

Interdependence of phosphorus, nitrogen, potassium and magnesium translocation by the ectomycorrhizal fungus *Paxillus involutus*

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Summary

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- Translocation is shown of phosphorus, nitrogen, potassium and magnesium to a P-deficient host from ectomycorrhizal fungal hyphae.
- Mycorrhizal (with *Paxillus involutus*) and nonmycorrhizal P-deficient spruce (*P. abies*) seedlings were grown in a two-compartment sand-culture system. Hyphal translocation of nutrients from the inner compartment (penetrated only by hyphae) to the host was measured using mass balance (for N, P and K) or stable isotope (¹⁵N and ²⁵Mg) methods.
- Addition of P to the hyphal compartment strongly stimulated hyphal growth, and this also increased both seedling P status and growth. Hyphae translocated nonlimiting elements in addition to P, contributing 52, 17, 5 and 3–4%, respectively, to total P, N, K or Mg plant uptake. The potential role of the ectomycorrhizal mycelium in K acquisition was demonstrated. Translocation to mycorrhizal seedlings of N, K and Mg was strongly reduced when hyphal P-fluxes ceased; this translocation of nonlimiting nutrients depended on simultaneous translocation of P.
- The ectomycorrhizal mycelium has an active role in P acquisition from sources not available to roots. Nutrient fluxes within fungal hyphae are interdependent and strong coupling of N, K and Mg fluxes with long-distance P translocation in the mycorrhizal mycelium occurs.

Key words: external mycelium, magnesium translocation, nitrogen translocation, *Paxillus involutus*, phosphorus translocation, *Picea abies*, potassium translocation.

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Introduction

The external mycorrhizal mycelium plays a crucial role in nutrient uptake of plants (Marschner & Dell, 1994; Smith & Read, 1997). This has predominantly been shown for arbuscular mycorrhizas (e.g. George *et al.*, 1992; Johansen *et al.*, 1993). For ectomycorrhizas, although a number of tracer studies have demonstrated the potential of ectomycorrhizal hyphae to increase N or P nutrition of their hosts (e.g. Melin & Nilsson, 1950, 1952; Finlay & Read, 1986; Kammerbauer *et al.*, 1989; Ek *et al.*, 1994), direct evidence supporting a significant role of the external mycelium in mineral nutrition of forest

tree seedlings is still scarce. Recently, using a compartmental culture technique, in which hyphal nutrient uptake could be isolated from root uptake, we have demonstrated that the external mycelium of *Paxillus involutus* contributed significantly to N and P nutrition of N and P deficient Norway spruce seedlings (Brandes *et al.*, 1998). However, as the seedlings were deficient in both N and P it was unclear from this experiment whether N or P limitation were the driving forces for mycelial foraging and translocation from patches of increased nutrient supply. Hyphal foraging for P, independently of N, may be important in P limited ecosystems to maximize P uptake from a heterogeneous soil environment.

Phosphate taken up by ectomycorrhizal fungi may readily be incorporated into polyphosphates located in fungal vacuoles (Ashford *et al.*, 1994; Gerlitz & Gerlitz, 1997). As the pH may range between 4.3 and 7.5 in fungal vacuoles (Rost *et al.*, 1995), polyphosphates are strongly negatively charged. To neutralize these charges polyphosphates must be associated with cations. Positively charged nitrogen compounds, such as basic amino acids (e.g. arginine), or metal cations, for example K^+ , Mg^{2+} or Ca^{2+} may play a role in neutralizing these negative charges. Recently, microanalytical studies have provided direct evidence for the association of vacuolar P with K and Mg (Orlovich & Ashford, 1993; Bücking & Heyser, 1999), and have shown that Ca plays no significant role in this association.

Different mechanisms of hyphal translocation have been postulated but the importance of these remains unclear (Finlay, 1992). Ashford (1998) has hypothesized that vacuoles may be involved in longitudinal translocation in fungi. At present, the available evidence supports a role of the pleiomorphic vacuolar system only within hyphal tips and their involvement in long-distance transport is less certain. Nevertheless, if indeed translocation occurs within motile vacuoles or 'pumping' of vacuolar contents through a tubular vacuole system, a strong coupling of K, Mg and possibly N compounds with P translocation may be expected. Thus, elements may even be translocated when not in short supply at the sink location (the host plant). Although translocation of mineral nutrients, such as N, P, Rb (for K), Ca (Finlay, 1992) or Mg (Jentschke *et al.*, 2000), has been studied in ectomycorrhizal systems, only a few investigations have looked at simultaneous translocation of more than one nutrient.

The aim of this study was to demonstrate: that hyphae of ectomycorrhizal plants deficient only in P forage for P and translocate it to the host, thereby increasing plant growth; and that translocation of elements not in short supply in the host plant, such as N, K or Mg, may be coupled to P translocation.

Materials and methods

Plant material and inoculation

Seeds of *Picea abies* (L.) Karst. were surface sterilized by incubation in 3% (w/w) H_2O_2 solution for 12 h followed by 45 min in 20% (w/w) H_2O_2 then rinsed with sterile distilled water and germinated on water agar. The fungus *Paxillus involutus* (Batsch) Fr. 533, isolated from a fruitbody collected at an acidified Norway spruce stand in northern Germany (Schlechte, 1986), was maintained on modified Melin-Norkrans medium (Marx, 1969). When seedlings were 3 wk old, they were transferred into a sterile perlite culture system (modified after Bigg & Alexander, 1981) and inoculated with *P. involutus* or remained uninoculated. The seedlings were grown for 9 wk in perlite (Marschner *et al.*,

1996) and then transferred to a two-compartment culture system described in detail by Brandes *et al.* (1998). Briefly, culture vessels were constructed from PVC tubes with diameters of 10 and 15 cm forming a cylindrical inner compartment and a ring-shaped outer compartment. Four windows, which were covered with a 45- μm nylon net, were cut into the inner tube. Hyphae, but not roots, can penetrate this net. The vessels were filled with acid-washed quartz sand and automatically irrigated with nutrient solution (composition see below). The nutrient solution was added at a rate of approx. 115 ml d^{-1} to the outer (root) compartment and approx. 34 ml d^{-1} to the inner (hyphal) compartment. The excess nutrient solution drained freely from the sand. The addition of a greater solution volume to the root compartment promoted mass flow from the plant to the hyphal compartment, and prevented mass flow in the opposite direction. Six seedlings were planted into the outer compartment of each vessel. Twelve vessels with nonmycorrhizal and eight with seedlings colonized by *P. involutus* were set up. Vessels were kept in a climatic chamber at 20°C, 60% rh and continuous light with a photosynthetic photon flux density of 300 $\mu mol m^{-2} s^{-1}$. During the initial 4 wk, nutrient solution (composition as in Marschner *et al.*, 1996) was added only to the root compartment. The nutrient solution contained 300 $\mu M NH_4NO_3$, 50 $\mu M Na_2SO_4$, 100 $\mu M K_2SO_4$, 30 $\mu M KH_2PO_4$, 60 $\mu M MgSO_4$, 130 $\mu M CaSO_4$, 5 $\mu M MnSO_4$, 5 $\mu M FeCl_3$, 5 $\mu M H_3BO_3$, 0.1 $\mu M Na_2MoO_4$, 0.1 $\mu M ZnSO_4$, 0.1 $\mu M CuSO_4$, 120 $\mu M HCl$ (pH 3.9). The vessels were randomized weekly to minimize variance in temperature and intensity of light. After 4 wk, two plants were harvested from each of the growth vessels to determine the initial content of nutrients in the plants.

Experimental design

After the initial harvest of plants, irrigation of the hyphal compartments of all the culture vessels was started. The experiment was a complete two-factorial design with factors mycorrhiza (nonmycorrhizal, with *P. involutus*) and P addition to the hyphal compartment (without P addition, with P addition). An additional treatment with nonmycorrhizal plants and P addition to both the root and hyphal compartment was included. This treatment will be referred to as 'mass flow control'. Each treatment was replicated four times. All treatments had the following nutrient concentrations for both plant and hyphal compartment in common: 900 : 300 $\mu M NH_4NO_3$ (plant : hyphal compartment), 200 $\mu M K_2SO_4$, 10/200 $\mu M Na_2SO_4$ (plant : hyphal compartment), 60 $\mu M MgSO_4$, 130 $\mu M CaSO_4$, 10 $\mu M MnSO_4$, 7.8 $\mu M Fe$ -Ethylenediamine-di(o-hydroxyphenylacetic acid), 5 $\mu M H_3BO_3$, 0.1 $\mu M Na_2MoO_4$, 0.1 $\mu M ZnSO_4$, 0.1 $\mu M CuSO_4$. Root compartment nutrient solutions had low P concentrations (15 $\mu M KH_2PO_4$) to limit plant growth. Nutrient solutions of the hyphal compartments contained 180 μM

KH_2PO_4 or no P, according to the treatment. Potassium concentrations in the hyphal compartments were, thus, 580 μM or 400 μM in the +P or -P treatment, respectively. The NH_4NO_3 for the hyphal compartments was enriched in $^{15}\text{NH}_4^+$ (10 atom percent, Sigma) to trace N uptake from the hyphal compartment. The root compartments received approx. 207 $\mu\text{mol } ^{14}\text{N}$, and the hyphal compartments 19 $\mu\text{mol } ^{14}\text{N}$ and 1 $\mu\text{mol } ^{15}\text{N d}^{-1}$. Five weeks after the start of the experimental period, the nutrient solution supplied to the hyphal compartment was additionally labelled with ^{25}Mg (97 atom percent) at 60 μM (Kuhn *et al.*, 2000) substituting nonlabelled Mg. The labelled solution was supplied for 6 wk until the end of the experiment.

For the nonmycorrhizal plants in the mass flow control, the amount of P supplied to the root compartment was similar to the total P input (hyphal + root compartment) in treatments with P addition to the hyphal compartment. They received 5.2 $\mu\text{mol P per d}$ at a concentration of 45 μM . All input solutions had a pH of 3.9.

Calculation of seedling nutrient uptake and uptake from root and hyphal compartments

Uptake rates of N, P, K and Mg of the seedlings at the end of the experiment (week 11) were calculated as the products of relative growth rates at week 11 and plant nutrient contents at the final harvest. Relative growth rates were determined by linear regressions of shoot length measured weekly. Shoot length is strongly and significantly linearly correlated with shoot dry weight (data not shown). The simple model used for assessment of uptake rates assumes that both root/shoot ratios and tissue nutrient concentrations remain constant.

Uptake of P, NH_4^+ , NO_3^- , and K from the plant and hyphal compartments over the 11-wk experimental period was determined by a mass balance approach as described previously (Brandes *et al.*, 1998). Briefly, aliquots of input and output solutions were pooled, by individual vessels and compartments, over time. The pooled samples were analysed for P and K using inductively coupled plasma atomic emission spectroscopy (Spectroflame, Spectro Analytical Instruments, Kleve, Germany) as well as for NH_4^+ , NO_3^- , NO_2^- and total N using standard autoanalyser procedures (Cenco Instrumenten BV, Breda, The Netherlands). Concentrations of NO_2^- and organic N, calculated as the remainder of total N minus NH_4^+ and NO_3^- , were negligible in all solutions analysed. Uptake from the plant or from the hyphal compartment was calculated from the difference between nutrient contents of input and output solutions of the corresponding compartment. As nutrients were also transported with the directed mass flow from the root compartment to the hyphal compartment, calculated nutrient uptake rates were corrected for these nutrient fluxes ($\text{flux}_{\text{RC-HC}}$), using the following equation:

$$\text{flux}_{\text{RC-HC}} = c_{\text{transfer}} \times (V_{\text{HC out}} - V_{\text{HC in}}) \quad \text{Eqn 1}$$

where (c_{transfer} , concentration of the nutrient of interest in the transfer solution; $V_{\text{HC out}}$, volume of solution draining from the hyphal compartment; $V_{\text{HC in}}$, volume of nutrient solution added to the hyphal compartment.) c_{transfer} can safely be assumed to range between concentrations of input and output solutions of the root compartment. In a conservative estimation c_{transfer} was assumed to equal the output concentration of the root compartment. Thus, this calculation tended to underestimate uptake from the hyphal compartment.

In calculating nutrient uptake, the influence of microorganisms was not taken into account. However, the plant and hyphal compartments were covered with lids which prevented algal growth, and reduced immobilization of nutrients by algae. Low levels of organic N in the output solutions and results from a previous experiment (Brandes *et al.*, 1998) suggested that microbial transformations of N were not important under the experimental conditions used. Microbial immobilization of N, P and K may have led to an overestimation of N, P and K uptake. However, the validity of the calculation was checked by comparison with the N, P and K contents in the plant.

Harvest and analysis of the plant material

After 11 wk of treatment, plants were separated into young and old needles, stems, coarse and fine roots. The percentage of mycorrhizal root tips was determined by counting a representative sample with the aid of a stereo microscope. All parts of the plants were dried to a constant weight at 70°C and homogenized with a ball mill. For determination of P, K and Mg, plant material was wet ashed using 65% (w/w) HNO_3 in closed Teflon vessels under high pressure at 180°C. Concentrations of P, K and Mg were determined by inductively coupled plasma atomic emission spectroscopy (Spectroflame, Spectro Analytical Instruments, Kleve, Germany). For the determination of Mg isotopic ratios ($^{25}\text{Mg} : ^{24}\text{Mg}$, $^{26}\text{Mg} : ^{24}\text{Mg}$), an inductively coupled plasma mass spectrometer (ELAN 6000, Perkin Elmer, Norwalk, Connecticut, USA) equipped with a microconcentric nebuliser (MicroMist with mini-cyclonic Cinnabar spray chamber, Glass Expansion, Australia) was used. The determination of magnesium isotopic ratios was verified using the isotope standard reference material (NBS 980). The measured isotopic ratios for magnesium were in good agreement with the certified values ($^{25}\text{Mg}/^{24}\text{Mg} = 0.1266$ and $^{26}\text{Mg}/^{24}\text{Mg} = 0.1393$). The per cent ^{25}Mg of total Mg was calculated from $^{25}\text{Mg}/^{24}\text{Mg}$ and $^{26}\text{Mg}/^{24}\text{Mg}$ isotopic ratios. For the calculation of total ^{25}Mg transported by hyphae, the atom percentage ^{25}Mg excess was calculated by subtracting the natural abundance of ^{25}Mg (10.00%) (Weast, 1989) from the measured value. The C and N concentrations were determined with a C/N

analyser (Na1500, Carlo-Erba, Milan, Italy), atom percentage ^{15}N by mass spectrometry. The atom% ^{15}N excess was calculated by subtracting the atom percentage ^{15}N of plants which had not received any ^{15}N label (namely 0.365) from the measured atom %.

Hyphal density in the sand was determined by a direct counting method as described previously (Brandes *et al.*, 1998). Statistical analysis was carried out using one-way ANOVA (SAS Institute, 1987). Significance of differences between means was tested using the Tukey test ($P \leq 0.05$; SAS Institute, 1987). To increase homogeneity of variance if necessary, the data were log transformed before statistical analysis. *t*-tests (SAS Institute, 1987) were performed on differences between measured and calculated plant N, P and K contents to check the validity of the mass balance approach. The significance of calculated hyphal uptake rates (uptake rate > 0 , mass balance data) was checked using *t*-tests.

Results

Mycorrhizas and plant growth

Mycorrhizas were well established on plants inoculated with *P. involutus*. The percentage of mycorrhizal root tips ranged from 86% in the treatment without P to 93% in the treatment with P added to the hyphal compartment. No mycorrhizal tips were found on noninoculated plants.

Root dry weight was not affected by P addition to the hyphal compartment (Fig. 1). Mycorrhizal plants with P added to the hyphal compartment had significantly greater shoot dry weights than plants of all the other treatments (Fig. 1), except for nonmycorrhizal plants of the mass flow control treatment, which had slightly higher dry weights (i.e. 3.5 g per vessel and 2.0 g per vessel for shoots and roots, respectively.) However, the differences between the mass flow

control treatment and the mycorrhizal seedlings with P addition were not significant ($P \leq 0.05$).

Hyphal distribution

In nonmycorrhizal treatments, hyphal density was $< 0.01 \text{ m g}^{-1}$ dry sand in both compartments. Hyphal density in the root compartment of the mycorrhizal treatments tended to be higher in the treatment without P addition (Fig. 2), though the difference was not significant. In the hyphal compartment, hyphal density in the treatment without P addition declined with distance from the nylon net. By contrast, in treatments with additional P, hyphal density peaked in the centre of the hyphal compartment where the nutrients were applied. In this region, hyphal density was significantly (approx. 3 times) greater in the treatment with P addition than without.

Phosphorus uptake and hyphal P translocation

P addition to the hyphal compartment increased P concentrations (Fig. 3) and contents (data not shown) of roots and shoots in the mycorrhizal but not in the nonmycorrhizal seedlings.

The calculation of uptake from the root compartment showed a higher P uptake in mycorrhizal than in nonmycorrhizal treatments (Table 1). P addition to the hyphal compartment had no effect on root compartment P uptake. Hyphal uptake was only significant in the treatment with mycorrhiza and P addition to the hyphal compartment (Table 1). In all other treatments, values tended to be negative as the directed mass flow transported more nutrients from the plant to the hyphal compartment than accounted for by the correction method. In the mycorrhizal treatment with P addition there was no significant difference between

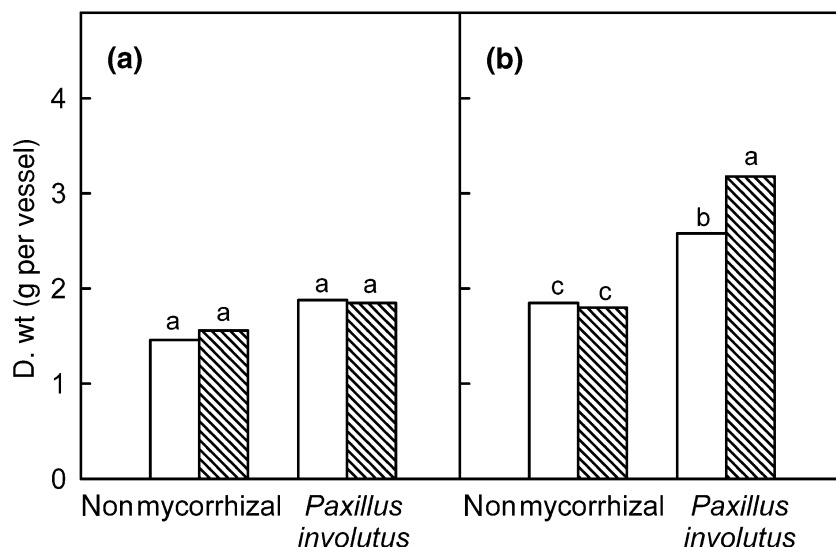


Fig. 1 (a) Root and (b) shoot d. wt of nonmycorrhizal or mycorrhizal (*Paxillus involutus*) Norway spruce (*Picea abies*) seedlings grown in a two-compartment culture system without P (open bar) or with P (hatched bar) added to the hyphal compartment. Each vessel of the two-compartment culture system contained four plants. Means ($n = 4$) followed by different letters are significantly different (Tukey, $P \leq 0.05$).

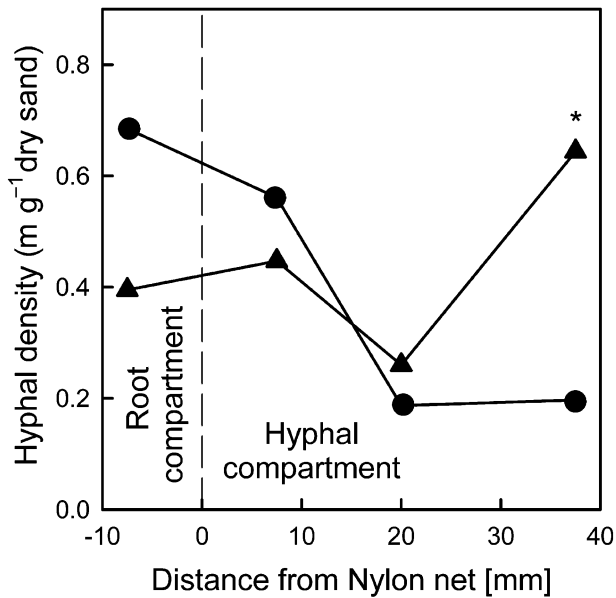


Fig. 2 Hyphal density of *Paxillus involutus* as affected by P addition (closed circle, -P; closed triangle, +P). P was added to the centre of the hyphal compartment, at 5 cm from the nylon net. Background values of hyphal density measured in nonmycorrhizal treatments were < 1 cm g⁻¹ dry sand. Asterisk, significant difference between treatments (ANOVA, $P \leq 0.05$).

P contents calculated from solution input and output and P contents measured in the plant material. Thus, for this treatment the balance approach yielded reasonable estimates. During the experiment, hyphal P uptake contributed 52% to total P uptake.

Nitrogen, potassium and magnesium tissue concentrations and plant uptake

While P addition to the root compartment decreased N

concentrations of old needles in nonmycorrhizal seedlings (mass flow control), the reverse pattern was observed in both young and old needles of mycorrhizal seedlings when P was added to the hyphal compartment (Table 2). Improved P nutrition tended to increase N concentrations of stems, coarse roots and fine roots in both nonmycorrhizal and mycorrhizal seedlings. In all compartments, N concentrations were higher in mycorrhizal seedlings with P addition than in nonmycorrhizal seedlings with P addition to the root compartment (mass flow control), though differences were only significant for fine and coarse roots (Table 2).

K and Mg concentrations in old needles ranged from 4.5 to 5.2 mg g⁻¹ and from 0.7 to 1.0 mg g⁻¹, respectively (data not shown). In young needles and stems, mean K concentrations were 10 and 7 mg g⁻¹, respectively, differences between treatments were not significant ($P \leq 0.05$). Mg concentrations in these compartments in all treatments were approx. 0.7 or 1.2 mg g⁻¹, respectively. In coarse and fine roots, Mg concentrations ranged from 0.6 to 0.7 mg g⁻¹; K concentrations were approx. 4.5–5 mg g⁻¹ or 8–10 mg g⁻¹. Neither K nor Mg concentrations in roots were significantly ($P \leq 0.05$) affected by treatments (data not shown).

As a result of relatively high P concentrations, molar N : P ratios were lowest in the mycorrhizal plants with P addition (Table 2), but still above the optimal N : P ratio of 12 determined by Ingestad (1979). Differences between these seedlings and nonmycorrhizal plants of the mass-flow control were only significant in fine roots however (Table 2). Molar K : P and Mg : P ratios followed a similar pattern (data not shown), in all cases exceeding the optimal ratios of 2.5 or 0.4 (Ingestad, 1979).

Nutrient uptake rates calculated from seedling growth rates and tissue nutrient concentrations at the final harvest are shown in Table 3. Except for P, nutrient supply to the root compartment exceeded uptake rates by at least 80%.

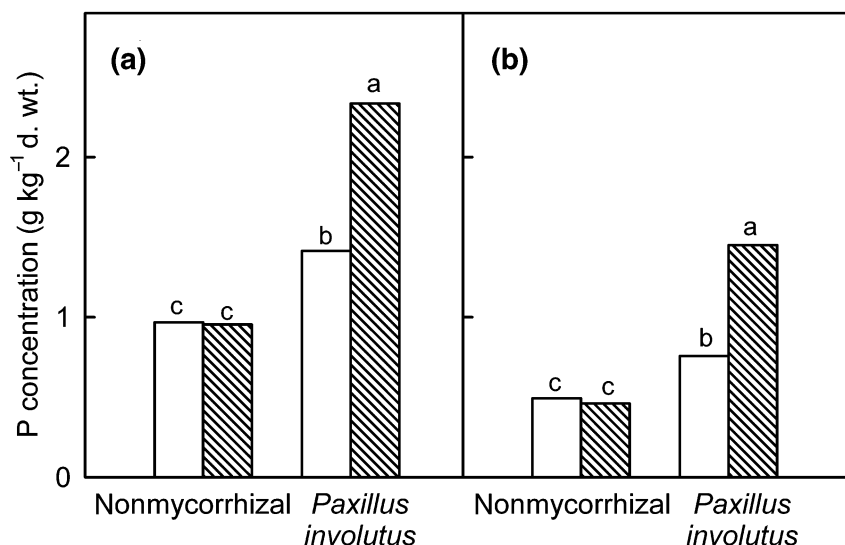


Fig. 3 (a) Root and (b) shoot P concentrations of nonmycorrhizal or mycorrhizal (*Paxillus involutus*) Norway spruce seedlings (*Picea abies*) grown in a two compartment culture system -P (open bars) or +P (hatched bars) added to the hyphal compartment. Means followed by different letters are significantly different (Tukey, $P \leq 0.05$).

Table 1 Initial P content, uptake from nutrient solutions and final P contents of mycorrhizal (with *Paxillus involutus*) or nonmycorrhizal Norway spruce (*Picea abies*) seedlings grown in a two-compartment culture system with or without P addition to the hyphal compartment

Treatment		Initial P content (mg per vessel) week 0	P uptake (mg per vessel) week 0 – week 11		Final P content (mg per vessel) week 11	
			Plant compartment	Hyphal compartment	Calculated	Measured
Nonmycorrhizal	–P	1.0a	2.7b	–1.2b	2.5bc	2.4
	+P	0.8a	2.7b	–1.7b	1.7c	2.3
<i>P. involutus</i>	–P	1.0a	3.1a	–0.8b	3.4b*	4.6*
	+P	1.2a	3.2a	3.7a	8.0a	8.9

Each vessel of the two-compartment culture system contained four plants. Final P contents were either calculated from input and output of P in solution and initial P contents or measured directly in the plant material. Means ($n = 4$) within one column followed by different letters are significantly different (Tukey, $P \leq 0.05$). *, significant difference between calculated and final nutrient content (t -test, $P \leq 0.05$).

Table 2 Effect of P addition on N concentrations in needles, stems and roots of nonmycorrhizal and mycorrhizal (*Paxillus involutus*) Norway spruce (*Picea abies*) seedlings and tissue N : P ratios

Compartment	Tissue N concentration (g kg^{-1} d. wt) or N : P ratio (mol mol^{-1})				
	Nonmycorrhizal		Mass flow control	<i>P. involutus</i>	
	–P	+P		–P	+P
Tissue N concentration					
Fine roots	11.8c	11.6c	14.8b	16.3b	19.2a
Coarse roots	6.6b	5.6b	6.9b	7.0b	8.6a
Stem	10.3ab	9.7ab	10.7ab	8.6b	11.8a
Old needles	14.1a	14.8a	11.3bc	10.7c	12.2b
Young needles	11.7a	11.3a	10.8a	9.2b	11.6a
Tissue N : P ratio					
Fine roots	25a	25a	19b	23a	16c
Coarse roots	26a	24a	17bc	19b	15c
Stem	33a	34a	20bc	22b	15c
Old needles	78a	78a	30bc	37b	27c
Young needles	45ab	70a	19b	23ab	15b

The additional P was supplied to the hyphal compartment or to the roots of nonmycorrhizal seedlings (mass flow control). Data were log-transformed before statistical analysis. Back-transformed means ($n = 4$) within rows with different letters are significantly different (Tukey test, $P \leq 0.05$).

Table 3 Calculated net uptake rates of N, P, K and Mg of nonmycorrhizal and mycorrhizal (with *Paxillus involutus*) *Picea abies* seedlings and nutrient addition rates to the root compartment at the end of the experiment (week 11)

Treatment	Relative growth rate (% d^{-1})	Net uptake or supply rate of nutrients ($\mu\text{mol per vessel d}^{-1}$)			
		Nitrogen	Phosphorus	Potassium	Magnesium
Nonmycorrhizal					
–P	0.8	21	0.6	4.7	0.9
+P	0.8	21	0.6	5.0	0.9
MFC	1.4	65	3.1	14.9	2.6
<i>P. involutus</i>					
–P	0.9	33	1.3	7.1	1.3
+P	1.4	69	4.0	13.4	2.1
Nutrient supply to root compartment		207 (207)*	1.7 (5.2)	24.7 (28.2)	6.9 (6.9)

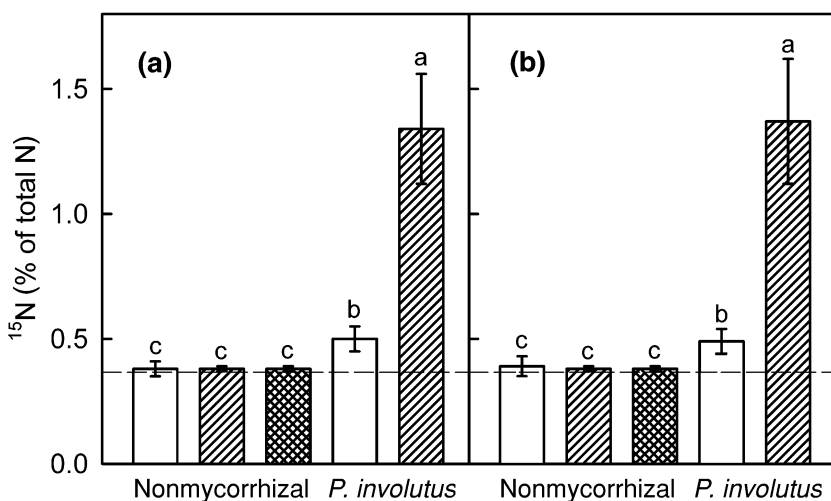
*values in brackets are for the mass flow control.

Table 4 Initial N content, N uptake from nutrient solutions and final N contents (mg per vessel) of mycorrhizal (with *Paxillus involutus*) or nonmycorrhizal Norway spruce (*Picea abies*) seedlings grown in a two-compartment culture system with or without P addition to the hyphal compartment

Treatment		Initial nitrogen content week 0	Nitrogen uptake week 0 – week 11		Final nitrogen content week 11	
			Root compartment	Hyphal compartment	Calculated	Measured
Nonmycorrhizal	–P	8.1a	34.9b	–5.7b	37.2b	39.1b
	+P	6.5a	37.0b	–6.5b	37.0b	39.3b
<i>P. involutus</i>	–P	7.1a	44.2ab	–4.3b	47.0b	52.2b
	+P	8.3a	55.5a	11.4a	75.2a	69.4a

Each vessel of the two-compartment culture system contained 4 plants. Final N contents were either calculated from input and output of N compounds in solution and initial N contents or measured directly in the plant material. Means ($n = 4$) within one column followed by different letters are significantly different (Tukey, $P \leq 0.05$). Differences between final calculated and measured nutrient contents were not significant (t -test, $P \leq 0.05$).

Fig. 4 (a) ^{15}N root and (b) shoot concentrations in nonmycorrhizal or mycorrhizal (*Paxillus involutus*) Norway spruce (*Picea abies*) seedlings grown in a two-compartment culture system without P (open bars) or with P (hatched bars) added to the hyphal compartment. Mycorrhizal seedlings with P added to the root compartment (cross-hatched bars, mass flow control) were used as additional control. The nutrient solution in the hyphal compartment was labelled with $^{15}\text{NH}_4\text{NO}_3$ (10 atom %). Broken line indicates abundance of ^{15}N in control plants not treated with ^{15}N . Data were log-transformed before statistical analysis. The figure shows back-transformed means of four replicate vessels. Values followed by different letters are significantly different (Tukey, $P \leq 0.05$).



Hyphal translocation of nitrogen, potassium and magnesium

Hyphal N translocation was determined by both an N mass balance approach and ^{15}N labelling. The calculation of uptake from the root compartment showed a higher N uptake in mycorrhizal seedlings compared to nonmycorrhizal ones (Table 4). Hyphal N uptake was only significant in mycorrhizal seedlings with P addition to the hyphal compartment. In all other treatments values were negative as a result of passive N transfer from the plant to the hyphal compartment being underestimated by the correction method used. There was no significant difference between N contents calculated from solution input and output and N contents determined in the plant material. Thus, the balance approach yielded reasonable estimates. Total hyphal N uptake ($\text{NH}_4^+ + \text{NO}_3^-$, based on total N measurements) contributed 17.0%

to total N uptake in the mycorrhizal seedlings with P addition. Hyphal NH_4^+ or NO_3^- uptake was 8.3 or 3.1 mg per vessel, contributing 12.4% or 4.6% to total N uptake, respectively.

The nutrient solution of the hyphal compartment was labelled with $^{15}\text{NH}_4\text{NO}_3$. In nonmycorrhizal seedlings including the mass flow control, ^{15}N concentrations in shoots and roots (Fig. 4) were only slightly raised above control values (namely 0.365 atom percent, seedlings harvested before ^{15}N labelling commenced), indicating that physical N transport in the sand by mass flow and diffusion was low. Concentrations of ^{15}N in mycorrhizal seedlings without P addition were significantly higher than in nonmycorrhizal seedlings of the mass flow control, indicating significant hyphal N translocation. Addition of P to the hyphal compartment strongly and significantly increased ^{15}N concentrations in the mycorrhizal seedlings 2.5-fold. ^{15}N excess in these seedlings was 0.68 mg, corresponding to 7.1 mg of plant N derived

Treatment	Excess ^{15}N in seedling (mg per vessel)	$^{14}\text{NH}_4^+ + ^{15}\text{NH}_4^+$ uptake from hyphal compartment (mg per vessel)	Percentage of N uptake from hyphal compartment*
Nonmycorrhizal -P	0.009c	0.09c	0.2c
Nonmycorrhizal +P	0.006c	0.06c	0.1c
Nonmycorrhizal (mass flow control)	0.009c	0.09c	0.1c
<i>Paxillus involutus</i> -P	0.070b	0.73b	1.6b
<i>Paxillus involutus</i> +P	0.676a	7.02a	11.5a

Nonmycorrhizal and mycorrhizal seedlings were grown in a two-compartment culture system with $^{15}\text{NH}_4\text{NO}_3$ added (10 atom percent ^{15}N) to the hyphal compartment for 11 weeks. Nitrogen ($^{14}\text{N} + ^{15}\text{N}$) uptake from the hyphal compartment was calculated by dividing excess ^{15}N in the seedlings by the ^{15}N atomic excess of the labelling solution (9.63%). Means ($n = 4$) within columns with different letters are significantly different (Tukey test, $P \leq 0.05$). *, total N uptake = final N content - initial N content (Table 4) = 100%.

Table 5 ^{15}N uptake of nonmycorrhizal and mycorrhizal (with *Paxillus involutus*) Norway spruce (*Picea abies*) seedlings from the hyphal compartment

Treatment	Initial K content (mg per vessel) week 0	K uptake week 0 - week 11		Final K content (mg per vessel) week 11		
		Plant compartment	Hyphal compartment	Calculated	Measured	
Nonmycorrhizal	-P	4.4a	22.7b	-4.3b	22.8b	24.4b
	+P	3.3a	26.2ab	-5.4b	24.1b	25.6b
<i>P. involutus</i>	-P	3.2a	27.4ab	-5.6b	25.0b	28.9ab
	+P	4.0a	32.6a	1.9a	38.5a	37.3a

Table 6 Initial K content, uptake from nutrient solutions and final K contents of mycorrhizal (with *Paxillus involutus*) or nonmycorrhizal Norway spruce (*Picea abies*) seedlings grown in a two-compartment culture system with or without P addition to the hyphal compartment

Each vessel of the two-compartment culture system contained four plants. Final K contents were either calculated from input and output of K in solution and initial K contents or measured directly in the plant material. Means ($n = 4$) within one column followed by different letters are significantly different (Tukey, $P \leq 0.05$). Differences between final calculated and measured nutrient contents were not significant (t -test, $P \leq 0.05$).

from $^{14}\text{NH}_4^+$ and $^{15}\text{NH}_4^+$ in the hyphal compartment (Table 5). Hyphal NH_4^+ uptake, therefore, contributed 11.5% to total N uptake. This estimate was in reasonable accordance with the balance approach.

The K balance revealed that a minimum of 1.9 mg K per vessel was taken up from the hyphal compartment in the seedlings with P addition (Table 6). A t -test showed that this estimate was significantly different from zero ($P \leq 0.05$). In all other treatments hyphal K uptake was not significant. There was no significant difference between K contents calculated from solution input and output and K contents determined in the plant material. Thus, the balance approach yielded reasonable estimates. Hyphal K uptake contributed 5.5% to total K uptake in the mycorrhizal seedlings with P addition.

The nutrient solution of the hyphal compartment was labelled with ^{25}Mg during the final 6 weeks of the experimental period. In nonmycorrhizal seedlings including the mass flow control, ^{25}Mg concentrations in fine roots were

indistinguishable from natural abundance (10.00%; Weast, 1989), indicating that physical Mg transport in the sand by mass flow and diffusion was low (Fig. 5). Mycorrhizal seedlings without P addition had similar ^{25}Mg concentrations in all compartments as nonmycorrhizal seedlings of the mass flow control, suggesting that ^{25}Mg translocation through the external mycelium was negligible in this treatment. When P was added to the hyphal compartment, we detected ^{25}Mg in plant tissues significantly in excess of natural abundance. The percent of ^{25}Mg excess decreased with increasing distance from the plant uptake site (i.e. from approx. 4% in fine roots to approx. 1% in old needles; Fig. 5). In total, the mycorrhizal seedlings with P addition took up 90 μg ^{25}Mg in excess of the natural abundance during the 6 wk labelling period.

Molar proportions of translocated elements (+P treatment) were approx. 100 : 18 : 8 : 1 for N : P : K : Mg, respectively (Table 7).

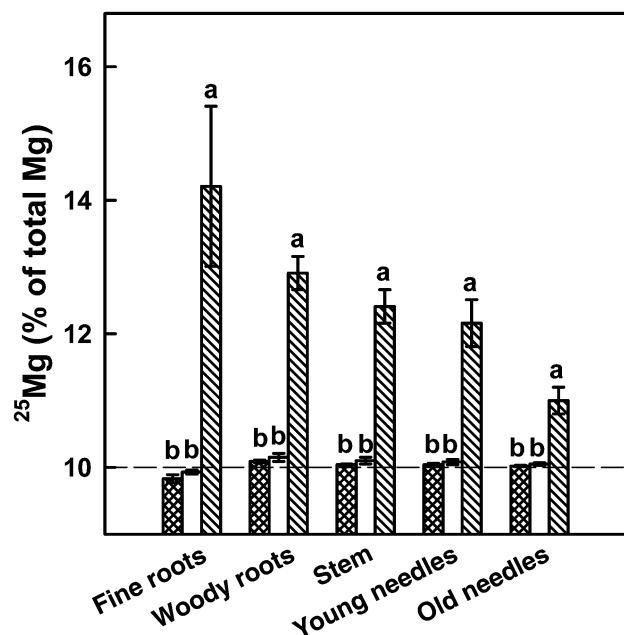


Fig. 5 Concentration of ²⁵Mg (expressed as percentage ²⁵Mg of total Mg) in tissues of nonmycorrhizal and mycorrhizal (with *Paxillus involutus*) Norway spruce (*Picea abies*) seedlings after labelling the hyphal compartment in the culture system with ²⁵Mg for 6 wk. Nonmycorrhizal seedlings (cross-hatched columns) had P added to the root compartment, mycorrhizal seedlings were without (open columns) or with (hatched columns) P added to the hyphal compartment. The broken line indicates the natural abundance of ²⁵Mg (10.0%). Bars, SE. Means ($n = 4$) followed by different letters are significantly different (Tukey, $P \leq 0.05$).

Table 7 Hyphal translocation of elements in mycorrhizal (with *Paxillus involutus*) *Picea abies* seedlings during the 11-wk experimental period

Treatment	Hyphal translocation ($\mu\text{mol per vessel}$)			
	Nitrogen	Phosphorus	Potassium	Magnesium
-P	70	n.d.	n.d.	n.d.
+P	660	120	50	7

P and K translocation amounts are from mass balance calculations. N and Mg translocation were determined by stable isotope labelling. Translocation of N was corrected for NO_3^- uptake, assuming that hyphae in all treatments took up NH_4^+ and NO_3^- at a fixed ratio of 2.7 : 1. Translocation of Mg was corrected for the shorter time period of ²⁵Mg labelling (6 wk), assuming that translocation rates were constant over the experiment. nd, not detected.

Discussion

Addition of P available only to mycorrhizal hyphae strongly stimulated hyphal growth at the location of P application. The hyphae took up and translocated significant amounts

of P to their P-deficient host plant and strongly improved host P status. Improved P nutrition, in turn, stimulated seedling growth. This series of events in our experiment provides direct evidence for the active role of the external ectomycorrhizal mycelium in foraging for P and stimulation of host P nutrition and growth. Our data confirm earlier results that showed simultaneous hyphal foraging for N and P ameliorated N and P deficiency in mycorrhizal Norway spruce seedlings (Brandes *et al.*, 1998). As the seedlings in our experiment were solely deficient in P the results demonstrate that P deficiency alone may trigger hyphal foraging and translocation of P from P-rich sites.

Paxillus involutus hyphae, besides P, translocated significant amounts of N, K and Mg to the host plant. Based on both ¹⁵N labelling and mass balance data, hyphal NH_4^+ acquisition contributed 12% to total plant N uptake. Ek *et al.* (1994) found NO_3^- translocation in *P. involutus* hyphae connected to *Picea abies* and *Betula pendula* in presence of NH_4^+ was approx. 50% of NH_4^+ translocation (measured at pH 4). Our estimates of NO_3^- translocation based on N mass balance confirm these figures. Thus, total hyphal N translocation including NO_3^- amounted to 17% of total plant N uptake and, in absolute numbers, exceeded P translocation five-fold (Table 7). Potassium translocation was of a similar order of magnitude to P translocation, though estimates based on mass balance were somewhat less precise, as K uptake was relatively small compared to K fluxes through the system. Yet, hyphal K acquisition was significant and contributed a minimum of 6% to total plant uptake. These data confirm the potential role of the ectomycorrhizal mycelium in K acquisition, which has so far only been demonstrated using radioisotopes of Rb as analogue for K (Finlay, 1992). Potassium fluxes in our experiment were in the same order of magnitude as P fluxes, confirming the earlier results of Finlay (1992). Magnesium translocation by the external mycelium, estimated by stable isotope labelling, was approx. 3–4% of total host Mg uptake as published in detail elsewhere (Jentschke *et al.*, 2000). In absolute terms, Mg fluxes through the hyphal network were lowest of all element fluxes determined, one or two orders of magnitude lower than P or N fluxes, respectively (Table 7).

The nutrient solution added to the root compartment was designed to limit plant growth by P. The molar ratio of N : P, K : P, Mg : P and other element : P ratios by far exceeded critical values for spruce seedlings determined by Ingestad (1979). Phosphorus limitation was directly evident from the strong growth response of nonmycorrhizal plants receiving extra P (mass-flow-control plants). Even in these plants, supply of nutrients other than P was still super-optimal as relevant element : P ratios, measured in both nutrient solutions and plant tissues, exceeded critical values (Ingestad, 1979). Mycorrhizal plants which had access to a similar amount of P as the fertilized nonmycorrhizal seedlings were similar in size,

but had slightly lower N : P ratios (but still in the optimal range) than seedlings of the mass flow control. In addition, N supply to the roots exceeded N uptake by a factor of 3, suggesting that N was not limiting growth. Similar estimations for K and Mg indicate that these nutrients were also not in short supply. Thus, here we demonstrate, for the first time to our knowledge, a cotransport of limiting (P) and nonlimiting nutrients (N, K, Mg) in the ectomycorrhizal mycelium.

Different mechanisms of hyphal translocation have been postulated but the relative importance of these under different conditions and for different elements remains unclear (Finlay, 1992). However, there is increasing evidence that P translocation in ectomycorrhizal hyphal systems occurs by active translocation rather than by transpiration-driven mass flow of water or diffusion (Timonen *et al.*, 1996; Ashford, 1998). If translocation is indeed an active process, it may then be expected for reasons of energy efficiency that it is controlled by external factors such as the host nutritional status and demand. This may be achieved either indirectly by varying growth of the external mycorrhizal mycelium (Wallander & Nylund, 1992; Arnebrant, 1994), or directly by selectively down-regulating translocation processes for nutrients not in short supply. The translocation of significant amounts of nutrients not limiting host growth (N and K) suggests that these nutrient fluxes were not strongly down-regulated. In a similar experiment (Brandes *et al.*, 1998), in which the same symbionts but a different nutrient regime were used, we found that the molar ratio of N to P transported through the mycorrhizal mycelium was 12. As the seedlings were deficient in both N and P, the higher relative N transfer (N : P ratio 12 vs 5 as found in this experiment) suggests that translocation may indeed be affected by host demand, albeit moderately. Acquisition of N not needed at the time of uptake, however, may be stored in the host plant and may provide an additional N source in times with insufficient N supply. Thus, in terms of long-term plant success, the simultaneous delivery of N, P and other nutrients by the external mycorrhizal mycelium may be an advantageous strategy.

When no P was supplied to the hyphal compartment and, thus, no P was translocated to the host plant, the transfer of N, K, and Mg decreased or ceased. As both the growth rate of seedlings, which determines nutrient demand, and the hyphal density in the hyphal compartment differed between +P and -P mycorrhizal seedlings, care must be taken in interpreting these results. However, while seedling N uptake during the experimental period and hyphal density were only reduced to 74% (Table 4, final minus initial seedling N content) or 33% (Fig. 1) of the values in the +P seedlings, respectively, nitrogen transfer was reduced to 10% of the rate with simultaneous P transfer (i.e. much more than expected from the combination of the reductions ($0.74 \times 0.33 = 24\%$)). Potassium translocation may additionally

have been influenced by differences in K supply to the hyphal compartment between -P and +P seedlings; however, these differences (approx. 30%) were probably too small to explain the strong reduction in K translocation in -P mycorrhizal seedlings. Mg translocation completely ceased when no P was translocated. Although our experiment does not provide conclusive evidence, it is likely that these changes were a result of a strong coupling of N and especially Mg and K fluxes to P translocation. Finlay (1992) suggested that N transported as positively charged amino acid, such as arginine, may play a role in neutralizing negative charges of simultaneously translocated (poly)phosphates. In fact, microanalytical studies have provided evidence for association of N and P compounds in mycorrhizal fungal vacuoles (Turnau *et al.*, 1993; Kottke *et al.*, 1995). As N translocation may exceed P translocation severalfold (Table 7), it is, however, clear that only a small portion of N as arginine would be needed to neutralize negative charges of P compounds. In *P. involutus*, however, arginine as a transport form of N may play no role at all (Finlay, 1992; Ek *et al.*, 1994). Thus, in this fungus, negative charges from translocated P compounds must be neutralized by other means. Our data support the idea that K and, to a minor extent, Mg may act as neutralizing agents. Ashford (1998) has hypothesized that longitudinal P transport in fungi may be carried out through the tubular vacuole system, which is also present in *P. involutus* (Rees *et al.*, 1994). As X-ray microanalysis has repeatedly shown that P in fungal vacuoles is mainly associated with K and Mg (Orlovich & Ashford, 1993; Bücking & Heyser, 1999), translocation of vacuolar contents through the tubular system would most likely require K and Mg fluxes to be strongly coupled with P translocation.

This work has confirmed the active role of ectomycorrhizal fungal hyphae in acquisition of P in P deficient Norway spruce seedlings. Although not in short supply, the hyphae also translocated N, K and Mg to the host plant. The translocation of nonlimiting nutrients depended upon the simultaneous translocation of P. This supports the idea that nutrient fluxes within fungal hyphae are interdependent and that for reasons of charge neutralization cation fluxes may be strongly coupled to P translocation.

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References

- Arnebrant K. 1994. Nitrogen amendments reduce the growth of extramatrical ectomycorrhizal mycelium. *Mycorrhiza* 5: 7–15.
- Ashford AE. 1998. Dynamic pleiomorphic vacuole systems: Are they endosomes and transport compartments in fungal hyphae? *Advances in Botanical Research* 28: 119–159.
- Ashford AE, Ryde S, Barrow KD. 1994. Demonstration of a short chain polyphosphate in *Pisolithus tinctorius* and the implications for phosphorus transport. *New Phytologist* 126: 239–247.
- Bigg WL, Alexander IJ. 1981. A culture unit for the study of nutrient uptake by intact mycorrhizal plants under aseptic conditions. *Soil Biology and Biochemistry* 13: 77–78.
- Brandes B, Godbold DL, Kuhn AJ, Jentschke G. 1998. Nitrogen and phosphorus acquisition by the mycelium of the ectomycorrhizal fungus *Paxillus involutus* and its effect on host nutrition. *New Phytologist* 140: 735–743.
- Bücking H, Heyser W. 1999. Elemental composition and function of polyphosphates in ectomycorrhizal fungi – an X-ray microanalytical study. *Mycological Research* 103: 31–39.
- Ek H, Andersson S, Arnebrant K, Söderström B. 1994. Growth and assimilation of NH_4^+ and NO_3^- by *P. involutus* in association with *Betula pendula* and *Picea abies* as affected by substrate pH. *New Phytologist* 128: 629–637.
- Finlay R. 1992. Uptake and translocation of nutrients by ectomycorrhizal fungal mycelia. In: Read DJ, Lewis DH, Fitter AH, Alexander IJ, eds. *Mycorrhizas in ecosystems*. Wallingford, UK, CAB International, 91–97.
- Finlay RD, Read DJ. 1986. The Structure and function of the vegetative mycelium of ectomycorrhizal plants. II. The uptake and distribution of phosphorus by mycelial strand interconnecting host plants. *New Phytologist* 103: 157–165.
- George E, Häussler KU, Vetterlein D, Gorgus E, Marschner H. 1992. Water and nutrient translocation by hyphae of *Glomus mosseae*. *Canadian Journal of Botany* 70: 2130–2137.
- Gerlitz TGM, Gerlitz A. 1997. Phosphate uptake and polyphosphate metabolism of mycorrhizal and non-mycorrhizal roots of pine and of *Suillus bovinus* at varying external pH measured by *in vivo* P-31-NMR. *Mycorrhiza* 7: 101–106.
- Ingestad T. 1979. Mineral nutrient requirements of *Pinus sylvestris* and *Picea abies* seedlings. *Physiologia Plantarum* 45: 373–380.
- Jentschke G, Brandes B, Kuhn AJ, Schröder WH, Becker JS, Godbold DL. 2000. The mycorrhizal fungus *Paxillus involutus* transports magnesium to Norway spruce seedlings. Evidence from stable isotope labeling. *Plant and Soil* 220: 243–246.
- Johansen A, Jakobsen I, Jensen ES. 1993. External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. 3. Hyphal transport of ^{32}P and ^{15}N . *New Phytologist* 124: 61–68.
- Kammerbauer H, Agerer R, Sandermann Jr H. 1989. Studies on ectomycorrhiza. XXII. Mycorrhizal rhizomorphs of *Telephora terrestris* and *Pisolithus tinctorius* in association with Norway spruce (*Picea abies*): formation in vitro and translocation of phosphate. *Trees* 3: 78–84.
- Kottke I, Holopainen T, Alanen E, Turnau K. 1995. Deposition of nitrogen in vacuolar bodies of *Cenococcum geophilum* Fr. mycorrhizas as detected by electron energy loss spectroscopy. *New Phytologist* 129: 411–416.
- Kuhn AJ, Schröder WH, Bauch J. 2000. The kinetics of calcium and magnesium entry into mycorrhizal spruce roots. *Planta* 210: 488–496.
- Marschner H, Dell B. 1994. Nutrient uptake in mycorrhizal symbiosis. *Plant and Soil* 159: 89–102.
- Marschner P, Godbold DL, Jentschke G. 1996. Dynamics of lead accumulation in mycorrhizal and non-mycorrhizal Norway spruce (*Picea abies* (L.) Karst.). *Plant and Soil* 178: 239–245.
- Marx DH. 1969. The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. I. Antagonism of mycorrhizal fungi to root pathogenic fungi and soil bacteria. *Phytopathology* 59: 153–163.
- Melin E, Nilsson H. 1950. Transfer of radioactive phosphorus to pine seedlings by means of mycorrhizal hyphae. *Physiologia Plantarum* 3: 88–92.
- Melin E, Nilsson H. 1952. Transport of labelled nitrogen from an ammonium source to pine seedlings through mycorrhizal mycelium. *Svensk Botanisk Tidskrift* 46: 281–285.
- Orlovich DA, Ashford AE. 1993. Polyphosphate granules are an artefact of specimen preparation in the ectomycorrhizal fungus *Pisolithus tinctorius*. *Protoplasma* 173: 91–102.
- Rees B, Shepherd VA, Ashford AE. 1994. Presence of a motile tubular vacuole system in different phyla of fungi. *Mycological Research* 98: 985–992.
- Rost FWD, Shepherd VA, Ashford AE. 1995. Estimation of vacuolar pH in actively growing hyphae of the fungus *Pisolithus tinctorius*. *Mycological Research* 99: 549–553.
- SAS Institute. 1987. *SAS/STAT™ Guide for personal computers, version 6 edition*. Cary, NC, USA: SAS Institute Inc., 549–640.
- Schlechte G. 1986. Zur Mykorrhizapilzflora in geschädigten Forstbeständen. *Zeitschrift für Mykologie* 52: 225–232.
- Smith SE, Read DJ. 1997. *Mycorrhizal symbiosis*. London, UK: Academic Press.
- Timonen S, Finlay RD, Olsson S, Söderström B. 1996. Dynamics of phosphorus translocation in intact ectomycorrhizal systems: non-destructive monitoring using a β -scanner. *FEMS Microbiology Ecology* 19: 171–180.
- Turnau K, Kottke I, Oberwinkler F. 1993. *Paxillus involutus* – *Pinus sylvestris* mycorrhizae from heavily polluted forest. 1. Element localization using electron energy loss spectroscopy and imaging. *Botanica Acta* 106: 213–219.
- Wallander H, Nylund JE. 1992. Effects of excess nitrogen and phosphorus starvation on the extramatrical mycelium of ectomycorrhizas of *Pinus sylvestris* L. *New Phytologist* 120: 495–503.
- Weast RC. 1989. *Handbook of chemistry and physics, 70th edn*. Cleveland, OH, USA: Chemical Rubber Co.