Mycorrhiza (2005) 15: 65–70
DOI 10.1007/s00572-004-0337-y

SHORT NOTE

Minna-Maarit Kytöviita

Role of nutrient level and defoliation on symbiotic function: experimental evidence by tracing $^{14}C/^{15}N$ exchange in mycorrhizal birch seedlings

Received: 30 January 2004 / Accepted: 15 October 2004 / Published online: 19 November 2004

Abstract High nutrient availability and defoliation generally reduce ectomycorrhizal colonization levels in trees, but it is not known how this affects the functional aspects of mycorrhizal symbiosis. It was therefore investigated whether (1) defoliation or increasing substrate N availability reduce C allocation from the plant to the fungus and N allocation from the fungus to the plant (symbiotic resource exchange), (2) symbiotic resource exchange depends on relative N and P availability, and (3) fungal N translocation to plant and plant C allocation to fungus are interdependent. Birch (*Betula pendula*) seedlings were grown in symbiosis with the ectomycorrhizal fungus *Paxillus involutus* at five times excess N, or at five times excess N and P for 6 weeks. One-half of the plants were defoliated and the plant shoots were allowed to photosynthesize $^{14}CO_2$ while the fungal compartment was exposed to $^{14}NH_4$. After 3 days, the ^{14}C of plant origin in fungal tissues and ^{15}N of fungal origin in plant tissues were quantified. Nutrient availability had no observable effect on symbiotic resource exchange in non-defoliated systems. Defoliation reduced symbiotic N acquisition by plants at all levels of nutrient availability, with the reduction being most marked at higher N availability, indicating an increased tendency in the symbiotic system to discontinue resource exchange after defoliation at higher fertility levels. The concentration of ^{14}C in extramatrical mycelium correlated significantly with the concentration of ^{15}N in birch shoots. The results support the assumption that N delivery to the host by the mycorrhizal fungus is dependent on C flow from the plant to the fungus, and that exchanges between the partners are reciprocal. No significant reductions in root ^{14}C content as a response to defoliation were observed, indicating that defoliation specifically reduced allocation to fungus, but not markedly to roots.

Keywords Herbivory · Ectomycorrhiza · Nitrogen · Phosphorus

M.-M. Kytöviita (✉)
Microbial Ecology, Lund University,
Ecology Building,
223 62 Lund, Sweden
e-mail: minna-maarit.kytoviita@oulu.fi
Tel.: +358-8-5531523
Fax: +358-8-5531061

Present address:
M.-M. Kytöviita
Department of Biology, Oulu University,
90014 Oulu, Finland

Introduction

While intensive mycorrhizal colonization of tree roots under natural conditions is the rule, it has been convincingly shown that high levels of mineral nutrient availability decrease the intensity of root colonization by ectomycorrhizal fungi (Wallenda and Kottke 1998). The exact mechanisms that control plant carbon (C) allocation to root symbionts and those that affect fungal nutrient delivery to the plant remain unresolved, and it is not known how increased nutrient availability leads to decreased levels of root symbionts. Generally, plants allocate resources to optimize acquisition of growth-limiting factors, which results in smaller root-to-shoot ratios at higher nutrient levels (Ericsson 1995). However, it is not self-evident that a reduction in root size should result in reduced relative amounts of root symbionts.

In addition to high levels of nutrients, consumption of foliage by herbivores also results in reduced mycorrhizal colonization intensity (Cullings et al. 2001; Del Vecchio et al. 1993; Gehring et al. 1997; Gehring and Whitham 1991, 1994, 1995; Rossow et al. 1997). Herbivores remove an important portion of tree foliage in boreal forests; it has been estimated that up to 8% of the annual foliage production may be consumed by phytophagous insects alone (Mattson and Addy 1975). During years of insect outbreak, a much higher proportion of foliage is consumed (Mattson and Addy 1975). Although not indisputably shown, it is logical to assume that a reduction in fungal colonization of roots after herbivory is the end result of changes in resource allocation between the symbionts.

Specifically, it has been suggested that defoliated plants with reduced C acquisition rate would reduce C allocation to root symbionts (Gehring and Whitham 2002).

Defoliation results in several changes in plant physiology: it increases root-to-shoot ratio, decreases transpiration through foliage and, as many nutrients are translocated by the gradient in plant water potential (Marschner 1995), it may reduce transpiratory drive of nutrient uptake. Due to increased root-to-shoot ratio, water acquisition and cytokinin levels in remaining leaves increases, which may be part of the mechanism responsible for the increase in photosynthetic rates in the remaining leaves (Trumble et al. 1993). These compensatory processes, together with remobilization of stored C reserves, alleviate C deficiency after defoliation. Depending on plant C reserves and the severity of defoliation, no effects on roots may be observed (Dickmann et al. 1996) or, at the other extreme, root death may occur (Ruess et al. 1998) after loss of foliage. It is currently unknown how defoliation affects plant C allocation to mycorrhizal root symbionts. Theoretically, plant investment to root symbionts should decrease when photosynthetic nutrient use efficiency is not high enough to meet the C cost of symbiosis-mediated resources (Tuomi et al. 2001). Tentatively, the mechanisms accountable for reduced plant resource investment to mycorrhiza at high nutrient availability and after defoliation could be the same (Tuomi et al. 2001).

In the present work, the function of a mycorrhizal system exposed to increasing nutrient levels and defoliation is investigated. Both increasing nutrient levels and defoliation were predicted to result in decreasing resource exchange defined as symbiosis-mediated nitrogen (N) acquisition by the plant, and fungal net acquisition of plant recent photosynthate. More specifically, it was tested whether (1) defoliation and increasing substrate N availability both reduce resource exchange between symbionts, (2) symbiotic resource exchange depends on relative N and phosphorus (P) availability, and (3) fungal N translocation to plant and plant C allocation to fungus are interdependent. The first point examines how resource exchange between the symbionts is affected by altering the resource level. The second point investigates whether not only one nutrient (N), but also other nutrients, such as P, could be involved in the regulation of resource exchange between symbionts. The third aspect elucidates whether resource delivery by the partner is conditional on symbiotic resource acquisition in return.

Materials and methods

Plant material and pregrowing conditions

Mycorrhizal associations between *Betula pendula* (L.) (seed lot 91T005, Skogforsk Sävar, Sweden) and *Paxillus involutus* (Batsch) Fr (ATCC 20017, isolate no 87.017 in the collection of the Department of Microbial Ecology, Lund University) were synthesized by the method described by Duddridge (1986), as modified by Finlay et al. (1988). In short, the birch seedlings were grown with their root systems closed in Petri dishes filled with peat: vermiculite (1:4) moistened with half-strength Modified Melin Norkrans solution and inoculated with the fungus. The plants were grown in a greenhouse and natural light was supplemented with artificial light to give a minimum of 150–300 μmol $m^{-2}s^{-1}$ during the light period, and the air temperature varied between 15 and 35°C. At 4 months old, the seedlings were transferred to small Plexiglas growth units (12×12 cm) containing a 2- to 3-mm-thick layer of non-sterile peat. At 5 months old, the plants were transferred to similar larger (20×20 cm) units (three plants per unit) and 1 month later retransferred to new units (one plant per unit). During the growth period, the root systems of the plants in the Plexiglass units were sprayed with small amounts of half-strength Ingestad nutrient solution (Ingestad 1979) and with tap water to keep the substrate moist. Ingestad solution was used because it delivers nutrients in ratios that optimize tree growth (Ingestad 1979).

Nutrient and defoliation treatments

At 8.5 months old, the seedlings were transferred into polystyrene observation chambers (25×25×2.5 cm) filled with peat:sand (2:1, v:v) mixture. The sand was collected from a pine forest and semi-sterilized by heating in a microwave oven twice for 5 min. To each chamber, 154 g dry weight substrate was added. The pH of the substrate was 3.7 and organic matter content 13%. The plants in observation chambers were transferred into a biotrone with fully controlled environmental variables (18/15°C, 60/80% RH, 18 h 300 μmol $m^{-2}s^{-1}$ light/6 h darkness) and were allocated to one of three nutrient treatments: (1) additions of full-strength Ingestad nutrient solution containing 1.8 mM N and 0.12 mM P (1N1P), (2) as (1) but with a solution containing five times more N (5N1P), (3) as (1) but with a solution containing five times more N and 5 times more P (5N5P).

In treatments (2) and (3), the substrate was amended with 2 mg N and 2 mg N +0.3 mg P, respectively, before planting the seedlings into the observation chambers. The N was added as NH_4NO_3 and P as Na_2HPO_4. A total of 20 ml Ingestad nutrient solution was added in increasing doses during a 6-week period. Consequently, the total amount of N and P added was (1) 1 mg N and 0.15 mg P, (2) 7 mg N and 0.15 mg P, (3) 7 mg N and 1 mg P. Assuming 1 g cm^{-3} soil density and 30 cm soil depth, these nutrient additions are equivalent to 20 kg N and 2.9 kg P ha^{-1} for the 1N1P treatment and 136 kg N and 19 kg P ha^{-1} for the 5N5P treatment. One-third of each observation chamber was separated by a plastic barrier over and below which only fungal mycelium was allowed to grow, forming an exclusive fungal compartment. At the end of the 6-week period, the fungus colonized most of the substrate in all mycorrhizal treatments and covered the area below the barrier almost completely. At this point, five plants were defoliated and five plants remained as

non-defoliated controls in each nutrient treatment. Defoliation was conducted by cutting off one-half of each leaf at the time of last nutrient addition. Calculated as the proportion of the total amount of foliage remaining at the end of the experiment, the amount cut off varied between 44 and 47%.

Isotope feeding procedure

Three days after defoliation, the plants were exposed to ^{14}C and ^{15}N isotopic tracers simultaneously. To the exclusively fungal compartment was added 1 mg ^{15}N (99% atom excess) in 5 ml 200 mM solution of $(^{15}NH_4)_2SO_4$. Immediately thereafter, the plant shoots were enclosed in plastic chambers (260 cm^3) into which 9.2 μg ^{14}C was released as $^{14}CO_2$ (1.5 MBq) by adding 500 μl 15% lactic acid to 40 μl 20 mM $NaH^{14}CO_3$. The plastic incubation chambers were removed after 5 h; the defoliated plants were estimated to have assimilated all added label well within this time. The plants were allowed to translocate the isotopes for 72 h under a photoperiod of 16 h 120 μmol $m^{-2}s^{-1}$ and 8 h darkness.

Harvesting and chemical analyses

At the end of the experiment, the shoots were cut off and the root system gently washed under running water until no substrate was visible. All plant parts were dried in an oven at 80°C for 24 h, weighed and milled. The fungal mycelium was harvested by carefully collecting fungal hyphae with forceps from the substrate surface, including areas below and above the barrier. Radioactivity in plant and fungal material was measured by burning the samples in a sample oxidizer (Model 304, Packard); the $^{14}CO_2$ was trapped in a Carbosorb, mixed with Permafluor and counted (Tri-Carb 2100TR, Packard) at the Department of Plant Ecology, Lund University. Total N and ^{15}N in the shoots and roots were analyzed using an Automated Nitrogen Carbon Analyzer-Mass Spectrometer (ANCA-MS, Europa Scientific, Sercon, Crewe, UK) at the Swedish University of Agricultural Sciences (Uppsala, Sweden). For roots and shoots, ^{14}C and ^{15}N are expressed as dissociations per minute (dpm) $plant^{-1}$ and microgram $plant^{-1}$, respectively. Fungal ^{14}C concentrations are expressed as dpm (mg dry weight)$^{-1}$.

Data analysis

Effect of the nutrient levels (1N1P, 5N1P, 5N5P) and defoliation (yes/no) on plant parameters were analyzed by two-factor ANOVA. Plant total ^{15}N content data were log-transformed to reduce heterogeneity of variances among treatments. Correlations were analyzed with Spearmans test. All analyses were conducted using the SPSS statistical package (SPSS 1998).

Results

Increasing nutrient availability increased plant mass, and defoliation reduced it (Table 1). The concentration of recently photosynthesized C, as indicated by the total amount of radioactivity recovered in plants after the 72 h trace period, was relatively variable and neither defoliation nor nutrient level affected the amount of ^{14}C in plant

Table 1 Total biomass, root-to-shoot ratio, nitrogen percentage (N %) in the shoots (includes leaves and stem) and roots, ^{15}N enrichment in shoots and roots, total plant ^{15}N content (μg/plant) and plant ^{14}C content (dpm, dissociations per minute $\times 10^6$ per plant) in 10-month-old non-defoliated (control) and defoliated seedlings of *Betula pendula*, and ^{14}C concentration (dpm mg dry weight^{-1}) in the extramatrical mycelium of the symbiotic fungus *Paxillus involutus*. The seedlings were exposed to one of three nutrient levels (1N1P, 5N1P or 5N5P). The values are means ±1SE, n=5. P values indicate results from a two-factor ANOVA with nutrient level and defoliation treatment as fixed factors. Interactions were not significant in any case

	1N1P		5N1P		5N5P		P	
	Control	Defoliated	Control	Defoliated	Control	Defoliated	Nutrient	Defoliation
Total mass (mg)	552±33	440±56	755±64	577±19	751±68	521±24	<0.01	<0.01
Root:shoot ratio	0.66±0.08	0.93±0.14	0.49±0.04	0.76±0.07	0.47±0.05	0.80±0.07	0.09	<0.01
Shoot N%	1.78±0.03	1.72±0.12	1.97±0.8	2.01±0.08	2.08±0.08	2.17±0.08	<0.01	0.74
of which ^{15}N (%)	0.40±0.16	0.21±0.11	0.43±0.21	0.03±0.00	0.40±0.30	0.02±0.01	0.86	0.03
Root N%	1.06±0.03	1.14±0.05	1.14±0.05	1.39±0.11	1.30±0.08	1.41±0.04	<0.01	0.01
of which ^{15}N (%)	0.87±0.25	0.46±0.29	0.62±0.25	0.07±0.03	0.67±0.36	0.03±0.00	0.32	0.01
Plant ^{15}N content (μg)	42.5±13.0	23.2±15.2	54.4±23.6	4.7±0.12	67.2±48.4	2.5±0.5	0.29	0.01
of which in shoot (%)	53±7	47±7	66±5	55±7	56±7	57±5	0.30	0.34
of which in root (%)	47±7	53±7	34±5	45±7	44±7	43±5	0.30	0.34
Plant ^{14}C content (dpm)	26.5±7.6	22.0±5.5	28.2±4.3	34.4±7.7	25.3±4.3	29.3±5.0	0.49	0.69
of which in shoot (%)	91±2	91±2	95±1	95±1	94±2	97±1	0.01	0.37
of which in root (%)	9±2	9±2	5±1	5±1	6±2	3±1	0.01	0.37
Fungal ^{14}C concentration (dpm/mg)	509±203	ND[a]	778±314	61±25	652±268	102±44	0.93	0.02

[a]Not determined

tissues significantly (Table 1). Shoots presented a strong sink strength for photosynthates, as indicated by the high concentration of ^{14}C in the shoots in comparison to the small amounts measured in the roots (Table 1). Additions of nutrients did not reduce the amount of ^{14}C measured in the mycelium of *P. involutus*, whereas defoliation reduced significantly the ^{14}C concentration in the mycelium in the 5 N treatments (Table 1). Unfortunately, it was not possible to analyze the ^{14}C concentration in mycelium connected to 1N1P defoliated seedlings. The growth pattern of the mycelium on the substrate varied in observation chambers, but this variance could not be attributed to any specific treatment, and the lack of sufficient mass for analysis in one of the six treatments is probably a random effect. The fungal growth rate was not visually different among nutrient treatments so that the fungal mycelium filled two-thirds of the surface of the root observation chambers and colonized the fungal compartment at the same time. There were no systematic differences in the appearance of the fungus between nutrient treatments, and no sclerotia or other specific features were observed. Mycorrhizal colonization percentage of the root tips was over 95% in all plants.

The amount of ^{15}N recovered in plants was between 0.1 and 10% of the total amount of ^{15}N applied 3 days earlier to the mycelial compartment. Translocation of ^{15}N to the shoots did not correlate with the N concentration of the shoots (r=−0.296, P=0.112) and, despite the different masses and N concentrations of the shoots in the different nutrient treatments, the shoot ^{15}N concentration and plant ^{15}N content in the non-defoliated plants was similar at all nutrient levels (Table 1). In contrast, defoliation significantly reduced ^{15}N translocation from the fungus to the plant (Table 1).

The concentration of ^{14}C in roots and that in fungal mycelium showed no correlation (r=0.157, P=0.474). Furthermore, there was no correlation between the root ^{14}C and the plant ^{15}N content (r=0.292, P=0.117). In contrast, the concentration of ^{14}C in the mycelium correlated significantly with the total ^{15}N content of plants (r=0.733, P<0.01) and with the ^{15}N concentration in the shoots (r=0.701, P<0.01).

Discussion

Increasing nutrient availability in itself had no observable effects on mycorrhizal functioning in the present study although reductions in numbers of mycorrhizal root tips (Menge and Grand 1978; Newton and Pigott 1991), growth of extramatrical mycelium (Arnebrant 1994) and numbers of fruitbodies (Brandrud 1995) have been reported previously in response to fertilization. Nitrogen delivery from *P. involutus* to the birch seedlings was not reduced by increasing N, or N and P, levels in the present work. Apparent fungal growth rate as well as extramatrical mycelium ^{14}C concentration were also similar across nutrient treatments. Increasing nutrient assimilation by mycorrhizal fungus has been reported to increase its respiratory loss of C (Ek 1997), so that, all other factors remaining constant, extramatrical ^{14}C concentrations should have decreased in the five N treatments if *P. involutus* increasingly assimilated the nutrients. The fact that nutrient additions did not affect ^{14}C concentration in the mycelium of *P. involutus* suggests that nutrient uptake by the fungus was unaltered by the present nutrient levels. This is supported by that fact that N translocation by the fungus to the plant was also not affected by increasing nutrient levels. Jentschke et al. (2001) report that translocation of N from *P. involutus* to *Picea abies* L. was dependent on simultaneous translocation of P. In contrast, the present results indicate that translocation of N from *P. involutus* to *Betula pendula* was independent of the level of P addition. This suggests either that P levels applied in the present experiment were sufficient to permit unaffected N translocation, or that the difference is due to the different experimental conditions, such as the plant species or fungal strain used. In the present work, the fungal growth rate was seemingly unaffected by the nutrient additions, whereas the plant shoot biomass was increased at the higher N level. This indicates that the balance between N and C acquisition rate was maintained by increasing C assimilating structures at higher N levels, rather than by reducing N acquiring organs such as roots and root symbionts.

In a similar experiment in which transfer of N from an organic source (alanine) by *P. involutus* in systems exposed for 9 weeks to 1N1P, 5N1P and 5N5P nutrient levels was measured, exposure to 5N5P significantly reduced N translocation to *B. pendula* (Kytöviita and Arnebrant 2000). In the current experiment, using an inorganic N source (NH_4), 6 weeks exposure to the different nutrient levels did not affect fungal N translocation to the plant. In both cases, the plant-mycorrhiza systems were in a similar active growth phase. Therefore, it seems likely that the higher total amount of nutrients added was the reason for the reduced fungal N translocation in the experiment by Kytöviita and Arnebrant (2000), although the possibility that the different N sources (alanine vs. NH_4) contributed to the difference cannot be entirely ruled out. In both experiments, the ^{15}N delivery from the fungus to the plant and ^{14}C translocation from the plant to the fungus correlated significantly, confirming the reciprocity of exchanges between the partners.

According to the model of Tuomi et al. (2001), nutrient additions should result in reduced C allocation to a mycorrhizal fungus when the plant cannot efficiently utilize the increased nutrient flow from belowground systems. The nutrient additions in the present work were equivalent to about 20 and 136 kg N ha^{-1} in the 1N and 5N treatments, respectively. Between 15 and 35 kg N ha^{-1} is enough to cause reductions in fungal sexual reproduction, and 40–60 kg N ha^{-1} generally reduces the relative amount of mycorrhizal root tips in ectomycorrhizal roots (Wallenda and Kottke 1998). However, the nutrients were supplied in a continuous manner in the present work and therefore may not compare with large single doses given

in forest fertilization practices. Furthermore, birch seedlings have great growth and nutrient use potential, which may also explain the lack of any observable effects of nutrient addition on symbiotic features in the present experiment. The seedlings grew larger and had higher N% in the 5N treatments, and it seems that the plants were able to utilize the increased nutrient flow in C assimilation and growth. Although common in natural nutrient-poor conditions, *P. involutus* is frequently fruiting in forests exposed to high nutrient inputs (Ohenoja 1978). Therefore, the symbiosis between birch and *P. involutus* may be described as being 'tolerant' to nutrient additions. However, it is necessary to better define the mechanisms of tolerance and intolerance of the mycorrhizal symbiosis to nutrient additions. If tolerance is linked to the plant's ability to utilize nutrients in the production of carbohydrates, then mycorrhizal fungi associated with coniferous trees should be less tolerant than those associated with deciduous species since photosynthetic nutrient use efficiency is lower in conifers as compared to deciduous trees (Reich et al. 1995). Fertilization has been reported to increase *P. involutus* fruitbodies in both deciduous and conifer forests (Brandrud 1995), which suggests that mycorrhizal fungal identity is also important in symbiotic nutrient tolerance. In comparison to some other ectomycorrhizal fungi, *P. involutus* transfers large quantities of N to the host plant (Bidartondo et al. 2001; Ek 1997). Whether this characteristic is part of the tolerance mechanism remains to be explored.

In the present study, birch seedlings reduced allocation of recent photosynthates to *P. involutus* after defoliation. The uptake of N by ectomycorrhiza is dependent on carbohydrate supply (Ek 1997), as energy and C skeletons are needed to convert inorganic N to organic compounds, and uptake and translocation of P within fungal hyphae is most likely also to require energy (Ashford et al. 1994). Therefore, it seems logical that if plant C supply to a fungal symbiont is reduced, the fungus would respond by reducing nutrient uptake and translocation to the host plant. In fact, defoliation reduced translocation of N to *B. pendula* by *P. involutus* in the present study. It is not possible to elucidate from the experimental system used whether it was fungal uptake of N or only transfer to the host that was reduced. The fungus connected to defoliated plants translocated only 5–50% of the quantity translocated to non-defoliated hosts. The role of nutrient levels was not dominant and defoliation reduced symbiotic N acquisition by plants at all levels of nutrient availability. Nevertheless, the reduction was most marked at higher N availability (5N), indicating an increased tendency of the symbiotic system to discontinue resource exchange after defoliation at higher fertility levels.

As a result of defoliation, both ^{14}C in the mycelium of *P. involutus* and symbiosis-mediated N in *B. pendula* seedlings were reduced. This supports the assumption that N delivery to the host by the mycorrhizal fungus is dependent on C flow from the plant to the fungus. These results further indicate that (1) defoliation reduced allocation of recently fixed C to the symbiotic fungus, and (2) C reserves were not used to support mycorrhizal function. If stored C were used to support symbiotic nutrient delivery after defoliation, the concentrations of ^{14}C in the fungus and ^{15}N in the plant would not correlate. However, in the present work, these two markers of photosynthate allocation and fungal nutrient delivery, respectively, showed a strong correlation. This contrasts with studies with large trees in which it has been shown that large tree stubs may allocate stored C to support ectomycorrhizal symbionts after the aboveground part has been destroyed by fire (Langley et al. 2002). No significant reductions in root ^{14}C content as a response to defoliation were observed in the present study, indicating that defoliation specifically reduced allocation to fungus, but not markedly to roots. This result is in agreement with those obtained with *Populus* seedlings where P uptake by mycorrhizal roots was reduced by half while uptake by nonmycorrhizal roots was unaffected by 5 days dark incubation (Bücking and Heyser 2001). Exposure to ozone also reduced photosynthesis and symbiosis-mediated N acquisition in *Pinus halepensis*, whereas root N acquisition was not impaired (Kytöviita et al. 2001). Respiratory losses of ^{14}C (not estimated in the present work) are unlikely to affect the above conclusions as it is highly improbable that plant defoliation would affect the relative respiration rate of ^{14}C by the fungus.

It may be asked whether herbivory reduces the effectiveness of mycorrhizal fungi as plant mutualists. The amount of nutrients delivered to the host in return for the photosynthates allocated to the fungus is generally considered an important measure of the efficiency of the symbiosis (Tinker et al. 1994). In the present study, defoliation markedly reduced symbiotic resource exchange, which may indicate that symbiosis becomes less important as a means of plant resource acquisition in disturbed systems. This agrees favorably with reports of reduced mycorrhiza-mediated nutrient acquisition in comparison to root nutrient acquisition in stressed seedlings (Bücking and Heyser 2001; Kytöviita et al. 2001) as well as with reduced mycorrhizal colonization rates after simulated defoliation or natural herbivory in ectomycorrhizal trees Cullings et al. 2001; Del Vecchio et al. 1993; Gehring et al. 1997; Gehring and Whitham 1991, 1994, 1995; Rossow et al. 1997).

Acknowledgements I wish to thank Kristina Arnebrant, Solbritt Andersson, Roger Finlay, Gunn Hansson, Ann-Margret Sonnerfelt and Håkan Wallander in the Department of Microbial Ecology at Lund University for practical help and advice. This work was funded by the Academy of Finland.

References

Arnebrant K (1994) Nitrogen amendments reduce the growth of extramatrical ectomycorrhizal mycelium. Mycorrhiza 5:7–15

Ashford AE, Ryde S, Barrow KD (1994) Demonstration of a short chain polyphosphate in *Pisolithus tinctorius* and the implications for phosphorus transport. New Phytol 126:239–247

Bidartondo MI, Ek H, Wallander H, Söderström B (2001) Do nutrient additions alter carbon sink strength of ectomycorrhizal fungi? New Phytol 151:543–550

Brandrud TE (1995) The effects of experimental nitrogen addition on the ectomycorrhizal fungus flora in an oligotrophic spruce forest at Gårdsjön, Sweden. For Ecol Manage 71:111–122

Bücking H, Heyser W (2001) Microautoradiographic localization of phosphate and carbohydrates in mycorrhizal roots of *Populus tremula* × *Populus alba* and the implications for transfer processes in ectomycorrhizal associations. Tree Physiol 21:101–107

Cullings KW, Vogler DR, Parker VT, Makhija S (2001) Defoliation effects on the ectomycorrhizal community of a mixed *Pinus contorta*/ *Picea engelmannii* stand in Yellowstone Park. Oecologia 127:533–539

DelVecchio TA, Gehring CA, Cobb NS, Whitham TG (1993) Negative effects of scale insect herbivory on the ectomycorrhizae of juvenile pinyon pine. Ecology 74:2297–2302

Dickmann DI, Nguyen PV, Pregitzer KS (1996) Effects of irrigiation and coppicing on above-ground growth, physiology, and fine-root dynamics of two field-grown hybrid poplar clones. For Ecol Manage 80:163–174

Duddridge JA (1986) The development and ultrastructure of ectomycorrhiza. IV. Compatible and incompatible interactions between *Suillus grevillei* (Klotzsch) Sing, and a number of ectomycorrhizal hosts in vitro in the presence of exogenous carbohydrate. New Phytol 103:465–471

Ek H (1997) The influence of nitrogen fertilization on the carbon economy of *Paxillus involutus* in ectomycorrhizal association with *Betula pendula*. New Phytol 135:133–142

Ericsson T (1995) Growth and shoot:root ratio of seedlings in relation to nutrient availability. Plant Soil 168–169:205–214

Finlay RD, Ek H, Odham G, Söderström B (1988) Mycelial uptake, translocation and assimilation of nitrogen from ^{15}N-labelled ammonium by *Pinus sylvestris* plants infected with four different ectomycorrhizal fungi. New Phytol 110:59–66

Gehring CA, Whitham TG (1991) Herbivore-driven mycorrhizal mutualism in insect-susceptible pinyon pine. Nature 353:556–557

Gehring CA, Whitham TG (1994) Interactions between above-ground herbivores and the mycorrhizal mutualists of plant. Trees 9:251–255

Gehring CA, Whitham TG (1995) Duration of herbivore removal and environmental stress affect the ectomycorrhizae of pinyon pines. Ecology 76:2118–2123

Gehring CA, Whitham TG (2002) Mycorrhizae-herbivore interactions: population and community consequences. In: van den Heijden MGA, Sanders I (eds) Mycorrhizal ecology. Springer, Berlin Heidelberg New York, pp 295–320

Gehring CA, Cobb NS, Whitham TG (1997) Three-way interactions among ectomycorrhizal mutualists, scale insects, and resistant and susceptible pinyon pines. Am Nat 149:824–841

Ingestad T (1979) Mineral nutrient requirements of *Pinus silvestris* and *Picea abies* seedlings. Physiol Plant 45:373–380

Jentschke G, Brandes B, Kuhn AJ, Schröder WH, Godbold DL (2001) Interdependence of phosphorus, nitrogen, potassium and magnesium translocation by the ectomycorrhizal fungus *Paxillus involutus*. New Phytol 149:327–337

Kytöviita M-M, Arnebrant K (2000) Effects of inorganic nitrogen and phosphorus supply on nitrogen acquisition from alanine by birch seedlings in symbiosis with *Paxillus involutus*. Plant Soil 219:243–250

Kytöviita M-M, Le Thiec D, Dizengremel P (2001) Elevated CO_2 and ozone reduce nitrogen acquisition by *Pinus halepensis* from its mycorrhizal symbiont. Physiol Plant 111:305–312

Langley JA, Drake BG, Hungate BA (2002) Extensive belowground carbon storage supports roots and mycorrhizae in regenerating scrub oaks. Oecologia 131:542–548

Marschner H (1995) Mineral nutrition of higher plants. Academic Press, Cambridge, UK

Mattsson WJ, Addy ND (1975) Phytophagous insects as regulators of forest primary production. Science 190:515–522

Menge JA, Grand LF (1978) Effect of fertilization on production of epigeous basidiocarps by mycorrhizal fungi in loblolly pine plantations. Can J Bot 56:2357–2362

Newton AC, Pigott CD (1991) Mineral nutrition and mycorrhizal infection of seedling oak and birch. II. The effect of fertilizers on growth, nutrient uptake and ectomycorrhizal infection. New Phytol 117:45–52

Ohenoja E (1978) Mushrooms and mushroom yields in fertilised forests. Ann Bot Fenn 15:38–46

Reich PB, Kloeppel BD, Ellsworth DS, Walters MB (1995) Different photosynthesis-nitrogen relation in deciduous hardwood and evergreen coniferous tree species. Oecologia 104:24–30

Rossow LJ, Bryant JP, Kielland K (1997) Effects of above-ground browsing by mammals on mycorrhizal infection in an early successional taiga ecosystem. Oecologia 110:94–98

Ruess RW, Hendrick RL, Bryant JP (1998) Regulation of fine root dynamics by mammalian browsers in early successional alaskan taiga forests. Ecology 79:2706–2720

SPSS (1998) SPSS 8.0.1. SPSS, Chicago, Ill.

Tinker PB, Durall DM, Jones MD (1994) Carbon use efficiency in mycorrhizas: theory and sample calculations. New Phytol 128:115–122

Trumble JT, Kolodny-Hirsch DM, Ting IP (1993) Plant compensation for arthropod herbivory. Annu Rev Entomol 38:93–119

Tuomi J, Kytöviita M-M, Härdling R (2001) Cost efficiency of nutrient acquisition and the advantage of mycorrhizal symbiosis for the host plant. Oikos 92:62–70

Wallenda T, Kottke I (1998) Nitrogen deposition and ectomycorrhizas. New Phytol 139:169–187