Mycorrhiza (1997) 7:89–94 © Springer-Verlag 1997

ORIGINAL PAPER

J. Baar

Ectomycorrhizal root growth in scots pine stands in response to manipulation of litter and humus layers

Accepted: 19 March 1997

Abstract The effect on ectomycorrhizal root growth in a nitrogen-enriched planted stand of Scots pine (*Pinus sylvestris* L.) on podzolic sandy soil to manipulation of litter and humus layers (removal, doubling and control treatments) was examined, and compared to ectomycorrhizal root growth in an untreated naturally established Scots pine stand on nutrient-poor non-podzolic sandy soil. Half a year after manipulation of litter and humus layers in the planted stand, ingrowth-cores to a depth of 60 cm were installed in both stands. Scots pine roots were sampled four times during two growing seasons. Ectomycorrhizal roots were found at all sampled soil depths to 60 cm in all plots. Root growth and ectomycorrhizal development were greater in the naturally established stand than in all plots in the planted stand. Numbers of ectomycorrhizal root tips in the litter and humus removal plots were generally higher than in the control plots in the planted stand until May 1992. Doubling litter and humus did not significantly affect root length or the numbers of ectomycorrhizal root tips. The $N_{dissolved}$, NH_4^+ and NO_3^- concentrations and the organic matter content in the upper 5 cm of the mineral soil in the planted stand on podzolic sandy soil were generally higher and the pH significantly lower than in the naturally established stand on non-podzolic sandy soil. Root growth and ectomycorrhizal development in the secondary stand may have been negatively affected by the chemical composition of the podzolic sandy soil.

Key words Below ground · Ectomycorrhizal fungi · Ingrowth core · Litter and humus layer · *Pinus sylvestris*

J. Baar[1]
Biological Station of the Agricultural University Wageningen, Kampsweg 27, 9418 PD Wijster, The Netherlands

Present address:
[1] Department of Plant and Microbial Biology, 341 Koshland Hall, University of California, Berkeley CA 94720-3102, USA
Fax: +1-510-642-4995; e-mail: baar@mendel.berkeley.edu

Introduction

Over the last several decades, forests in The Netherlands have experienced high levels of atmospheric nitrogen deposition. An intensive livestock industry has raised ammonia emissions from manure causing increased nitrogen depositions (Pearson and Stewart 1993). Average immission values of ammonia now range from 40 to 80 kg ha^{-1} $year^{-1}$ with local extremes to 100 kg ha^{-1} $year^{-1}$ (Draaijers et al. 1989; Dijk et al. 1989). As a result, litter and humus have accumulated because of increased foliage production and decreased rates of decomposition of nitrogen-enriched litter and humus (Kuyper 1988; Markkola and Ohtonen 1988; Ohtonen and Markkola 1991). This accumulation is particularly evident in stands of Scots pine (*Pinus sylvestris* L.) typified by thick layers of nitrogen-rich litter and humus (Klap and Schmidt 1992; Baar and ter Braak 1996). The grass *Deschampsia flexuosa* (L.) Trin. has increased enormously in Scots pine stands as a result of enhanced nitrogen deposition (Tamm 1991; Klap and Schmidt 1992). In these stands, Arnolds (1991) noted a decline of species and sporocarps of ectomycorrhizal fungi during this century.

Both litter and grass appear to affect sporocarp production: litter depth and ectomycorrhizal sporocarp abundance are negatively correlated (de Vries et al. 1985) and sporocarps of ectomycorrhizal fungi were scarce in Scots pine stands dominated by the grass *D. flexuosa* (Veerkamp and Kuyper 1993).

Below ground, ectomycorrhizal development also appears to be negatively affected by increasing litter accumulation e.g., the reduced development of ectomycorrhizal root tips of conifer seedlings (Alvarez et al. 1979; Schoeneberger and Perry 1983; Rose et al. 1983; Baar and de Vries 1995). Markkola and Ohtonen (1988) noted that development of several ectomycorrhizal root types were reduced by thick layers of nitrogen-enriched humus, e.g., *Dermocybe*, *Hebeloma* and *Piloderma*.

Recent studies have reported that removal of litter, humus and sod (herbaceous and grass roots), called "sod-removal", restored the ectomycorrhizal flora above ground in nitrogen-enriched Scots pine stands in The Netherlands (Baar and Kuyper 1993; de Vries et al. 1995). The enhancement, however, was smaller in stands on podzolic sandy soils than on non-podzolic sandy soils (Baar 1996).

To study the effects of thick litter and humus on the numbers of species and sporocarps of ectomycorrhizal fungi, sods were added to several planted Scots pine stands in The Netherlands (Baar and ter Braak 1996). Sod-addition refers to the addition of litter, humus and sods to the existing litter and humus layers in Scots pine stands to simulate thick (4–10 cm) ectorganic layers present in 50- to 70-year-old planted stands. Sod-addition had no significant effects on numbers of species and sporocarps of ectomycorrhizal fungi above ground in Scots pine stands in The Netherlands (Baar and ter Braak 1996).

The studies by Alvarez et al. (1979), Schoeneberger and Perry (1983), Rose et al. (1983), Markkola and Ohtonen (1988) and Baar and de Vries (1995) focused on effects of litter and humus layers on ectomycorrhizal development of conifers in the top layers of the soil. Although some studies have addressed root growth and ectomycorrhizal development in deeper soil layers in Scots pine stands (Persson 1979, 1980a, 1984; Ahlström et al. 1988), effects of accumulated litter and humus on ectomycorrhizal development in deeper soil layers have not been studied. Therefore, the present study examined effects of manipulation of litter and humus layers in a planted nitrogen-enriched Scots pine stand on ectomycorrhizal root growth in the mineral soil layers at a soil depth of 0–60 cm compared with that in an untreated, naturally established nitrogen-poor Scots pine stand over time.

The objectives of this study were to investigate (1) whether removal of litter, humus layers and herbaceous vegetation decreases ectomycorrhizal development in the mineral soil within a year of treatment, (2) whether addition of litter and humus layers affects ectomycorrhizal development in the mineral soil, and (3) whether ectomycorrhizal development in naturally established Scots pine stands on nutrient-poor non-podslic sandy soil is greater than in planted nitrogen-enriched Scots pine stands on podzolic sandy soil.

Materials and methods

Site description

Two Scots pine stands in The Netherlands were selected which differed in soil nitrogen concentrations. The nitrogen-poor stand (N) is located in the drift sand area Hulshorsterzand (52° 21'N, 5° 44'E) and consists of an overstory of naturally established 15- to 20-year-old trees with a sparse understory of mosses and lichens (*Cladonia* spp.). The soil of this natural stand is nutrient-poor and sandy (Haplic Arenosol, FAO-Unesco 1988). The nitrogen-rich stand (P) is located in the Dwingeloo forest (52° 50' N, 6° 25'E). Until 1920, the site of this planted stand was *Calluna* heathland. Then *Pinus sylvestris*, *Quercus robur* L. and *Picea abies* (L.) Karsten were planted. In 1974, the stand was clearcut and replanted with *Pinus sylvestris*. The understory vegetation is dominated by the grass *Deschampsia flexuosa*; the herb *Ceratocapnos claviculata* (L.) Lidèn and the grass *Molinia caerulea* (L.) Moench. also occur. The soil of this planted stand is a podzolic sandy soil (Haplic Podzol, FAO-Unesco, 1988).

Treatments

In 1990, four plots (15 × 15 m), homogenous in vegetation type, were randomly selected in the naturally established stand on nitrogen-poor sandy soil and received no treatment. Twelve plots (15 × 15 m), also homogenous in vegetation type, were randomly selected in the nitrogen-enriched planted stand. In May and June 1990, litter and humus layers and the herbaceous vegetation in the planted stand were removed from four plots down to the mineral soil. The litter, humus and herbaceous vegetation of four additional plots were covered by the removed sods, simulating the thick (4–10 cm) organic layers present in 50- to 70-year-old stands (Baar and ter Braak 1996). The four remaining plots were left untreated and served as controls. Also in spring 1990, the few naturally occurring deciduous ectomycorrhizal trees were cut down and Scots pine seedlings were removed every year to insure that only ectomycorrhizal fungi associated with adult Scots pine trees were present.

Root sampling

In January and February 1991, four independent sample points were randomly selected in each plot at least 2.5 m from the margin. After removing the herbaceous vegetation, litter and humus, mineral soil was sampled to a depth of 60 cm and sieved (2-mm sieve). Sieving the humus layers in the secondary stand plots was impossible because of the thick root growth of *D. flexuosa*, so soil from the grass rhizosphere was excluded from the experimental soil insertion.

Within 2 weeks of sieving, the sieved and root-free mineral soil was inserted by profile where the soil had been removed, and was covered with humus. The ingrowth of new roots into the originally root-free cores was subsequently measured. One core (cylindrical soil coring tube, 4.1 cm diameter, 11.2 cm long) from each plot was removed to a depth of 60 cm without replacement in May 1991, November 1991, May 1992 and November 1992. The soil was sieved (2-mm sieve) and sand grains were rinsed from the roots. Dead roots, defined as desiccated, shrunken and highly fragile, were removed. After cleaning, the roots were stored in a glutaraldehyde buffer (Alexander and Bigg 1981).

In each sample, root length was determined according to Newman (1966) and the numbers of ectomycorrhizal root tips were counted. Ectomycorrhizal root tips were defined either as roots containing a clearly visible mantle, external mycelium and no root hairs, or as root tips having less than three root hairs. This working definition of mycorrhizal and non-mycorrhizal roots was based on extensive screening of roots before the actual counting: from microscopic observations, it appeared that root tips with one or two poorly developed root hairs always possessed a Hartig net and that root tips with three or more root hairs had no Hartig net. No attempts were made to identify the ectomycorrhizal root tips to fungal species.

Root length and numbers of ectomycorrhizal root tips were recalculated to a soil volume of 100 cm^3. Ramification indices (Meyer 1987) of ectomycorrhizal root tips (numbers of ectomycorrhizal root tips per cm root) and frequencies of ectomycorrhizal root tips (numbers of ectomycorrhizal root tips/total numbers of root tips × 100) were calculated.

Chemical analysis of soils

In April 1993, the upper 5 cm of the mineral soil was sampled in all plots by using cores (3.4 cm diameter) after removing the herbaceous vegetation, litter and humus layers. Most of the ectomycorrhizal roots were assumed to be in the upper 5 cm of the mineral soil. Ten samples collected randomly per plot were mixed and dried at 40 °C. After drying, the soil samples were sieved (2-mm sieve). Each sample was analyzed chemically for inorganic nutrients according to the $CaCl_2$ method described by Houba et al. (1990). $N_{dissolved}$, NH_4^+, NO_3^-, P and K^+ were analyzed in 0.01 M $CaCl_2$ extracts and the pH ($CaCl_2$) was determined. $N_{dissolved}$ includes NH_4^+, NO_3^- and organic nitrogen extracted by 0.01 M $CaCl_2$. Organic matter content of the mineral soil samples was determined by loss-on-ignition.

Statistics

Data were analyzed by analysis of variance (one-way ANOVA). If necessary, log transformation was used to obtain normality. Differences among means were evaluated by the Tukey-test (Sokal and Rohlf 1981) with a Bonferroni correction.

Results

Root growth and ectomycorrhizal development

No roots were found in any plots at the first sample date, May 1991. In November 1991 and May 1992, the number of ectomycorrhizal root tips and ramification index to a soil depth of 60 cm were significantly higher in the plots in the natural stand on nitrogen-poor sandy soil than in the plots in the nitrogen-enriched planted stand on podzolic sandy soil (Table 1). Also in November 1991 and May 1992, the root length and frequency of ectomycorrhizal root tips were generally higher in the natural stand plots than in the planted stand plots. Unfortunately, no comparisons could be made between root development in the natural and planted stand plots in November 1992; cores installed in the natural stand plots could not be harvested because someone had removed the labels.

In November 1991, the ramification index and frequency of ectomycorrhizal root tips to a soil depth of 60 cm were significantly higher in the sod-removed plots than in the sod-added plots (Table 1). In November 1991 and May 1992, the number of ectomycorrhizal root tips was generally higher in the sod-removed plots than in the control and sod-added plots indicating enhanced ectomycorrhizal development after sod removal. Root parameters did not differ between treatments in the planted stand in November 1992.

Root length and number of ectomycorrhizal root tips in the control plots generally decreased with depth (Fig. 1). This root profile from 0–60 cm indicated that

Table 1 Root length in 100 cm^3 soil (*RL*), number of ectomycorrhizal root tips in 100 cm^3 soil (*MT*), ramification index (*RI*) and frequency of ectomycorrhizal root tips (*FR*) in the soil to a depth of 60 cm. Differences between treatments at each sample time are indicated by different letters ($P<0.05$, Tukey) (*A* sod added, *C* control, *R* sod removed, *N* natural stand, *P* planted stand, *sed* standard error of difference)

November 1991	RL	MT	RI	FR
N-C	626.3b	2086.0b	18.8c	88.9b
P-C	203.4a	141.5a	4.0ab	41.9ac
P-R	265.8ab	424.7a	8.9b	67.8bc
P-A	297.4ab	202.5a	2.2a	29.6a
sed	422.4	1373.3	6.3	34.0
May 1992				
N-C	569.6a	2221.6b	23.3b	92.9b
P-C	226.6a	286.1a	8.2a	81.5ab
P-R	346.1a	400.4a	7.4a	81.6ab
P-A	284.4a	245.8a	4.3a	73.0a
sed	322.3	1080.8	10.5	22.7
November 1992				
N-C	–	–	–	–
P-C	926.9a	1793.6a	8.5a	72.8a
P-R	1064.7a	1515.0a	8.6a	75.2a
P-A	773.5a	1441.7a	9.8a	68.2a
sed	893.5	1657.3	5.3	23.7

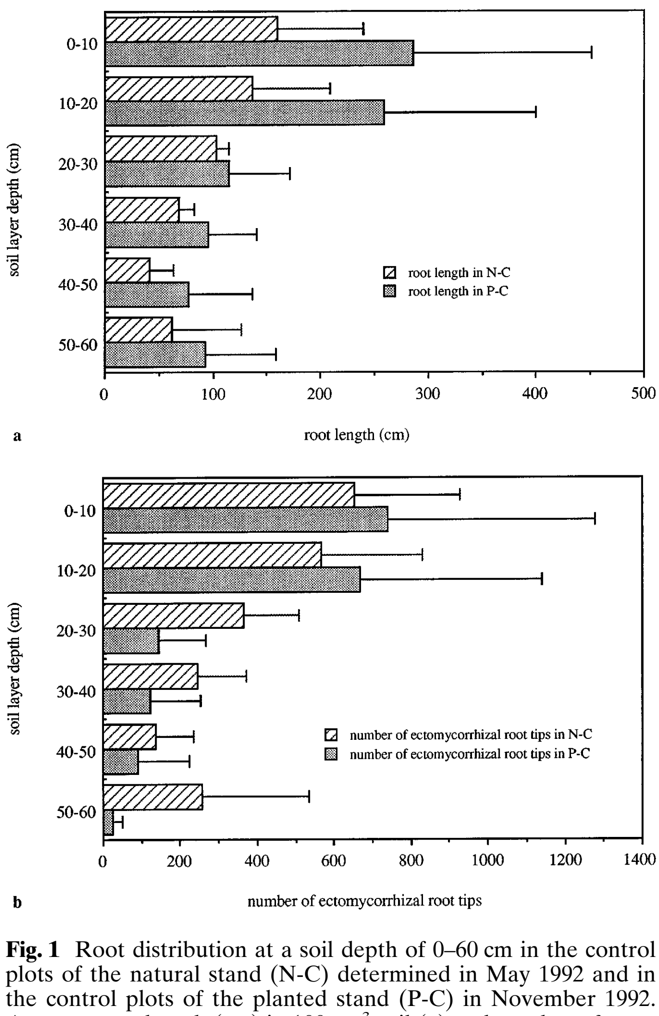

Fig. 1 Root distribution at a soil depth of 0–60 cm in the control plots of the natural stand (N-C) determined in May 1992 and in the control plots of the planted stand (P-C) in November 1992. Average root length (cm) in 100 cm^3 soil (**a**) and number of ectomycorrhizal root tips in 100 cm^3 soil (**b**) are presented per 10 cm soil layer

most Scots pine roots occurred in the upper soil layers.

All root parameters in the planted stand plots significantly increased from November 1991 to November 1992, except for the ramification index and frequency of ectomycorrhizal root tips in the sod-removed plots (Table 2).

Chemical composition of soils

Concentrations of $N_{dissolved}$, NH_4^+ and NO_3^- and organic matter content in the upper mineral soil in all planted stand plots on podzolic sandy soil in 1993 were generally higher than in the natural stand plots on non-podzolic sandy soil. In addition, pH in the planted stand plots was significantly lower than in the natural stand plots (Table 3). The NO_3^- concentrations in the upper mineral soil in the sod-added plots were significantly higher than in the remaining plots, but much lower than the NH_4^+ concentrations in all cases.

Discussion

The number of ectomycorrhizal root tips was higher in the nutrient-poor natural stand plots than in the nitrogen-enriched planted stand plots. In nutrient-poor soils, relatively large root systems, high fine root biomass and abundant ectomycorrhizal root tips have been found by several investigators (Keyes and Grier 1981; Wästerlund 1989; Bowen 1984; Olsthoorn 1991). The elongated root length and high number of ectomycorrhizal root tips in the mineral soil in the natural stand plots may also have been the result of a shift of root growth from humus to deeper soil layers, which would enhance water uptake (Feil et al. 1988; Persson 1992). Ectomycorrhizal formation has long been known to enhance nitrogen and phosphate uptake (Bledsoe and Zasosky 1983; Finlay 1989; Finlay et al. 1989).

Table 2 Comparison of root parameters in November 1991 and November 1992. Abbreviations as in Table 1

	RL	MT	RI	FR
N-C	–	–	–	–
P-C	*	*	*	*
P-R	*	*	ns	ns
P-A	*	**	***	**

* $P<0.05$, Tukey-test
** $P<0.001$
*** $P<0.0001$
ns not significant

Table 3 Nutrient concentrations (mg kg^{-1}), pH and organic matter content (*OM*, %) of the upper 5 cm of mineral soil sampled in April 1993. Significant differences between treatments are indicated by different letters ($P<0.05$, Tukey-test). Abbreviations as in Table 1

Plot	N_{diss}	NH_4^+	NO_3^-	P	K^+	pH	OM
N-C	3.7a	0.6a	0.1a	0.5a	25.4a	3.9b	0.9a
P-C	13.3b	3.3ab	1.5b	0.2a	15.4a	3.3a	4.5b
P-R	12.2b	3.2ab	1.0ab	0.2a	15.5a	3.4a	4.4b
P-A	16.8b	5.7b	2.9c	0.6a	16.9	3.2a	5.4b
sed	6.8	2.8	1.3	1.1	41.7	0.2	3.2

The low number of ectomycorrhizal root tips in the podzolic sandy soil was associated with the relatively high nitrogen concentrations and organic matter content and low pH in the upper 5 cm of the mineral soil. Baar and de Vries (1995) found a reduced number of ectomycorrhizal root tips of Scots pine seedlings in the podzolic soil of a secondary stand in 1992. Persson (1980b) noted a low ramification index in podzolic mineral soil of a 15- to 20-year-old Scots pine stand primarily because of formation and elongation of fast-growing roots.

In the present study, the highest number of ectomycorrhizal root tips was found in the upper 20 cm of the mineral soil, which is similar to earlier findings (Meyer 1973; Persson 1980b; Rastin et al. 1990). Ectomycorrhizal root tips, however, were found to a soil depth of 60 cm in nearly all plots. Several investigators have reported ectomycorrhizal root tips of Scots pine at depths of 1.5 m and 1.9 m (Werlich and Lyr 1957; Lobanow 1960). In The Netherlands, ectomycorrhizal root tips of *Pseudotsuga menziesii* (Mirb.) Franco were found at depths of up to 1.2 m, although not in large amounts (Olsthoorn and Tiktak 1991).

Ectomycorrhizal development rapidly followed root invasion, as indicated by the presence of ectomycorrhizal root tips at depths of 0–60 cm in the sod-removed plots. Mycelia of ectomycorrhizal fungi associated with Scots pine roots, specifically *Laccaria bicolor* (Maire) P.D. Orton, apparently survived in deeper layers of the mineral soil after sod-removal (Baar et al. 1994).

The progression of root invasion and ectomycorrhizal development in the 60-cm soil cores from the planted stand indicated recovery of root growth by the Scots pine trees after removal of litter, humus layers and herbaceous vegetation. This root invasion occurred simultaneously with an increase in above-ground species and sporocarps of ectomycorrhizal fungi in sod-removed plots, as noted by Baar (1996). In November 1992, however, the numbers of ectomycorrhizal root tips in the sod-removed plots did not differ from those in the control plots in the planted stands. Baar (1996) noted from field observations in these plots that the numbers of species and sporocarps of ectomycorrhizal fungi in the sod-removed plots were higher than in the control plots in 1992.

The occurrence of ectomycorrhizal fungi below ground was compared with that above ground because contrasting results have been reported. Jansen and de Nie (1988) noted a positive correlation between numbers of ectomycorrhizal root tips in the upper layers of the soil and sporocarp abundance in Douglas fir stands.

In contrast, Termorshuizen and Schaffers (1991) found no such correlation in Scots pine stands. The results of the present study suggest that the number of ectomycorrhizal root tips is poorly related to sporocarp abundance and that sporocarp formation does not necessarily reflect numbers (Dahlberg 1991) and activity (Wallander 1992) of ectomycorrhizal root tips.

The root parameters measured from samples of the humus layers and upper mineral soil in Germany and The Netherlands (Ritter and Tölle 1978; Kuyper 1990; Termorshuizen 1991) were lower than those measured in this study. Higher numbers of ectomycorrhizal root tips, however, were reported for Scots pine forests in Norway (142/cm^3 humus; Timmermann 1994) and Finland (16–35/cm^3 humus; Ohtonen et al. 1990) than in this study (5–8/cm^3 upper mineral soil). Species and sporocarps of ectomycorrhizal fungi in The Netherlands have declined due to high nitrogen input from air pollution (Arnolds 1991; Termorshuizen 1993). Negative correlations between ectomycorrhizal development and increasing nitrogen content of the soils have been found by several investigators (Blaschke 1981; Ohtonen et al. 1990; Termorshuizen and Ket 1991).

Use of the ingrowth technique may underestimate root growth (Nadelhoffer et al. 1985; Vogt and Persson 1991; Hendricks et al. 1993; Fahey and Hughes 1994). Nevertheless, the present study suggests that the ingrowth core technique can be used in comparative studies. Use of this technique revealed that sod-removal increased root growth and ectomycorrhizal development in Scots pine stands for at least 2 years after the treatment and that sod-addition had no effect.

In conclusion, root growth and ectomycorrhizal development recovered to a soil depth of 60 cm in a nitrogen-enriched planted Scots pine stand on podzolic soil after sod-cutting. The measured root parameters, however, were larger in the natural stand on non-podzolic sandy soil than in a nitrogen-enriched planted stand on podzolic sandy soil. Root growth and ectomycorrhizal development might have been favored by the nutrient-poor conditions of the soil in the natural stand plots.

Acknowledgements This is communication 578 of the Biological Station Wijster. The investigations were supported by the Foundation for the Life Sciences (SLW), which is subsidized by the Netherlands Organization for Scientific Research (NWO). I thank B.W.L. de Vries, F.W. de Vries and M. Oude Elferink for their practical help and Dr K. Gerow for his advice on the statistical analysis. And I am grateful to Drs. L. Brussaard, M. Christensen, A. Olsthoorn, P.D. Stahl and N.L. Stanton for reading the manuscript.

References

Ahlström K, Persson H, Börjesson I (1988) Fertilization in a mature Scots pine (*Pinus sylvestris* L.) stand. Effects on fine roots. Plant Soil 106:179–190

Alexander IJ, Bigg WL (1981) Light microscopy of ectomycorrhizas using glycol methacrylate. Trans Br Mycol Soc 77:425–429

Alvarez IF, Rowney DL, Cobb FW Jr (1979) Mycorrhizae and growth of white fir seedlings in mineral soil with and without organic layers in a California forest. Can J For Res 9:311–315

Arnolds E (1991) Decline of ectomycorrhizal fungi in Europe. Agric Ecosyst Environ 35:209–244

Baar J (1996) The ectomycorrhizal flora of primary and secondary stands of *Pinus sylvestris* in relation to soil conditions and ectomycorrhizal succession. J Veg Sci 7:497–504

Baar J, Braak CJ ter (1996) Ectomycorrhizal sporocarp abundance as affected by manipulation of litter and humus layers in Scots pine stands of different age. App Soil Ecol 4:61–73

Baar J, Kuyper TW (1993) Litter removal in forests and effect on mycorrhizal fungi. In: Pegler DN, Boddy L, Ing B, Kirk PM (eds) Fungi of Europe: investigation, recording and mapping. Kew Gardens, London, pp 275–286

Baar J, Vries FW de (1995) Effects of manipulation of litter and humus layers on ectomycorrhizal colonization potential in Scots pine stands of different age. Mycorrhiza 5:267–272

Baar J, Ozinga WA, Kuyper TW (1994) Spatial distribution of *Laccaria bicolor* genets reflected by sporocarps after removal of litter and humus layers in a *Pinus sylvestris* forest. Mycol Res 98:726–728

Blaschke H (1981) Veränderung bei der Feinwurzelentwicklung in Weisstannenbeständen. Forstwiss Centralbl 100:190–195

Bledsoe CS, Zasosky RJ (1983) Effects of ammonium and nitrate on growth and nitrogen uptake by mycorrhizal Douglas-fir seedlings. Plant Soil 71:445–454

Bowen GD (1984) Tree roots and the use of soil nutrients. In: Bowen GD, Nambiar EKS (eds) Nutrition of plantation forests. Academic Press, London, pp 147–179

Dahlberg A (1991) Ectomycorrhiza in coniferous forests: structure and dynamics of populations and communities. PhD thesis, Swedish University of Agricultural Sciences, Uppsala, Sweden

Dijk HFG van, Creemers RCM, Rijniers JPLWM, Roelofs JGM (1989) Impact of artificial ammonium-enriched rainwater on soils and young coniferous trees in a greenhouse. I. Effect on soils. Environ Pollut 62:317–366

Draaijers GPJ, Ivens WPMF, Bos MM, Bleuten W (1989) The contribution of ammonia emissions from agriculture to the deposition of acidifying and eutrophying compounds on stands. Environ Pollut 60:55–66

Fahey TJ, Hughes JW (1994) Fine root dynamics in a northern hardwood forest ecosystem, Hubbard Brook Experimental Forest, NH. J Ecol 82:533–548

FAO-Unesco (1988) Soil map of the world. Revised legend. World Soil Resources, Report 60. Food and Agricultural Organisation of the United Nations, Rome

Feil W, Kottke I, Oberwinkler F (1988) The effect of drought on mycorrhizal production and very fine root system development of Norway spruce under natural and experimental conditions. Pant Soil 108:221–231

Finlay RD (1989) Functional aspects of phosphorus uptake and carbon translocation in incompatible ectomycorrhizal associations between *Pinus sylvestris* and *Suillus grevillei* and *Boletinus cavipes*. New Phytol 122:185–192

Finlay RD, Ek H, Odham G, Söderström B (1989) Uptake, translocation and assimilation of nitrogen from ^{15}N-labelled ammonium and nitrate sources by intact ectomycorrhizal systems of *Fagus sylvatica* with *Paxillus involutus*. New Phytol 113:47–55

Hendricks JJ, Nadelhoffer KJ, Aber JD (1993) Assessing the role of fine roots in carbon and nutrient cycling. Trends Ecol Evol 8:174–178

Houba VJG, Novozamsky I, Lexmond TM, Lee JJ van der (1990) Applicability of 0.01 M $CaCl_2$ as a single extraction solution for the assessment of the nutrient status of soils and other diagnostic purposes. Commun Soil Sci Plant Anal 21:2281–2290

Jansen AE, Nie HW de (1988) Relations between mycorrhiza and fruitbodies of mycorrhizal fungi in Douglas fir plantations in the Netherlands. Acta Bot Neerl 37:243–249

Keyes MR, Grier CC (1981) Above- and belowground net production in 40-year-old Douglas fir stands on low and high productivity sites. Can J For Res 11:599–605

Klap JM, Schmidt P (1992) Maatregelen om effekten van eutrofiëring en verzuring in bossen tegen te gaan. (English summary) Hinkeloord reports 2:1–140 Department of Forestry, Agricultural University Wageningen, The Netherlands

Kuyper ThW (1988) The effect of forest fertilization on the abundance and diversity of ectomycorrhizal fungi. In: Jansen AE, Dighton J, Bresser AHM (eds) Ectomycorrhizas and acid rain. Commission of the European Communities, Brussel, pp 146–149

Kuyper TW (1990) Some effects of forest fertilization on ectomycorrhizal fungi and ectomycorrhizas: implications for tree responses to nitrogen pollution. In: Persson H (ed) Above and below-ground interactions in forest trees in acidified soils. Air Pollution Research Report 32. Commission of the European Communities, Brussel, pp 66–73

Lobanow NW (1960) Mykotrophie der Holzpflanzen. Wissenschaftliche Verlagsgesellschaft, Berlin

Markkola AM, Ohtonen R (1988) The effect of acid deposition on fungi in forest humus. In: Jansen AE, Dighton J, Bresser AHM (eds) Ectomycorrhizas and acid rain. Commission of the European Communities, Brussel, pp 122–126

Meyer FH (1973) Distribution of ectomycorrhizae in native and man-made forests. In: Marks GC, Kozlowski TT (eds) Ectomycorrhizae. Academic Press, New York, pp 79–102

Meyer FH (1987) Der Verzweigungsindex, ein Indikator für Schäden am Feinwurzelsystem. Forstwiss Centralbl 106:84–92

Nadelhoffer KJ, Aber JD, Melilo JM (1985) Fine roots, net primary production and soil nitrogen availability: a new hypothesis. Ecology 66:1377–1390

Newman EI (1966) A method of estimating the total length in a root sample. J Appl Ecol 3:139–145

Ohtonen R, Markkola AM (1991) Biological activity and amount of FDA mycelium in mor humus of Scots pine stands (*Pinus sylvestris* L.) in relation to soil properties and degree of pollution. Biogeochemistry 13:1–26

Ohtonen R, Markkola AM, Heinonen-Tanski H, Fritze H (1990) Soil biological parameters as indicators of changes in Scots pine forests (*Pinus sylvestris* L.) caused by air pollution. In: Kauppi P, Anttilla P, Kenttamies K (eds) Acidification in Finland. Springer Verlag, Berlin, pp 373–393

Olsthoorn AFM (1991) Fine root density and root biomass of Douglas-fir stands on sandy soils in The Netherlands. 1. Root biomass in early summer. Neth J Agric Sci 39:49–60

Olsthoorn AFM, Tiktak A (1991) Fine root density and root biomass of two Douglas-fir stands on sandy soil in the Netherlands. 2. Periodicity of fine root growth and estimation of belowground carbon allocation. Neth J Agric Sci 39:61–77

Pearson J, Stewart GR (1993) Tansley Review No. 56. The deposition of atmospheric ammonia and its effects on plants. New Phytol 125:283–305

Persson H (1979) Fine-root production, mortality and decomposition in forest ecosystems. Vegetatio 41:101–109

Persson H (1980a) Spatial distribution of fine-root growth, mortality and decomposition in a young Scots pine stand in Central Sweden. Oikos 34:77–87

Persson H (1980b) Fine-roots dynamics in a Scots pine stand with and without near-optimal nutrient and water regimes. Acta Phytogeog Suec 68:101–110

Persson H (1984) The dynamic fine roots of forest trees. In: Agren GI (ed) State and change of forest ecosystems. Indicators in current research. Swedish University of Agricultural Sciences, Uppsala, Sweden. Report No. 13, pp 193–204

Persson H (1992) Factors affecting fine-root dynamics of trees. Suo 43:163–172

Rastin N, Schlechte G, Hüttermann A (1990) Soil macrofungi and some soil biological, biochemical investigations on the upper and lower slope of a spruce forest. Soil Biol Biochem 22:1039–1947

Ritter G, Tölle H (1978) Stickstoffdüngung in Kieferbeständen und ihre Wirkung auf Mykorhizabildung und Fruktifikation der Symbiosepilze. Beitr Forstwiss 12:162–166

Rose SL, Perry DA, Pilz D, Schoeneberger MM (1983) Allelopathic effects of litter on the growth and colonization of mycorrhizal fungi. J Chem Ecol 9:1153–1162

Schoeneberger MM, Perry DA (1983) The effect of soil disturbance on growth and ectomycorrhizae of Douglas-fir and western hemlock seedlings: a greenhouse bioassay. Can J For Res 12:343–353

Sokal RR, Rohlf FJ (1981) Biometry, 2nd edn. Freeman, San Francisco

Tamm CO (1991) Nitrogen in terrestrial ecosystems. Springer Verlag, Berlin

Termorshuizen AJ (1991) Succession of mycorrhizal fungi in stands of *Pinus sylvestris* in the Netherlands. J Veg Sci 2:555–564

Termorshuizen AJ (1993) The influence of nitrogen fertilisers on ectomycorrhizas and their fungal carpophores in young stands of *Pinus sylvestris*. For Ecol Manage 57:179–189

Termorshuizen AJ, Ket PC (1991) Effects of ammonium and nitrate on mycorrhizal seedlings of *Pinus sylvestris*. Eur J For Pathol 21:404–413

Termorshuizen AJ, Schaffers A (1991) The decline of carpophores of ectomycorrhizal fungi in stands of *Pinus sylvestris* L. in The Netherlands: possible causes. Nova Hedwigia 53:267–289

Timmermann V (1994) The mycorrhizal status of Norway spruce under different nitrogen loads. A comparative study of three oligotrophic forest stands in Northern Europe along a regional and an experimentally induced local nitrogen gradient. Candidate scientific thesis in ecological botany, University of Oslo

Veerkamp MT, Kuyper ThW (1993) Mycological investigations in Dutch forest reserves. In: Broekmeyer MEA, Vos W, Koop H (eds) European forest reserves. Pudoc, Wageningen, pp 127–143

Vogt KA, Persson H (1991) Measuring growth and development of roots. In: Hinckley T, Lassoie JP (eds) Techniques and approaches in forest ecophysiology. CRC Press, Boca Raton, Fla, pp 477–501

Vries BWL de, Jansen AE, Barkman JJ (1985) Verschuivingen in het soortenbestand van fungi in naaldbossen van Drenthe, 1958–1983. In: Arnolds E (ed) Veranderingen in de paddestoelenflora (mycoflora). Wetensch Meded Kon Ned Natuurhist Ver, Hoogwoud, pp 74–83

Vries BWL de, Jansen E, Dobben H, Kuyper TW (1995) Partial restoration of fungal and plant species diversity by removal of litter and humus layers in stands of Scots pine in The Netherlands. Biodivers Conserv 4:156–164

Wallander H (1992) Regulation of ectomycorrhizal symbiosis in *Pinus sylvestris* L. seedlings. Influence on mineral nutrition. PhD thesis, University of Uppsala, Sweden

Wästerlund I (1989) Hur påverkar skogens skötsel förekomsten av storsvampars fruktkroppar? Sven Bot Tidskr 83:103–112

Werlich I, Lyr H (1957) Über die Mykorhizaausbildung von Kiefer (*Pinus sylvestris* L.) und Buche (*Fagus sylvatica* L.) auf verschiedenen Standorten. Arch Forstwes 6:1–23