fine-root production are higher in young Scots pine stands than in a mature ones. The respiration costs increases furthermore considerable with age of the trees. The mechanisms resulting in the rapid disappearance of roots upon death are of great interest since a high amount of carbon is involved.

Carbon economy

An indication of the importance of root production in the total carbon flow may be obtained from carbon budgets (Ågren and Axelsson 1979, Ågren et al. 1980, Table 1). The root production may then be obtained as a rest fraction if growth, respiration and the net photosynthesis are measured. The carbon budget reveals that 13-57 % of the carbon that is assimilated annually by Scots pine trees of varying age is used for the growth of root systems; the related respiration cost is 5-25 %. Investigations by Raich and Nadelhoffer (1989) show that live-root respiration can be a major contributor to the total soil respiration, up to one-third to two-third of the annual carbon release from the forest soil.

The carbohydrate storage capacity in tree roots is generally fairly high (as much as 5-25 % of the dry weight consists of starch - Ericsson and Persson 1980). Within fine roots variation in starch content is considerable, also due to varying diameter (Wargo 1976, Ericsson and Persson 1980). It may be concluded that fine-root growth leads to a reduction in starch concentration (Ericsson and Persson 1980). Marshall and Waring (1985) developed a model to predict fine-root production and turnover from soil tem-

Table 1. Annual carbon budget for an average tree in eight forest stands in Jädraås, Central Sweden (Ågren and Axelsson 1979). Estimates are given as g C year-1.

Stand age	17	32	40	56	83	90	106	122
Net photosynthesis	1551	2738	6315	3348	7914	9880	10695	11653
Roots <5 mm	885	307	790	1075	4300	4619	4276	6479
	(57%)	(11%)	(13%)	(32%)	(54%)	(47%)	(40%)	(56%)
Growth	592	1861	4186	1768	2848	4067	4992	4115
	(38%)	(68%)	(66%)	(53%)	(36%)	(41%)	(47%)	(35%)
Respiration	74	570	1338	504	765	1194	1427	1059
	(5%)	(21%)	(21%)	(15%)	(10%)	(12%)	(13%)	(9%)

perature and starch depletion of the fine roots. The following hypotheses were tested (i) that the growth of fine roots is accompanied by starch accumulation rather than depletion; (ii) that a fully developed fine root meets its maintenance requirements wholly from its starch and sugar reserves, and (iii) that the root dies when its starch and sugar reserves are exhausted. From the work by the latter authors one may conclude: that initial starch concentration and soil temperature are key variables estimating fine-root turnover and fine-root biomass.

The general lack of insight into the relationship between above- and belowground production in forests results from the relatively few reliable measurements of belowground production (cf. Santantonio et al. 1977, Santantonio and Hermann 1985, Persson 1980). Fine-root production and mortality can occur simultaneously in small soil volumes and can therefore not be separated on time or space (Santantonio and Grace 1987). Only limited attempts have been made so far to test their statistical precision of existing data (Persson 1979). Estimation of fine-root production is usually a part of studies to quantify total stand production in plantations and natural forests. Although most studies have been comparative e.g. good versus poor sites (Keyes and Grier 1981), quantity and form of available nitrogen (Finér 1992, Helmisaari 1990, Nadelhoffer et al. 1985) few studies have so far been experimental. Results from minirhizotrone investigations (Fig. 1) on the cumulative fine-root production and mortality in the control and "optimum" fertilized plots with liquid fertilizers" (IF at Skogaby, cf. Persson et al. in press) make it clear that the production and mortality proceeded much more rapidly in the IF-plots than in the C-plots. Results from related studies suggest that a greater amount or proportion of the total net primary production goes to the fine roots, when site conditions are less favourable for growth (Persson et al. in press).

Data on fine-root growth in response to the presence of plant nutrients in the vicinity of the tree roots are available from experimentally manipulated areas from many field experiments in Sweden (cf. e.g. Ahlström et al. 1988, Persson 1979, Persson and Ahlström 1990, Persson et al. in press). From these areas has been shown that an increased needle mass in stands with a high nitrogen supply often corresponds to a reduced amount of fine roots and mycorrhizal frequency. Liquid fertilization using drop tubes indicates a positive effect on fine root growth in the area nearest the drop tube. Other experimental treatments such as liming and compensatory fertilization may result in both negative and positive effects on the growth and development of fine roots, depend-

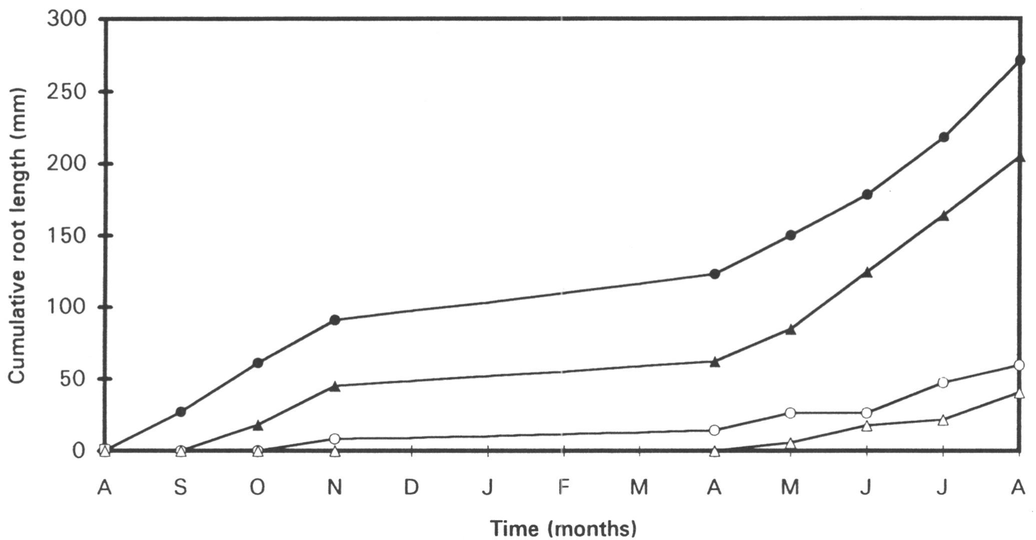

Figure 1. Cumulative fine-root production and mortality (mm) in different treatment plots from August 1991 to August 1992 at Skogaby to a depth of 30 cm. The estimates were obtained on the 1.35 * 1.8 cm area of observation ▲ = control and • = LI treament areas. Filled symbols refer to production and unfilled symbols refer to mortality.

ing on the soil type and the dose at which the liming or fertilization agents are applied (Persson and Ahlström 1994).

Investigations of long term effects of forest liming on fine-root growth dynamics show a tendency to increased specific root length and slightly thinner roots (Clemensson-Lindell and Persson 1992). Similar effects have furthermore been shown by liquid fertilization (Persson 1980). Liming, however, does not seem to have a persistent long term effect on the fine-root development (Persson and Ahlström 1994). N-fertilization in most cases will cause persistent negative effects on both fine-root and mycorrhizal development (Ahlström et al. 1988, Persson and Ahlström 1990).

A sufficient water uptake by some part of the root system can provide the necessary water for the whole tree. Therefore, some roots of the same root system may grow through dry zones when the water uptake and supply is guaranteed by other roots. In dry soils, roots have a tendency to grow towards more humid zones and are generally found at greater depth than in moist soils (Lyr and Hoffmann 1967, Persson et al. in press). In dry sites the whole soil profile appears to be more occupied by fine roots than in wet sites, although the total fine-root production may not differ significantly (cf. Santantonio 1979). Roots are forced to penetrate large soil volumes, often against mechanical resistance of densely packed soil layers (cf. Clemensson-Lindell and Persson 1992). Root members in compacted soil are often less branched and have thicker long root tips, compared to normal conditions. In a mixed forest stand (two, three species or more, the tree-root system may

overcome the soil resistance better and grows deeper than in monocultural forests.

Root damage

A destabilization of the root systems in the forest soil frequently occurs as a result of human activities (cf. Puhe et al. 1986). Root damage is often observed as a decline in the amount of living fine-roots, an increase in the amount of dead versus live fine roots (a lower live/dead ratio) and an increasing amount of dead medium and coarse roots. The most important factors which may cause a reduction in fine-root growth and mycorrhizal development are: (i) ion-imbalances, *viz.* high nitrogen/nutrient ratios; (ii) Al toxicity, *viz.* elevated Al/cation ratios and (iii) an increased sensivity of the root systems to environmental stress (drought, wind-break, nutrient shortage, etc.).

The processes of ion uptake are dependent of the degree of penetration of fine roots and mycorrhizas into the soil. If the uptake process is hampered, the growth of the whole tree may be affected. Root damage, in this context, may underline a predisposing stress, thus reducing water and mineral nutrient uptake. Root damage often seems to be related to poor soil conditions with generally low nutrient availability (Ulrich 1990).

Future root investigations

Many major environmental problems result from the lack of understanding of the part played by tree roots, in the total carbon flow (Persson 1991). New pathways of carbon and mineral nutrient cycling require further assessment. Some important areas for future root investigations in forest ecosystems are:

i) Carbon and mineral nutrient allocation pattern in trees.
ii) How long the nutrients remain in any of the belowground compartments (i.e. the resistence time)?
iii) Are there physiological differences between young and old trees in the growth dynamics of fine roots?
iv) Links between soil, rhizosphere and fine-root chemistry.
v) Exudation from tree roots.
vi) The uptake by mycorrhizal roots.
vii) The pattern of distribution and movement of water in the forest soil and in the rhizosphere.

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Effect of shadelight quality on dry weight allocation and mycorrhizal development in Scots pine seedlings

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Introduction

In their natural habitat, plants are exposed to continual changes in the light environment. Shading of canopies or neighbouring plants not only reduces the total amount of radiation (total flux density), but also alters the composition of light due to selective spectral absorption and reflection of leaves, particularly in the red and far red region. Low red/far-red photon ratios (R/FR) characteristic of shading or neighbouring vegetation are sensed by plants through phytochromes. The perception of low R/FR usually promotes stem elongation and alters the biomass allocation - e.g. reduced leaf/stem dry weight ratio and increased shoot/root ratio.

Root system growth and formation of mycorrhiza are dependent on carbohydrate supply from the shoot. Any effect of light quality on the allocation of resources might affect root development. Low light intensities decrease ectomycorrhizal formation (Björkman 1942, Ekwebelam and Reid 1983), and this effect has been attributed to carbohydrate availability. To the best of our knowledge, the effect of light quality on the growth of the root system and mycorrhiza development has not been studied before, but the results discussed above indicate that such an effect could be possible.

The aim of this study was to examine early changes in dry weight allocation, morphology and formation of mycorrhizas in

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Scots pine (*Pinus sylvestris* L.) seedlings in relation to light quality.

Material and methods

Pinus sylvestris L. seedlings were grown from seeds in a substrate containing 1 part sand and 2 parts unfertilised peat (v/v), in a greenhouse (26/15 °C max. day / min. night temperatures and 18 hours light regime from natural light plus metal halide lamps). Within a randomised design, four seedlings per treatment within each of six blocks were placed under the following light quality treatments: control and supplemental far-red (FR+). Starting 1 wk after germination, FR light was provided by an additional far-red sidelight source, filtered through two glass cuvettes containing water (to prevent difference in temperature) and red plus blue polyester films. The photosynthetic photon flux density (I^{PAR}) at the top of the plants was approx. 250 μmol m^{-2} s^{-1}. A complete nutrient solution (modified Ingestad solution, Riddoch et al. 1991) was provided at a rate of 1.2 mg N wk^{-1} per seedling throughout the whole experiment. Forty two days after germination, all plants were inoculated with *Suillus bovinus* (strain K4 ex Robin Sen), by placing fungal culture (Hagem agar) in direct contact with fine roots to ensure availability of inoculum.

The height of plants was measured to the nearest millimetre with a ruler twice a week throughout the experiment. Seedlings were harvested 93 d after the light treatment was started. The root systems were carefully washed and two subsamples at 30 - 50 and 105 - 125 mm depths were taken from each seedling. The subsamples were observed under a stereomicroscope for total count of short root tips and characterisation of mycorrhizas. The development of mycorrhizas was assessed by microscopy after staining (Koske and Gema 1989), and root length was estimated by the grid intersection method according to Tennant (1975). Short root tip classification included the following categories: non mycorrhizal tips; developing mycorrhiza (mantle not yet developed, but Hartig net visible after staining); mycorrhiza with mantle and/or external mycelium. Using the dry weight of the subsamples and of the whole root system, estimates of root length and numbers of short root tips and mycorrhizas in the whole plant were calculated. The root subsamples as well as the remaining root system, stem and needles were dried at 65° C for 48 hours before weighing.

Results

Light quality had a significant effect on the growth of young Scots pine seedlings: FR+ increased stem height by 17% (P = 0.010*) (Table 1). The total dry weight of the plant was 564 mg in control and 482 mg in FR+ (P = 0.073), but the dry weight ratio of stem was larger in the FR+ and that of roots was smaller (Fig. 1). Total root length (data not shown) and specific root length (Table 1) were not affected by FR+ treatment.

Mycorrhizal categories as percentage of the total number of short root tips and the number of root tips expressed per unit of length were not affected by light quality (Table 1). However, the estimated total number of short root tips and developing mycorrhizas per seedling were somewhat smaller in the FR+ seedlings. There were very few mycorrhizas with mantle and/or external mycelium (Table 1 and 2).

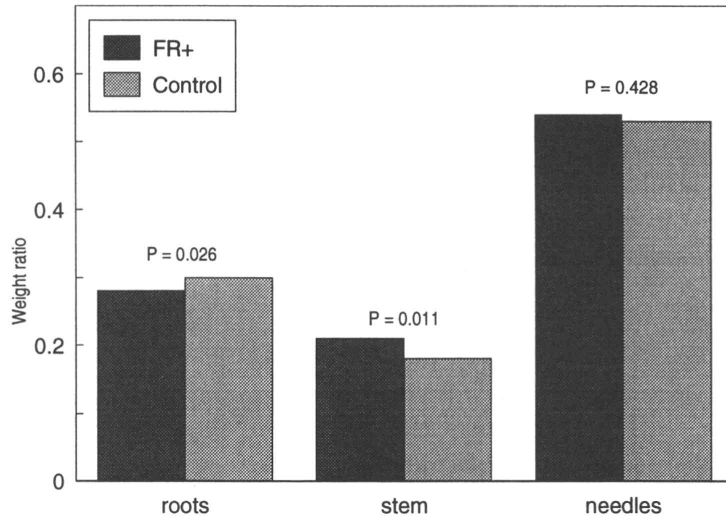
Table 1. Stem length, specific root length, number of short root tips per root length unit and mycorrhizal categories as percentage of the total number of short root tips. Probabilities from ANOVA.

	CONTROL	FR+	P
Stem length (mm)	107.02	124.84	0.010
Specific root length ($m\ g^{-1}$)	71.4	73.8	0.632
Short roots (tips m^{-1})	175	189	0.200
Non mycorrhizal tips (%)	0.27	0.14	0.434
Developing mycorrhiza (%)	99.5	99.56	0.995
Mycorrhiza with mantle and/ or external mycelium (%)	0.02	0.01	0.593

Table 2. Estimated total number of short root tips and mycorrhizas per seedling. Probabilities from ANOVA.

	CONTROL	FR+	P
Total short root tips	2184	1591	0.096
Non mycorrhizal tips	4.09	1.61	0.190
Developing mycorrhiza	2167	1573	0.096
Mycorrhiza with mantle and/ or external mycelium	0.40	0.37	0.578

Figure 1. Dry weight ratio of Scots pine seedling as affected by supplementary FR+.



Discussion

The Scots pine seedlings used in this study responded to FR light by increasing stem elongation, a common "shade avoidance" behaviour, observed in many herbaceous and tree species including Monterey pine (Warrington et al. 1989). In contrast to previous studies on herbaceous plants (Ballaré et al. 1991, Smith 1994), dry weight allocation to needles did not differ between light treatments, however in FR+ seedlings allocation to stems increased at the expense of roots. This suggests that, in this case, increased stem elongation does represent a cost in terms of carbon allocation, but to roots rather than to needles. This different behaviour could be related to differences in life span and competition strategies of annual and perennial plants.

Reduction in the number of short root tips and developing mycorrhiza paralleled the decrease in root biomass. Root morphology or the stage of development of mycorrhizas did not change due to FR+. As Ekwebelam and Reid (1983) found an increase in the percent of mycorrhizas with increasing light intensity, our results suggest that the effects of light quality and of total irradiance on mycorrhizas are different.

The ability of Scots pine seedlings to sense changes in light quality could enhance success in competition with other plants. However, one should bear in mind that allocation to roots and aboveground parts is strongly affected by soil nutrients and water availability.

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Biomass and turnover of roots in a mesotrophic fen

Timo Saarinen

Introduction

Estimates of the below-ground biomass and production of mire plants are scarce. Recently reported values both from a minerotrophic fen (Sjörs 1991) and an ombrotrophic bog (Wallén 1986) indicate that over 90 % of the total biomass may be located below-ground.

The aim of this work was to measure the vertical distribution of biomass and production of vascular plants in a mesotrophic fen. Special attention was paid to fine roots and deep growing roots and their significance to peat accumulation.

Material and methods

The study site is located on the Suurisuo mire complex, Janakkala, southern Finland. A mesotrophic fen community dominated by *Carex rostrata* and *Potentilla palustris* was chosen for the study.

The indirect ^{14}C labelling method described by Wallén (1986) was applied to estimate the below-ground biomass of the vascular plants in the uppermost 30 cm in June 1992. The deepest roots (between 30-230 cm) were sorted manually from cores collected in June 1993. The ^{14}C turnover method developed by Milchunas and Lauenroth (1992) was modified to measure the below-ground productivity. Long-term ^{14}C sample plots established in July 1992 were sampled regularly. ^{14}C activities were analysed both in structural and non-structural carbon fractions of roots and the proportion of labelled fine roots were calculated with autoradiography. Below-ground production was estimated by dividing

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Fig. 1. Vertical distribution of biomass of *Carex rostrata* and *Potentilla palustris* in a mesotrophic fen of Suurisuo mire complex, Janakkala, southern Finland.

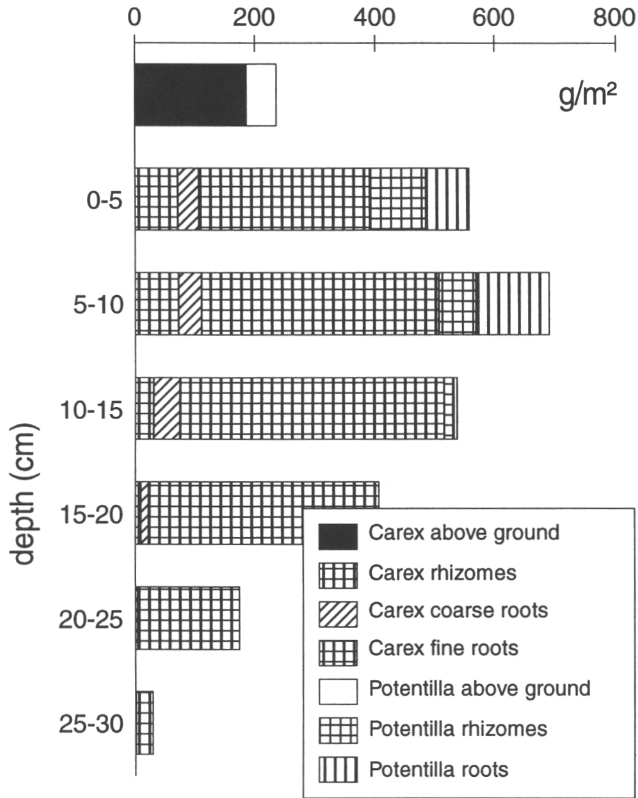
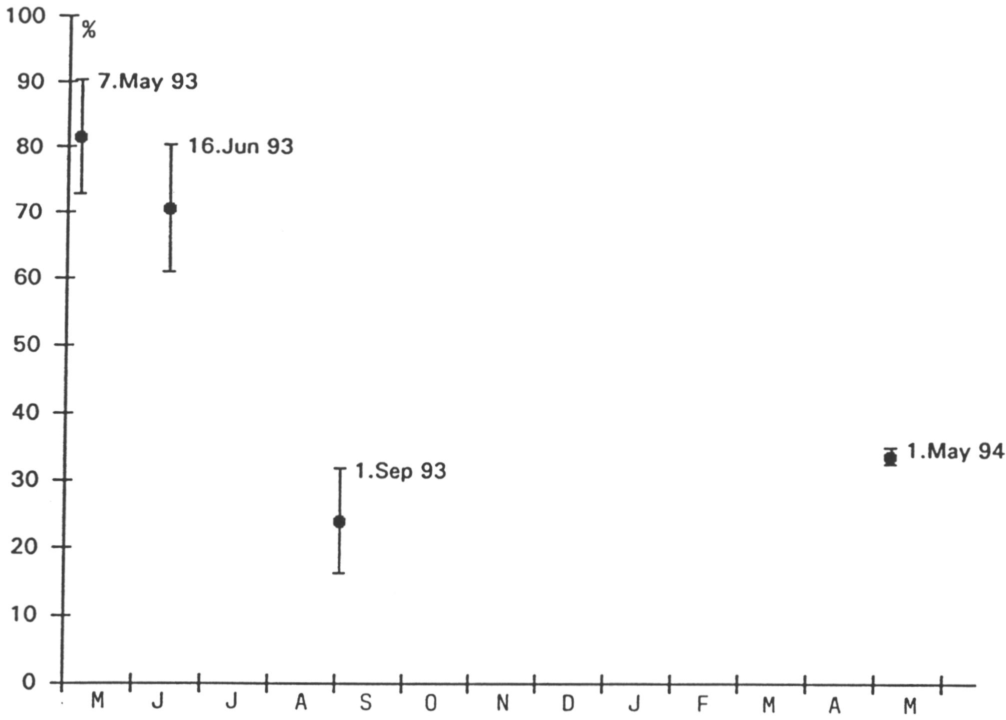


Fig. 2. Percentage of ^{14}C labelled fine roots of *Carex rostrata*. The sample plots were pulse labelled in July 1992.



biomass by ^{14}C turnover time. Above-ground biomass and production were measured using demographic methods. Individual shoots were tagged on 10 sample plots in May 1993. The length of the shoots was measured and new shoots tagged monthly during the growing seasons. Biomass was calculated using the relationship between length and weight of shoots.

Results and discussion

The total living biomass of *Carex* and *Potentilla* was 2280 g m^{-2} and 420 g m^{-2}, respectively (Fig. 1). The below-ground biomass of both species was considerably high, no less than 92% and 88% of the total biomass *Carex* and *Potentilla*, respectively. Fine roots comprised 78% of the biomass of *Carex*.

The living roots of *Carex* reached at least the depth of 230 cm. This is clearly deeper than the values reported in earlier studies (e.g. Metsävainio 1931). As decomposition of peat is slow in catotelm, even the relatively small biomass of these deepest roots (68 g m^{-2} between 30 and 230 cm) may affect the accumulation of peat.

In the beginning of May 1993, the majority of the ^{14}C label of fine roots (labelled in July 1992) was in the structural carbon fraction. The label in living roots may disappear only when old roots die and new roots develop. During the summer period (May-September), the percentage of the labelled fine roots decreased from 81 % to 24 %, which refers to a relatively rapid turnover of fine roots (Fig. 2). However, no decrease could be observed in the winter time (September-May).

Due to a relatively rapid turnover of fine roots, the annual production of *Carex rostrata* is high (1340 g $m^{-2}a^{-1}$) in this fen

Table 1. Biomass and production of *Carex rostrata* in a mesotrophic fen on Suurisuo mire complex, Janakkala, southern Finland.

	Biomass (g m^{-2})	Turnover (a^{-1})	Production (g $m^{-2}a^{-1}$)
Shoots	185	-	176
Rhizomes	185	0.47	87
Coarse roots	135	0.47	63
Fine roots	1717	0.59	1014

(Table 1). With an average carbon content of 48%, there is a carbon input of approximately 640 g C m^{-2} a^{-1}. Fine roots contribute to 76 % of the total production.

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Microbial biomass in the rhizosphere of trees

Aino Smolander, Ilari Lumme and Heljä-Sisko Helmisaari

Introduction

The rhizosphere can be defined as that part of the soil adjacent to a plant root, which is different from the surrounding soil because of the chemical, physical and biological activity of plant root. The effect of root is caused by several processes such as water and nutrient uptake, gas exchange and excretion of organic compounds. There is evidence that plants can stimulate microbial activity through the supply of organic subtrate in root exudates, but they may also limit microbial activity through depletion of mineral nutrients (Bååth et al. 1978, van Veen et al. 1989, Parmelee et al. 1993).

The aim was to study the effect of tree roots on soil microbial biomass and activities related to carbon and nitrogen cycles. Preliminary microbial biomass results are discussed in this paper.

Materials and methods

Pot experiment

The effect of the roots of Norway spruce (*Picea abies* Karst.) seedlings on soil microbial biomass was studied in connection with nitrogen allocation studies using ^{15}N isotope (Lumme 1994). Three-year-old potted seedlings of a Norway spruce clone were grown for 3 months in acid sandy soil. Nitrogen ($(NH_4)_2SO_4$) was applied to the soil in the seedling pot and in the corresponding pots without seedlings twice a week, the total amount of nitrogen applied being 200 mg N per seedling, corresponding to approximately 40 kg/ha/year. Soil without plants was

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treated similarly. At the end of the experiment, roots were separated from the soil by hand picking, and microbial biomass in the remaining soil was determined using the fumigation extraction (FE) technique (Vance et al. 1987).

Field studies

The gradual development of rhizosphere microbial population with the growing roots and mycorrhizas is under study in some Norway spruce and Scots pine (*Pinus sylvestris* L.) stands using the root ingrowth core method (Persson 1990). In this method, a nylon net mesh is inserted into the hole in soil, made by a cylindrical corer, and filled with sorted and rootless mineral soil. After one to four growing seasons, the cores are removed, and the biomass of roots determined. In the present study, roots were separated from the soil by hand picking, and microbial biomass in the remaining soils was determined using both the fumigation extraction (Vance et al. 1987) and substrate induced respiration (SIR) (Andersson and Domsch 1978) techniques, as described earlier by Smolander et al. (1994).

Results and discussion

Soil microbial biomass was almost six-fold in pots planted with spruce seedlings compared to those without seedlings (Fig. 1). The increase in soil microbial biomass C in planted pots indi-

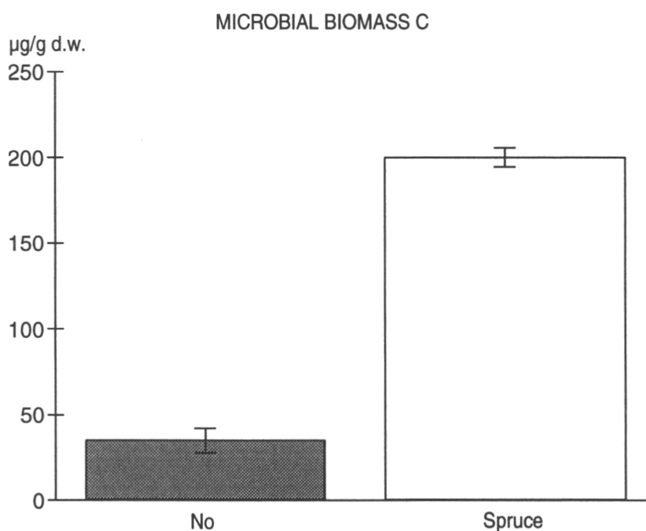

Figure 1. FE-derived microbial biomass C in sandy soil where Norway spruce seedlings had grown for 3 months, and in corresponding soil without plants. Mean ± SD for 3 replicate pots.

cated the importance of plant derived substrate in the mineral soil with a low organic matter content. Accordingly, Parmelee et al. (1993) showed the positive effect of pine roots on microbial biomass in mineral soil but got opposite results in organic soil.

Results of the first sampling of root ingrowth cores showed a slight decrease with depth in both FE- and SIR-derived microbial biomass C. No clear relationship between root biomass and microbial biomass was yet observed.

In the pot experiment and field studies, soil microbial biomass values are probably underestimations because part of mycorrhiza and the microbes attached to roots and mycorrhiza are not included. A few replicate root ingrowth cores with roots were also subjected to microbial biomass determination; removal of roots had decreased the SIR-derived biomass C values 0-26 %.

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A new hypothesis to explain the negative influence of nitrogen on ectomycorrhizal development

Håkan Wallander

Abstract

Nutrient uptake by forest trees is largely dependent on their associated ectomycorrhizal fungi. The presence of extramatrical mycelium produced by ectomycorrhizal fungi allows trees to exploit a larger soil volume. Elevated levels of nitrogen strongly inhibit the development of extramatrical mycelium.

To explain reduced ectomycorrhizal development under conditions of high levels of N supply, it is suggested that the the fungus consumes the available carbohydrate in order to take up and assimilate nitrogen. Only if there is a surplus of carbohydrates, fungal mycelium and fruit bodies can be produced. The present hypothesis proposes that it is the fungus, rather than the host which adjusts its carbon allocation patterns to the N supply.

Introduction

Ectomycorrhizal roots are characterized by fungal hyphae growing between root cortical cells to form a Hartig net and by several layers of hyphae ensheathing the root to form a mantle (a good overview of mycorrhizal structure and function is provided by Harley and Smith 1983). An extramatrical mycelium is radiating out from the mantle into the soil. In this way, the soil volume exploited by the tree is increased substantially (Read et al. 1985,

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Harley 1989). It has been suggested that the host allocates less carbohydrate to the mycobiont at high levels of nutrient supply owing to a greater demand for carbon by the growing shoots under such conditions (originally proposed by Björkman 1942). In the present paper, an alternative hypothesis is presented that may explain nitrogen effects on mycorrhizal development.

Influence of nitrogen supply on production of extramatrical mycelium

In general, high nitrogen availability, due to anthropogenic N deposition (Termorshuizen and Schaffers 1991, Arnolds 1988) or forest fertilization (Wästerlund 1982, Ohenoja 1988) reduces the production of ectomycorrhizal fruit bodies. However, fruit body production does not necessarily reflect the amount or activity of ectomycorrhizal roots.

We have for a long time investigated mineral nutrient effects on mycorrhizal development in a steady-state, semi-hydroponic cultivation system. Fungal biomass of mycorrhizal seedlings have consistently been reduced at elevated levels of N supply (Nylund and Wallander 1989, Wallander and Nylund 1991,1992). This reduction was more pronounced for extramatrical mycelium than for fungal biomass in mycorrhizal roots (Wallander and Nylund 1992). Whereas mycorrhizal roots continued to develop, but at a lower rate, the growth of extramatrical mycelium was completely inhibited and in some cases the mycelium even died when supplied with excess nitrogen. Similar results were found by Arnebrant (1994) who showed that the growth of extramatrical mycelium of an unidentified white ectomycorrhizal fungus was totally inhibited once it reached peat amended with 1, 2 or 4 mg N g^{-1} ($(NH_4)_2SO_4$ or $NaNO_3$) whereas *Paxillus involutus* colonized peat with N amendments of up to 4 mg N g^{-1}. Gorissen *et al.* (1991) found that Douglas-fir seedlings fertilized with 200 kg N ha^{-1} had the same mycorrhizal frequency as seedlings fertilized with 50 kg N ha^{-1}, but that the respiration of labelled ^{14}C by root and fungal tissue was reduced by 60% in the high N treatment. As these investigators pointed out, this reduction could have been due to a decrease in fungal activity at high N levels that was not revealed by counting mycorrhizal root tips.

Possible ways to explain the negative influence of nitrogen on extramatrical mycelium

Increasing the levels of N supply may have a number of effects on carbon flow between host and fungus. Björkman (1942) suggested that the host would allocate less carbohydrate to the mycobiont at high levels of nitrogen and phosphorus availability owing to a greater demand for carbon by growing shoots under such conditions (Fig. 1 a,b). By contrast, we found that carbohydrate pools in roots increased in response to elevated N levels; still, mycorrhizal development was reduced (Wallander and Nylund 1991). This finding indicates that fungal growth was inhibited for reasons other than carbohydrate deficiency. Björkman (1942) assumed that nitrogen was assimilated in the host and that all carbohydrates transferred to the fungus were used for its development. However, since then it has been convincingly demonstrated that ammonium is assimilated in the fungal tissue (France and Reid 1983, Finlay et al. 1988). Host carbohydrates allocated to

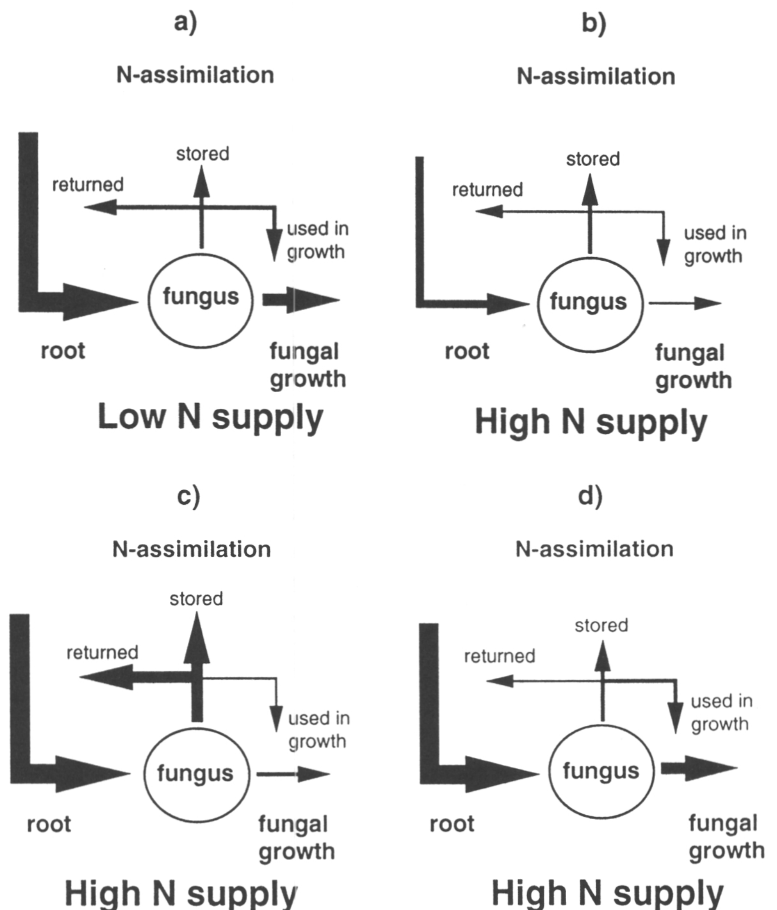

Figure 1. Hypothetical patterns of carbon flow between host and fungus at low or high levels of N supply. a) At a low level of N supply most host carbon is used for fungal growth. At high N supply levels a number of effects on carbon flow are possible: b) Total carbon flow to the fungus is reduced, leading to reduced fungal growth. c) The fungus is forced to allocate more carbon to N-assimilation and less to fungal growth. N availability does not influence total carbon flow to the fungus. d) The fungus has a low N-assimilation rate and can maintain a high rate of fungal growth at elevated levels of N supply.

the mycorrhizal fungus are used in growth processes and as carbon skeletons and energy sources in the process of ammonium assimilation. The assimilated N is either used in growth processes by the fungus, stored as amino acids or protein in the fungal mantle or returned to the host in the form of amino acids (Fig. 1). In our work we found that growth of the extramatrical mycelium of *L.bicolor* resumed once the N supply had been lowered (Wallander and Nylund 1992). These findings suggest that an excess supply of nitrogen might not be detrimental to the fungus. Thus when faced with an oversupply of nitrogen the fungus might allocate its carbohydrate reserves to amino acid biosynthesis, whereas at low levels of nitrogen supply the carbohydrates could be used for growth. The hypothesis can be expressed as follows: Ectomycorrhizal fungi are adapted to N-limited environments. Host carbohydrates available to the fungus are preferably used to assimilate ammonium-N into amino acids. The surplus of the carbohydrates can be used to produce fungal mycelium and fruit bodies.

In contrast with the general suggestion that elevated N levels inhibit mycorrhizal fungi, it has been shown that *Lactarius rufus*, *Laccaria laccata* and *P. involutus,* among other species, can substantially increase sporocarp production after N additions (Ohenoja 1978, 1988). N-tolerant fungi may reduce the allocation of host carbon to N assimilation in other (unknown) ways in order to favour fungal growth (Fig. 1d).

To test the hypothesis we are now investigating a number of strains of *P. involutus* and *Suillus variegatus*. The main question is: Do ectomycorrhizal fungi which are more sensitive to nitrogen have a high rate of N uptake and a high rate of translocation of assimilated N to the host. And vice versa: Do nitrogen tolerant species have a possibility to reduce the N uptake and N translocation when the N input is high?

In conclusion: It seems clear that mycorrhizal fungi allocate more carbon to the process of N assimilation (carbon skeletons and energy) when N supply levels are high, leading to reduced fungal growth. However, the response of fungal species are likely to differ in this respect (see Fig. 1), depending on their capacity to assimilate N, their sensitivity to toxic levels of N and, probably, on other unknown factors as well.

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Mycorrhizal root colonization and ergosterol content in an experimentally polluted subarctic birch-pine forest

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Introduction

Deposition of Cu and Ni and acid rain have had a considerable effect on plant communities in the subarctics (Kozlov et al. 1993). Both arbuscular mycorrhiza (AM, Gildon and Tinker 1983) and ectomycorrhiza (Denny and Wilkins 1987) influence the uptake of heavy metals by plants. If pollution is changing the degree of mycorrhizal root colonization, the response of plants can be in correlation with it's mycorrhizal status.

The aim of this study was to gather preliminary information about the effects of acid rain and Cu-Ni pollution induced changes to ectomycorrhiza and AM of the two common plant species of a subarctic forest.

Materials and methods

The study was conducted near the Kevo Subarctic Research Station in Finnish Lapland. In June, 1991, 4x4 m plots with a similar field layer vegetation located in a mountain birch forest were randomly assigned to different treatments. A factorial design incorporated two factors - acid and heavy metal treatments. Each

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treatment combination was replicated five times. Treatments were applied by irrigating plots twice a week (about 5 mm/irrigation event) during the periods 20 July - 27 August 1991 and 11 June - 28 August 1992. Control plots received water from lake Kevojärvi (pH about 7). The acid water was acidified with sulphuric acid to pH 3. CUNI water was prepared by adding Cu (as $CuSO_4$) and Ni (as $NiSO_4$) to the raw water to give a deposition/irrigation event of 8.3 and 5.0 mg/m^2 for Cu and Ni, respectively.

The mycorrhiza of two relatively abundant plant species - *Betula pubescens* ssp. *tortuosa* and *Linnaea borealis* - were studied. During July 20-28,1992, roots were sampled from each study plot (20 in total). Mycorrhizal root tips of mountain birches were collected. For studying AM, root samples of *L. borealis* were taken. In all cases, root samples of one species from one experimental plot were pooled because it was impossible to separate individuals.

The ergosterol assay was used to quantify mycorrhiza. The analysis was restricted to birch root tips displaying a predominating smoke gray pinnate type of ectomycorrhiza characteristic of *Lactarius* spp. (Ingleby et al. 1990). Ergosterol was measured according to Nylund and Wallander (1992) using HPLC. In addition, roots of *L. borealis* were stained by trypan blue, the percent of root colonization was determined by microscope slide method. The data were analyzed by a mixed ANOVA model, in which metal and acid rain were fixed effects and study area was random and nested within two others.

Results and discussion

Based on ANOVA, none of the factors had a significant ($p<0.05$) impact on the ergosterol content of the *Lactarius* mycorrhiza of mountain birch (Tables 1 and 2). However, the influence of acid rain treatment was marginally significant at the level 0.077 - resulting in clearly lower content of ergosterol. No statistically significant effect of the heavy metal treatment alone or in combination with the acid rain treatment was apparent. In plots receiving heavy metals, the content of ergosterol in root tips was slightly lower than in irrigated control although the lowest recorded mean concentration was detected in the acid rain and CuNi treated plots.

AM colonization of the roots of *L. borealis* was very sparse (2-3 % of the root length). The percent colonization was strongly influenced by acid rain treatment; significantly lower root coloni-

Table 1. Content of ergosterol (μg/mg) in birch root tips (A) and in roots of *Linnaea borealis* (B) and percent root colonization of *L.borealis* (C) in case of four experimental treatments (IR - irrigated control, A3 - simulated acid rain, M - metal (Cu and Ni) treatment, A3M - combination of acid rain and metal treatments), mean and standard error.

	A	B	C
IC	2.45 ± 0.37	0.99 ± 0.17	2.37 ± 0.52
A3	1.92 ± 0.43	0.97 ± 0.16	1.53 ± 0.63
M	2.29 ± 0.30	0.53 ± 0.08	1.87 ± 0.78
A3M	1.58 ± 0.26	1.35 ± 0.29	1.66 ± 0.68

Table 2. Impact of experimental acid rain and Cu-Ni treatments on mycorrhizal status of mountain birch and *Linnaea borealis* - results of ANOVA. A3 - simulated acid rain, M - metal (Cu and Ni) treatment, B - experimental area (block). A3 and M are fixed effects and B(A3*M) is random and nested within A3 and M.

Source of variation	DF	F value	P
Content of ergosterol in mycorrhiza root tips of Betula pubescens ssp. tortuosal			
A3	1	3.57	0.077
M	1	0.81	0.380
B(A3*M)	15	0.00	0.480
A3*M	1	1.03	0.956
Content of ergosterol in rootlets of Linnaea borealis			
A3	1	0.00	0.980
M	1	0.55	0.594
B(A3*M)	15	1.03	0.660
A3*M	1	0.00	0.958
Percent colonization of rootlets of Linnaea borealis by AM			
A3	1	11.14	0.001
M	1	1.34	0.247
B(A3*M)	15	16.87	0.000
A3*M	1	4.02	0.046

zation was observed in acid treated plots. Heavy metal treatment had no significant effect on percent colonization, though the mean colonization rate was lower in metal treated plots (Table 2).

When the content of ergosterol in rootlets of *L. borealis* was considered, very different results were observed. The content of ergosterol in *L. borealis* was more than two fold lower than in birch root samples. According to the ANOVA, none of the factors had a significant influence on the ergosterol content. Heavy metal treatment resulted in a slightly lower content. We did not find any significant correlation ($r=0.094$, $p=0.700$) between the percentage of root colonization and the content of ergosterol.

Our results suggest a negative impact of acid rain on mycorrhizal root colonization of two common plant species in a subarctic forest. Still, the ergosterol analysis did not give satisfactory results in case of *L. borealis*, which is probably due to nonselective nature of this method. Rootlets of *L. borealis* were surrounded and sometimes also penetrated by different hyphae. Under the light microscope only mycorrhizal fungi were counted, but chemical method, in turn, takes all these fungi into account.

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