ECTOMYCORRHIZAL FUNGI OF SCOTS PINE AS AFFECTED BY LITTER AND HUMUS

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ECTOMYCORRHIZAL FUNGI OF SCOTS PINE AS AFFECTED BY LITTER AND HUMUS

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STELLINGEN

1. Plaggen in Grove-dennenbossen leidt tot herstel van de ectomycorrhizaflora en kan worden toegepast als beheersmaatregel.
   Dit proefschrift.

2. Het aantal soorten ectomycorrhizaschimmels is negatief gecorreleerd met de concentratie anorganische stikstof in de bodem.
   Dit proefschrift.

3. Het voorkomen van vruchtlichamen van ectomycorrhizaschimmels in Grove-dennenbossen in Nederland is niet in overeenstemming met de indeling in "early-" and "late-stage" fungi, het C-S-R-concept en de classificatie volgens epidemiologische kenmerken.
   Dit proefschrift.
   Deacon, J.W., Donaldson, S.J. and Last, F.T. 1983. Plant Soil 71: 257-262.
   Grime, J.P. 1985. In: The population structure of vegetation (Ed. White, J.), pp. 503-514. Junk, Dordrecht.
   Mason, P.A., Last, F.T., Pelham, J. and Ingleby, K. 1982. For. Ecol. Manag. 4: 19-39.
   Newton, A.C. 1992. Mycorrhiza 2: 75-79.

4. De huidige maat voor de vitaliteit van Grove-dennebomen aan de hand van uiterlijke kenmerken geeft onvoldoende informatie over de groei en fysiologie van deze bomen.
   Dit proefschrift.
   Van den Ancker, J.A.M., Evers, P.W., Maessen, P.P.Th.M., Oterdoom, J.H. and Van den Tweel, P.A. 1987. Ned. Bosbouwtijds. 59: 405-417.

5. Niet de kwantiteit, maar de kwaliteit van organische stof op en in de bosbodem is in de eerste plaats bepalend voor het voorkomen van vruchtlichamen van ectomycorrhizaschimmels in Grove-dennenbossen.

6. De oplossing van het mestprobleem in Nederland is niet het injecteren van mest in de bodem, maar het grondig reduceren van de veestapel.

7. Diervriendelijke produkten kan men beter in de mond nemen, dan dat men er de mond vol van heeft.

8. Na het onbezoldigd promoveren van vrouwen volgt het onbezoldigd promoveren van allochtonen, gehandicapten en uiteindelijk mannen.

9. Om het aantal studenten aan de universiteiten te verminderen verdient selectie aan de poort de voorkeur boven de tempobeurs.

10. In Nederland wegen ervaring en capaciteiten van mensen van ± 45 jaar en ouder ruimschoots op tegen de hoge arbeidsproduktiviteit van jongere mensen.

11. Het is merkwaardig dat hoofdzakelijk mensen met vaste banen spreken over flexibilisering van de arbeid voor mensen zonder vaste banen.

12. In Noord- en Centraal-Australië kunnen "kangoeroevangers" op auto's beter worden omgedoopt tot "koeievangers".

Stellingen behorend bij het proefschrift "Ectomycorrhizal fungi of Scots pine as affected by litter and humus".
Jacqueline Baar, Wageningen, 9 juni 1995.

ABSTRACT

Removal of litter and humus layers and herb vegetation dominated by the grass *Deschampsia flexuosa* ("sod-cutting") in Scots pine (*Pinus sylvestris*) stands enhanced numbers of species and sporocarps of ectomycorrhizal fungi, particularly in middle-aged and old stands. Three and a half years after sod-cutting soil conditions and the ectomycorrhizal flora in a middle-aged secondary stand on non-podzolic sandy soil came to closely resemble the nutrient-poor soil conditions and rich ectomycorrhizal flora in a spontaneously established Scots pine stand in a drift sand area. Sporocarps of ectomycorrhizal species, among which *Cantharellus cibarius*, *Coltricia perennis*, *Rhizopogon luteolus*, *Suillus bovinus* and *Tricholoma albobrunneum* were observed in the sod-cut plots, and not in the control plots. Sod-cutting on a podzolic soil was less effective due to higher nutrient concentrations and lower pH in the mineral soil compared to non-podzolic soil. After sod-cutting on podsolic and non-podsolic sandy soil, the tree roots recovered in the mineral soil up to a depth of 60 cm. The ectomycorrhizal colonization potential also increased after the treatment.

Addition of litter and humus layers to existing ectorganic layers ("sod-adding") in young and middle-aged stands did not affect numbers of species and sporocarps of ectomycorrhizal fungi and ectomycorrhizal development in the mineral soil up to a soil depth of 60 cm, but reduced ectomycorrhizal colonization potential.

Ectomycorrhizal succession during stand development is mainly driven by soil processes, whereas tree ageing plays a less important role.

Laboratory experiments were carried out to provide a lower-level explanation for the results of the field experiments. Development of *Laccaria bicolor*, *R. luteolus* and *S. bovinus* on Scots pine seedlings on forest soils in growth chambers was largely in accordance with field observations. Extracts of Scots pine needles and shoots and roots of the grass *D. flexuosa* containing considerable amounts of nitrogen and phenolic compounds reduced development of *Laccaria proxima* and *R. luteolus* in pure culture. Sporocarps of these fungi were mainly found in young Scots pine stands or in sod-cut plots. *Paxillus involutus* and *Xerocomus badius* were less sensitive to needle and grass extracts, although sporocarps of those species were observed in older stands. *Laccaria bicolor*, which was insensitive to needle and grass extracts, could persist as mycelium in grassy Scots pine stands, but tremendously increased sporocarp production after sod-cutting.

The results of the field and laboratory experiments show that sod-cutting in Scots pine stands with thick nitrogen-rich litter and humus layers leads to a (partial) restoration of the ectomycorrhizal flora.

Keywords: ectomycorrhiza, humus, litter, nitrogen, phenolics, *Pinus sylvestris*, restoration management, sod-cutting, succession

A sod-cut plot in a Scots pine stand planted in 1974 (S3).

CONTENTS

VOORWOORD

Een ieder, die me op enigerlei wijze geholpen heeft bij het onderzoek en het tot stand komen van dit proefschrift, wil ik bedanken voor zijn of haar hulp. Een aantal van hen wil ik met naam noemen.

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Jacqueline
30 april 1995.

GENERAL INTRODUCTION

(ECTO)MYCORRHIZAL FUNGI

A mycorrhizal fungus usually lives with its plant partner in a balanced intimate association from which both derive benefit. The fungus enlarges the root system of the plant and takes up water and nutrients from the soil. The host plant supplies the mycorrhizal fungus with carbon. There are several kinds of mycorrhizas: a.o. ericoid, ecto- and arbuscular mycorrhizas (Jackson and Mason, 1984).

Trees belonging to *Pinaceae*, *Betulaceae*, *Corylaceae*, *Fagaceae* and *Salicaceae* form ectomycorrhizas. An ectomycorrhizal root tip (ectomycorrhiza) consists of a fungal sheath surrounding the tree root (mantle) and hyphae of the fungus between the cortical cells of the root (Hartig net). The structure of the mantle and the extent of outgrowth of hyphae into the soil (extramatrical hyphae) depend on the fungal species (Harley and Smith, 1983; Jackson and Mason, 1984). Coniferous trees have large numbers of ectomycorrhizal root tips and roots without ectomycorrhizal root tips are rare (Termorshuizen, 1990; Dahlberg, 1991). Most ectomycorrhizal fungi take up inorganic nutrients, but it has been shown that some ectomycorrhizal fungi can utilize nutrients from organic sources (Dighton, 1991; Read, 1991). Ectomycorrhizal fungi furthermore increase resistance of plants against water shortage, aluminium and heavy metals, enhance protection against soil pathogens and produce phytohormones (Marks and Kozlowski, 1973).

Arnolds and De Vries (1989) listed 652 fungal species of the 3400 macromycetes known from the Netherlands as forming ectomycorrhizal root tips. The majority of the ectomycorrhizal fungi belongs to the Basidiomycetes (Harley, 1989). Most ectomycorrhizal fungi form epigeous sporocarps and some hypogeous sporocarps. Ectomycorrhizal fungi belong to several families, e.g. *Boletaceae*, *Russulaceae*, *Amanitaceae*, *Cortinariaceae*, *Tricholomataceae* (epigeous species) or to a number of unrelated taxa in the Gasteromycetes (hypogeous species). Only very few ectomycorrhizal fungi like *Cenococcum geophilum* do not form sporocarps, but only sclerotia.

SUCCESSION OF ECTOMYCORRHIZAL FUNGI

The ectomycorrhizal species composition changes with ageing of trees and forests. Some ectomycorrhizal fungi mainly occur in young (< 15 years) stands, others in older (> 50 years) stands (Ricek, 1981; Termorshuizen, 1991; Keizer and Arnolds, 1994). Species richness of ectomycorrhizal fungi increases with stand age until canopy closure is reached and

litter begins to accumulate, and thereafter it decreases (Dighton and Mason, 1985; Jansen, 1991). In The Netherlands this pattern has been observed in Douglas fir and Scots pine stands (Jansen, 1991; Termorshuizen, 1991).

Deacon et al. (1983) introduced the concept of ectomycorrhizal succession with "early-" and "late-stage" fungi. "Early-stage" fungi can readily infect isolated seedlings growing in unsterile soils, whereas "late-stage" fungi cannot, at least not outside forests (Deacon et al., 1983; Dighton and Mason, 1985). "Early-stage" fungi are characteristic of very young trees (< 4 years) and are replaced by "late-stage" fungi (Dighton and Mason, 1985). Last et al. (1987) described "early-stage" fungi as fungi adapted to mineral soil resources and to competition with other ectomycorrhizal fungi in soils with little or no tree litter. In contrast, "late-stage" fungi are able to grow in soils with accumulated litter and are hypothesized to derive nutrients from organic material. This classification is a special case of the r-K concept. r-Selected organisms have a short life expectancy and a high intrinsic rate of increase contrary to K-selected organisms (MacArthur and Wilson, 1967; Pianka, 1970). Grime (1985) expanded the r-K selection to C-S-R selection as many organisms do not fit in the r-K spectrum. The C-S-R concept recognizes three strategies: combative (C), stress tolerant (S) and ruderal (R). These strategies can be used to describe behaviour of organisms, but cannot be used to classify organisms. Strategies may vary under different circumstances or at different stages of the life-cycle (Dahlberg, 1991). Since these classifications have a number of limitations, particularly the lack of precision of terminology adopted and of general applicability, Newton (1992) proposed a functional classification of ectomycorrhizal fungi based on epidemiological characteristics. He suggested that ectomycorrhizal succession can be explained by the relative ability of different fungi to colonize and spread from different sources of inoculum such as spores and mycelial spread.

DECLINE OF ECTOMYCORRHIZAL FUNGI

In The Netherlands many species of ectomycorrhizal fungi in coniferous stands situated on nutrient-poor soils have decreased during this century (Arnolds, 1985; Arnolds, 1991; Arnolds and Jansen, 1992). Derbsch and Schmitt (1987) reported a similar decline of ectomycorrhizal fungi in Germany. The decline of ectomycorrhizal fungi in older stands exceeds that during natural succession, and is most likely caused by air pollution (Arnolds, 1991).

In The Netherlands numbers of species and sporocarps of ectomycorrhizal fungi in 50- to 80-year-old Scots pine stands in areas with heavy air pollution by nitrogen were reduced compared to the beginning of this century and compared to stands in areas with less air pollution (Termorshuizen and Schaffers, 1987; Termorshuizen, 1991). Several ectomycorrhizal species characteristic of *Cladonia-Pinus* forests on dry, acidic, nutrient-poor sandy soils such as *Cantharellus cibarius*, *Hygrophorus hypotheius*, *Suillus luteus* and

Tricholoma portentosum have decreased (Arnolds, 1991; Termorshuizen, 1991). Abundunce and diversity of species and sporocarps of ectomycorrhizal fungi were high in young (< 15 years) Scots pine stands compared to those in old stands. However, ectomycorrhizal fungi, among which *H. hypotheius*, *S. luteus* and *T. portentosum*, occurring in these young stands frequently occur in undisturbed, old stands in Scandinavia (Termorshuizen, 1991). Similar observations of reduced species richness and abundance of ectomycorrhizal fungi have been made in stands of *Pseudotsuga menziesii* in The Netherlands in areas with high nitrogen pollution by Jansen and De Vries (1988).

A decrease of ectomycorrhizal species diversity in Douglas fir stands was observed both above and below ground (Jansen and De Nie, 1988). This pattern was not clear in Scots pine stands (Termorshuizen and Schaffers, 1991).

DECLINE OF ECTOMYCORRHIZAL FUNGI IN RELATION TO AIR POLLUTION

Several decades ago in The Netherlands a strongly intensifying livestock industry began to raise ammonia emissions and hence, depositions of NH_3 and NH_4^+. Nowadays ammonia is the most important air pollutant in The Netherlands (Van Dijk, 1993; Pearson and Stewart, 1993). Average immission values of ammonia range from 40 to 80 kg ha^{-1} yr^{-1} with local extremes around 100 kg ha^{-1} yr^{-1} (Van Aalst, 1984; Draaijers et al., 1989; Van Dijk et al., 1989). These extreme immissions have a fertilizing effect, especially on nitrogen-poor soils. Ammonia immissions are highest in sandy areas (Van Dijk, 1993). Excess of ammonium leads to ammonium accumulation and shortages in magnesium, potassium and phosphorus in soil and needles of coniferous trees (Roelofs et al., 1985; Van Dijk and Roelofs, 1988).

Numbers of sporocarps of ectomycorrhizal fungi and ectomycorrhizal root tips are highest in soils with low levels of available nutrients (Jansen and Dighton, 1990). Mineral nitrogen is usually in short supply in the ectorganic layers where most ectomycorrhizal root tips and mycelia occur (Dighton, 1991). Input of nitrogen due to air pollution directly reduces growth of most ectomycorrhizal fungi except for nitrophilous species (Jansen and Dighton, 1990). Termorshuizen and Schaffers (1991) noted that numbers and dry weight of sporocarps of ectomycorrhizal fungi in 50- to 80-year-old Scots pine stands were strongly negatively correlated with NH_3 and NO_x. Low numbers of species of ectomycorrhizal fungi were observed in Douglas fir stands older than 20 years in areas with high nitrogen deposition (Jansen and De Vries, 1988). These observations are in accordance with reduction of numbers of species and sporocarps of ectomycorrhizal fungi as a result of nitrogen fertilization (Ritter and Tölle, 1978; Wästerlund, 1982a; Ohenoja, 1988; Shubin, 1988; Termorshuizen, 1993). Nitrogen fertilization appears to inhibit fructification to a larger extent than formation of ectomycorrhizal root tips, and the decrease of sporocarps of ectomycorrhizal fungi seems to be larger in older stands than in young stands before canopy closure (Kuyper, 1989; Termorshuizen and Schaffers, 1991). The effects are most pronounced on dry and nutrient-

poor soils (Ohenoja, 1988).

In experiments *in vitro* with inoculated Scots pine seedlings, formation and abundance of ectomycorrhizal root tips were also reduced by high external nitrogen concentrations (Termorshuizen and Ket, 1991; Wallander and Nylund, 1991).

DECLINE OF ECTOMYCORRHIZAL FUNGI IN RELATION TO LITTER AND HUMUS ACCUMULATION

As a result of nitrogen deposition from the air, the build-up of litter and humus layers in stands on originally nutrient-poor acidic soil proceeds at a faster rate. Simultaneously decomposition of nitrogen-rich recalcitrant litter and humus is reduced (Kuyper, 1988; Markkola and Ohtonen, 1988). A negative effect of thick humus layers polluted with nitrogen and sulphur from the air on ectomycorrhizal root tips has been observed by Markkola and Ohtonen (1988). De Vries et al. (1985) noted significant negative correlations between numbers of ectomycorrhizal species above ground and thickness of litter and humus layers in The Netherlands.

The grass *Deschampsia flexuosa* has enormously increased in Scots pine stands in The Netherlands, partly as a result of changed soil chemistry due to nitrogen deposition (Tamm, 1991; Van der Werf, 1991), partly due to natural succession (Fanta, 1992). Negative correlations have been found between the abundance of macromycete sporocarps and grass cover (Barkman, 1976; Veerkamp and Kuyper, 1992).

Kallenbach (cited in Grosse-Brauckmann and Grosse-Brauckmann, 1978) noted that management practices such as removal of litter positively affected ectomycorrhizal fungi, especially hydnaceous fungi. Termorshuizen (1991) counted 94 times more sporocarps of ectomycorrhizal fungi on a small spot where unknowns illegally had removed litter and humus layers and herb vegetation in a Scots pine stand in The Netherlands. Removal of litter and humus layers and herb vegation dominated by *D. flexuosa* ("sod-cutting") on small sites in old (40-80 years) Scots pine stands increased numbers of species and sporocarps of ectomycorrhizal fungi two years after the treatment. Sporocarps of *Amanita gemmata*, *C. cibarius*, *Rhizopogon luteolus*, *Suillus bovinus* were observed in the sod-cut plots in higher numbers than in the control plots (De Vries et al., 1995). These species have declined in The Netherlands during the last decades and are threatened nowadays (Arnolds, 1989; Arnolds, 1991).

These findings suggest negative effects of accumulated litter and humus on ectomycorrhizal fungi. Accumulation of pollutants, mainly nitrogen in The Netherlands, to toxic concentrations or the formation of recalcitrant nitrogen-phenolics in litter and humus layers may explain the decline of ectomycorrhizal fungi (Kuyper, 1988). However, the underlying mechanisms between litter and humus accumulation and the occurrence of ectomycorrhizal fungi are unknown.

DECLINE OF ECTOMYCORRHIZAL FUNGI AND TREE VITALITY

Termorshuizen and Schaffers (1987) and Jansen and De Vries (1988) noted positive correlations between numbers of sporocarps of ectomycorrhizal fungi and tree vitality of Scots pine and Douglas fir. Positive correlations were also found between occurrence of ectomycorrhizal root tips and tree vitality (Meyer, 1988; Jansen and De Vries, 1988). Reduced tree vitality is considered an important cause of decline of ectomycorrhizal fungi below and above ground (Meyer, 1984; Gulden and Høiland, 1985; Arnolds, 1988). Reduced tree vitality may cause a decrease of ectomycorrhizal development and changed soil chemistry may lead to diminished ectomycorrhizal development and hence reduced uptake of cations causing reduced tree vitality. However, relative importance of above- and below-ground processes due to air pollution and interactions between the two are still unclear.

SCOTS PINE (PINUS SYLVESTRIS)

For several reasons, the experiments of the present study were carried out with *P. sylvestris*. Scots pine is able to grow on nutrient-poor soils and is quantitatively the most important tree species in Dutch forests in the Pleistocene region.

Scots pine occurred in North West Europe 9500 B.C. and dominated the eastern part of The Netherlands. After 7500 B.C. Scots pine was replaced by deciduous trees and disappeared (Weeda et al., 1985). Since the 19th century Scots pine has often been planted on former drift sands and heathlands. The soils were sometimes fertilized with acorns, compost or ground basic slag. During stand development the poorly growing and deformed trees were felled at regular time intervals in order to give other trees more space and light. Scots pine stands are often clear-felled for silvicultural reasons before trees reach maximum age. Scots pine stands planted on clear-felled sites with developed humus profiles are indicated with secondary stands. Nowadays, the understorey vegetation in these stands is often dominated by the grass *D. flexuosa* (Weeda et al., 1985). In drift sand areas in The Netherlands spontaneously grown (primary) Scots pine stands are still found. The understorey vegetation of the young stands consists mainly of *Cladonias* and mosses (Weeda et al., 1985). A rich ectomycorrhizal flora has been reported from these stands by Termorshuizen (1991).

Other reasons to study Scots pine are that ectomycorrhizal fungi associated with Scots pine stands in The Netherlands are well known as a result of a monitoring study by Termorshuizen (1990) and a fertilization experiment by Kuyper and De Vries (1990). Experience with removal of litter and humus and herb vegation in 40- to 80-year-old Scots pine stands was obtained in a small-scale study by De Vries et al. (1995). Furthermore, Scots pine seedlings are relatively easily grown and can be inoculated with ectomycorrhizal fungi which is useful in laboratory experiments.

OBJECTIVES OF THE STUDY

The main objectives of the present study were:

1. to investigate whether ectomycorrhizal fungi are negatively affected by litter and humus layers. In these layers complex organic substances, a.o. humus compounds and phenolics, nitrogen compounds originating from air pollution, and their interaction products might exert allelopathic effects on those fungi (Kuyper, 1988; Arnolds, 1991). Litter and humus accumulate with ageing of trees and stands and are mixed with mineral soil when clear-cut areas are replanted (Nohrstedt et al., 1989; Arnolds, 1991).

2. to investigate whether ectomycorrhizal fungi decrease as a result of reduced tree vitality.

If indeed litter and humus layers negatively influence ectomycorrhizal fungi, manipulation of these layers might be used as a means for restoration of the ectomycorrhizal flora in Scots pine stands. Removal of litter and humus layers may change ectomycorrhizal species diversity in stands with thick litter and humus layers towards that in stands with thin litter and humus layers as observed in spontaneously grown stands in drift sand areas (Termorshuizen, 1991).

OUTLINE OF THIS THESIS

In chapters 2 and 3 a field experiment is described in which litter and humus layers in Scots pine stands of different age were manipulated. The main objectives of this experiment were to investigate whether removal of litter and humus layers raises the numbers of species and sporocarps of ectomycorrhizal fungi and whether addition of litter and humus reduces these numbers. The results of this experiment are discussed in relation to soil chemistry, ectomycorrhizal succession and tree vitality. Chapter 4 is devoted to development of ectomycorrhizal root tips of Scots pine in relation to soil chemistry and stand age. In chapter 5 the effects of manipulation of litter and humus layers on ectomycorrhizal inoculum potential of Scots pine are described. In chapter 6 a laboratory study is described in which the effects of different forest soils on development of several ectomycorrhizal fungi associated with Scots pine seedlings were studied. The ectomycorrhizal development is discussed in relation to soil chemistry. In chapter 7 attention is paid to the effects of extracts of Scots pine needles and the grass *D. flexuosa* containing large amounts of nitrogen and phenolics on the growth of ectomycorrhizal fungi *in vitro*. The performance of ectomycorrhizal fungi on solid media with different glucose concentrations as carbon source, with different concentrations of ammonium as inorganic nitrogen source and different concentrations of glycine as organic nitrogen source is decribed in chapter 8. The ectomycorrhizal performance is determined on the basis of growth rate, biomass and fractal geometry. Chapter 9 is focused on the population structure of *Laccaria bicolor* in plots where litter and humus layers had been removed.

THE OCCURRENCE OF SPOROCARPS OF ECTOMYCORRHIZAL FUNGI AS AFFECTED BY MANIPULATION OF LITTER AND HUMUS LAYERS IN SCOTS PINE STANDS OF DIFFERENT AGE

SUMMARY

In a field study litter and humus layers and herb vegetation were removed ("sod-cutting") in stands of Pinus sylvestris *of different age in 1990. Removed litter and humus were added on existing ectorganic layers ("sod-addition") in young and middle-aged stands. Control treatments were present. Surveys carried out in 1993 showed that removal of litter and humus layers enhanced numbers of species and sporocarps of ectomycorrhizal fungi mainly in middle-aged and old stands. The enhancement was smaller in stands on podzolic sandy soil. Addition of litter and humus layers did not affect numbers of species and sporocarps of ectomycorrhizal fungi. In 1993, chemical analyses showed a reduction of the* $N_{dissolved}$, NH_4^+, NO_3^- *and P concentrations and an increase of pH of ectorganic layers as a result of sod-cutting. Nutrient concentrations in ectorganic layers and needles were not affected by sod-adding. The occurrence of sporocarps of ectomycorrhizal fungi is discussed in relation to soil conditions and nutrient concentrations of recently formed tree needles. The present study indicates that the occurrence of ectomycorrhizal species above ground is strongly affected by soil nitrogen concentrations.*

INTRODUCTION

Numbers of species and sporocarps of ectomycorrhizal fungi declined in stands of *Pinus sylvestris* L. in The Netherlands during the last decades. This decline is especially evident in older stands and differs clearly from the natural decline in species and sporocarps of ectomycorrhizal fungi during stand succession (Termorshuizen and Schaffers, 1991). In the same period litter accumulation and development of the humus profile have accelerated associated with atmospheric deposition of nitrogen (Klap and Schmidt, 1992). Also the grass *Deschampsia flexuosa* (L.) Trin. increased in Scots pine stands in The Netherlands. This increase, larger than during natural succession, was caused by increased nitrogen availability associated with atmospheric deposition (Tamm, 1991; Van der Werf, 1991).

A strong negative correlation between ectomycorrhizal species richness and thickness of humus layers was found by De Vries et al. (1985). Several investigators noted higher numbers of species and sporocarps of ectomycorrhizal fungi at sites without accumulation

of litter and humus (Termorshuizen, 1990; Tyler, 1991; Keizer and Arnolds, 1994). Veerkamp and Kuyper (1993) noted that hardly any sporocarps of ectomycorrhizal fungi were observed in Scots pine stands with an understorey vegetation dominated by *D. flexuosa*.

The aims of the present study were to investigate whether 1. removal of litter and humus layers and herb vegetation increases numbers of species and sporocarps of ectomycorrhizal fungi in Scots pine stands of different age; 2. adding litter and humus on top of the existing ectorganic layers decreases numbers of species and sporocarps of ectomycorrhizal fungi and 3. high nutrient concentrations in the mineral soil and in the needles of the trees negatively affect occurrence of species and sporocarps of ectomycorrhizal fungi.

MATERIALS AND METHODS

Treatments

In spring 1990, 6 stands of *P. sylvestris* of different age located in the northeastern part of The Netherlands were selected (Table 1). The stands were situated on former stand or heathland sites. In May and June 1990, litter and humus layers and herb vegetation of 4 plots (15 * 15 m) were removed ("sod-cutting") in each of 2 young (S1 and S2) and in each of 2 middle-aged stands (S3 and S4; Table 1). Four plots (20 * 20 m) were sod-cut in each of 2 old stands (S5 and S6; Table 1). In each of all stands 4 untreated plots were used as controls. In each of the young and middle-aged stands litter and humus layers of 4 plots (15 * 15 m) were covered by sods ("sod-addition") simulating thick litter and humus layers as present in old stands. The few naturally occurring deciduous ectomycorrhizal trees were cut down. Newly established Scots pine seedlings were removed yearly.

Description of the ectomycorrhizal flora

In 1993, sporocarps of ectomycorrhizal fungi were systematically searched in all plots except for margins of 2.5 m wide, three times at three week intervals during the period September - November. Caps of the sporocarps were removed in order to avoid double counting. Sporocarps were collected for identification when necessary. Taxonomy is according to Moser (1983).

Soil and needle analysis

The thickness of the litter and humus layers together was determined in all plots in 1993. These layers were defined as ectorganic layers.

In April 1993, ten samples (98.2 cm^3) were taken from the bottom 5 cm of the humus layers on top of the mineral soil in control and sod-added plots. Ten samples of 5 cm litter or fewer when fewer litter was present, were collected in the sod-cut plots. In all

plots the upper 5 cm mineral soil was sampled after removing the herb vegetation, litter and humus layers. These layers were sampled because ectomycorrhizal root tips and mycelia occur mainly in humus layers and upper mineral soil (Persson, 1980a; Ponge, 1990; Rastin et al., 1990; Chapter 4). The ten samples collected per plot were mixed and dried at 40°C. After drying, the ectorganic layer samples were ground. The mineral soil samples were sieved over a 2 mm sieve to remove organic material such as roots. Each sample was analysed chemically for inorganic nutrients. The amounts of $N_{dissolved}$, NH_4^+, NO_3^-, P and K^+ were analysed in 0.01*M* $CaCl_2$ extracts and the $pH(CaCl_2)$ was determined (Houba et al., 1990). The organic matter content of the mineral soil samples was determined by loss-on-ignition.

In November 1993, the dormant season, needles of the current year were sampled in all plots. Needles from the fifth to the tenth whorl from the top of the Scots pine trees were collected. Needles from 10 randomly selected trees per plot were sampled in S1, S2 and S3. Needles from 5 randomly selected trees were sampled in S4 and needles from 1 randomly selected tree in S5 and S6 because of the height of the trees (15-20 m). The collected needles were dried at 70°C and ground. Samples were digested in H_2SO_4, salicylic acid and H_2O_2. N and P were measured colorimetrically. K and Ca by atomic emission and Mg by absorption spectrometry.

Table 1. Characteristics of studied Scots pine stands in 1990. C = control, S = sod-cut and A = addition of sods.

Characteristics	S1	S2	S3	S4	S5	S6
Stand	Dwingeloo	Dwingeloo	Dwingeloo	Smilde	Smilde	Dwingeloo
Coordinates	52° 49'N 6° 28'E	52° 50'N 6° 27'E	52° 50'N 6° 25'E	52° 53'N 6° 18'E	52° 53'N 6° 18'E	52° 49'N 6° 26'E
History	pine stand	pine stand	pine stand	pine stand	pine stand	heathland
Year of planting	1987	1980	1974	1963	1940	1924
Internal cover canopy (%)	2	29	19	13	4	7
Soil (FAO-Unesco, 1988)	Haplic Arenosol	Haplic Podzol	Haplic Podzol	Haplic Arenosol	Haplic Arenosol	Haplic Arenosol
Size plot (m^2)	225	225	225	225	400	400
Treatments	C, S, A	C, S, A	C, S, A	C, S, A	C, S	C, S
Replicates	4	4	4	4	4	4

Herb vegetation

The abundance of *D. flexuosa* was recorded in June 1992 and the percentage cover was estimated.

Statistical tests

Data were analysed by analysis of variance (one-way ANOVA), if necessary after log transformation. Differences among means were evaluated with the LSD-test (Sokal and Rohlf, 1981). Differences in estimated cover of *D. flexuosa* were not normally distributed after arcsin transformation and tested with the Mann-Whitney U test (Siegel and Castellan, 1988).

Uni- and multivariate analyses

Correlation between chemical composition as well as thickness of the ectorganic layers in 1993 and species composition of ectomycorrhizal fungi in all plots in 1993 were analysed by Canonical Correspondence Analysis (CCA, CANOCO) (Jongman et al., 1987). Nutrient concentrations, pH and thickness of the ectorganic layers were used as environmental explanatory variables because the treatment was manipulation with litter and humus layers. The numbers of sporocarps of each species received a figure according to a logarithmic scale: 0 sporocarps = 0, 1-3 sporocarps = 1, 4-10 sporocarps = 2, 11-30 sporocarps = 3, 31-100 sporocarps = 4, etc. (Arnolds, 1992). Standard options of CANOCO were used when data were processed. It was tested whether the influence of a variable on the species composition of the ectomycorrhizal fungi was significantly different from random by using the Monte Carlo permutation test (Økland and Eilertsen, 1994).

The numbers of species of ectomycorrhizal fungi in 1993 were correlated with the nutrient concentrations of ectorganic layers in 1993 as well as with the nutrient concentrations of the sampled Scots pine needles.

RESULTS

Ectomycorrhizal fungi

In the sod-cut plots of all stands except for S1 significantly higher numbers of species and sporocarps of ectomycorrhizal fungi were recorded than in the control plots (Table 2). The total number of ectomycorrhizal species was highest in the sod-cut plots in S4, S5 and S6 (Table 3). Sod-cutting changed the species composition in middle-aged and old stands in 1993 (Table 3). Sporocarps of several species among which *Amanita gemmata* (Fr.) Bertillon, *Coltricia perennis* (L.: Fr.) Murr., *Russula adusta* (Pers.: Fr.) Fr. and *Tricholoma albobrunneum* (Pers.: Fr.) Kumm. were observed in the sod-cut plots in the middle-aged and old stands only, not in the control plots.

Sod-adding did not significantly affect numbers of species and sporocarps compared to the control plots (Table 2).

The average numbers of species and sporocarps of ectomycorrhizal fungi significantly differed between the stands in many cases (Table 2). In the control plots in S1 and S5 higher numbers of species were found than in the control plots in the remaining stands. In the sod-cut plots in the older stands S4, S5 and S6 significantly higher numbers of species were observed than in the sod-cut plots in the younger stands S1, S2 and S3. A relationship between stand age and numbers of sporocarps could not be established.

Table 2. Numbers of species per plot and numbers of sporocarps per 1000 m^2 in Scots pine stands of different age in 1993. C = control, S = sod-cut and A = sod-added. Significant effects of sod-cutting and sod-adding compared to control are indicated by * ($p < 0.05$, LSD) or ns (not significant). Significant differences between stands are indicated by different letters ($p < 0.05$, LSD).

	Species per plot						Sporocarps / 1000 m^2					
	S		C		A		S		C		A	
S1	5.8 *a*	ns	5.0 *c*	ns	3.8 *b*		10203 *b*	ns	8625 *e*	ns	7580 *d*	
S2	5.8 *a*	*	2.5 *ab*	ns	2.5 *ab*		4050 *a*	*	218 *c*	ns	208 *b*	
S3	5.0 *a*	*	0.5 *a*	ns	1.3 *a*		4128 *a*	*	143 *b*	ns	23 *a*	
S4	7.8 *b*	*	2.8 *ac*	ns	1.5 *ab*		3553 *a*	*	1223 *cde*	ns	873 *c*	
S5	8.5 *b*	*	4.5 *ac*		–		4566 *a*	*	548 *cd*		–	
S6	9.5 *b*	*	2.5 *ab*		–		2616 *a*	*	82 *a*		–	

Sporocarps of several species among which *Dermocybe croceocona* (Fr.) Mos., *Dermocybe semisanguinea* (Fr.) Mos. and *Gomphidius roseus* (Fr.) Fr. were only observed in S1. Other species such as *Laccaria bicolor* (R. Maire) P.D. Orton, *L. proxima* (Boud.) Pat. and *L. hepaticus* Plowr. fructified in relatively large amounts in all stands (Table 3).

Table 3. Relative abundance (%) of ectomycorrhizal species in control (C), sod-cut (S) and sod-added (A) plots in the 6 studied Scots pine stands of different age in 1993. n = total number of sporocarps. Species have been arranged in a sequence of decreasing affinity for sod-cut plots.

Stand		C	S	A	n
S1	*Amanita rubescens* Pers.: Fr.	0	100	0	1
	Suillus bovinus (L.: Fr.) Kuntze	13	87	0	24
	Laccaria bicolor (R. Maire) P.D. Orton	8	84	8	25
	Gomphidius roseus (Fr.) Fr.	25	75	0	4
	Thelephora terrestris Willd.: Fr.	38	54	8	2039
	Lactarius hepaticus Plowr.	57	39	4	44
	Laccaria proxima (Boud.) Pat.	29	36	35	8125
	Dermocybe semisanguinea (Fr.) Mos.	40	27	33	45
	Dermocybe croceocona (Fr.) Mos.	100	0	0	4
	Rhizopogon luteolus Fr.	100	0	0	1
	Dermocybe crocea (Schaeffer) Mos.	50	0	50	2
	Paxillus involutus (Batsch: Fr.) Fr.	50	0	50	2
	Inocybe brevispora Huijsman	0	0	100	1
	Xerocomus badius (Fr.: Fr.) E.J. Gilbert	0	0	100	1
	Xerocomus subtomentosus (L.: Fr.) Quél.	0	0	100	1
	Total number of species	11	8	10	15
S2	*Inocybe brevispora*	0	100	0	10
	Thelephora terrestris	1	99	0	163
	Inocybe lacera (Fr.: Fr.) Kumm.	0	98	2	46
	Laccaria bicolor	0	96	4	444
	Laccaria proxima	2	94	4	378
	Lactarius rufus (Scop.: Fr.) Fr.	11	89	0	532
	Lactarius hepaticus	9	75	16	196
	Xerocomus subtomentosus	0	8	92	13
	Xerocomus badius	0	0	100	8
	Total number of species	4	8	6	9
S3	*Laccaria bicolor*	0	100	0	1388
	Laccaria proxima	0	100	0	108
	Inocybe lacera	0	100	0	34
	Lactarius rufus	0	100	0	14
	Amanita rubescens	0	100	0	2
	Inocybe brevispora	0	100	0	2
	Russula ochroleuca Pers.	0	100	0	1
	Lactarius hepaticus	12	83	5	122
	Xerocomus badius	0	83	17	6
	Total number of species	1	9	2	9
S4	*Suillus bovinus*	0	100	0	113
	Inocybe lacera	0	100	0	29
	Coltricia perennis (L.: Fr.) Murr.	0	100	0	13
	Cortinarius fusisporus Kühner	0	100	0	13
	Inocybe brevispora	0	100	0	6
	Laccaria proxima	0	100	0	6
	Paxillus involutus	0	100	0	2
	Russula adusta (Pers.: Fr.) Fr.	0	100	0	2
	Amanita gemmata (Fr.) Bertillon	0	100	0	1
	Amanita rubescens	0	100	0	1
	Rhizopogon luteolus	0	100	0	1
	Russula vesca Fr.	0	100	0	1

continued

		C	S	A	n
	Scleroderma citrinum Pers.: Pers.	0	100	0	1
	Tricholoma albobrunneum (Pers.: Fr.) Kumm.	0	100	0	1
	Laccaria bicolor	5	95	0	756
	Russula emetica Pers.: Fr.	33	67	0	3
	Xerocomus badius	57	43	0	7
	Lactarius hepaticus	38	33	29	1178
	Lactarius rufus	50	0	50	2
	Lactarius necator (J.F. Gmel.: Fr.) Pers.	100	0	0	1
	Total number of species	6	18	2	20
S5	*Cortinarius fusisporus*	0	100	–	293
	Laccaria proxima	0	100	–	132
	Inocybe lacera	0	100	–	30
	Rhizopogon luteolus	0	100	–	21
	Amanita gemmata	0	100	–	7
	Amanita rubescens	0	100	–	3
	Amanita muscaria (L.: Fr.) Pers.	0	100	–	1
	Russula ochroleuca	0	100	–	1
	Laccaria bicolor	0	99	–	2782
	Thelephora terrestris	2	98	–	125
	Lactarius rufus	14	86	–	14
	Xerocomus badius	21	79	–	29
	Lactarius hepaticus	39	61	–	1127
	Inocybe ovatocystis Bours. & Kühn.	50	50	–	2
	Paxillus involutus	88	12	–	16
	Russula emetica	93	7	–	15
	Lactarius necator	100	0	–	1
	Russula paludosa Britz.	100	0	–	1
	Leccinum scabrum (Bull.: Fr.) S.F. Gray	100	0	–	2
	Total number of species	10	16	–	19
S6	*Laccaria bicolor*	0	100	–	412
	Inocybe lacera	0	100	–	212
	Thelephora terrestris	0	100	–	37
	Rhizopogon luteolus	0	100	–	4
	Amanita gemmata	0	100	–	4
	Paxillus involutus	0	100	–	4
	Amanita rubescens	0	100	–	3
	Inocybe brevispora	0	100	–	3
	Amanita muscaria	0	100	–	2
	Cortinarius fusisporus	0	100	–	1
	Lactarius necator	0	100	–	1
	Russula aeruginea Lindbl.	0	100	–	1
	Russula parazurea J. Schff. ex J. Schff.	0	100	–	1
	Laccaria proxima	2	98	–	1399
	Russula ochroleuca	14	86	–	22
	Lactarius rufus	14	86	–	7
	Lactarius hepaticus	15	85	–	266
	Xerocomus badius	21	79	–	33
	Total number of species	5	18	–	18

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Table 4. Nutrient concentrations (mg kg^{-1}), pH and thickness (TH, cm) of ectorganic layers (humus in control and sod-added plots, litter in sod-cut plots) sampled in April 1993. Significant differences of S (= sod-cut) and A (= sod-added) compared with C (= control) in a stand are indicated by * ($p < 0.05$, LSD). Significant differences between control treatments in stands of different age are indicated by different letters ($p < 0.05$, LSD).

	N_{diss}	NH_4^+	NO_3^-	P	K^+	pH	TH
S1-S	20.3 *	6.0	0.1	2.8 *	28.6 *	3.3 *	2.6 *
S1-C	64.0 *a*	23.3 *a*	0.4 *a*	8.4 *a*	85.8 *ab*	3.0 *b*	6.5 *ab*
S1-A	70.4	45.0	0.7 *	10.6	77.5 *	2.9	7.5
S2-S	121.6	33.0	3.8	12.3	92.0	3.2	2.3 *
S2-C	108.3 *ab*	38.1 *ab*	5.2 *b*	12.2 *ab*	85.1 *a*	2.9 *ab*	7.6 *b*
S2-A	128.5	52.9	9.6	10.3	31.4	2.9	8.2
S3-S	83.6 *	40.3	2.2 *	11.1	121.1	3.1 *	2.4 *
S3-C	143.3 *b*	59.9 *cd*	8.3 *c*	15.1 *b*	163.6 *abc*	2.8 *a*	7.1 *ab*
S3-A	151.9	68.6	15.6 *	12.0	148.8	2.8	7.5
S4-S	47.9 *	16.6 *	1.7	4.2 *	95.9 *	3.4 *	2.0 *
S4-C	151.8 *b*	65.1 *d*	3.0 *ab*	11.7 *ab*	186.4 *b*	3.1 *c*	6.1 *a*
S4-A	88.0 *	36.1 *	5.8 *	8.8 *	155.8 *	2.9 *	6.9 *
S5-S	41.2 *	17.9 *	1.9	4.6 *	78.3 *	3.3 *	1.9 *
S5-C	103.9 *ab*	58.5 *bcd*	2.5 *ab*	12.1 *ab*	225.5 *c*	2.9 *ab*	7.9 *b*
S6-S	37.8	14.3 *	1.6 *	5.9 *	121.3	3.6 *	1.5 *
S6-C	73.4 *a*	43.5 *abc*	4.9 *b*	9.2 *a*	231.7 *c*	3.0 *abc*	10.4 *c*

Soil and needle analyses

The $N_{dissolved}$, NH_4^+, P and K^+ concentrations in the ectorganic layers in the sod-cut plots in nearly all stands were lower than those in the ectorganic layers in the control plots (Table 4). In all plots the pH was extremely low varying from 2.8 to 3.6. The pH of the ectorganic layers was significantly higher in all sod-cut plots than in control plots except for S2. Three and a half year after sod-cutting the ectorganic layers were still significantly thinner than the litter and humus layer in the control plots. The highest concentrations of $N_{dissolved}$ were found in the control plots in S3 and S4 as compared with the other stands. The highest concentrations of K^+ were found in the control plots in S5 and S6 compared

to the other stands. NO_3^- concentrations were much lower than those of NH_4^+ in all cases.

The $N_{dissolved}$ concentrations in the mineral soil of the sod-cut plots in S1, S4, S5 and S6, i.e. on the non-podzolic sandy soils, were significantly lower than the concentrations in control plots (Table 5). The highest nutrient concentrations and the lowest pH in the mineral soil in the control plots were found in S2 and S3, i.e. on the podzolic sandy soils.

Table 5. Nutrient concentrations (mg kg^{-1}), pH and organic matter content (OM, %) of mineral soil sampled in April 1993. Significant differences of S (= sod-cut) and A (= sod-added) compared with C (= control) in a stand are indicated by * ($p < 0.05$, LSD). Significant differences between control treatments in stands of different age are indicated by different letters ($p < 0.05$, LSD).

	N_{diss}	NH_4^+	NO_3^-	P	K^+	pH	OM
S1-S	3.5 *	0.2 *	0.1	0.2 *	4.6 *	3.8	1.5 *
S1-C	6.7 a	1.3 a	0.1 a	0.9	10.5 a	3.5 ab	2.5 b
S1-A	5.8	0.8	0.3	1.0	12.7	3.6	2.0
S2-S	8.8	2.1	0.6	0.6	10.1	3.5	3.5
S2-C	10.7 b	2.3 b	1.0 bc	0.9	10.8 a	3.3 a	4.0 b
S2-A	10.2	3.1	1.0	1.1	10.1	3.4	2.7
S3-S	12.2	3.2	1.0	0.2	15.5	3.4 *	4.4
S3-C	13.3 b	3.3 b	1.5 c	0.2	15.4 b	3.3 a	4.5 b
S3-A	16.8	5.7	2.9 *	0.6	16.9	3.2	5.4
S4-S	3.3 *	0.7	0.5	0.0	7.3 *	3.9 *	1.2
S4-C	5.9 a	1.4 a	0.6 ab	0.5	10.4 a	3.7 bc	1.4 a
S4-A	5.6	1.4	0.9 *	0.1	9.2	3.6 *	1.6
S5-S	3.2 *	1.1	0.6	0.1	7.9	3.9	1.0 *
S5-C	5.0 a	1.2 a	0.8 bd	0.3	10.2 a	3.9 c	1.2 a
S6-S	1.7 *	0.3	0.8	0.1	6.5	4.0 *	0.8 *
S6-C	5.5 a	1.6 a	1.0 cd	0.3	8.2 a	3.7 bc	1.4 a

The P, Ca and Mg concentrations in the 0.5-year-old Scots pine needles of trees in S3 on podzolic sandy soils were signifcantly reduced by sod-cutting (Table 6). The N and P concentrations in the needles of trees in S4 on non-podzolic sandy soil were significantly enhanced by sod-addition. A significantly higher K concentration was found in the needles of trees in the sod-cut plots in S6.

Table 6. Nutrient concentrations (g kg^{-1} DM) in 0.5-year-old needles sampled in November 1993. Significant differences of S (= sod-cut) and A (= sod-added) compared with C (= control) in a stand are indicated by * ($p < 0.05$, LSD). Differences between control treatments in stands of different age are indicated by different letters ($p < 0.05$, LSD).

	N	P	K	Ca	Mg
S1-S	14.39	1.57	5.80	2.07 *	0.83
S1-C	15.77 a	1.63 b	5.46 b	2.81 b	0.99 b
S1-A	17.58	2.00 *	5.28	2.86	0.99
S2-S	17.50	1.60	5.50	1.28	0.80
S2-C	17.13 ab	1.59 b	5.50 b	1.41 a	0.78 a
S2-A	20.17	1.73	6.17	1.56	0.77
S3-S	16.31	1.25 *	6.75	1.04 *	0.59 *
S3-C	19.25 b	1.58 b	6.18 b	1.66 a	0.68 a
S3-A	19.05	1.63	6.36	1.86	0.71
S4-S	15.20	1.29	5.99	1.61	0.74
S4-C	17.89 ab	1.34 a	5.68 b	1.28 a	0.81 ab
S4-A	19.32 *	1.51 *	5.66	1.32	0.76
S5-S	15.68	1.27	5.80	0.87 *	0.58
S5-C	15.36 a	1.33 a	5.50 b	1.66 a	0.76 a
S6-S	14.38	1.28	5.18 *	1.45	0.78
S6-C	16.25 a	1.27 a	4.29 a	1.36 a	0.67 a

Herb vegetation

The cover of *D. flexuosa* was significantly lower in the sod-cut plots than in the control and sod-added plots in all stands, except for that in S2 (Table 7). Differences between stands of different age were not observed except for significantly lower cover of *D. flexuosa* in the control plots in S2 compared to the control plots in S3, S5 and S6.

Table 7. The estimated cover (%) of *D. flexuosa* in June in 1992. Significant differences of S (= sod-cut) and A (= sod-added) compared with C (= control) in a stand are indicated by * ($p < 0.05$, MWU) or ns (not significant).

	S		C		A
S1	3.0	*	26.0 ab	ns	28.5
S2	4.5	ns	14.8 a	ns	7.5
S3	3.0	*	44.3 bc	ns	26.0
S4	3.0	*	37.0 ac	ns	39.5
S5	3.0	*	44.3 bc		–
S6	3.0	*	50.5 c		–

Uni- and multivariate analyses

The CCA-diagram of the ectomycorrhizal species with nutrient concentrations, pH and thickness of the ectorganic layers is shown in Figure 1. The eigenvalue of axis 1 = 0.28 and axis 2 = 0.18. The first two axes explain 70.0% of the variance. The species environment correlation of the first axis is 0.78 and is defined by K^+ ($r = 0.64$). A Monte Carlo permutation test showed that the first axis significantly contributed to the explained variance ($p < 0.01$). The second axis is correlated with the thickness of the ectorganic layers ($r = 0.71$), pH ($r = -0.71$), $N_{dissolved}$ ($r = 0.57$), P ($r = 0.54$) and NH_4^+ ($r = 0.51$). The explained variation in the numbers of species was significant for all environmental variables (Table 8).

The regression equation $Sp = -13.671 - 0.019 * Nh + 6.424 * pH$ adequately describes the relation between the number of species (Sp), the $N_{dissolved}$ concentration (Nh) ($R = 0.77$; $p < 0.01$) and the pH in the ectorganic layers ($R = 0.71$; $p < 0.001$). Stepwise, forward and backward regressions resulted in the same equation.

A significant linear relation was found between the logarithm of the number of species (Sp) and the N concentration of the needles Nn. The equation was: $\ln(Sp) = 4.302 - 0.176 * Nn$ ($R = 0.59$; $p < 0.01$). Stepwise, forward and backward regressions resulted in the same equation.

Table 8. Test of significance of explanatory variables. p = significance of contribution of constrained axis to explained variance in a Monte Carlo permutation test ** ($p < 0.01$). Variation explained = eigenvalue of constrained axis divided by the sum of all eigenvalues (total inertia) in CCA.

Variable	Variation explained	p	Variable	Variation explained	p
N_{diss}	0.05	**	K^+	0.07	**
NH_4^+	0.05	**	pH	0.04	**
NO_3^-	0.04	**	TH	0.05	**
P	0.04	**			

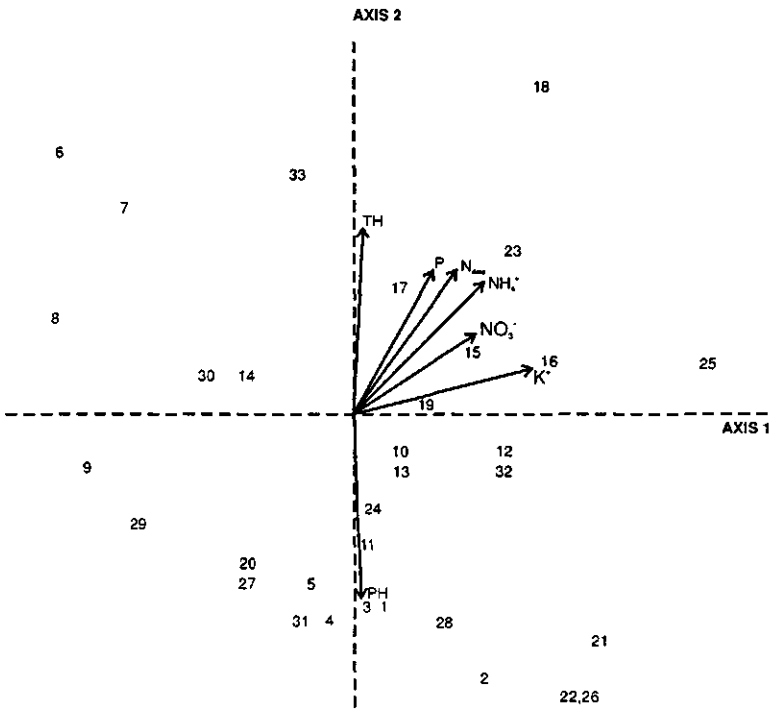


Fig. 1. CCA-diagram for ectomycorrhizal species and environmental variables (nutrient concentrations, thickness (TH) and pH of the ectorganic layers in all stands). 1 = *A. gemmata*, 2 = *A. muscaria*, 3 = *A. rubescens*, 4 = *C. perennis*, 5 = *C. fusisporus*, 6 = *D. crocea*, 7 = *D. croceocona*, 8 = *D. semisanguinea*, 9 = *G. roseus*, 10 = *I. brevispora*, 11 = *I. lacera*, 12 = *I. ovatocystis*, 13 = *L. bicolor*, 14 = *L. proxima*, 15 = *L. hepaticus*, 16 = *L. necator*, 17 = *L. rufus*, 18 = *L. scabrum*, 19 = *P. involutus*, 20 = *R. luteolus*, 21 = *R. adusta*, 22 = *R. aeruginea*, 23 = *R. emetica*, 24 = *R. ochroleuca*, 25 = *R. paludosa*, 26 = *R. parazurea*, 27 = *R. vesca*, 28 = *S. citrinum*, 29 = *S. bovinus*, 30 = *T. terrestris*, 31 = *T. albobrunneum*, 32 = *X. badius*, 33 = *X. subtomentosus*.

DISCUSSION

Sod-cutting enhanced numbers of species and sporocarps of ectomycorrhizal fungi especially in the middle-aged and old Scots pine stands. The regression equation describing the relation between numbers of species and pH and $N_{dissolved}$ in ectorganic layers and the correlations of the environmental variables $N_{dissolved}$ and NH_4^+ with the second axis in the CCA (Fig. 1) indicate that nitrogen concentrations in the ectorganic layers affected the ectomycorrhizal species composition. In The Netherlands average immission values of ammonia ranging from 40 to 80 kg ha^{-1} yr^{-1} and local extremes near sources around 100 kg ha^{-1} yr^{-1} have been reported (Draaijers et al., 1989; Van Dijk et al., 1989), which is in the same order of magnitude of amounts applied to agricultural systems as fertilizer (Pearson and Stewart, 1993). For returning to their former relatively nutrient-poor status of several decades ago atmospheric deposition of total nitrogen will have to be reduced to 6 kg ha^{-1} yr^{-1} (Van Breemen and Van Dijk, 1988). Removal of litter and humus layers and herb vegetation reduced the nitrogen concentrations in the stand soils, especially on the non-podzolic sandy soils (Table 4 and 5), indicating a partial return to relatively nutrient-poor soils. The reduction of nitrogen availability enhanced the fructification of ectomycorrhizal fungi. Earlier investigations showed a reduction of numbers of sporocarps and species in Scots pine stands following nitrogen fertilization (Wästerlund, 1982a; Ohenoja, 1988; Shubin, 1988; Termorshuizen, 1993).

The increase of pH in the sod-cut plots might have played a role too, as indicated by the correlation with the second axis in the CCA (Fig. 1) and by the regression equation describing the relation between numbers of species and pH and $N_{dissolved}$ in ectorganic layers. Shaw and Lankey (1994) noted a positive correlation between ectomycorrhizal species above ground and soil pH in young Scots pine stands.

The high correlation of the K^+ concentration in the ectorganic layers with axis 1 in the CCA (Fig. 1) indicates that K^+ concentrations affected the numbers of ectomycorrhizal species. However, potassium likely played a minor role compared to nitrogen. In several fertilization experiments in Scots pine stands weak positive or no effects on the numbers of ectomycorrhizal species due to potassium have been reported (Wästerlund, 1982a; Shubin, 1988). The high K^+ concentrations in the ectorganic layers more likely reflect the presence of *D. flexuosa*. Fanta (1992) noted that in early stages of succession *D. flexuosa* takes up potassium and accumulates this. In later stages of succession, the potassium concentrations of the humus layers increase due to decomposition of shoots of *D. flexuosa*.

Sod-cutting obviously caused removal of phenolics present in Scots pine needle and grass litter. Laboratory experiments showed sensitivity of isolates of several ectomycorrhizal fungi to realistic concentrations of phytotoxic components of extracts of Scots pine needles and shoots and roots of *D. flexuosa* including phenolics. However, isolates of *L. bicolor* were not sensitive to these extracts (Chapter 7) and nitrogen fertilization enhanced numbers of sporocarps of *L. bicolor* (Ohenoja, 1988). Sod-cutting

reduced the nitrophilous *D. flexuosa* as also found by De Vries et al. (1995). This reduction likely favoured the sporocarp formation of ectomycorrhizal fungi among which *L. bicolor*. In most Scots pine stands the grass *D. flexuosa* dominates the understorey vegetation in The Netherlands and forms a tight mat with a dense root system (Arnolds, 1991; Nabuurs, 1991) inhibiting fructification of ectomycorrhizal fungi.

In The Netherlands strongly threatened species among which *A. gemmata, C. perennis, R. adusta* and *T. albobrunneum* (Arnolds, 1989) were observed in the sod-cut plots in the middle-aged and old stands from 1991 to 1993. In the autumn of 1994, sporocarps of the strongly declined species *C. cibarius* were observed in a sod-cut plot in S4 for the first time (pers. obs.). In the past these species were common in *Cladonia*-rich Scots pine stands with thin humus layers on dry, mineral-poor, acidic non-podzolic sandy soils (Arnolds, 1991). During the last decades this stand type has disappeared and changed into *D. flexuosa*-rich Scots pine stands associated with high nitrogen input (Klap and Schmidt, 1992). Ectomycorrhizal species fructifying in Scots pine stands with thick humus layers at relatively nutrient-rich mineral soil such as *R. ochroleuca* and *X. badius* remained approximately constant and *L. hepaticus* seemed to have increased (Arnolds, 1991). In this study sporocarps of *L. hepaticus, L. rufus, R. emetica, R. ochroleuca* and *X. badius* were observed in all treatments. However, most sporocarps were found in the sod-cut plots in all stands. Besides removal of nitrogen and phenolics other environmental conditions such as changes in microclimate or different structure of the ectorganic layers might have stimulated fructification of ectomycorrhizal fungi.

The numbers of species and sporocarps of ectomycorrhizal fungi in the sod-added plots were not different from those in the control plots (Table 2). The cover of *D. flexuosa* in the sod-added plots did not differ from that in the control plots unfavourable for ectomycorrhizal fungi. Sod-adding did not affect the nutrient concentrations, pH and thickness of the humus layers (Table 4 and 5).

No positive effects of either sod-cutting or sod-adding on the sporocarp formation of ectomycorrhizal fungi in the youngest stand (S1) were found. The circumstances in the young stand were already favourable for fructification of ectomycorrhizal fungi due to mixing (undisturbed) humus layers with mineral soil at time of planting (Termorshuizen and Schaffers, 1987).

The low numbers of ectomycorrhizal fungi in the control plots in S2 and S3 (Table 3) coincided with the presence of litter and humus layers and *D. flexuosa* and high $N_{dissolved}$ and NH_4^+ concentrations and a high organic matter content of the mineral soil (Table 5). Presumably because of this, sod-cutting in these stands on podzolic sandy soil was less effective than in S4, S5 and S6 on non-podzolic sandy soil.

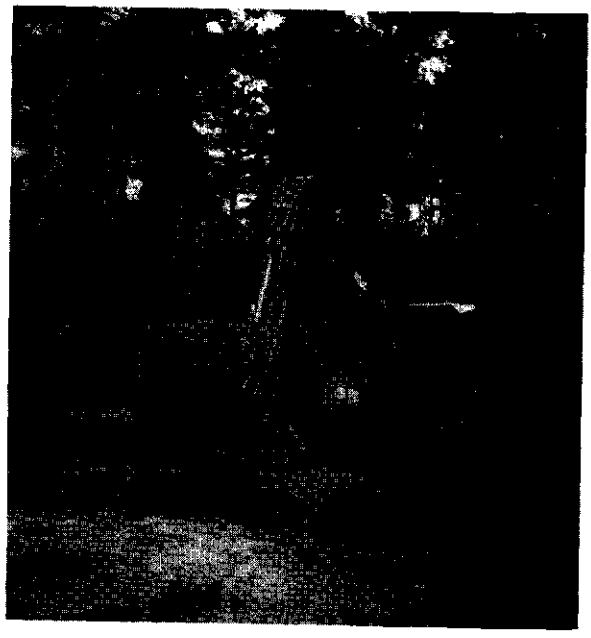
In the present study, it was decided to measure tree vitality by chemical analyses of the needles. The negative correlation between the numbers of ectomycorrhizal species and nitrogen concentration in the needles is in accordance with findings by Wallander and Nylund (1992). These investigators concluded that high nitrogen levels inhibit

ectomycorrhizal development indirectly, i.e. mediated through the host, rather than directly.

The high total numbers of species in the four sod-cut plots per stand (Table 3) compared to the average numbers of species per plot (Table 2) can be explained by the small size of the plots. Some species among which *A. gemmata*, *R. adusta* and *T. albobrunneum* formed sporocraps in low amounts and were only observed in one or two plots in a stand. Sporocarps of ectomycorrhizal species associated with deciduous trees like *Leccinum scabrum* were observed in the plots although all deciduous trees were removed from the plots. Roots of deciduous trees present outside the plots and/or mycelia have apparently grown in the plots.

The effects of sod-cutting on the nutrient concentrations of the ectorganic layers might have been overestimated and the effects of sod-adding underestimated. The specific density of the ectorganic layers in the control plots was higher than in the sod-cut plots and lower than in the sod-added plots (unpubl. results). Therefore, further research on nutrient concentrations, as well as nitrogen and phosphorus mineralization and fluxes for better understanding of influences of soil processes on the occurrence of sporocarps of ectomycorrhizal fungi is necessary. In addition, volumes of the various layers should be taken into account.

In the present study, it was shown that removal of litter and humus layers and herb vegetation enhanced the numbers of species and sporocarps of ectomycorrhizal fungi, especially in middle-aged and old Scots pine stands. Removal of litter and humus layers was less effective in stands on a podzolic sandy soil. High nitrogen concentrations, high organic matter content and low pH reduced the numbers of ectomycorrhizal species.



Mechanical sod-cutting in a Scots pine stand planted in 1924 (S6)

THE ECTOMYCORRHIZAL FLORA OF PRIMARY AND SECONDARY STANDS OF PINUS SYLVESTRIS IN RELATION TO SOIL CONDITIONS AND ECTOMYCORRHIZAL SUCCESSION

SUMMARY

Species composition and sporocarp abundance of ectomycorrhizal fungi in two middle-aged (15-20 years) primary stands of Pinus sylvestris *in the central part of The Netherlands were compared with those in two middle-aged (planted in 1963 and 1974) secondary stands in the northeastern part of The Netherlands. The stand planted in 1974 was situated on podzolic sandy soil and the stand planted in 1974 on non-podzolic sandy soil without prominent profile development. In the secondary stands litter and humus layers and herb vegetation were removed ("sod-cutting") in order to set back soil development. Control treatments were present. In 1991, 1992 and 1993 surveys showed that sod-cutting enhanced abundance and diversity of ectomycorrhizal fungi. However, the species richness and diversity in the primary stands were higher than in the secondary stands, also even in the sod-cut plots. High species richness and diversity were associated with low concentrations of nitrogen and high pH in the ectorganic layers and mineral soil.*

INTRODUCTION

As forests age litter and humus accumulate and ectomycorrhizal succession occurs (Last et al., 1987; Mason et al., 1987). Physiology of the trees and chemical composition of the forest soil change simultaneously during stand development, influencing the ectomycorrhizal flora. There is increasing evidence that changes in the ectomycorrhizal flora are largely driven by changes in the forest soil as suggested by Blasius and Oberwinkler (1989) and not so much by increase of tree age.

Frankland (1992) defined primary fungal succession as succession originating on a virgin surface, e.g. mineral soil without vegetation, and reflecting a pioneer situation. Secondary fungal succession follows disruption of a primary succession by a sudden event such as clearcutting or fire. Most studies of ectomycorrhizal succession have been focused on primary succession (Dighton and Mason, 1985; Last et al., 1987) and until so far little is known of secondary succession.

Termorshuizen (1991) observed higher numbers of ectomycorrhizal species in primary young (5-13 years) stands of *Pinus sylvestris* L. than in secondary stands of the same age.

Primary and secondary stands were situated on nutrient-poor non-podzolic sandy soils. In the primary young stands litter and humus layers were thin in contrast to the secondary young stands. Soil development can be set back by removal of litter and humus layers and herb vegetation ("sod-cutting"). Sod-cutting in secondary stands on non-podzolic and podzolic sandy soils resulted in thin litter and humus layers similar to those in primary stands and enhanced numbers of species and sporocarps of ectomycorrhizal fungi (Baar and Kuyper, 1993; De Vries et al., 1995). However, the enhancement was smaller in stands on podzolic sandy soils. The nitrogen concentrations and organic matter content of the podzolic sandy soil were higher and the pH lower than in non-podzolic sandy soils (Chapter 2).

The aims of the present study were 1. to investigate whether species richness and diversity of ectomycorrhizal fungi in primary middle-aged Scots pine stands are higher than in secondary middle-aged Scots pine stands; 2. to investigate whether species richness, diversity and species composition of ectomycorrhizal fungi in the sod-cut plots in secondary middle-aged stands on podzolic and non-podzolic sandy soils are similar to those in primary stands; 3. to investigate whether soil nutrient concentrations affect species richness, diversity and species composition of ectomycorrhizal fungi and 4. to compare primary ectomycorrhizal succession with secondary ectomycorrhizal succession.

MATERIALS AND METHODS

Scots pine stands

In spring 1990, two middle-aged primary stands of *P. sylvestris* (15-20 years, spontaneously grown) situated in the drift sand area Hulshorsterzand in the central part of The Netherlands (52° 21'N, 5° 44'E) (P1 and P2) and two middle-aged (planted in 1963 and 1974) secondary stands of *P. sylvestris* both situated in forestry Dwingeloo in the northeastern part of The Netherlands (52° 50' N, 6° 25'E and 52° 53' N, 6° 18'E) were selected (S3 and S4) for study. The primary stands have spontaneously established on drift sands, Haplic Arenosol (FAO-Unesco, 1988). Until 1920, the site where S3 was located was heathland. Then *P. sylvestris* mixed with *Quercus robur* L. and *Picea abies* (L.) Karsten was planted. In 1974, the stand was felled and replanted with *P. sylvestris*. The soil of S3 is a Haplic Podzol (FAO-Unesco, 1988). Until 1908, the site of S4 was drift sand, Haplic Arenosol (FAO-Unesco, 1988). In 1908, *P. sylvestris* was planted. In 1963, the trees were felled and the site was replanted with *P. sylvestris*.

In 1990, in each primary stand 4 plots (control plots of 15 * 15 m) and in each secondary stand 8 plots (4 control and 4 sod-cut plots of 15 * 15 m) were selected. In May and June 1990, the litter and humus layers and the herb vegetation of the four selected plots were removed ("sod-cutting") in each of the two secondary stands (S3 and S4). The few naturally established deciduous trees were chopped and Scots pine seedlings

were removed every year. The understorey vegetation in the primary stands was poor and consisted mainly of mosses and *Cladonia* species. Before sod-cutting the understorey vegetation in the secondary stands was dominated by the grass *Deschampsia flexuosa* (L.) Trin. In S3, *Ceratocapnos claviculata* (L.) Lidén and *Molinia caerulea* (L.) Moench and in S4, *Empetrum nigrum* L. also occurred.

Description of the ectomycorrhizal flora

From 1990 - 1993, sporocarps of ectomycorrhizal fungi were systematically searched in all plots except for margins of 2.5 m wide, two (1991) or three (1990, 1992, 1993) times at three week intervals during the period September - November. The surveys were usually finished when a frost period started. Caps of the sporocarps were removed in order to avoid double counting. Sporocarps were collected for identification when necessary. Taxonomy is according to Moser (1983).

Soil analysis

The thickness of the litter and humus layers together was determined in all plots in 1993. These layers are defined as ectorganic layers.

In April 1993, ten samples (98.2 cm^3) were taken from the bottom 5 cm of the humus layers on top of the mineral soil in each of the control plots. Ten samples of 5 cm litter or fewer when fewer litter was present were collected in each of the sod-cut plots. In all plots, the upper 5 cm mineral soil was sampled after removing the herb vegetation and litter and humus layers. These layers were sampled because ectomycorrhizal root tips and mycelia occur mainly in the humus layers and upper mineral soil (Persson, 1980a; Ponge, 1990; Rastin et al., 1990, Chapter 4). The ten soil samples collected per plot were mixed and dried at 40°C. After drying the samples of the ectorganic layers were ground and the mineral soil samples were sieved over a 2 mm sieve in order to remove organic material such as roots. Each sample was analysed chemically for (plant) available nutrients. $N_{dissolved}$, NH_4^+, NO_3^-, P and K^+ were analysed in 0.01*M* $CaCl_2$ extracts and the pH($CaCl_2$) was determined (Houba et al., 1990). The organic matter content of the mineral soil samples was determined by loss-on-ignition.

Herb vegetation

The abundance of the grass *D. flexuosa* was recorded in June 1992 and the cover was estimated.

Diversity indices

Average numbers of sporocarps per 1000 m^2 and average numbers of species per plot were calculated for the four years of investigation to show the effects of sod-cutting over the years. Two diversity indices, the Berger-Parker index and Margalef's index, describing different aspects of diversity, were calculated for 1993 to show differences in

diversity in the plots in the final year of research. The Berger-Parker index (d) relates the number of individuals of the most abundant species (N_{max}) to the total number of individuals (N):

$$d = N_{max}/N$$

(Magurran, 1988). In the present study a sporocarp was considered as an individual. An increase of the reciprocal value of this index indicates an increase in diversity and a reduction of the most dominant species. Margalef's index (D_{MG}) is based on the number of species (S) recorded and the total number of individuals summed over all species (N):

$$D_{MG} = (S-1)/\ln N$$

Statistical tests

Data were analysed by analysis of variance (one-way ANOVA), if necessary after transformation to obtain normality. Differences among means were evaluated with the LSD-test (Sokal and Rohlf, 1981). Differences in cover of *D. flexuosa* were tested with Mann-Whitney U-test (Siegel and Castellan, 1988).

Clustering and ordination

The numbers of sporocarps of each species received a figure according to a logarithmic scale: 0 sporocarps = 0, 1-3 sporocarps = 1, 4-10 sporocarps = 2, 11-30 sporocarps = 3, 31-100 sporocarps = 4, etc. (Arnolds, 1992).

The divisive clustering technique two-way indicator species analysis (TWINSPAN) was used to detect similar fungal groups (Jongman et al., 1987). Maximum density of sporocarps during a year was used to include all ectomycorrhizal species observed over the four years of investigation. Standard options of TWINSPAN were used for processing the data (Hill, 1979).

Correlation between chemical composition or thickness of the ectorganic layers and species composition of ectomycorrhizal fungi in all plots in 1993 were analysed by Canonical Correspondence Analyis (CCA, CANOCO) (Jongman et al., 1987). $N_{dissolved}$, NH_4^+, NO_3^-, P and K^+ concentrations, pH and thickness of the ectorganic layers were used as environmental explanatory variables because the treatment was manipulation with litter and humus. Standard options of CANOCO were used when data were processed (Ter Braak, 1990). It was tested whether the influence of a variable on the species composition of the ectomycorrhizal fungi was significantly different from random by using the Monte Carlo permutation test (Økland and Eilertsen, 1994).

RESULTS

Ectomycorrhizal fungi

In P1 and P2 significantly higher numbers of ectomycorrhizal species were recorded than in the control and sod-cut plots in S3 and S4 from 1990 until 1993 (Fig. 1). Significantly higher numbers of species in the sod-cut plots in S4 than in S3 were observed in 1993 only.

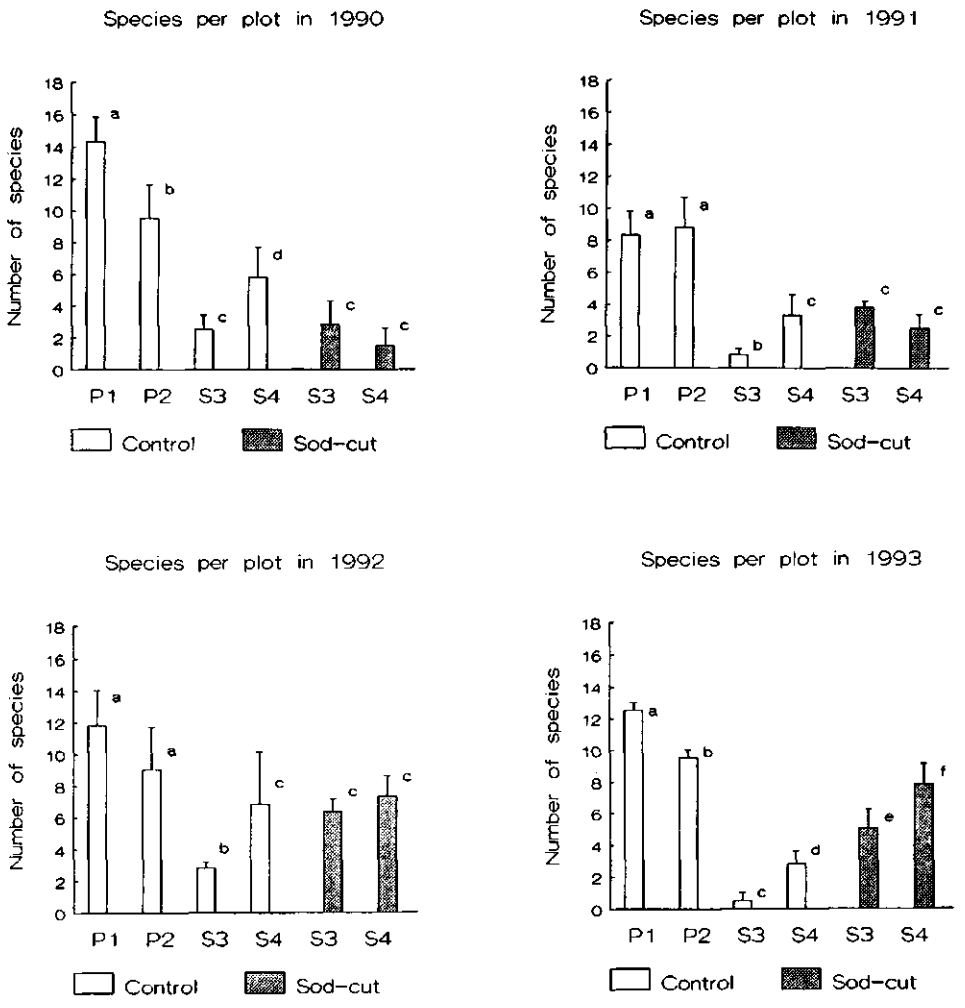


Fig. 1. Numbers of species per plot in 1990, 1991, 1992 and 1993 in the primary (P1 and P2) and secondary (S3 and S4) stands. Significant differences between treatments are shown by different letters ($p < 0.05$, LSD).

In 1993, the diversity of ectomycorrhizal fungi in P1 and P2 was significantly higher than in the control and sod-cut plots in S3 and S4 (Table 1).

Table 1. Average scores of Berger-Parker index (inverse) and Margalef's index in 1993. C = control and S = sod-cut. The higher the index, the higher the diversity. Significant differences between treatments are indicated by different letters ($p < 0.05$, LSD) and standard error of difference (s.e.d.).

	Berger-Parker index	Margalef's index
P1	2.6 cd	2.4 e
P2	2.9 d	1.7 d
S3-C	0.5 a	0.0 a
S4-C	1.1 ab	0.4 ab
S3-S	1.4 ab	0.7 b
S4-S	1.8 bc	1.2 c
s.e.d.	1.0	0.4

The Twinspan analysis resulted in 5 clusters (Table 2). Cluster 1 consisted of P2 plots, cluster 2 of P1 plots, cluster 3 of S4-S and some S4-C plots, cluster 4 mainly of S3-S plots and cluster 5 mainly of S3-C plots. The ectomycorrhizal species composition of P1 and P2 differed from that in the control and sod-cut plots in S3 and S4. Sporocarps of 12 species like *Hygrophorus hypotheius* (Fr.: Fr.) Fr., *Sarcodon imbricatus* (L.: Fr.) Karst., *Suillus luteus* (L.: Fr.) S.F. Gray and *Tricholoma focale* (Fr.) Ricken were only observed in P1 and P2. Sod-cutting changed the ectomycorrhizal species composition in both S3 and S4, but more so in S4, i.e. on non-podzolic sandy soil (Table 2). Sporocarps of several species such as *Coltricia perennis* (L.: Fr.) Murr. and *Tricholoma albobrunneum* (Pers.: Fr.) Kumm. were observed in the sod-cut plots in S4, on non-podzolic sandy soil, but not in S3, on podzolic sandy soil. The ectomycorrhizal composition of S4-S plots had more in common with the plots in P1 and P2 and that of S3-S with S3-C than the sod-cut plots in the two stands with each other.

Soil analysis

In 1993, the $N_{dissolved}$, NH_4^+ and K^+ concentrations in ectorganic layers in the control plots in S3 and S4 were generally higher than those in the sod-cut plots and in the plots in P1 and P2 (Table 3). The NO_3^- concentrations in the ectorganic layers in the control plots in S3 were highest. The NO_3^- concentrations in the ectorganic layers were much lower

Table 2. Ectomycorrhizal fungi in primary (P1 and P2) and secondary (S3 and S4) stands. Maximum density of sporocarps during a year over the four years of investigation was assigned a figure according to a logarithmic scale: 1-3 sporocarps = 1, 4-10 sporocarps = 2, 11-30 sporocarps = 3, etc. (Arnolds, 1992). C = control and S = sod-cut.

Plot	P	P	P	P	P	P	P	P	S	S	S	S	S	S	S	S	S	S	S	S	S	S
	2	2	2	2	1	1	1	1	4	4	4	4	4	4	3	3	3	3	4	3	3	3	3
Treatment	C	C	C	C	C	C	C	C	S	S	S	S	C	C	C	S	S	S	S	C	C	C	C	C
TWINSPAN cluster	1	1	1	1	2	2	2	2	3	3	3	3	3	3	4	4	4	4	5	5	5	5	5
Boletus edulis	2	1																					
Suillus luteus	2	1	1																				
Tricholoma flavovirens	5	4	4	4	4	4	4	4															
Amanita muscaria	4	2	7	1	2	3	3	2															
Hebeloma cylindrosporum		1	3	1	4	4	3	2															
Suillus variegatus		1	1																				
Hygrophorus hypoteius		1	2	2																			
Dermocybe croceocona				2	2	2	2	1															
Tricholoma portentosum							3	2															
Tricholoma focale							2																
Sarcodon imbricatus							2																
Lactarius glyciosmus						4	4																
Suillus bovinus	2	3	3	2	1	3		4	5														
Rhizopogon luteolus	4	3	3	4	3	3	2	3	3	1													
Tricholoma alboburneum		1	3	2	2	4	3	2	2		1												
Thelephora terrestris	6	5	2	3	5	3		4		3			3	2									
Inocybe lacera	3	4			1	2			2	6		1				4		3					
Amanita gemmata		1								1		1											
Dermocybe crocea				2	1	4	3	4					1										
Cortinarius fusisporus					4	1	2	1		2		2	2										
Gomphidius roseus								2	2														
Russula vesca									1														
Coltricia perennis										3		1	2										
Russula adusta											1	1											
Lactarius necator											1		1										
Inocybe ovatocystis														1									
Scleroderma citrinum												1											
*Cantharellus cibarius**												2											
Xerocomus badius												2	2		1			1	2	3	1		
Russula ochroleuca																		1	3		2	1	
Amanita rubescens											2	3	2		1	1	1	2	1	1			
Russula emetica				1			2	2	1				3	1									
Lactarius hepaticus			1	3	3	2		2	5	5	4	5	6	6	5	4	4	4	4	6	5	3	2	2
Paxillus involutus	1	1	1		3	3	4	2	1	1	2		1	1		2		1					
Inocybe brevispora	3	3			1	3	2		1	1	3						1	1	1				
Laccaria proxima	5	2	2	1		2		3	4	2	2	3	5	2	4	5	2	1	2	1			3
Laccaria bicolor	4		2		3	3	2	4	6	5	5	4	4	1	4	4	6	6	6	2		1	1	1
Lactarius rufus	5	4	5	6	4	4	4	4					2	1	2	3	3	4	4	3	2	1	3	4

* *C. cibarius* was observed in 1994 for the first time.

continued

Total numbers of species (N_s) recorded over the period 1990-1993.

Stand	P1	P2	S3-C	S4-C	S3-S	S4-S
N_s	23	22	7	10	14	21

than NH_4^+ concentrations. Although the pattern is less clear than for the nitrogen concentrations in the ectorganic layers, the P and K^+ concentrations were generally highest in the secondary stand control plots, followed by the sod-cut plots on podzolic sandy soil. The chemical composition of the ectorganic layers in the sod-cut plots on non-podzolic sandy soils was generally more similar to that in the primary stand plots. The same, but inverse, relationships were observed for pH (Table 3). The ectorganic layers were thickest in the control plots in the secondary stand and thinnest in the sod-cut plots.

Table 3. Nutrient concentrations (mg kg^{-1}), pH and thickness (TH, cm) of ectorganic layers (humus in control and sod-added plots, litter in sod-cut plots) sampled in April 1993. C = control and S = sod-cut. Significant differences between treatments are indicated by different letters ($p < 0.05$, LSD) and standard error of difference (s.e.d.).

	N_{diss}	NH_4^+	NO_3^-	P	K^+	pH	TH
P1	40.2 ab	19.6 a	0.7 a	9.4 c	112.5 bd	3.2 b	4.0 b
P2	31.4 a	10.9 a	0.5 a	2.5 a	52.4 a	3.4 d	4.4 b
S3-C	143.3 c	59.9 bc	8.3 d	15.1 d	163.6 cd	2.8 a	7.1 c
S4-C	151.8 c	65.1 c	3.0 c	11.7 cd	186.4 c	3.1 c	6.1 c
S3-S	83.6 b	40.3 b	2.2 bc	11.1 cd	121.1 b	3.1 c	2.4 a
S4-S	47.9 ab	16.6 a	1.7 b	4.2 b	95.9 ab	3.4 d	2.0 a
s.e.d.	45.9	20.6	0.3	5.3	54.9	0.1	1.0

In 1993, the $N_{dissolved}$, NH_4^+ and NO_3^- concentrations and organic matter content in the mineral soil in the control and sod-cut plots in S3 on podzolic sandy soil were generally higher than in the plots in S4, P1 and P2 on non-podzolic sandy soil (Table 4). The NO_3^- concentrations in the mineral soil were much lower than NH_4^+ concentrations. The pH in the control and sod-cut plots in S3 on podzolic sandy soil was lower than in the plots in S4, P1 and P2 on non-podzolic sandy soil. The chemical composition of the mineral soil in the sod-cut plots on non-podzolic sandy soils was generally more similar to that in the primary stand plots.

Table 4. Nutrient concentrations (mg kg^{-1}), pH and organic matter content (OM, %) of mineral soil sampled in April 1993. C = control and S = sod-cut. Significant differences between treatments in stands are indicated by different letters ($p < 0.05$, LSD) and standard error of difference (s.e.d.).

	N_{diss}	NH_4^+	NO_3^-	P	K^+	pH	OM
P1	3.7 a	0.6 a	0.1 a	0.5	25.4	3.9 c	0.9 a
P2	3.5 a	0.6 a	0.2 ab	0.2	18.9	4.0 c	0.8 a
S3-C	13.3 b	3.3 b	1.5 d	0.2	15.4	3.3 a	4.5 b
S4-C	5.9 a	1.4 a	0.6 bcd	0.5	10.4	3.7 b	1.4 a
S3-S	12.2 b	3.2 b	1.0 d	0.2	15.5	3.4 ab	4.4 b
S4-S	3.3 a	0.7 a	0.5 ac	0.0	7.3	3.9 c	1.2 a
s.e.d.	3.6	1.4	0.4	0.6	1.0	0.1	0.3

Herb vegetation

The estimated cover of *D. flexuosa* in the control plots in S3 and S4 was significantly higher than in the remaining plots (Table 5).

Table 5. Estimated cover (%) of the grass *D. flexuosa* in P1, P2, S3 and S4 in June 1992. C = control and S = sod-cut. Differences between treatments are shown by different letters ($p < 0.05$, MWU).

	P1	P2	S3-C	S4-C	S3-S	S4-S
Cover (%)	0.8 a	0.0 a	44.3 c	37.0 c	3.0 b	3.0 b

Ectomycorrhizal fungi in relation to soil chemistry

The CCA-diagram of the ectomycorrhizal species and nutrient concentrations and pH of the ectorganic layers is shown in Fig. 2. The eigenvalue of axis 1 = 0.46 and axis 2 = 0.26. The first two axes explain 75.4% of the variance. A Monte Carlo permutation test showed that the first axis significantly contributed to the explained variance ($p < 0.01$). The species-environment correlation of the first axis is 0.92 and is defined by NO_3^- (r = 0.85), $N_{dissolved}$ (r = 0.64) and NH_4^+ (r = 0.61). The second axis is correlated with pH (r = −0.60), P (r = 0.59) and K^+ (r = 0.51). The explained variation in numbers of species was significant for all environmental variables except for thickness of ectorganic layers (Table 6).

Table 6. Test of significance of explanatory variables. P = significance of contribution of constrained axis to explained variance in a Monte Carlo permutation test ** ($p < 0.01$), * ($p < 0.05$) and ns (not significant). Variation explained = eigenvalue of constrained axis divided by the sum of all eigenvalues (total inertia) in CCA.

Variable	Variation explained	P	Variable	Variation explained	P
$N_{dissolved}$	0.11	**	K^+	0.11	**
NH_4^+	0.10	**	pH	0.09	**
NO_3^-	0.17	**	TH	0.06	ns
P	0.08	*			

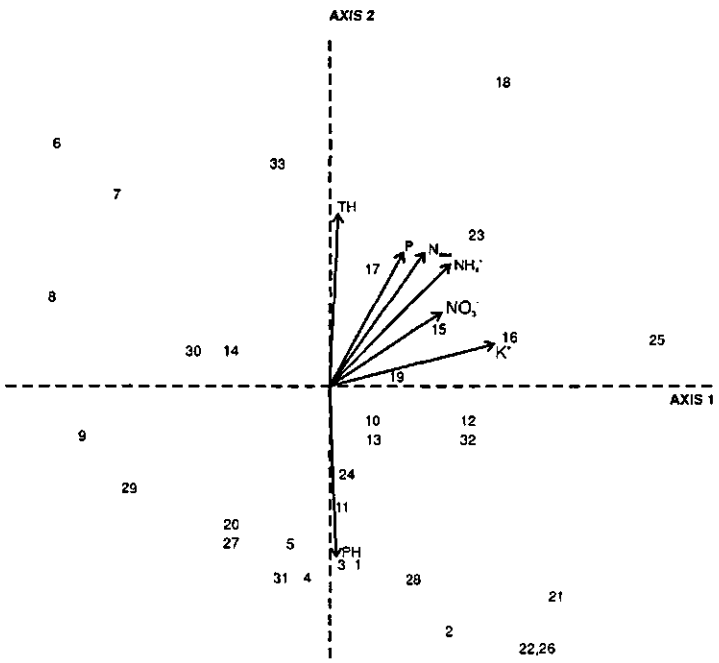


Fig 2. CCA-diagram for the ectomycorrhizal species and nutrient concentrations and pH of the ectorganic layers. 1 = *A. gemmata*, 2 = *A. muscaria*, 3 = *A. rubescens*, 4 = *C. perennis*, 5 = *C. fusisporus*, 6 = *D. crocea*, 7 = *D. croceocona*, 8 = *H. cylindrosporum*, 9 = *I. brevispora*, 10 = *I. lacera*, 11 = *L. bicolor*, 12 = *L. proxima*, 13 = *L. glyciosmus*, 14 = *L. hepaticus*, 15 = *L. necator*, 16 = *L. rufus*, 17 = *P. involutus*, 18 = *R. luteolus*, 19 = *R. adusta*, 20 = *R. emetica*, 21 = *R. ochroleuca*, 22 = *R. vesca*, 23 = *S. citrinum*, 24 = *S. bovinus*, 25 = *S. luteus*, 26 = *S. variegatus*, 27 = *T. terrestris*, 28 = *T. albobrunneum*, 29 = *T. focale*, 30 = *T. portentosum*, 31 = *T. flavovirens*, 32 = *X. badius*.

DISCUSSION

The higher numbers of ectomycorrhizal species and the diversity indices in the primary stands compared to those in the control plots in the secondary stands (Fig. 1 and Table 1) were associated with low nitrogen concentrations in the ectorganic layers (Table 3). Also the low cover of *D. flexuosa* in the primary stands compared to the control plots in the secondary stands might have favoured the occurrence of ectomycorrhizal species. The species composition in the primary stand plots differed from that in the control plots of the secondary stands (Table 1). Sporocarps of ectomycorrhizal species threatened in The Netherlands and characteristic for dry, mineral-poor, acidic sandy soil such as *S. imbricatus*, *T. albobrunneum* and *Tricholoma portentosum* (Fr.) Quél. (Arnolds, 1989; Arnolds, 1991) were observed only in the primary stands. Similar ectomycorrhizal species composition in 20- to 30-year-old Scots pine stands in Finland and Estonia were reported by Hintikka (1988) and Kalamees and Silver (1988), respectively. The understorey vegetation of the stand in Estonia was dominated by *Cladonia* species and in Finland by *Vaccinium* spp. while *D. flexuosa* was absent.

Sod-cutting in S4, i.e. on non-podzolic sandy soil, increased the average numbers of ectomycorrhizal species and the Margalef diversity index three and a half years after the treatment (Fig. 1 and Table 1). Removal of the thick ectorganic layers and herb vegetation dominated by *D. flexuosa* caused removal of nutrients, phenolics and an increase of pH (Baar and Kuyper, 1993; Chapter 2).

The species composition of the sod-cut plot in S4 on non-podzolic sandy soil was most similar to that in the primary stands (Table 1). *Amanita gemmata* (Fr.) Bertillon, *Rhizopogon luteolus* Fr., *Suillus bovinus* (L.: Fr.) O. Kuntze and *T. albobrunneum* were observed in the sod-cut plots in S4 and in the primary stands. The correlations of the environmental variables $N_{dissolved}$, NH_4^+, NO_3^- and pH with the first and second axes in the CCA (Fig. 2) indicate that nitrogen concentrations and pH in the ectorganic layers affected the ectomycorrhizal species composition. The nutrient concentrations and pH of the ectorganic layers and mineral soil in the sod-cut plots on non-podzolic sandy soil were nearly similar to those in the primary stands (Table 3).

Besides species observed in the primary stands different species threatened in The Netherlands colonized the sod-cut plots in S4, on non-podzolic sandy soil. The low nutrient concentrations in the ectorganic layers and mineral soil (Table 3 and 4) were likely favourable for ectomycorrhizal fungi and mycelia were able to establish in the sod-cut plots. *Cantharellus cibarius* Fr., *C. perennis* and *Russula adusta* (Pers.: Fr.) Fr. are known as common species in young, middle-aged and old Scots pine stands with thin humus layers on dry, mineral-poor, acidic sandy soils (Hintikka, 1988; Kalamees and Silver, 1988; Termorshuizen, 1991). Sporocarps of these species were observed in other Scots pine stands in the northeastern part of The Netherlands (pers. obs.). Jansen and Van

Dobben (1987) noted that sporocarps of *C. cibarius* were observed on well-drained soils with a low content of organic matter. They also suggested that decrease of soil pH reduces numbers of sporocarps of *C. cibarius*. The significant increases of pH in ectorganic layers and mineral soil after sod-cutting (Table 3 and 4) might have been the main effect that positively affected *C. cibarius*.

Sod-cutting increased the numbers of ectomycorrhizal species in S3 on podzolic sandy soil in 1993, but less so than in S4 on non-podzolic sandy soil. This is likely caused by the fact that the nitrogen concentrations and organic matter content of this podzolic mineral soil after four years were still as high and the pH as low as in the control plots in S3 (Table 4). Termorshuizen (1993) noted that nitrogen fertilization reduced numbers of sporocarps and species of ectomycorrhizal fungi in Scots pine stands. Negative correlations between diversity of ectomycorrhizal species and organic matter content of the soil and positive correlations between species diversity and soil pH in Scots pine stands were noted by Shaw and Lankey (1994).

Another explanation for the absence of sporocarps of some ectomycorrhizal species in the sod-cut plots on sandy and podzolic soil is that chances were rather small that ectomycorrhizal fungi, not occurring in the neighbourhood of the secondary stands, e.g. *H. hypotheius* or *T. focale*, colonized the relatively isolated sod-cut plots by spores. Even if some species established in the sod-cut plots by spore dipersal, they might have a weak competitive or mycelium expanding ability when other mycelia are already present as suggested by Dahlberg and Stenlid (1990).

The high diversity indices of the primary stands in 1993 indicate that none of the species recorded was dominant. The low Berger-Parker indices of the control and sod-cut plots in the middle-aged stands indicate dominance of species, i.c. *Laccaria bicolor* (R. Maire) P.D. Orton in the sod-cut plots and *Lactarius hepaticus* Plowr. in the control plots (Table 1). The dominance of *L. bicolor* can be explained by the survival of mycelia in the mineral soil, in old rotten stumps not removed or by saprotrophic survival on rotten wood and dead roots (Chapter 9) as well as by the ability to respond rapidly to environmental changes (Såstad and Jensen, 1993). *Lactarius hepaticus* is known as a species fructifying in Scots pine stands with thick humus layers on relatively nutrient-rich soil (Arnolds, 1991).

Sporocarps of ectomycorrhizal fungi associated with deciduous trees like *Lactarius necator* (J.F. Gmel.: Fr.) Pers. and *Russula vesca* Fr. were observed in the plots although all deciduous trees had been removed from the plots. These species might be associated with deciduous trees outside the plots.

The ectomycorrhizal species diversity in the secondary stand plots was lower than, and the species composition different from that in the primary stand plots (Tables 1 and 2) indicating that ectomycorrhizal primary succession differs from secondary succession. Termorshuizen (1991) also noted higher species richness and different species composition in primary Scots pine stands than in secondary stands. Most of the nine species mentioned

by Termorshuizen (1991) as differential and exclusive species for young (8-13 years) spontaneously grown Scots pine stands, such as *A. gemmata*, *Boletus edulis* Bull.: Fr., *Lactarius glyciosmus* (Fr.: Fr.) Fr. and *T. portentosum*, were observed in the middle-aged primary stand plots. Termorshuizen (1991) indicated four ectomycorrhizal species as exclusive for second or third rotation young (6-11 years) planted stands among which *A. rubescens* and *C. perennis*. In the present study sporocarps of these species were observed in plots in S3 and S4, irrespective of treatment (Table 2).

During succession of Scots pine stands *D. flexuosa* establishes when F-horizons are formed, i.e. after 30-40 years (Fanta, 1992), which explains the very low cover of *D. flexuosa* in the primary 15- to 20-year-old stands in the present study (Table 5). Once established, *D. flexuosa* dominates the understorey vegetation of Scots pine stands for 50-60 years (Fanta, 1992). In the present study the humus profiles in the secondary stands were 80 years old and dominated by *D. flexuosa* (Table 5). The establishment and dominance of *D. flexuosa* in Scots pine stands was further enhanced by high nitrogen deposition due to air pollution (Tamm, 1991; Van der Werf, 1991).

In conclusion, sod-cutting in secondary stands on mineral poor non-podzolic sandy soil changed soil conditions towards those in primary stands causing species richness and diversity of ectomycorrhizal fungi to become more similar to those of primary stands. Sod-cutting in secondary stands on podzolic sandy soil was not as effective due to relatively high nutrient concentrations and organic matter content. Primary ectomycorrhizal succession differed from secondary succession due to differences in age of humus profiles and hence differences in chemical composition of ectorganic layers and mineral soil.

Manual sod-cutting in a Scots pine stand planted in 1974 (S3).

ECTOMYCORRHIZAL ROOT GROWTH IN SCOTS PINE STANDS OF DIFFERENT AGE IN RESPONSE TO MANIPULATION OF LITTER AND HUMUS LAYERS AND SOIL CHEMISTRY

SUMMARY

Root growth and ectomycorrhizal development in the mineral soil in a spontaneously grown primary middle-aged (15-20 years) stand of Scots pine (Pinus sylvestris L.) *on non-podzolic sandy soil was compared with those in a secondary middle-aged Scots pine stand (planted in 1974) on podzolic sandy soil and with those in a primary old Scots pine stand (planted in 1924) on non-podzolic sandy soil. Litter and humus layers and herb vegetation were removed ("sod-cutting") in the secondary middle-aged and primary old stands in 1990. Removed litter and humus were added to existing ectorganic layers ("sod-addition") in the secondary middle-aged stand. Control treatments were present. In January and February 1991, ingrowth-cores were installed to a depth of 60 cm. Scots pine roots were sampled five times during two growing seasons. Ectomycorrhizal root tips were found at all sampled soil depths up to 60 cm in all plots. Root length and numbers of ectomycorrhizal root tips in the sod-cut plots in the middle-aged secondary stand were generally larger than in the control plots until May 1992. From November 1991 to November 1992 root length and numbers of ectomycorrhizal root tips in the sod-cut plots in the primary old stand were larger than in the control plots. Sod-addition did not affect root length and the numbers of ectomycorrhizal root tips. Root length and numbers of ectomycorrhizal root tips in the middle-aged primary stand plots on non-podzolic sandy soil were larger than in the middle-aged secondary stand plots on podzolic sandy soil and in the primary old stand plots on non-podzolic sandy soil. The* $N_{dissolved}$, NH_4^+, NO_3^- *and* K^+ *concentrations and the organic matter content in the upper 5 cm of the mineral soil in the plots on podzolic sandy soil were significantly higher and the pH significantly lower than in the plots in the primary middle-aged and old stands. Small root length and low numbers of ectomycorrhizal root tips were associated with high nitrogen concentrations in the upper mineral soil and high tree age.*

INTRODUCTION

Removal of litter and humus layers and herb vegetation ("sod-cutting") reduced numbers of species and sporocarps of ectomycorrhizal fungi above ground in planted stands of Scots pine (*Pinus sylvestris* L.) of different age half a year after the treatment (Baar and Kuyper, 1993), but enhanced those three and a half years after the treatment (Chapter 2). The

enhanced numbers of sporocarps were generally higher than those in spontaneously grown 15- to 20-year-old Scots pine stands on nutrient-poor non-podzolic sandy soil. However, the enhanced numbers of species above ground were lower than in the spontaneously grown 15- to 20-year-old Scots pine stands, especially in stands on podzolic sandy soil and in old (> 60 years) stands. Addition of litter and humus on top of the existing litter and humus layers in Scots pine stands of different age did not significantly affect the numbers of species and sporocarps of ectomycorrhizal fungi above ground (Chapter 2). The numbers of ectomycorrhizal species above ground in Scots pine stands were negatively associated with high inorganic nitrogen concentrations and organic matter content and low pH in the mineral soil (Chapter 2).

The present study presents below-ground data on the effects of manipulation of litter and humus layers in Scots pine stands on root length and development of ectomycorrhizal root tips over time. Only few studies on root growth and ectomycorrhizal development in deeper soil layers in Scots pine stands have been carried out (Persson, 1979; Persson, 1980a; Persson, 1984; Ahlström et al., 1988).

Therefore the aims of the present study were to investigate 1. whether removal of litter and humus layers and herb vegetation decreases root growth and numbers of ectomycorrhizal root tips in the mineral soil within a year after the treatment and later on increases; 2. whether addition of litter and humus layers does not affect root growth and numbers of ectomycorrhizal root tips in the mineral soil; 3. whether root growth and numbers of ectomycorrhizal root tips in the mineral soil in a middle-aged spontaneously grown Scots pine stand on non-podzolic sandy soil are higher than those in a middle-aged planted Scots pine stand on podzolic sandy soil and in an old planted stand on non-podzolic sandy soil; 4. whether high nutrient concentrations in the mineral soil negatively affect root growth and ectomycorrhizal development.

MATERIALS AND METHODS

Site description

A middle-aged (15-20 years) primary Scots pine stand (P1) situated in the drift sand area Hulshorsterzand in the central part of The Netherlands (52° 21'N, 5° 44'E), and a middle-aged secondary stand (S3) and an old primary Scots pine stand (S6) both situated in the forestry Dwingeloo in the northeastern part of The Netherlands (52° 50' N, 6° 25'E and 52° 49' N, 6° 26'E) were selected for study. The primary middle-aged stand has spontaneously established on drift sand (Haplic Arenosol, FAO-Unesco, 1988). Until 1920, the S3 site was *Calluna* heathland. Then *P. sylvestris* mixed with *Quercus robur* L. and *Picea abies* (L.) Karsten was planted. In 1974, the stand was felled and replanted with *P. sylvestris*. The soil of S3 is a podzolic sandy soil (Haplic Podzol, FAO-Unesco, 1988). Around 1850, the site where S6 was located, was drift sand (Haplic Arenosol, FAO-Unesco, 1988). In 1924, the

site had developed into *Calluna* heathland, and was planted with *P. sylvestris*.

In 1990, 4 plots were selected in P1 (control plots of 15 * 15 m), 12 plots were selected in S3 and 8 plots in S6. In May and June 1990, the litter and humus layers and the herb vegetation of 4 plots of 15 * 15 m in S3 and of 4 plots of 20 * 20 m in S6 were removed. In S3, the litter and humus layers of 4 plots (15 * 15 m) were covered by sods ("sod-addition"), simulating thick litter and humus layers present in old stands. In both stands, 4 untreated plots were used as controls. The few naturally regenerated deciduous trees were cut down and Scots pine seedlings were removed every year. The understorey vegetation in P1 was poor and consisted mainly of mosses and *Cladonia* species. Before sod-cutting, the understorey vegetation in S3 and S6 was dominated by the grass *Deschampsia flexuosa* (L.) Trin. In S3, *Ceratocapnos claviculata* (L.) Lidén and *Molinia caerulea* (L.) Moench and in S6 *C. claviculata* and *Dryopteris dilatata* (Hoffm.) A. Gray also occurred.

Root sampling

The root corer used in the present study was a cylindrical soil coring tube, 4.1 cm in diameter and 11.2 cm long with a sharp serrated edge for cutting roots. The corer was driven into the soil with a 2 kg hammer.

In January and February 1991, in each plot 5 sample points were randomly selected at least 2.5 m from the margin. After removing the litter and humus layers and herb vegetation mineral soil was sampled to a depth of 60 cm and sieved over a 2 mm sieve. It was impossible to sieve the humus layers in S3 and S6 as many roots of *D. flexuosa* occurred in these layers. The sieved and root-free mineral soil was put in the holes where the soil had been sampled and covered with some humus. Roots could freely grow inside. In May 1991, August 1991, November 1991, May 1992 and November 1992, four cores per treatment with sieved soil were sampled for measuring the ingrowth of new roots in the originally root-free cores. The samples were sieved over a 2 mm sieve. Sand-grains were removed from the roots in a glass box containing water. Dead roots, defined as desiccated, shrunken and highly fragile roots, were also 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 and aerial mycelium and no root hairs, or as root tips having less than three root hairs (Termorshuizen, 1991). No attemps were made to identify the ectomycorrhizal root tips to fungal species.

Root length and numbers of ectomycorrhizal root tips were recalculated to a volume of 100 cm^3 per soil layer of 10 cm. Ramification indices (Meyer, 1987b) of ectomycorrhizal root tips, numbers of ectomycorrhizal root tips cm^{-1} root, and frequencies of ectomycorrhizal root tips (100% * numbers of ectomycorrhizal root tips/total numbers of root tips) were calculated per layer of 10 cm.

Chemical analysis of soils

Soil samples were taken in April 1993. Spring, before the start of the growing season, is the best time to take soil samples. In all plots, the upper 5 cm of the mineral soil was sampled by using cores of 98.2 cm^3 after removing the herb vegetation, litter and humus layers. It was assumed that a relatively large amount of ectomycorrhizal roots and mycelia occur in the upper mineral soil (Persson, 1980a). The ten samples collected per plot were mixed and dried at 40°C. After drying the mineral soil samples were sieved over a 2 mm sieve. Each sample was analysed chemically for (plant) available nutrients according to the $CaCl_2$-method. The amounts of $N_{dissolved}$, NH_4^+, NO_3^-, P and K^+ were analysed in 0.01*M* $CaCl_2$ extracts and the pH($CaCl_2$) was determined (Houba et al., 1990). The 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 after transformation to obtain normality. Differences among means were evaluated with the LSD-test (Sokal and Rohlf, 1981)

RESULTS

Root growth and ectomycorrhizal development

In May 1991, no roots were found in any plots except in the sod-cut plots in S6 where the root length was small (Table 1).

In August 1991, root length in the sod-cut plots in S3 and in the primary stand plots with thin litter and humus layers was significantly larger than in the control plots in the S3 and S6-plots up to a soil depth of 20 cm (Table 1). The numbers of ectomycorrhizal root tips and ramification indices of the S3-S and P1-C plots were significantly higher than in the S3-C plots up to a soil depth of 10 cm (Tables 2 and 3). This indicates recovery of the ectomycorrhizal root tips of the Scots pine trees in the S3-S after sod-cutting in the upper mineral soil. In August 1991, root length was also significantly larger in the S3-S plots than in the S3-C and S6-plots at a soil depth from 20-50 cm (Table 1). The numbers of ectomycorrhizal root tips were significantly higher in the S3-S than in the S3-C plots at the soil depth from 20-30 and 50-60 cm (Table 2). This indicates that also recovery of ectomycorrhizal root tips occurred in the sod-cut plots in S3-S in deeper soil layers. The ramification indices of S6-S were significantly higher than of S6-C at a soil depth from 20-40 cm (Table 3). This indicates recovery of ectomycorrhizal root tips of the Scots pine trees in the sod-cut plots in S6 in deeper soil layers.

In November 1991, root length and numbers of ectomycorrhizal root tips were significantly larger in the P1-C plots than in the S3-C and S6-C plots in the upper 20 cm of the mineral

Table 1. Average root length in 100 cm^3 soil per layer of 10 cm. Differences between treatments at each sample time are indicated by different letters ($p < 0.05$, LSD). P1 = primary middle-aged stand, S3 = secondary middle-aged stand and S6 = primary old stand. C = control, S = sod-cut and A = sod-added.

Depth (cm)	Plot	May '91	Aug '91	Nov '91	May '92	Nov '92
0-10	P1-C	0.0	80.2 c	112.4 c	159.7 b	–
	S3-C	0.0	7.0 a	14.8 b	47.6 a	286.1 b
	S3-S	0.0	50.9 bc	60.6 bc	54.1 a	235.8 b
	S3-A	0.0	22.0 ab	96.9 c	73.9 ab	222.5 b
	S6-C	0.0	4.5 a	2.6 a	36.1 a	33.6 a
	S6-S	1.7	3.6 a	8.3 ab	108.4 ab	35.5 a
10-20	P1-C	0.0	66.6 b	112.4 b	136.2 b	–
	S3-C	0.0	7.0 a	18.8 a	48.5 a	259.4 b
	S3-S	0.0	50.9 b	60.6 ab	54.7 ab	230.8 b
	S3-A	0.0	22.0 a	59.7 ab	69.2 ab	201.4 b
	S6-C	0.0	4.4 a	2.7 a	32.1 a	33.1 a
	S6-S	1.4	4.5 a	8.3 a	106.7 ab	35.5 a
20-30	P1-C	0.0	17.4 ab	101.6 b	102.6 d	–
	S3-C	0.0	8.4 a	27.3 a	26.8 ac	115.1 bc
	S3-S	0.0	33.2 b	37.8 a	52.2 b	194.0 c
	S3-A	0.0	17.7 ab	31.7 a	41.5 bc	93.1 ab
	S6-C	0.0	5.0 a	2.9 a	3.0 a	28.6 a
	S6-S	1.1	11.3 a	33.0 a	68.4 b	37.2 ab
30-40	P1-C	0.0	14.0 a	97.7 b	68.1 cd	–
	S3-C	0.0	9.9 a	38.4 ab	22.4 ab	95.2 b
	S3-S	0.0	34.6 b	31.3 a	51.2 cd	161.8 c
	S3-A	0.0	18.6 ab	57.7 ab	34.6 bc	85.0 ab
	S6-C	0.0	4.9 a	8.0 a	2.3 a	28.4 a
	S6-S	1.1	11.6 a	27.9 a	37.7 bc	36.8 a
40-50	P1-C	0.0	7.6 a	95.6 b	40.9 bc	–
	S3-C	0.0	12.6 a	48.6 ab	34.4 b	77.9 ab
	S3-S	0.0	45.1 b	40.2 ab	64.8 ac	120.6 b
	S3-A	0.0	18.2 a	37.4 ab	21.6 ab	76.6 ab
	S6-C	0.0	2.5 a	12.5 a	5.9 a	24.5 a
	S6-S	1.3	10.9 a	10.6 a	21.1 ab	41.3 a
50-60	P1-C	0.0	7.8 bc	106.4 b	62.0 bc	–
	S3-C	0.0	20.1 cd	60.5 ab	46.8 bc	93.2 ab
	S3-S	0.0	38.8 d	35.4 a	69.1 c	121.8 b
	S3-A	0.0	6.7 b	14.1 a	30.3 bc	94.8 ab
	S6-C	0.0	0.0 a	23.7 a	4.6 a	23.2 a
	S6-S	1.5	3.0 ab	5.6 a	18.5 ab	43.8 ab

Table 2. Average numbers of ectomycorrhizal root tips in 100 cm^3 soil per layer of 10 cm. Differences between treatments at each sample time are indicated by different letters ($p < 0.05$, LSD) or ns (not significant). P1 = primary middle-aged stand, S3 = secondary middle-aged stand and S6 = primary old stand. C = control, S = sod-cut and A = sod-added.

Depth (cm)	Plot	May '91	Aug '91	Nov '91	May '92	Nov '92
0-10	P1-C	0.0	141.2 b	404.5 b	651.4 b	–
	S3-C	0.0	4.7 a	11.4 a	81.1 a	740.7 b
	S3-S	0.0	125.3 b	114.5 a	87.4 a	446.0 b
	S3-A	0.0	20.1 a	119.1 a	90.9 ab	551.8 b
	S6-C	0.0	15.6 a	0.3 a	63.8 a	48.3 a
	S6-S	0.0	10.6 a	28.4 a	155.4 ab	98.8 a
10-20	P1-C	0.0	96.3 bc	404.5 d	566.0 b	–
	S3-C	0.0	4.7 a	14.8 c	78.6 a	666.6 c
	S3-S	0.0	125.3 ac	114.5 bd	85.8 a	434.5 abc
	S3-A	0.0	20.1 ab	63.3 cd	82.2 a	500.1 bc
	S6-C	0.0	15.1 a	1.1 a	44.7 a	55.4 a
	S6-S	0.0	16.1 a	28.4 b	172.0 a	98.8 ab
20-30	P1-C	0.0	13.2 ab	308.4 c	365.0 c	–
	S3-C	0.0	7.8 a	18.8 ab	45.0 b	145.8 ab
	S3-S	0.0	64.1 b	64.9 bc	55.6 b	280.1 b
	S3-A	0.0	15.6 ab	8.2 ab	39.3 b	185.1 ab
	S6-C	0.0	18.7 ab	1.6 a	3.8 a	62.7 a
	S6-S	0.0	55.3 ab	97.2 bc	201.0 bc	124.7 ab
30-40	P0-C	0.0	16.4 ns	295.6 b	245.7 d	–
	S3-C	0.0	7.9 ns	25.0 a	31.0 bc	123.4 ns
	S3-S	0.0	44.0 ns	53.7 ab	54.6 c	206.7 ns
	S3-A	0.0	9.4 ns	7.6 a	17.9 b	111.1 ns
	S6-C	0.0	20.1 ns	14.1 a	2.1 a	65.5 ns
	S6-S	0.0	52.6 ns	83.3 ab	81.8 cd	140.8 ns
40-50	P1-C	0.0	24.2 ns	327.1 b	135.5 d	–
	S3-C	0.0	9.8 ns	38.2 a	27.9 bc	91.7 ns
	S3-S	0.0	43.9 ns	43.2 a	64.2 cd	88.5 ns
	S3-A	0.0	3.7 ns	3.5 a	10.1 b	41.4 ns
	S6-C	0.0	10.0 ns	11.2 a	0.8 a	53.9 ns
	S6-S	0.0	44.7 ns	18.1 a	22.6 bd	107.0 ns
50-60	P1-C	0.0	21.0 bc	345.9 c	257.9 d	–
	S3-C	0.0	4.4 ab	33.3 ab	22.4 c	25.4 a
	S3-S	0.0	45.4 c	33.8 b	52.5 cd	59.3 ab
	S3-A	0.0	1.5 a	0.7 a	5.4 b	52.2 ab
	S6-C	0.0	0.0 a	3.4 ab	1.7 a	41.4 ab
	S6-S	0.0	5.9 ab	9.2 ab	22.1 cd	111.9 b

Table 3. Ramification index per layer of 10 cm. Differences between treatments at each sample time are indicated by different letters ($p < 0.05$, LSD) or ns (not significant). P1 = primary middle-aged stand, S3 = secondary middle-aged stand and S6 = primary old stand. C = control, S = sod-cut and A = sod-added.

Depth (cm)	Plot	May '91	Aug '91	Nov '91	May '92	Nov '92
0-10	P1-C	0.0	2.0 b	3.0 b	4.9 b	–
	S3-C	0.0	0.6 a	0.8 ab	1.7 ab	2.7 b
	S3-S	0.0	2.3 b	2.0 ab	1.9 a	2.2 ab
	S3-A	0.0	0.5 a	2.2 ab	1.2 a	2.6 b
	S6-C	0.0	1.8 ab	0.2 a	1.8 a	1.1 a
	S6-S	0.0	1.1 ab	2.4 ab	2.1 ab	2.0 ab
10-20	P1-C	0.0	1.4 ab	3.0 c	4.8 b	–
	S3-C	0.0	0.6 a	0.8 ab	1.8 a	2.6 ns
	S3-S	0.0	2.3 ab	2.0 bc	1.8 a	2.2 ns
	S3-A	0.0	0.5 a	0.8 ab	1.2 a	2.5 ns
	S6-C	0.0	1.6 ab	0.5 a	0.7 a	1.4 ns
	S6-S	0.0	3.4 b	1.7 abc	2.4 ab	2.0 ns
20-30	P1-C	0.0	1.1 a	3.0 bc	3.5 c	–
	S3-C	0.0	0.6 a	0.6 ab	2.0 bc	1.2 ns
	S3-S	0.0	1.9 a	1.8 c	0.8 ab	1.6 ns
	S3-A	0.0	0.7 a	0.3 a	0.9 ab	2.1 ns
	S6-C	0.0	1.6 a	0.9 ab	0.4 a	1.2 ns
	S6-S	0.0	5.2 b	3.3 c	2.8 c	2.4 ns
30-40	P1-C	0.0	1.7 ab	3.0 b	3.4 c	–
	S3-C	0.0	0.5 a	0.6 a	1.4 ab	1.1 ns
	S3-S	0.0	1.3 a	1.6 a	1.0 ab	1.4 ns
	S3-A	0.0	0.4 a	0.1 a	0.5 a	1.4 ns
	S6-C	0.0	1.4 a	0.9 a	1.3 ab	2.0 ns
	S6-S	0.0	4.4 b	3.2 b	2.8 bc	2.4 ns
40-50	P1-C	0.0	2.2 ns	3.8 b	3.3 b	–
	S3-C	0.0	0.8 ns	0.9 a	0.8 a	0.7 ab
	S3-S	0.0	1.2 ns	0.8 a	0.9 a	0.7 ab
	S3-A	0.0	0.2 ns	0.1 a	0.3 a	0.6 a
	S6-C	0.0	1.4 ns	0.5 a	0.5 a	2.1 b
	S6-S	0.0	1.9 ns	1.5 a	3.8 b	2.0 b
50-60	P1-C	0.0	2.0 b	3.1 c	2.8 c	–
	S3-C	0.0	0.3 a	0.4 ab	0.5 a	0.2 a
	S3-S	0.0	1.3 ab	0.7 ab	0.7 ab	0.6 ac
	S3-A	0.0	0.1 a	0.0 a	0.2 a	0.7 ac
	S6-C	0.0	0.0 a	0.2 a	0.6 a	2.4 b
	S6-S	0.0	0.5 a	1.5 b	2.2 bc	1.9 bc

Table 4. Frequency of ectomycorrhizal root tips per layer of 10 cm. Differences between treatments at each sample time are indicated by different letters ($p < 0.05$, LSD) or ns (not significant). P1 = primary middle-aged stand, S3 = secondary middle-aged stand and S6 = primary old stand. C = control, S = sod-cut and A = sod-added.

Depth (cm)	Plot	May '91	Aug '91	Nov '91	May '92	Nov '92
0-10	P1-C	0.0	80.0 ns	88.2 b	93.5 ns	–
	S3-C	0.0	40.2 ns	60.7 ab	89.1 ns	91.0 ns
	S3-S	0.0	76.6 ns	77.2 b	81.4 ns	94.0 ns
	S3-A	0.0	34.9 ns	63.2 ab	87.8 ns	78.9 ns
	S6-C	0.0	64.6 ns	25.0 a	65.8 ns	94.4 ns
	S6-S	0.0	44.0 ns	69.7 ab	84.0 ns	72.4 ns
10-20	P1-C	0.0	83.4 ns	88.2 b	93.5 b	–
	S3-C	0.0	40.2 ns	55.5 ab	89.6 b	91.1 ns
	S3-S	0.0	34.9 ns	54.0 ab	88.0 b	79.8 ns
	S3-A	0.0	76.6 ns	77.2 ab	81.3 b	93.7 ns
	S6-C	0.0	58.6 ns	37.5 a	42.0 a	93.8 ns
	S6-S	0.0	68.9 ns	69.7 ab	84.7 ab	72.4 ns
20-30	P1-C	0.0	53.6 ns	88.2 b	94.4 b	–
	S3-C	0.0	51.8 ns	34.8 a	88.7 b	77.7 ns
	S3-S	0.0	77.5 ns	72.9 ab	84.4 ab	83.1 ns
	S3-A	0.0	80.5 ns	26.3 a	84.8 ab	75.8 ns
	S6-C	0.0	54.2 ns	66.5 ab	47.2 a	66.1 ns
	S6-S	0.0	72.4 ns	68.1 ab	91.8 ab	68.9 ns
30-40	P1-C	0.0	84.1 ns	89.1 c	93.2 b	–
	S3-C	0.0	45.2 ns	30.1 ab	84.5 ab	75.0 ns
	S3-S	0.0	67.0 ns	73.7 bc	84.4 ab	76.9 ns
	S3-A	0.0	57.6 ns	14.5 a	73.6 a	64.2 ns
	S6-C	0.0	32.2 ns	64.4 bc	93.8 b	88.1 ns
	S6-S	0.0	71.0 ns	68.2 bc	91.2 b	67.9 ns
40-50	P1-C	0.0	69.9 ns	90.1 c	92.9 ns	–
	S3-C	0.0	51.9 ns	41.6 ab	73.3 ns	67.4 ns
	S3-S	0.0	65.9 ns	56.8 ac	80.4 ns	55.6 ns
	S3-A	0.0	33.7 ns	10.3 a	54.3 ns	51.9 ns
	S6-C	0.0	32.2 ns	46.8 ac	70.0 ns	91.3 ns
	S6-S	0.0	43.1 ns	69.0 bc	89.9 ns	67.8 ns
50-60	P1-C	0.0	71.5 ns	89.3 c	89.8 ns	–
	S3-C	0.0	28.2 ns	28.9 a	63.0 ns	34.7 ns
	S3-S	0.0	73.6 ns	49.1 ac	77.5 ns	48.0 ns
	S3-A	0.0	20.9 ns	9.0 a	49.9 ns	59.2 ns
	S6-C	0.0	0.0 ns	35.1 ab	65.7 ns	87.3 ns
	S6-S	0.0	20.3 ns	77.5 bc	88.6 ns	66.3 ns

soil (Tables 1 and 2). At the same soil depth the ramification index of P1-C was significantly larger than in S6-C (Table 3). This suggests that thick litter and humus layers in S3 and S6 reduced ectomycorrhizal formation. At a soil depth from 20-60 cm the numbers of ectomycorrhizal root tips were significantly higher in the P1-C plots than in the S3-C and S6-C plots (Table 2) and the frequencies of ectomycorrhizal root tips were significantly higher in the P1-C plots than in the S3-C and S3-A plots (Table 4) suggesting that the presence of thick litter and humus layers negatively affected ectomycorrhizal development in deeper soil layers too. At the same soil depth root length was significantly larger in the P1-C plots on non-podzolic sandy soil than in the S6-plots on non-podzolic sandy soil (Table 1) indicating that root growth of old trees was reduced.

. In November 1991 and in May 1992, the root length and numbers of ectomycorrhizal root tips in the P1-C plots on non-podzolic sandy soil between 0 and 60 cm were generally higher than in the S3 plots on podzolic sandy soil, irrespective of treatment.

In May 1992, in the upper 20 cm of the mineral soil, the numbers of ectomycorrhizal root tips in the P1-C plots were significantly higher than in the S3-S plots (Table 2). The numbers of ectomycorrhizal root tips in the S6-S plots were generally higher than in the S3-S plots in the mineral soil from 0-20 cm (Table 2). This suggest that after an increase of root growth due to sod-cutting, ectomycorrhizal development in the podzolic sandy soil was reduced. The root length and numbers of ectomycorrhizal root tips in P1-C plots were significantly higher than in the S6-C plots at a soil depth from 20-60 cm (Tables 1 and 2). At the same soil depth the numbers of ectomycorrhizal root tips were significantly higher in the P1-C plots than in the S3-C and S3-A plots (Table 2) showing that in the deeper soil layers ectomycorrhizal development in podzolic sandy soil was also diminished.

In November 1992, the root length and numbers of ectomycorrhizal root tips in the S3-plots were significantly larger than in the S6-plots from 0-20 cm and 0-10 cm, respectively (Tables 1 and 2). This shows that root growth and ectomycorrhizal development of middle-aged Scots pine trees on podzolic sandy soil was larger than those of old trees on non-podzolic sandy soil from May 1992 to November 1992 in the upper 20 cm despite the relatively high nitrogen concentrations, high organic matter content and low pH. A similar pattern was observed for the soil layers from 20-60 cm, although less clear.

Chemical composition of soils

In 1993, the $N_{dissolved}$ and NH_4^+ concentrations and organic matter content in the upper mineral soil in all plots in S3 on podzolic sandy soil were significantly higher than in P1 and S6 (Table 5). The NH_4^+ and NO_3^- concentrations in the upper mineral soil in the sod-added plots in S3 were significantly higer than in the remaining plots. The NO_3^- concentrations were much lower than the NH_4^+ concentrations in all cases. The pH in the mineral soil in all plots in S3 was significantly lower than in the P1 and S6 plots. The chemical composition of the mineral soil in the control and sod-cut plots in S6 on non-podzolic soil was generally similar to that of the middle-aged primary stand plots.

Table 5. 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 ($p < 0.05$, LSD-test) or ns (not significant). P1 = primary middle-aged stand, S3 = secondary middle-aged stand and S6 = primary old stand. C = control, S = sod-cut and A = sod-added.

Plot	N_{diss}	NH_4^+	NO_3^-	P	K^+	pH	OM
P1	3.7 a	0.6 a	0.1 a	0.5 ns	25.4 ab	3.9 bc	0.9 ab
S3-C	13.3 bc	3.3 b	1.5 c	0.2 ns	15.4 ab	3.3 a	4.5 c
S3-S	12.2 b	3.2 b	1.0 c	0.2 ns	15.5 ab	3.4 a	4.4 c
S3-A	16.8 c	5.7 c	2.9 d	0.6 ns	16.9 b	3.2 a	5.4 c
S6-C	5.5 a	1.6 a	1.0 bc	0.3 ns	8.2 ab	3.7 b	1.4 b
S6-S	1.7 a	0.3 ab	0.8 b	0.1 ns	6.5 a	4.0 c	0.8 a

DISCUSSION

Tree roots in the ectorganic layers were removed together with removal of litter and humus layers (Baar and Kuyper, 1993). The larger root length and ectomycorrhizal development in the sod-cut plots compared to those in the control plots in the secondary middle-aged stand until May 1992 and in the old primary stand until November 1992 indicate recovery of root growth by the Scots pine trees after removal of litter and humus layers. The high ramification indices of the sod-cut plots in the primary old stand in November 1991 and May 1992, nearly similar to those of the primary middle-aged stand plots, suggest increase of ectomycorrhizal development below ground due to sod-cutting. This is in accordance with the increase of species and sporocarps of ectomycorrhizal fungi in the sod-cut plots in the primary old stand in 1993 (Chapter 2). In contrast, in May and November 1992 the numbers of ectomycorrhizal root tips in the sod-cut plots in the secondary middle-aged stands did not differ from those in the control plots, although the numbers of species and sporocarps of ectomycorrhizal fungi in the sod-cut plots were much higher than in the control plots (Chapter 2). This suggests that the numbers of ectomycorrhizal root tips below ground and numbers of sporocarps of ectomycorrhizal fungi above ground are not necessarily related (Wallander, 1992; Chapter 5).

Ectomycorrhizal development occurred as indicated by the presence of ectomycorrhizal root tips up to a depth of 60 cm in the sod-cut plots in S3 and S6 in August 1991 (Table 2). After sod-cutting, mycelia of ectomycorrhizal fungi associated with Scots pine roots have survived in deeper layers of the mineral soil as suggested for *Laccaria bicolor* (Maire) P.D. Orton (Chapter 9).

Sod-addition in the secondary stand plots increased root length and numbers of ectomycorrhizal root tips in the upper 20 cm of the mineral soil until May 1992. Roots from the humus on top of the cores might have grown into the cores. However, sod-addition did not affect root length and numbers of ectomycorrhizal root tips in deeper soil layers up to a soil depth of 60 cm.

Root length, numbers of ectomycorrhizal root tips, ramification indices and ectomycorrhizal frequencies in the mineral soil in the middle-aged primary stand plots on non-podzolic sandy soil were generally larger than those in the secondary stand plots on podzolic sandy soil and than those obtained from samples in the humus layers and upper mineral soil by several investigators (Ritter and Tölle, 1978; Kuyper, 1990; Termorshuizen, 1991). However, higher numbers of ectomycorrhizal root tips than those in the present study were determined in Scots pine forests in Norway (142 cm^{-3} humus) and in Finland (16-35 cm^{-3} humus; Timmermann, 1994; Ohtonen et al., 1990). High nitrogen input associated with air pollution in The Netherlands has been identified as an important cause of the decline of ectomycorrhizal fungi (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).

Numbers of ectomycorrhizal root tips up to a soil depth of 60 cm in the middle-aged primary stand plots on non-podzolic sandy soil were higher than those in the secondary stand plots on podzolic sandy soil in November 1991 and in May 1992. In nutrient-poor soils relatively large roots systems, high fine root biomass and great abundance of ectomycorrhizal root tips have been found (Keyes and Grier, 1981; Wästerlund, 1982b; Bowen, 1984; Olsthoorn, 1991). Ectomycorrhizal formation enhances nitrogen and phosphate uptake (Bledsoe and Zasosky, 1983; Finlay, 1989; Finlay et al., 1989). The large root length and high numbers of ectomycorrhizal root tips in the mineral soil in the middle-aged primary stand plots can also be explained by a shift of root growth from humus layers to deeper soil layers enhancing water uptake (Feil et al., 1988; Persson, 1992).

The low numbers of ectomycorrhizal root tips in the podzolic sandy soil are in accordance with earlier findings and are associated with the high nitrogen concentrations in the upper 5 cm of the mineral soil (Table 5). Persson (1980b) noted lower ramification indices in a podzolic mineral soil in a 15- to 20-year-old Scots pine stand. Mainly formation and elongation of fast growing long roots took place in the podzolic soil. Reduced numbers of ectomycorrhizal root tips of Scots pine seedlings were found in the podzolic soil in S3 in 1992 (Chapter 5). Arnebrant and Söderström (1992) noted that large amounts of nitrogen decreased ectomycorrhizal development of Scots pine seedlings.

The numbers of ectomycorrhizal root tips and the ramification indices in the primary old stand control plots were smaller than those in the primary middle-aged stand plots in November 1991 and May 1992 (Tables 3 and 4). Jansen (1991) reported lower numbers of ectomycorrhizal root tips in humus layers in older ($>$ 40 years) Douglas fir stands compared to those in young ($<$ 10 years) stands. Older coniferous trees may take up less nutrients as

shown for nitrogen and potassium (Cole and Rapp, 1981).

In the present study the highest numbers of ectomycorrhizal root tips were found in the upper 20 cm of the mineral soil which is in accordance with earlier findings (Meyer, 1973; Persson, 1980b; Rastin et al., 1990). However, ectomycorrhizal root tips were found up to a soil depth of 60 cm in nearly all plots irrespective of treatment. Several investigators reported ectomycorrhizal root tips of *P. sylvestris* at a depth of 1.5 m and 1.9 m (Werlich and Lyr, 1957; Lobanow, 1960). In The Netherlands, ectomycorrhizal root tips of Douglas fir were found at soil depths up to 1.2 m (Olsthoorn and Tiktak, 1991).

The use of the ingrowth technique might underestimate root growth (Nadelhoffer et al., 1985; Vogt and Persson, 1991; Hendricks et al., 1993; Fahey and Hughes, 1994). However, the use of this technique in the present study revealed that sod-cutting increased root growth and ectomycorrhizal development in Scots pine stands until two years after the treatment and that sod-addition did not affect those.

In conclusion, root growth and ectomycorrhizal development up to a soil depth of 60 cm in middle-aged and old Scots pine stands on podzolic and non-podzolic soil recovered after sod-cutting. However, the recovery of the Scots pine roots retarded in the podzolic soil in May 1992. Sod-addition did not affect root growth and ectomycorrhizal development. The small root length and low numbers of ectomycorrhizal root tips in the secondary stand plots on podzolic sandy soil compared to the primary middle-aged stand on non-podzolic soil can be explained by the high nitrogen concentrations, high organic matter content and low pH in the mineral soil. The small root length and low numbers of ectomycorrhizal root tips in the primary old stands on non-podzolic soil compared to the primary stand plots were associated with the high tree age. It can be concluded that small root length and low numbers of ectomycorrhizal root tips are associated with high nitrogen concentration in the upper mineral soil and high tree age.

EFFECTS OF MANIPULATION OF LITTER AND HUMUS LAYERS ON ECTOMYCORRHIZAL COLONIZATION POTENTIAL IN SCOTS PINE STANDS OF DIFFERENT AGE

SUMMARY

Effects of manipulation of litter and humus layers (removal, doubling and control treatments) on the colonization potential of ectomycorrhizal fungi were studied in two secondary stands of Pinus sylvestris *(planted in 1974 and 1987) in The Netherlands. Five-month-old, sterile-grown Scots pine seedlings, inoculated with* Laccaria bicolor, Paxillus involutus *or* Rhizopogon luteolus *and non-inoculated seedlings were used as baits. The seedlings were harvested after one growing season. For comparison, sporocarps of ectomycorrhizal fungi were also investigated. Genus composition on the seedlings was independent of initial inoculum, but determined by both treatment and age of the stands. In both stands, removal of litter and humus layers increased, and addition of organic material decreased the numbers of ectomycorrhizal types on the seedlings. Not all indigenous genera were observed by either outplanting seedlings or sporocarp surveys.*

INTRODUCTION

In a previous study, the effects of removal of litter and humus layers on ectomycorrhizal fungi were assessed (Baar and Kuyper, 1993). Removal of litter, humus and the herbaceous vegetation by sod-cutting (removal of the organic top soil and vegetation) was found to raise the numbers of species and sporocarps compared to the control plots in young (< 15-year-old) and middle-aged (15- to 50-year-old) secondary Scots pine stands. In the same stands, addition of ectorganic layers by adding sods, simulating thick litter and humus layers of old (> 50 years) stands, reduced the numbers of species and sporocarps compared to the control plots.

Ectomycorrhizal fungi, however, are not observed when they do not form sporocarps, so sporocarp surveys have to be repeated for several years. In the present study, pine seedlings were used as baits to investigate the colonization potential of ectomycorrhizal fungi including non-sporocarp forming species, as affected by soil treatments (Stenström, 1990; Arnebrant, 1991; Dahlberg and Stenström, 1991).

The objectives of the present study were 1. to determine effects of manipulation of litter and humus layers on the ectomycorrhizal colonization capacity, 2. to investigate replacement of inoculated fungi by indigenous fungi and 3. to compare the results of

baiting with those of sporocarp surveys.

MATERIALS AND METHODS

Study sites

The study was carried out in two secondary stands (S1 planted in 1987 and S3 in 1974) of *P. sylvestris* L. both situated in forestry Dwingeloo in the northeastern part of The Netherlands (52° 49' N, 6° 28' E and 52° 50' N, 6° 25' E, respectively) in 1992. Until 1939, the site of S1 was drift sand, Haplic Arenosol (FAO-Unesco, 1988) with patches heathland. Then *Picea sitchensis* (Bong.) Carr. mixed with *Castanea sativa* Miller, *Fagus sylvatica* L., *Betula* spp. and *Prunus* spp. was planted. In 1987, this stand was felled and replanted with *P. sylvestris*. Until 1920, the site of S3 was heathland. Then *P. sylvestris* mixed with *Quercus rubra* L. and *Picea abies* (L.) Karsten was planted. In 1974, the stand was felled and replanted with *P. sylvestris*. The soil of S3 is podzolic (Haplic Podzol, FAO-Unesco, 1988).

In May 1990, litter and humus layers of 4 plots (15 * 15 m) per stand were removed ("sod-cutting"). At the same time, ectorganic material was added to 4 plots ("sod-adding"), simulating ageing of the humus profile, and 4 control plots were selected. The few naturally regenerated deciduous trees were chopped and Scots pine seedlings were removed every year.

In May 1992, the average thickness of the humus layers of the control and sod-added plots in S1 were 8 and 11 cm, respectively. The herbaceous understory vegetation of all plots in S1 consisted of *Deschampsia flexuosa* (L.) Trin., *Chamerion angustifolium* (L.) Holub and *Calluna vulgaris* (L.) Hull. The average thickness of the humus layers of the control and sod-added plots in S3 was 10 and 9 cm, respectively. The control and sod-added plots in S3 were dominated by *D. flexuosa*, *Ceratocapnos claviculata* (L.) Lidén en *Molinia caerulea* (L.) Moench. The herbaceous vegetation of the sod-cut plot consisted of *C. vulgaris*, *Carex pilulifera* L. and *D. flexuosa*.

Baiting procedure

Seeds of *P. sylvestris* originating from forestry Junne in the northeastern part of The Netherlands were sterilized in 15% H_2O_2 for 30 min. Sterilized seeds were germinated on sterile water agar containing 0.5% glucose in December 1991.

After germination, seedlings were inoculated or not with isolates of *Laccaria bicolor* (Maire) P.D. Orton, *Paxillus involutus* (Batsch: Fr.) Fr. or *Rhizopogon luteolus* Fr. *Laccaria bicolor* had been collected in the autumn of 1990 in a 50-year-old secondary stand of *P. sylvestris* (S5), located in the northeastern part of The Netherlands. *Paxillus involutus* and *R. luteolus* had been collected in the autumn of 1990 in a 15- to 20-year-old primary Scots pine stand (P1), located in the central part of The Netherlands. These fungi

were chosen because they reacted strongly on removal of litter and humus layers or accumulation of ectorganic material as shown by observations on sporocarps (Baar and Kuyper, 1993), and because these species can easily be cultured and inoculated. *Laccaria bicolor* and *R. luteolus* were positively affected by sod-cutting, whereas sporocarps of *P. involutus* were mainly found in the control plots.

Inoculated seedlings were grown *in vitro* on twice autoclaved (20 min, 110°C) peat, vermiculite and MMN solution (at 4:10:7 by volume) in closed glass tubes in a greenhouse from January to May 1992. Peat, vermiculite and MMN solution were used as substrate to stimulate ectomycorrhizal development. Non-inoculated seedlings were grown in small pots with sandy soil originating from the 15- to 20-year-old primary Scots pine stand (P1). Non-ectomycorrhizal seedlings developed better in sandy soil than in peat, vermiculite and MMN solution. Day temperature was kept at 21°C and night temperature at 8°C for 3 months, then day temperature became outdoor temperature while night temperature was kept at a minimum of 10°C.

In May 1992, 88 seedlings were planted out in one control plot, 88 seedlings in one sod-cut plot and 88 seedlings in one sod-added plot in S1. Four groups consisting of 22 seedlings each were planted at 4 sites in each plot. The distance between 2 sites was 8 m (Fig. 1). Each group of seedlings was covered with wire netting to protect them from grazing by animals. The same procedure was carried out in S3.

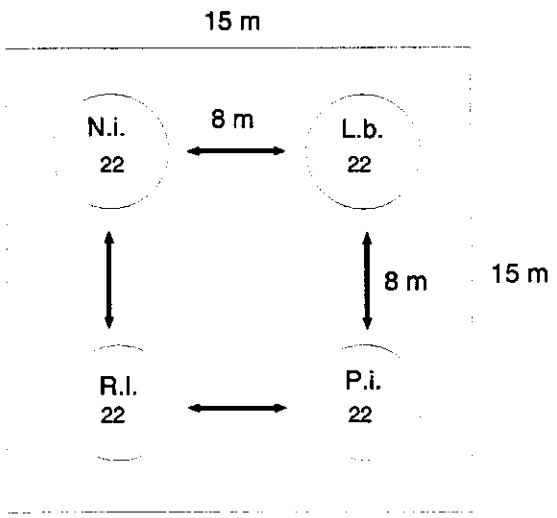


Fig. 1. Experimental design of one plot. N.i. = non-inoculated seedlings, L.b. = inoculated with *L. bicolor*, P.i. = inoculated with *P. involutus* and R.l. = inoculated with *R. luteolus*. 22 = number of outplanted seedlings.

Five months later, 10 surviving seedlings per site were harvested (or fewer when fewer seedlings survived). The root systems were cut off and stored in a glutaraldehyde buffer (Alexander and Bigg, 1981) until further analysis.

Determining ectomycorrhizal incidence

The root systems were studied using a dissecting microscope. The ectomycorrhizal root tips were divided in two groups on the basis of appearance. Well-developed ectomycorrhizal root tips with a smooth, relatively thick mantle were categorized as vital. Poorly developed ectomycorrhizal root tips with a dented and more or less wrinkled mantle or no distinct mantle were categorized as non-vital. Only well-developed ectomycorrhizal root tips could be identified on the basis of colour and morphology (Agerer, 1987; Ingleby et al., 1990). Types thus recognized generally correspond to fungal taxa at the genus level except for ITE3. Therefore, the terms below-ground ectomycorrhizal types and above-ground fungal genera will be treated as equivalent.

Root length per seedling was determined according to the line intersect method of Newman (1966). Total numbers of root tips, numbers of vital and non-vital ectomycorrhizal and non-ectomycorrhizal root tips per seedling were determined. The frequencies of ectomycorrhizal (vital and non-vital) and non-ectomycorrhizal root tips were calculated from numbers of non-ectomycorrhizal root tips/numbers of ectomycorrhizal root tips + numbers of non-ectomycorrhizal root tips. The frequencies of vital ectomycorrhizal root tips of each ectomycorrhizal type were also calculated, as well as the total number of root tips per centimeter root.

Survey of sporocarps

From the beginning of September until the end of October 1992 the plots of S1 and S3 were surveyed three times for sporocarps of ectomycorrhizal fungi. The caps of sporocarps were removed in order to avoid double counting.

Statistical analysis

Main effects of treatments and stand ages were tested with one-way ANOVA when the variables did not show interaction (Sokal and Rohlf, 1981). Data not normally distributed, even after log transformation, were analysed with the Kruskal-Wallis test (Siegel and Castellan, 1988).

RESULTS

Mycorrhizal types on the seedlings

The initial inoculum did not affect the composition of ectomycorrhizal types on the seedlings after one growing season. However, the composition of ectomycorrhizal types of the seedlings differed among the two stands. *Hebeloma*, *Rhizopogon* and *Thelephora* were only observed on seedlings in S1 and *Lactarius* and *Russula* were found only on seedlings planted in S3 (Table 1).

Table 1. Average frequency of ectomycorrhizal types (in %) of seedlings planted in S1 and S3. The ectomycorrhizal types on the seedlings similar to the initial inoculum are printed in bold. The species are abbreviated as follows: Aman = *Amanita* spp, Ceno = *Cenococcum* spp., Hebe = *Hebeloma* spp., ITE3 = ITE3, Lacc = *Laccaria* spp., Lact = *Lactarius* spp., Paxi = *Paxillus* spp., Rhiz = *Rhizopogon* spp., Russ = *Russula* spp. and Thel = *Thelephora* spp. C = control, S = sod-cut and A = sod-added. n = number of seedlings harvested.

	Ectomycorrhizal root tips				Vital root tips								
Initial inoculum	n	Non	Vit-	Vit+	Aman	Ceno	Hebe	ITE3	Lacc	Paxi	Rhiz	Russ	Thel
S1													
C: Non-inoculated	10	28.1	29.1	43.0	–	0.6	–	–	43.0	–	–	–	–
L. bicolor	3	39.1	22.8	38.1	–	–	–	–	**38.1**	–	–	–	–
P. involutus	7	16.1	13.9	70.0	–	–	–	–	66.4	**12.6**	–	–	–
R. luteolus	10	14.7	15.6	69.7	–	–	–	–	69.7	–	–	–	–
S: Non-inoculated	10	25.7	19.3	56.9	–	–	–	–	46.0	–	58.2	–	26.4
L. bicolor	3	13.0	32.8	54.1	–	–	31.9	–	**47.8**	–	7.8	–	–
P. involutus	8	18.0	23.9	58.1	24.7	–	–	–	47.8	–	**16.5**	–	–
R. luteolus	5	13.9	45.8	40.3	–	0.2	33.4	–	16.7	–	**8.8**	–	–
A: Non-inoculated	10	59.3	8.3	33.3	–	–	–	17.7	22.3	–	–	–	–
L. bicolor	10	32.8	13.7	53.6	–	–	–	5.8	**50.7**	–	–	–	–
P. involutus	10	11.4	22.7	65.9	–	–	–	–	65.9	–	–	–	–
R. luteolus	9	14.5	7.5	78.9	–	–	–	–	78.9	–	–	–	–

					Aman	Ceno	Hebe	ITE3	Lacc	Lact	Paxi	Rhiz	Russ
S3													
C: Non-inoculated	10	91.8	5.5	2.7	–	–	–	4.5	-	4.6	–	–	–
L. bicolor	10	79.1	17.3	3.6	–	–	–	–	**8.6**	8.6	–	–	1.8
P. involutus	10	58.9	41.1	–	–	–	–	–	-	–	–	–	–
R. luteolus	10	64.0	34.0	11.3	–	–	–	–	-	11.3	–	–	–
S: Non-inoculated	10	52.7	15.4	31.8	–	31.8	–	1.3	24.1	11.6	–	–	13.4
L. bicolor	10	51.3	30.9	27.0	–	2.9	–	–	**32.9**	35.1	–	–	–
P. involutus	10	53.5	41.8	4.7	4.9	–	–	–	-	1.0	**4.0**	–	3.9
R. luteolus	10	53.5	40.3	6.2	–	–	–	–	-	5.1	–	–	52.0
A: Non-inoculated	10	89.0	10.5	5.0	–	5.0	–	–	-	–	–	–	–
L. bicolor	10	68.2	30.9	8.3	–	–	–	–	**8.3**	–	–	–	–
P. involutus	10	58.5	41.5	–	–	–	–	–	-	–	–	–	–
R. luteolus	10	73.1	26.7	1.3	–	1.3	–	–	-	–	–	–	–

In S1, seedlings in all treatments had ectomycorrhizal root tips dominated by *Laccaria* (Table 1). The highest number (6) of ectomycorrhizal types was found in the sod-cut plot (Fig. 2). The lowest number of types (2) was observed in the sod-added plot.

In the sod-cut plot in S3, *Laccaria, Lactarius* and *Russula* were the most frequent types (Table 1). In the sod-added plot only two types were found, *Cenococcum* and *Laccaria*. The highest number (7) of ectomycorrhizal types was observed in the sod-cut plot, the lowest number (2) in the sod-added plot (Fig. 2).

Of the total number of seedlings, 14% and 2.3%, contained two and three ectomycorrhizal types, respectively, on the roots of the same seedling. No ectomycorrhizal root tips were found on 3.3% of the total number of surviving seedlings.

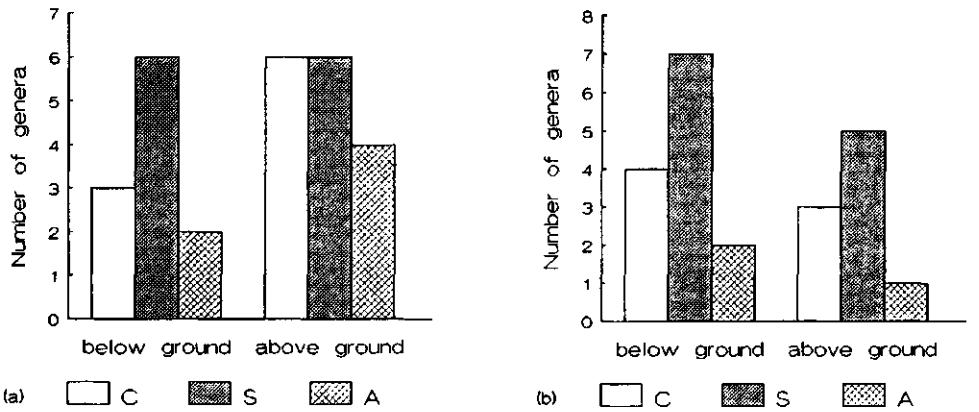


Fig. 2. Number of ectomycorrhizal genera observed on the roots of the seedlings (below ground) and as sporocarps (above ground) in S1 (a) and S3 (b) stands. C = control, S = sod-cut and A = sod-added.

Sporocarp survey

The numbers of species and genera over all treatments were higher in S1 than in S3 (Table 3). The highest number of genera (6) was found in the control and sod-cut plots of this stand (Table 3). In S3, the highest number of genera (5) was recorded in the sod-cut plot.

Root parameters

The root length, the numbers of root tips per seedling and the frequencies of vital ectomycorrhizal root tips of the seedlings were significantly higher ($p < 0.001$) in S1 than in S3 (Tables 1 and 2). The numbers of root tips per cm root of the non-inoculated

seedlings in S1 were significantly higher ($p < 0.001$) than in S3 (Table 1). The frequencies of occurrence of *Laccaria* were significantly higher ($p < 0.001$) in S1 than in S3.

Table 2. Average root length and total number of roots of the seedlings in S1 and S3. C = control, S = sod-cut and A = sod-added. n = number of seedlings harvested, Rl = root lenght, NrRt = number of root tips per seedling and Rt cm^{-1} = number of root tips per cm root per seedling.

	S1				S3			
Initial inoculum:	n	RL	NrRt	Rt cm^{-1}	n	RL	NrRt	Rt cm^{-1}
C: Non-inoculated	10	169±63	556.9±230.1	3.4±0.6	10	34±17	87.1±55.2	2.4±0.9
L. bicolor	3	68±15	151.5± 29.5	2.3±0.1	10	34±17	87.1±55.2	2.4±1.0
P. involutus	7	110±40	399.7±194.0	3.5±0.5	10	20± 4	60.6±31.3	2.9±1.0
R. luteolus	10	103±34	445.6±176.6	4.3±0.9	10	28± 8	82.7±52.9	2.9±1.6
S: Non-inoculated	10	87±28	393.0±159.1	4.5±1.0	10	39±15	129.2±39.2	3.5±0.7
L. bicolor	3	68±14	170.3± 65.6	2.5±0.5	10	54±31	132.6±96.3	2.2±0.8
P. involutus	8	70±29	224.1± 98.4	3.2±0.7	10	36±13	128.5±69.5	3.4±1.2
R. luteolus	5	72±32	271.0±113.0	3.8±0.7	10	41± 7	96.0±42.0	2.3±0.7
A: Non-inoculated	10	104±40	279.5±137.8	2.5±0.3	10	40±19	83.2±57.6	1.9±0.4
L. bicolor	10	94±52	287.0±201.1	2.9±0.6	10	53±20	139.1±59.3	2.5±0.5
P. involutus	10	100±31	319.2±152.7	3.0±0.8	10	33±13	105.4±67.0	3.3±2.1
R. luteolus	9	100±39	266.8±102.7	2.6±0.5	10	36±11	105.7±51.5	2.9±1.2

Comparison between baiting and sporocarp survey

Higher numbers of ectomycorrhizal genera were observed in control and sod-added plots in S1 by sporocarp survey than by baiting (Fig. 2). *Dermocybe*, *Gomphidius* and *Suillus* were only observed by sporocarp surveys and *Cenococcum*, *Hebeloma* and ITE3 only below-ground (Tables 1 and 3). Higher numbers of ectomycorrhizal genera were found in all treatments in S3 below ground than by investigating sporocarps (Fig. 2). The ectomycorrhizal genera *Amanita*, *Inocybe* and *Russula* were only observed by sporocarp surveys and *Cenococcum* and ITE3 only below ground (Tables 1 and 3).

Table 3. Number of sporocarps of ectomycorrhizal fungi in control (C), sod-cut (S) and sod-added (A) plots of S1 and S3 in 1992.

Species:	S1			S3		
	C	S	A	C	S	A
Amanita rubescens Pers.: Fr.	–	–	–	–	2	–
Dermocybe crocea (Schaeff.) Fr	–	3	–	–	–	–
Dermocybe semisanguinea (Fr.) Gill	–	2	2	–	–	–
Gomphidius roseus (Fr.) Fr.	1	–	–	–	–	–
Inocybe brevispora Huijsman	–	–	–	–	7	–
Inocybe lacera (Fr.: Fr.) Kumm	–	–	–	–	33	–
Laccaria bicolor	2	3	202	–	1301	–
Laccaria proxima (Boud.) Pat.	1648	943	723	2	–	–
Lactarius hepaticus Plowr.	–	–	–	14	74	1
Lactarius rufus (Scop.: Fr.) Fr.	–	–	–	2	40	–
Paxillus involutus	9	2	–	–	–	–
Rhizopogon luteolus	2	6	–	–	–	–
Russula ochroleuca Pers.	–	–	–	8	3	–
Suillus bovinus (L.: Fr.) O.Kuntze	13	39	1	–	–	–
Suillus variegatus (Sw.: Fr.) O. Kuntze	5	–	–	–	–	–
Thelephora terrestris Willd.: Fr.	889	414	294	–	–	–
Total number of sporocarps	2569	1412	1222	26	1460	1
Number of species	8	8	5	4	7	1
Number of genera	6	6	4	3	5	1

DISCUSSION

Ectomycorrhizal genus composition on the seedlings was independent of initial inoculum (Table 1). However, in two cases *Paxillus* was observed in low amounts which might be either inoculated or indigenous. In S1, *Paxillus* was mostly replaced by *Laccaria* which can be attributed either to direct competition among the fungi or to environmental factors like nutrient content of the soil (Stenström, 1990), differentially affecting the fungi. It is unclear whether inoculated or indigenous *Laccaria* and *Rhizopogon* were observed. It was not attempted to distinguish between inoculated and indigenous genera.

Manipulation of the litter and humus layers affected the ectomycorrhizal types on the seedlings. The highest numbers of vital ectomycorrhizal types were observed in the sod-cut plots (Table 1). These observations are in accordance with observations above ground (Table 3 and Fig. 2). Earlier investigations showed that sporocarps of ectomycorrhizal

fungi and species were positively affected by removal of litter and humus layers Kallenbach (cited in Grosse-Brauckmann and Grosse-Brauckmann, 1978); Termorshuizen, 1990; Tyler, 1991; Baar and Kuyper, 1993), probably because of removal of large amounts of nitrogen and phenolics with the litter and humus (Chapter 7).

The lowest number of ectomycorrhizal types was observed in the sod-added plots (Table 1). Addition of ectorganic material led to thick humus layers with high amounts of nitrogen and humus components like phenolics. A negative effect of thick humus layers on the ectomycorrhizal types *Dermocybe*, *Hebeloma* and *Piloderma croceum* was observed by Markkola and Ohtonen (1988), who suggested that this was partly due to high amounts of nitrogen in the humus layers. Arnebrant and Söderström (1992) showed that large amounts of nitrogen negatively affected numbers of ectomycorrhizal root tips on pine seedlings. A negative effect of the presence of organic layers on ectomycorrhizal root tips of seedlings of *Abies concolor* (Gord. et Glend.) Lindl. was likewise noticed by Alvarez et al. (1979). Schoeneberger and Perry (1983) found a negative effect of forest litter on ectomycorrhizal root tips of seedlings of *Pseudotsuga menziesii* (Mirb.) Franco.

The genus composition of the ectomycorrhizal fungi below and above ground differed among the two stands in all treatments (Table 1). *Laccaria*, presumably mainly consisting of *L. proxima* as shown by the results of the sporocarp survey (Table 3), dominated in all treatments in S1. Dominance of *L. proxima* in S1 can be explained by establishment from spore inocula when colonizing previously unforested sites (Newton, 1992). Ectomycorrhizal root tips of *Laccaria* in the sod-added plot in S1 may also belong to *L. bicolor* as the results of the sporocarp survey show (Table 3). *Laccaria bicolor* probably benefitted from nitrogen present in litter (Chapter 7). An increase of sporocarps of *L. bicolor* was recorded after fertilization in pine forests (Ohenoja, 1988). The most abundant ectomycorrhizal types on the seedlings in S3 were *Laccaria, Lactarius* and *Russula*. Ectomycorrhizal root tips of *Laccaria* in S3 presumably belong to *L. bicolor* as mainly sporocarps of *L. bicolor* were recorded.

Lactarius and *Russula*, only found in S3, were able to form ectomycorrhizal root tips with the outplanted seedlings (Tables 1 and 3). Fleming et al. (1986) likewise observed in a birch wood that young seedlings largely colonized by fungi were usually found near mature trees. A consequence of this way of infection is that young plants are rapidly integrated into an absorptive network of ectomycorrhizal mycelia, which is likely to be of considerable ecological significance (Read, 1991). A similar proposal was made by Arnebrant (1991) who observed the same ectomycorrhizal types on seedlings and on mature trees.

Cenococcum was found on the roots of non-inoculated seedlings in the sod-cut plot in S3 (Table 1). This observation is in accordance with findings of Dahlberg and Stenström (1991), who found *Cenococcum* on seedlings planted in mineral soil without humus in former Scots pine forests, and contradicts results of Meyer (1987a) and Markkola and

Ohtonen (1988) who noticed that ectomycorrhizal root tips of *Cenococcum* were abundant in thick nitrogen-rich humus layers.

The numbers of root tips per cm root were in accordance with findings by Arnebrant and Söderström (1992). The low numbers of root tips, the low root lengths (Table 2), the low frequencies of vital ectomycorrhizal root tips, high frequencies of non-ectomycorrhizal root tips (Table 1) and low number of root tips per cm root in all treatments in S3 are probably due to phenolic compounds in the ectorganic layer and to inorganic ammonium (Chapters 1, 2, 3 and 7) in the soil.

Several ectomycorrhizal fungi were not observed by either baiting or sporocarp survey. Genera such as *Cenococcum* and ITE3 never form sporocarps. And no sporocarps of *Hebeloma* were found although ectomycorrhizal root tips were observed. *Hebeloma* might have either colonized the baits and disappeared after harvesting the outplanted seedlings, or it might have formed ectomycorrhizal root tips with mature trees too and established permanently. Slow-growing fungi might not colonize the seedlings within one growing season even though they were present at high relative frequencies on the roots of mature trees (Arnebrant and Söderström, 1992). Dahlberg and Stenström (1991) noted that the numbers of ectomycorrhizal root tips of outplanted seedlings increased two and half times within two years. An alternative explanation is that some species that may grow rapidly but have just established in the sod-cut plots have too little mycelia to colonize the seedlings (Dahlberg and Stenström, 1991) in five months. Sporocarps of *Inocybe lacera* were recorded in low numbers in sod-cut plots in Scots pine stands and none in control plots 18 months after sod-cutting (Baar and Kuyper, 1993). This indicates colonization of the sod-cut plots by *I. lacera* by spores after sod-cutting. At the time the seedlings were planted only small-sized mycelia of *I. lacera* would be expected.

Baiting is a good method to determine the colonization potential of ectomycorrhizal fungi due to different treatments (Arnebrant and Söderström, 1992). Ectomycorrhizal genera of which no sporocarps were observed (no sporocarp formation, hypogeous genera) are also recorded by outplanting seedlings. Although it is possible to identify the ectomycorrhizal types of *P. sylvestris* to the genus level, in most cases it is still impossible to identify these types to the species level. A combination of observing sporocarps and roots of outplanted seedlings is a good way to study the effects of different treatments on physiologically active ectomycorrhizal fungi in the field. However, it would be worthwhile to leave the baits in the field for more than one growing season, enabling slower growing and just established fungi to colonize the seedlings.

The results of the present study demonstrate that manipulation of litter and humus layers strongly affects the ectomycorrhizal colonization capacity. What Termorshuizen (1991) concluded for the occurrence of sporocarps of ectomycorrhizal sporocarps in Scots pine forests of different ages also holds for mycorrhization of seedlings: it is not ageing of the trees but ageing of the forest soil which is likely to be the main factor determining ectomycorrhizal infection.

ECTOMYCORRHIZAL DEVELOPMENT ON SCOTS PINE (PINUS SYLVESTRIS L.) SEEDLINGS ON DIFFERENT SOILS

SUMMARY

The development of ectomycorrhizal root tips on Scots pine seedlings grown on different soils were studied. In perspex growth chambers Scots pine seedlings inoculated with Laccaria bicolor, Rhizopogon luteolus *and* Suillus bovinus *were grown on peat (T), humus originating from a 15- to 20-year-old primary Scots pine stand (H), humus (S) or podzolic soil (L) from a secondary Scots pine stand planted in 1974. After 9 weeks ectomycorrhizal development of* R. luteolus *and* S. bovinus *was good and that of* L. bicolor *poor on T. Numbers of vital ectomycorrhizal root tips of* S. bovinus *were significantly smaller on S and L than those on T. Frequencies of vital ectomycorrhizal root tips of* R. luteolus *on L were reduced compared to T. Ectomycorrhizal development of* L. bicolor *on L was significantly more extensive than on T. Ectomycorrhizal development of* L. bicolor, R. luteolus *and* S. bovinus *on Scots pine was associated with nutrient concentrations and pH in the soils.*

INTRODUCTION

Several investigators noted high numbers of ectomycorrhizal root tips in the humus layers and in the upper mineral soil in coniferous forests (Meyer, 1973; Persson, 1980a; Ponge, 1990; Rastin et al., 1990). According to Harley and Smith (1983) roots have a tendency to grow in the direction of newly accumulated litter and humus. Smaller numbers of ectomycorrhizal root tips also occur in the mineral soil up to a depth of 60 cm (Chapter 4).

In areas with high nitrogen input due to air pollution negative correlations have been observed between accumulation of humus and ectomycorrhizal species richness above and below ground (De Vries et al., 1985; Markkola and Ohtonen, 1988). Numbers of species and sporocarps of ectomycorrhizal fungi in secondary Scots pine stands on non-podzolic and podzolic sandy soil (Haplic Arenosol and Haplic Podzol, FAO-Unesco, 1988) with thick litter and humus layers were lower than in primary Scots pine stands with thin litter and humus layers on non-podzolic sandy soil. Nutrient concentrations in litter and humus layers in the secondary stands were higher than in the primary stands, and pH lower (Chapter 3). Removal of thick litter and humus layers ("sod-cutting") in secondary Scots pine stands enhanced the numbers of species and sporocarps of ectomycorrhizal fungi (Baar and Kuyper, 1993; De Vries et al., 1995). Species composition in sod-cut plots on non-podzolic sandy soil became

nearly similar to that in spontaneously grown Scots pine stands on nutrient-poor non-podzolic sandy soils with thin litter and humus layers. Removal of litter and humus layers in a Scots pine stand on podzolic sandy soil had a smaller effect on numbers of ectomycorrhizal species above ground, but tremendously increased numbers of sporocarps of *Laccaria bicolor* (Maire) P.D. Orton. The inorganic nitrogen concentrations and organic matter content in the podzolic sandy soil were higher than in the non-podzolic sandy soil and the pH lower (Chapter 2).

The general objective of the present study was to assess whether a causal relationship between ectomycorrhizal development and chemical composition of litter and humus layers and mineral soil exists. Therefore in the present study the development of different ectomycorrhizal species on different forest soils was studied. Peat was used as control substrate, poor in inorganic nutrients, because ectomycorrhizal species generally develop well on peat (Finlay and Read, 1986a,b; Read, 1991; Arnebrant, 1994). The hypotheses of the present study are: 1. development of different ectomycorrhizal species on humus originating from a primary stand is similar to that on peat; 2. development of different ectomycorrhizal species on humus originating from a secondary stand is reduced compared to that on peat and 3. development of different ectomycorrhizal species on podzolic sandy soil originating from a secondary stand is reduced compared to that on peat except for development of *L. bicolor*.

MATERIALS AND METHODS

Inoculation of seedlings with ectomycorrhizal fungi

Seeds of *Pinus sylvestris* L. originating from forestry Junne in the northeastern part of The Netherlands were surface-sterilized in 15% H_2O_2 and a droplet of Tween 80 for 30 min. Sterilized seeds were germinated on sterile water agar containing 0.5% glucose.

Isolates of *L. bicolor*, *Rhizopogon luteolus* Fr. and *Suillus bovinus* (L.: Fr.) O. Kuntze growing on MMN agar plates were used. *Laccaria bicolor* had been collected in a secondary stand of *P. sylvestris* planted in 1940 (S5), located in the northeastern part of the Netherlands, *R. luteolus* in a 15- to 20-year old primary Scots pine stand (P1), located in the central part of The Netherlands, *S. bovinus* in a secondary Scots pine stand planted in 1987 (S1), situated in the northeastern part of The Netherlands. Isolates of these fungi were used in the present study, because earlier observations showed that sporocarps of these fungi occur on soils with different chemical composition. High numbers of sporocarps of *R. luteolus* and *S. bovinus* were observed in primary Scots pine stands on non-podzolic sandy soil, but not in control and sod-cut plots in secondary Scots pine stands on podzolic sandy soil. Sporocarps of *L. bicolor* were found in high numbers in sod-cut plots in secondary Scots pine stands on non-podzolic and podzolic sandy soil and in low numbers in primary stands (Chapters 2 and 3). Furthermore, these species can easily be cultured and inoculated on Scots pine seedlings. Isolates of *L. bicolor* and *S. bovinus* originating from other Scots pine stands than where the soils were collected, were used because other isolates were not available.

After germination seedlings were transferred to Petri dishes with autoclaved (60 min, 110°C) peat, perlite and MMN-solution without glucose (at 1:2.5:1 per volume). A slit was made in the edge of the Petri dishes. Three seedlings were transferred to each Petri dish with their shoots protruding through the slit. A fourth part of the seedlings was not inoculated to serve as control seedlings. Each of the remaining seedlings was inoculated by putting six plugs of actively growing mycelium of a species around the root systems. The dishes were sealed with tape and anhydrous lanolin preventing contamination and water loss. The Petri dishes with non-inoculated and inoculated seedlings were placed vertically in small transient propagators inside a growth cabinet for 13 weeks until ectomycorrhizal root tips had developed. Day temperature was kept at 18°C for 18 hours and night temperature at 12°C for 6 hours. The relative humidity was 70% and the light exposure 0.102 $kJ.m^{-2}.s^{-1}$. Two weeks after inoculation 25 mg glucose in 5 ml demineralised water was added to Petri dishes with *L. bicolor* to stimulate early ectomycorrhizal development and 5 ml demineralised water to the remaining Petri dishes.

Inoculated seedlings on different soils

Non-sterile soils were used (Table 1) and sieved over a 2 mm sieve. Bottom plates of plexiglass growth chambers measuring 20 * 20 cm^2 were covered with 2-3 mm thick layers of the sieved soil according to Finlay and Read (1986a). One ectomycorrhizal seedling was laid out on the bottom plate and the plexiglas growth chamber was closed with an upper plate. Five replicates of seedlings with *R. luteolus* and *S. bovinus* were used and four replicates of seedlings with *L. bicolor* because no more seedlings with *L. bicolor* had developed. The non-inoculated seedlings could not be transferred from the Petri dishes to the plexiglass growth chambers because they all had died in the Petri dishes, giving an early indication of the all-importance of mycorrhization. The chambers were incubated in transient propagators which were placed in a growth cabinet under similar conditions as before, for 9 weeks.

Table 1. Origin of soils with abbreviations.

Scots pine stand	–	primary (P1)	secondary (S3)	secondary (S3)
Age (yr)	–	15-20	19	19
Year of planting	–	–	1974	1974
Soil type	peat	humus	humus	podzolic soil
Abbreviation	T	H	S	L

Analyses of ectomycorrhizal incidence

After 9 weeks, just before roots of the seedlings and ectomycorrhizal mycelia reached the edge of the growth chambers, root length was determined according to Newman (1966). Total numbers of vital ectomycorrhizal root tips per seedling were counted according to Termorshuizen (1991). Frequencies of vital ectomycorrhizal root tips (= 100% * numbers of vital ectomycorrhizal root tips/total numbers of root tips) were calculated.

Analysis of shoots

Subsequently shoots were cut off and dried at 70°C. Dry weight of the shoots was determined.

Needles from all seedlings per treatment were collected and ground. Composite samples were digested in H_2SO_4, salicylic acid and H_2O_2. N and P were measured colorimetrically.

Analysis of soils

Two samples of each soil were taken from unused material and dried at 40°C. The amounts of $N_{dissolved}$, NH_4^+, NO_3^-, P and K^+ were analysed in 0.01*M* $CaCl_2$ extracts and the pH($CaCl_2$) was determined (Houba et al., 1990). The organic matter content of the soils was determined by loss-on-ignition.

Statistical tests

Data were analysed by analysis of variance (one-way ANOVA), if necessary after transformation to obtain normality. Differences among means were evaluated with the LSD-test (Sokal and Rohlf, 1981). N and P concentrations in the needles were not statistically analysed because needles of all seedlings per treatment were pooled.

RESULTS

Analysis of ectomycorrhizal incidence

Ectomycorrhizal development of *R. luteolus* and *S. bovinus* was good, that of *L. bicolor* poor on T (Fig. 1a,b). The numbers of vital ectomycorrhizal root tips of *S. bovinus* on S was significantly smaller than that on T (Fig. 1a). The numbers of vital ectomycorrhizal root tips and frequencies of vital ectomycorrhizal root tips of *S. bovinus* on L were reduced compared to T and those of *L. bicolor* were enhanced (Fig. 1a,b). The frequencies of vital ectomycorrhizal root tips of *R. luteolus* on L were reduced compared to T (Fig. 1b).

Analysis of shoots

The dry weight of the shoots of the seedlings with *S. bovinus* was significantly lower on H, S and L than that on T (Fig. 2).

The N and P concentrations of the needles of all seedlings on L were low (Table 2). The

N concentrations in the needles of the seedlings with *L. bicolor* on all soils were lowest.

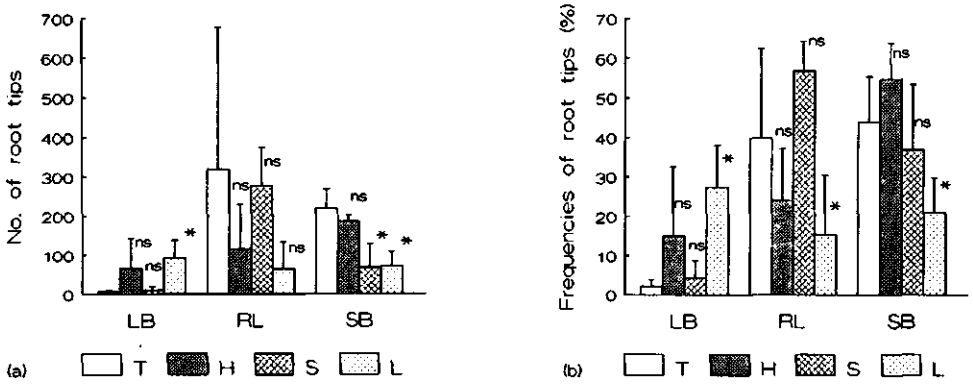


Fig. 1. Total numbers of vital ectomycorrhizal root tips (a) and frequencies of vital ectomycorrhizal root tips (b) of *L. bicolor* (LB), *R. luteolus* (RL) and *S. bovinus* (SB) on Scots pine seedlings on different forest soils compared to peat. T = peat, H = humus originating from a primary stand (P1), S = humus originating from a secondary stand planted in 1974 (S3) and L = podzolic sandy soil from S3. Differences between peat and the other treatments are indicated by * ($p < 0.05$, LSD) or ns (not significant).

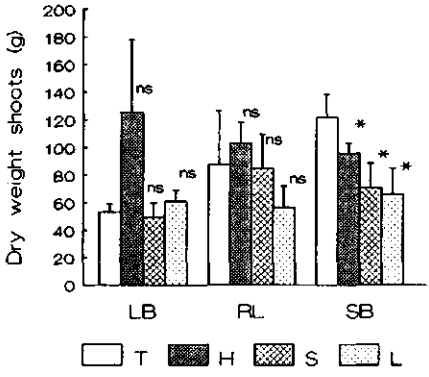


Fig 2. Dry weight of shoots of Scots pine seedlings with *L. bicolor* (LB), *R. luteolus* (RL) and *S. bovinus* (SB) on different forest soils compared to peat. P = peat, H = humus originating from a primary stand (P1), S = humus originating from a secondary stand planted in 1974 and L = podzolic sandy soil from S3. Differences between peat and the other treatments are indicated by * ($p < 0.05$, LSD) or ns (not significant).

Table 2. N and P concentrations (g kg^{-1} DM) in the needles of the seedlings grown on different soils at the end of the experiment. T = peat, H = humus from a primary stand, S = humus from a secondary stand planted in 1974 (S3) and L = podzolic sandy soil from S3. Statistical tests were not carried out because needles of all seedlings per treatment were pooled.

	L. bicolor		*R. luteolus*		*S. bovinus*	
Soil type	N	P	N	P	N	P
T	15.34	1.27	22.00	1.32	23.76	1.47
H	17.86	1.65	21.42	1.52	25.58	1.99
S	11.54	1.28	16.00	1.29	20.46	1.80
L	7.64	1.11	10.23	1.15	11.37	1.27

Analysis of soils

The $N_{dissolved}$ concentration and organic matter content in T were significantly higher than in the other soils (Table 3). The NH_4^+ and P concentrations in H and S were significantly higher than those in T. The pH in S was significantly lower than in the other soils. The NO_3^- concentration in H and L were significantly lower than in T. The $N_{dissolved}$ and P concentrations in L were significantly lower and the pH higher than in the remaining soils.

Table 3. Nutrient concentrations (mg kg^{-1}), pH and organic matter content (OM, %) in the different soils. T = peat, H = humus from a primary stand (P1), S = humus from a secondary stand planted in 1974 (S3) and L = podzolic sandy soil from S3. Differences between treatments are indicated by different letters ($p < 0.05$, LSD) and standard error of difference (s.e.d.).

Soil type	N_{diss}	NH_4^+	NO_3^-	P	K^+	pH	OM
T	368.6 d	17.7 a	25.4 c	2.3 b	74.5 ab	3.0 b	96.0 c
H	121.6 b	89.6 b	0.5 a	17.7 d	179.3 b	3.1 c	13.9 a
S	155.0 c	113.8 b	21.4 b	15.2 c	109.9 ab	2.8 a	31.1 b
L	9.9 a	2.4 a	2.0 a	0.2 a	7.9 a	3.9 d	5.2 a
s.e.d.	29.5	29.1	2.4	0.2	168.4	0.03	17.0

DISCUSSION

Ectomycorrhizal root tips of *R. luteolus* and *S. bovinus* on T developed according to expectations based on other studies when peat was used as substrate (Finlay and Read, 1986a,b). The $N_{dissolved}$, NH_4^+ and NO_3^- concentrations in T suggest that T contains high amounts of organic nitrogen (Table 3). Several investigators noted uptake of organic nitrogen by *S. bovinus* (Lundeberg, 1970; Abuzinadah and Read, 1986a,b; Read, 1991). The high NO_3^- concentration in peat apparently did not affect the ectomycorrhizal development of *S. bovinus* and *R. luteolus*. There was poor ectomycorrhizal development of *L. bicolor* on T. Finlay et al. (1992) reported that an isolate of *L. bicolor* was poor in using protein as a nitrogen source. However, in *an vitro* study *L. bicolor* was capable to grow on media with glycine as nitrogen source (Chapter 8). Ahmad et al. (1990) also noted that *L. bicolor* utilized different amino acids on liquid culture.

Ectomycorrhizal development of *L. bicolor* and *S. bovinus* on S was poor. The pH in S was lower than in T and the NH_4^+ concentrations higher (Table 3). Kamminga-Van Wijk (1991) and Jongbloed (1992) noted acid sensitivity of *L. bicolor*. Termorshuizen and Ket (1991) reported reduced ectomycorrhizal development on Scots pine seedlings as a result of ammonium fertilization *in vitro*. Wallander and Nylund (1991) and Arnebrant (1994) noted that *S. bovinus* was sensitive to excess of ammonium. Phenolics accumulated in S might also have decreased ectomycorrhizal development of *L. bicolor* and *S. bovinus*. Radial growth of the same isolate of *L. bicolor* was reduced by extracts of humus from a secondary Scots pine stand in an *in vitro* study (unpubl. results). Pellisier (1993) noted inhibited respiration of *L. laccata* (Scop.: Fr.) Berk. and Br. and *Cenococcum geophilum* (Fr.) Fr. caused by phenolic acids derived from humus originating from a stand of *Picea abies* (L.) Karst.

Ectomycorrhizal development of *R. luteolus* was not reduced on S compared to T, which does not fit with the sensitivity of *R. luteolus* to grass and Scots pine litter extracts as noted in chapter 7.

The ectomycorrhizal development of *R. luteolus* and *S. bovinus* was poor on L compared to T. The nutrient concentrations in L were lower than in T and likely too low for good development of *R. luteolus* and *S. bovinus*. However, the ectomycorrhizal development of *L. bicolor* on L, comparable to that of *R. luteolus* and *S. bovinus*, was larger than on T. Wallander and Nylund (1992) noted an increase of mycelial biomass of *L. bicolor* on Scots pine seedlings at low nitrogen concentrations (10-20 $mg.l^{-1}$). *Laccaria bicolor* possibly benefitted by the high pH in L too. Kamminga-Van Wijk (1991) reported best growth of *L. bicolor* associated with Douglas fir seedlings in hydroculture at pH 4.0.

The ectomycorrhizal development of *L. bicolor* on H and L and that of *S. bovinus* on H is in accordance with field observations of sporocarps of *L. bicolor* and *S. bovinus* (Termorshuizen, 1991; Chapter 2). These results suggest that *L. bicolor* and *S. bovinus* in Scots pine stands form sporocarps under ecological conditions that are conducive for optimum

ectomycorrhizal development. *Rhizopogon luteolus* formed high numbers of ectomycorrhizal root tips on S. However, sporocarps were not observed in Scots pine stands with thick litter and humus layers (Baar and Kuyper, 1993). Sporocarps of *R. luteolus* were mainly observed in Scots pine stands on nutrient-poor mineral soil with thin litter and humus layers or in plots where litter and humus were removed (Termorshuizen, 1991; Chapters 2 and 3). The poor ectomycorrhizal development of *R. luteolus* on H was in contrast with field obervations (Chapters 2 and 3). An explanation for these differences has not yet been found.

The nitrogen concentrations in the needles of the seedlings with *L. bicolor* were lower than in the needles of the seedlings with *R. luteolus* and *S. bovinus*. *Laccaria bicolor* apparently transported less nitrogen to the needles of the seedlings than the other fungi, because ectomycorrhizal development of *L. bicolor* was poorer than that of *R. luteolus* and *S. bovinus* on T, H and S. The pysiology of *L. bicolor* might also have affected the nitrogen uptake.

The numbers of vital ectomycorrhizal root tips of *L. bicolor* on H and L and of *R. luteolus* on the four soils were almost similar to the numbers of vital ectomycorrhizal root tips per seedling planted out in secondary Scots pine stands for five months (Chapter 5). Ectomycorrhizal root tips are likely more efficient in nutrient uptake than non-ectomycorrhizal tree roots as shown for NH_4^+ uptake by ectomycorrhizal roots of Sitka spruce seedlings (Alexander and Fairley, 1986). In the present study it was impossible to study growth of non-ectomycorrhizal seedlings because they died before the experiment started.

Ectomycorrhizal development might also have been affected by carbon demand of the fungi. Gibson and Deacon (1990) noted that ectomycorrhizal formation of several species among which *Laccaria proxima* (Boud.) Pat. and *Amanita muscaria* (L.: Fr.) Pers. associated with birch seedlings was affected by different levels of glucose.

In the present study seedlings were inoculated with one isolate per species and the results may not necessarily hold for the species in general. Different isolates reacted differently to the same treatments as shown by several investigators (Marx, 1981; Kieliszewska-Rokicka, 1992; Arnebrant, 1994).

In conclusion, the results of the present study suggest that nutrient concentrations and pH in the forest soil affect ectomycorrhizal development of *L. bicolor*, *R. luteolus* and *S. bovinus* on Scots pine. The ectomycorrhizal development of *L. bicolor* on podzolic sandy soil and on humus from a primary stand and that of *S. bovinus* on humus from a primary stand is in accordance with field observations of sporocarps of *L. bicolor* and *S. bovinus*. In contrast, *R. luteolus* formed high numbers of ectomycorrhizal root tips on humus from a secondary stand where no sporocarps were recorded.

STIMULATORY AND INHIBITORY EFFECTS OF NEEDLE LITTER AND GRASS EXTRACTS ON THE GROWTH OF SOME ECTOMYCORRHIZAL FUNGI

SUMMARY

Aqueous extracts of Scots pine needles, shoots and roots of the grass Deschampsia flexuosa *were investigated for their effects on the growth of isolates of ectomycorrhizal fungi. The growth rates of* Laccaria proxima *and* Rhizopogon luteolus *were negatively affected by the needle extracts. Only the high concentrations of the needle extracts had significant inhibitory effects on the growth rates of* Paxillus involutus *and* Xerocomus badius. *The growth rate of* Laccaria bicolor *was significantly enhanced by the needle extracts. Extracts of the shoots of* D. flexuosa *had inhibitory effects on the growth rates of* L. proxima, P. involutus *and* R. luteolus *and a significant enhancing effect on the growth rate of* L. bicolor. *The shoot extracts contained about 3-5 times more high molecular weight components, aliphatic acids and phenolics than the root extracts.*

INTRODUCTION

In old stands (50-80 years) of *Pinus sylvestris* L. in The Netherlands the numbers of sporocarps and species of ectomycorrhizal fungi have decreased during the last few decades, with the strongest decline in areas with high deposition of NH_3 (Termorshuizen and Schaffers, 1987). At the same time this atmospheric deposition has caused an increase of litter accumulation, development of a mor humus profile and an increase of the abundance of the grass *Deschampsia flexuosa* (L.) Trin. (Klap and Schmidt, 1992). Hardly any sporocarps of ectomycorrhizal fungi were found in Scots pine forests where *D. flexuosa* dominated the forest floor vegetation (Veerkamp and Kuyper, 1993; Chapters 2 and 3). Baar and Kuyper (1993) found an increase of ectomycorrhizal species in old Scots pine stands where litter and humus (and in some plots also the herbaceous vegetation dominated by *D. flexuosa*) had been removed. This suggests a negative effect of litter, humus or the grass *D. flexuosa* on the occurrence and growth of ectomycorrhizal fungi.

Negative effects of litter on ectomycorrhizal fungi were established by Melin (1946), Jarvis (1964), Olsen et al. (1971), Coté and Thibault (1988) and Rose et al. (1983). Sensitivity of ectomycorrhizal species to litter extracts seems to be associated with their ecological role. During succession litter around trees accumulates causing a change in the amounts and type of potentially allelochemical substances released from decomposing litter

which may influence a successional sequence of ectomycorrhizal fungi.

Deschampsia flexuosa might also play a causal role, for instance by a phytotoxic effect on the growth or sporocarp formation of ectomycorrhizal fungi. Under controlled conditions a depressing effect of the grass *Molinia caerulea* (L.) Moench on the numbers of ectomycorrhizas formed by *Laccaria bicolor* (Maire) P.D. Orton was found by Timbal et al. (1990) who suggested an allelopathic effect.

Therefore the effects of extracts of Scots pine needles on five ectomycorrhizal fungi characteristic for either young or old Scots pine stands were investigated. Additionally water-soluble components from shoot and root material of *D. flexuosa* were examined for their inhibitory effects on ectomycorrhizal fungi.

MATERIALS AND METHODS

Litter extract experiment

In the autumn of 1990 *Laccaria proxima* (Boud.) Pat. (F1) and *L. bicolor* (F3) were collected in a young stand (planted in 1987; S1) of *P. sylvestris*, *Xerocomus badius* (Fr.: Fr.) E.J. Gilb. (IS3) in a middle-aged stand (planted in 1963; S4) and *Paxillus involutus* (Batsch: Fr.) Fr. (IS8) in an old stand (planted in 1940; S5), all secondary stands located in the province of Drenthe, in the northern part of The Netherlands. *Rhizopogon luteolus* Fr. (F7) originated from a primary middle-aged Scots pine stand (15-20 years, P1) in the province of Gelderland, in the central part of The Netherlands. Extracts were prepared from needles that were collected in the autumn of 1990. The needles originated from the litter layer of an old Dutch Scots pine stand (planted in 1940) as well as from a 50-year-old fallen Scots pine tree. It was assumed that the content of phenolics of needles from a fallen tree differs from the phenolic content of needles picked from the forest floor. In the autumn of 1989, needles were collected from Scots pine trees in central Sweden. They are known to contain less nitrogen than Dutch needles. After sampling needles were dried (50°C, 48 hr) and roughly ground. An amount of 90 g ground needles was added to 675 ml distilled water. Extracts were made by shaking (22°C, 24 hr), autoclaved for 20 min (0.5 atm, 110°C) and added to Modified Melin-Norkrans agar medium in two concentrations: 7.5 ml extract in 100 ml MMN and 37.5 ml extract in 100 ml MMN [1% and 5% (w/v)], respectively. For inoculation, mycelial plugs of 6 mm in diameter were cut from the edge of fungal colonies on MMN agar and transferred to MMN media with the extracts and control media (no extracts added). Each treatment was replicated four times. The agar plates were kept at 22°C. The radial growth of a fungus was determined by measuring the colony diameter every 3 days as long as the growth of the controls was linear. The colony diameter of *L. bicolor* was measured for 31 days, of *L. proxima* for 32 days and of *X. badius* for 48 days. Measuring the colony diameter of *P. involutus* and *R. luteolus* ceased when the fungi reached the edge of the Petri dish (after 71 days).

Experiment with shoot and root extracts of D. flexuosa

Extracts were also made from shoots and roots of the grass *D. flexuosa*. The grass originated from a Scots pine stand planted in 1924 (S6) and was sampled in the autumn of 1991. The shoots and roots were separated and dried (50°C, 24hr). To 475 ml distilled water, 100 g dry matter of shoots or roots was added. Extracts of shoots and roots were made by shaking (22°C, 24 hr). The extracts were autoclaved for 20 min (0.5 atm, 110°C) and added to modified Melin-Norkrans agar medium in two concentrations: 7.5 ml extract in 100 ml MMN and 37.5 ml extract in 100 ml MMN [1.6% and 7.9% (w/v)], respectively. The same isolates of *L. proxima* (F1) and *R. luteolus* (F7) as used in the previous experiment were used. The isolates of *L. bicolor* (F3) and *P. involutus* (IS8) were replaced by isolates IS7 and F8, respectively, because isolates F3 and IS8 were not vital anymore. The experiment was carried out in the same way as the previous experiment. The colony diameter of *L. bicolor* was measured for 17 days, that of *L. proxima* for 44 days, that of *P. involutus* for 21 days and that of *R. luteolus* for 12 days.

Chemical analysis of the extracts

The concentrations of water-soluble phenolics (polyphenols and simple phenolics) in needle, shoot and root extracts were measured spectrophotometrically, using the Folin-Ciocalteu's reagent (Kuiters, 1987). Autoclaved extracts used in this experiment as well as untreated extracts were analysed to investigate the possible effects of autoclaving. Needle extracts were made from unground needles. Tannic acid was used as a standard and the concentration of water-soluble phenolics in the plant materials was expressed as mg TAE (tannic acid equivalent) g^{-1} dry matter (Kuiters, 1987). Total carbon and nitrogen were determined by a Carlo Erba elemental analyzer. Prior to analysis, samples were membrane filtered (0.45 μm). To get some further insight in the abundance and nature of organic compounds in the extracts, litter solutions were subjected to gel permeation chromatography using Sephadex G-25, with a separation range from 100 to 5000 dalton for dextrans (Kuiters and Mulder, 1992). A column of 20 * 1.6 cm i.d. was used. Of each sample, 0.5 ml was brought on the column, eluted with a 10 m*M* sodium acetate solution of pH 5.5 and the effluent was monitored for U.V. absorbance at 280 nm. This method allows a fractionation of dissolved components into a high (excluded) and a low (included) molecular weight fraction.

Statistical analysis

The growth rates of the fungi were analysed for homogeneity of variance by Bartlett's test. Normally distributed data were analysed by analysis of variance (MANOVA). Differences among means were evaluated with the LSD-test ($p < 0.05$) (Sokal and Rohlf, 1981). Data not normally distributed were tested with the Kruskal-Wallis and Mann-Whitney U-tests (Siegel and Castellan, 1988).

RESULTS

Litter extract experiment

The experiment ended before *L. bicolor*, *L. proxima* and *X. badius* reached the edge of the Petri dish and when the radial growth rate of the control fungi was no longer linear.

The growth rates of *L. proxima* and *R. luteolus* were significantly inhibited by both needle extract concentrations (Fig. 1). The higher concentration inhibited the growth of *L. proxima* completely. The higher concentrations of the needle extracts reduced the growth of *P. involutus*, but the lower concentration had a stimulatory effect. The extracts of the Swedish needles and the treatments with high concentrations reduced the growth of *X. badius* significantly. The low concentration of the Dutch needle extracts did not have any effect. The growth of *L. bicolor* was enhanced by the needle extracts, especially by those of Dutch origin.

Experiment with root and shoot extracts of D. flexuosa

The growth rates of the isolates of *L. proxima*, *R. luteolus* and *P. involutus* were strongly negatively affected by the extracts of the shoots (Fig. 2). The high concentrations of the shoot extracts totally inhibited the growth of the isolates of *L. proxima* and *R. luteolus*. The low concentration of the root extracts had significantly negative effects on the growth of *L. proxima*. Both concentrations of the root extracts reduced the growth of *P. involutus* significantly. The isolate of *L. bicolor* was not affected by the extracts except for the high concentration of the root extracts, which inhibited the growth.

Chemical composition of the needle and grass extracts

Total carbon and nitrogen in the plant and litter extracts are presented in Table 1. Among the needle materials, needles from the fallen tree contained more carbon and nitrogen relative to the needles picked from the forest floor and those collected from trees in Sweden. The extracts of Dutch needles from the fallen tree contained about 1.7 times more total carbon and about 10 to 15 times more total nitrogen than the extracts of the Swedish needles (Table 1). The extracts of Dutch litter contained about the same amount of total carbon and about 3 times more total nitrogen than the extracts of the Swedish needles.

Extracts from the shoot material from *D. flexuosa* had high concentrations of carbon and nitrogen. The shoot extracts of *D. flexuosa* contained about 3 times more total carbon and 1.7-1.8 more total nitrogen than the Dutch needles of a fallen tree (Table 1). The extracts of the shoots contained about 7 times more total carbon and about 3-6 time more total nitrogen than the extracts of the roots (Table 1).

Most phenolics were found in the extracts of shoot material from *D. flexuosa*. Concentrations in the needle litter materials were much lower (Fig. 3).

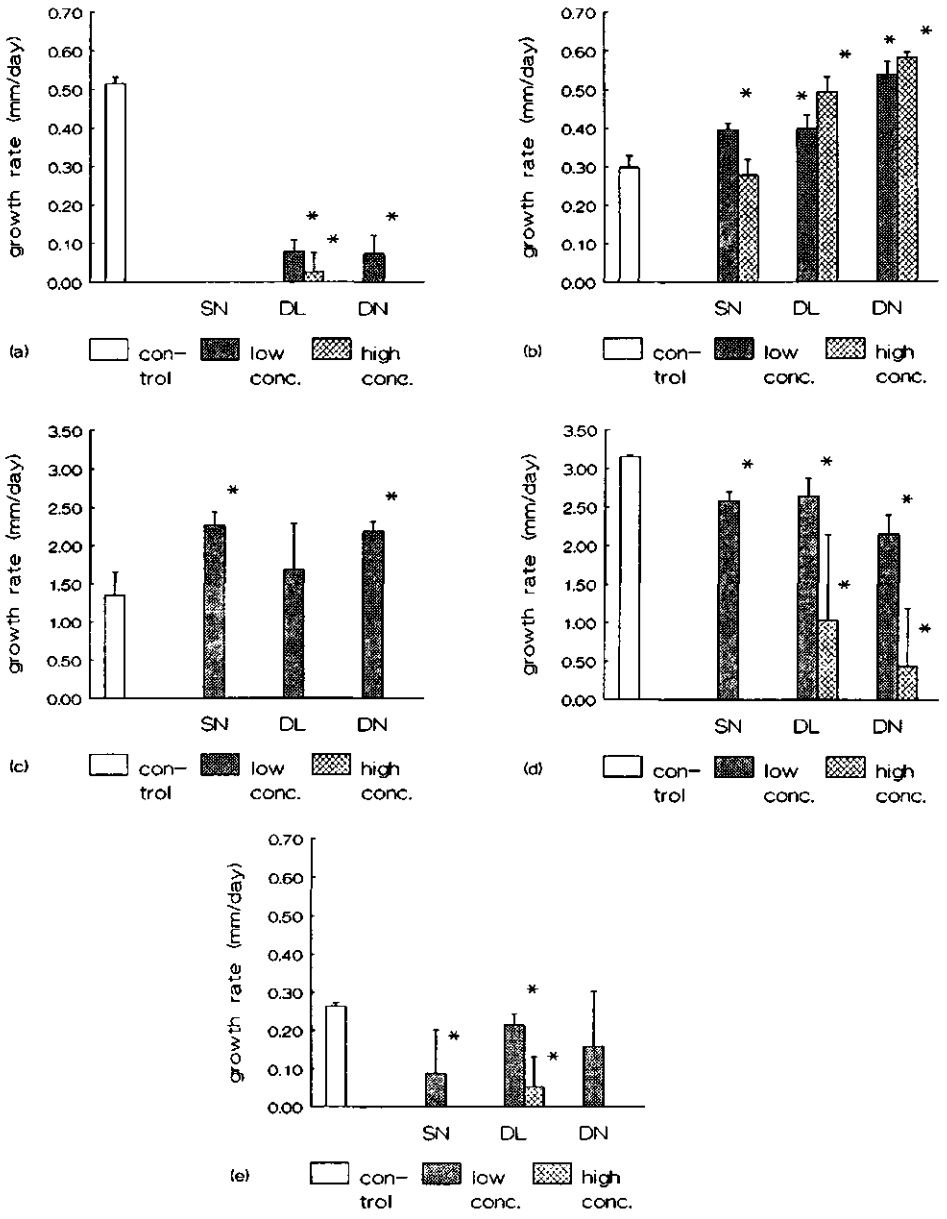


Fig. 1. Average growth of *L. proxima* (a), *L. bicolor* (b), *P. involutus* (c), *R. luteolus* (d) en *X. badius* (e) on MMN agar with extracts of needles and litter of *P. sylvestris* in two concentrations [1.6% and 7.9% (w/v)]. SN = Swedish needles, DN = Dutch needles and DL = Dutch litter. Significant differences with the control treatment are indicated by * ($p < 0.05$, MWU).

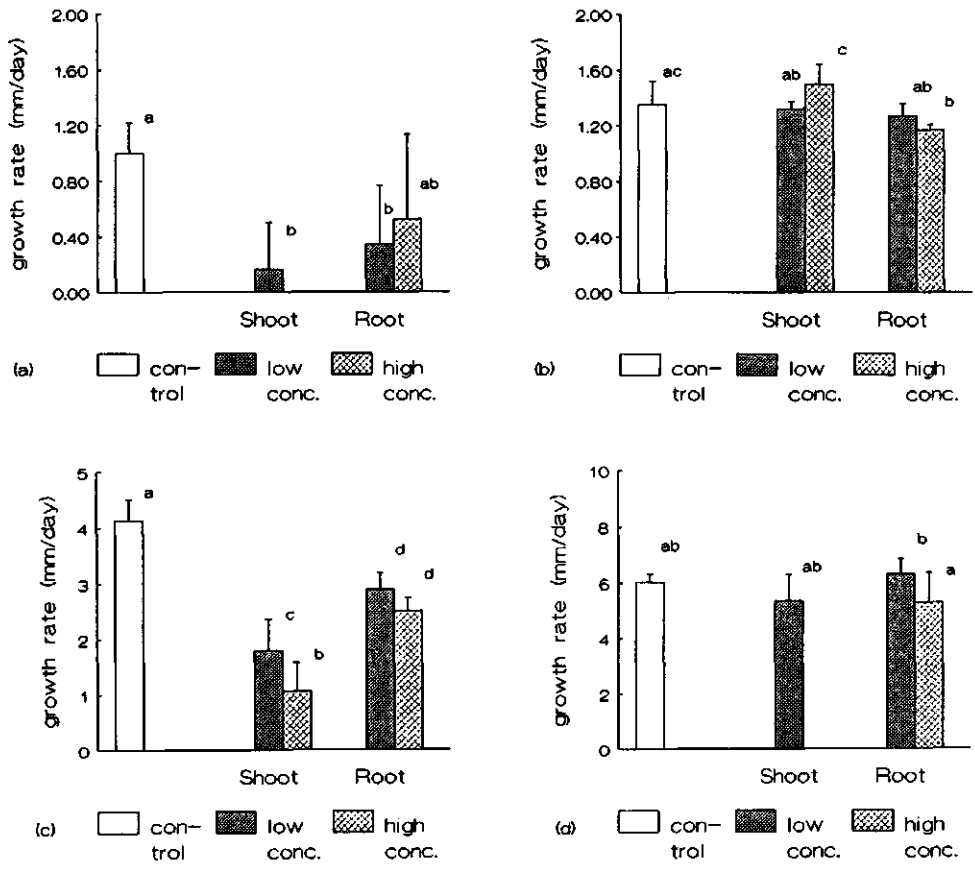


Fig. 2. Average growth of *L. proxima* (a), *L. bicolor* (b), *P. involutus* (c), *R. luteolus* (d) on MMN agar with grass extracts in two concentrations [1.6% and 7.9% (w/v)]. Pairwise comparisons between growth rates that are followed by different letters are significantly ($p < 0.05$, LSD and MWU) different.

The gel elution patterns of the grass and needle extracts after separation on Sephadex G-25 revealed two molecular weight groups of organic solutes. Organic components were eluted at or near the inclusion limit of the column (3 Kav), indicating that this group was composed of low-molecular weight compounds, presumably low-molecular weight aliphatic and phenolic acids. Substances of higher molecular weight were found near the exclusion limit of the column (0 Kav). This group occurred especially in extracts of shoots of *D. flexuosa*.

Autoclaving of the extracts resulted only in a small shift of the molecular weight distribution of the organic solutes.

Table 1. Total carbon and nitrogen (in mg l^{-1}) and water-soluble phenol concentration (in μg tannic acid equivalent mg^{-1} C) of the autoclaved and untreated needle extracts and extracts of the shoots and roots of *D. flexuosa*.

Extracts	autoclaved	total carbon mg l^{-1}	total nitrogen mg l^{-1}	phenols μg TAE mg^{-1} C
Swedish needles	+	1047	14	235.8
Swedish needles	−	990	10	245.1
Dutch needles	+	1755	151	62.0
Dutch needles	−	1712	157	54.5
Dutch litter	+	1144	39	301.6
Dutch litter	−	1080	38	329.0
Root	+	637	72	25.5
Root	−	788	45	44.9
Shoot	+	4535	250	85.6
Shoot	−	5663	288	97.1

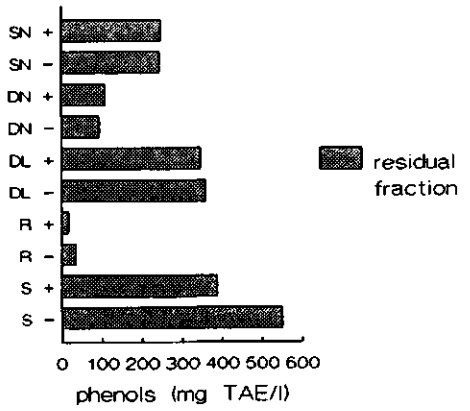


Fig. 3. The amount of phenols in needle and grass extracts in mg tannic acid equivalent l^{-1}. SN = Swedish needles, DN = Dutch needles, DL = Dutch litter, R = roots of *D. flexuosa* and S = shoots of *D. flexuosa*. + = autoclaved, − = untreated.

DISCUSSION

The sensitivity of isolates for needle and grass extracts studied was assessed on the basis of radial growth and not on the basis of biomass. Paustian and Schnürer (1987) proposed a model for fungal growth which is regulated so as to maximize hyphal length and, thereby, the extent of substrate exploitation. Their model implies that hyphal expansion might occur at the expense of an increase in biomass as was indeed found by Littke et al. (1984). Ecological considerations would make such a model quite plausible as radial growth can be (directly) translated to absorbing area of trees in the soil, whereas biomass probably reflects carbon drain.

It has to be realized that only one isolate per species was tested in the present study and the results do not hold for species in general. Earlier investigations by Marx (1981) and Kieliszewska-Rokicka (1992) showed that different isolates of one species reacted differently to the same treatments.

Laccaria proxima and *R. luteolus* were more sensitive to needle and grass extracts than *P. involutus* and *X. badius*. *Laccaria bicolor* was insensitive and its growth was stimulated by extracts of Dutch needles and shoots of *D. flexuosa*. *Laccaria bicolor* probably benefitted from nitrogen present in the shoots of *D. flexuosa*. *Laccaria bicolor* was stimulated in pine forests by fertilization (Ohenoja, 1988). Jongbloed and Borst-Pauwels (1990) showed that high concentrations of ammonium did not inhibit the growth rate of *L. bicolor* and increased the biomass.

Coté and Thibault (1988) confirmed different sensitivity of isolates of *L. proxima* and *L. bicolor* to leaf leachates of *Rubus idaeus* L. In that experiment too, *L. proxima* was far more sensitive than *L. bicolor*.

Ohenoja (1988) observed an increase in sporocarp production of *P. involutus* following nitrogen fertilization in forests. Kieliszewska-Rokicka (1992) noted that higher nitrogen levels in agar medium stimulated the growth of *P. involutus* isolates. In the present study *P. involutus* seemed to be more sensitive to phenolic components of extracts of shoots of *D. flexuosa* and Dutch needles than be benefitted by nitrogen present in these extracts.

The decision to use *L. bicolor*, *L. proxima*, *P. involutus*, *R. luteolus* and *X. badius* was based on practical considerations. These fungi occurred in fairly large amounts in Scots pine stands or reacted strongly to removal of litter and humus or accumulation of ectorganic material (Baar and Kuyper, 1993). These fungi can easily be cultured and grow relatively fast (about 0.3-1 mm day^{-1}). This selection may have biased the results.

The growth of ectomycorrhizal fungi on MMN-agar was affected by Scots pine needle and grass extracts. Analysis showed phenolic components present in the needle and grass extracts (Fig. 3). Several benzoic and cinnamic acids were also detected in water extracts of Scots pine needles by Blaschke (1979) and Kuiters and Sarink (1986). It is suggested that these components caused the growth rate effects on the fungi.

The concentrations of all extracts were realistic which enhances the possiblity to

translate the results of the present study to field observations. A Petri dish with MMN-agar medium and low concentration [1% (v/w)] of the Dutch needle extracts contained about 430 μg TAE g^{-1} water-soluble phenolics. Kuiters and Sarink (1986) measured 514 μg TAE g^{-1} dry weight water-soluble phenolics in litter of *P. sylvestris* and about 500-1500 μg TAE g^{-1} water-soluble phenolics in pine litter leachates. The content of phenolics in the extracts of the Swedish needles was comparable with the amount of phenolics in Dutch Scots pine needle leachates analysed by Kuiters and Sarink (1986). The extracts of the Dutch needles of a fallen tree contained a low amount of phenolics because the needles were not degraded. In forest soils organic matter accumulates with ageing of the stand. A higher humus content permits more polyphenols to be weakly bound to the humus complex and thus prevents them from leaching to deeper soil layers (Kuiters, 1987). The mycelium and ectomycorrhizal roots are situated in the humus layer and exposed to phenols.

Besides inhibition by leachates of Scots pine needles, negative effects of the grass *D. flexuosa* might be one of the possible causes of the decline of ectomycorrhizal fungi. In the present study the extracts of shoots significantly inhibited the growth of *L. proxima*, *P. involutus* and *R. luteolus* and the autoclaved extracts contained less phenolics than the untreated extracts (Fig. 3). It is still unclear which organic components are responsible for the negative effects caused by the grass extracts. Investigations by Coté and Thibault (1988) and Timbal et al. (1990) suggest that phenolics might play an important role. In the present experiment the shoot extracts which caused the largest reduction of the growth contained much more phenolics and high molecular weight components than the extracts of the grass roots and Swedish and Dutch needles (Fig. 3).

The high nitrogen content of the extracts of the shoots of *D. flexuosa* and Dutch needles may be explained by the atmospheric deposition of, on average, 50 kg ammonia ha^{-1} yr^{-1} in The Netherlands (Schneider and Bresser, 1988). The abundance of *D. flexuosa* in Scots pine forests is likely to inhibit ectomycorrhizal fungi by toxicity of plant components as noted by Jarvis (1964). However, the decline of *L. bicolor* in grassy Scots pine stands cannot easily be explained by this mechanism. Other causes might be a negative mechanical effect of *D. flexuosa* or competition for water or phosphate by mycorrhizal roots of *D. flexuosa*.

Field observations in untreated plots by Baar and Kuyper (1993) show that sporocarpes of *L. proxima* occurred in young Scots pine stands (S1 and S2) in high numbers. Sporocarps of *L. bicolor*, *P. involutus* and *X. badius* were mainly recorded in old stands (S5 and S6), where also low numbers of sporocarps of *L. proxima* were observed. Sporocarps of *R. luteolus* were not recorded in untreated plots of secondary stands of different age. The sensitivity of *L. proxima* and *R. luteolus* to the needle and grass extracts is in accordance with these field observations. The insensitivity of *L. bicolor* agreed with these field observations contrary to the reduction of the growth rate of *P. involutus* and *X. badius*.

The results of the present study indicate that phenolics affect ectomycorrhizal fungi. Further studies are needed for determining the exact nature of the phenolics and studying the effects of each phenolic on the growth of ectomycorrhizal fungi. Other undefined components of needle and grass extracts besides phenolics and nitrogen might have affected the growth of the tested ectomycorrhizal fungi. Determining these unknown components warrants further research.

However, the results of the present study suggest that the decrease of ectomycorrhizal fungi is not only due to high atmospheric deposition of ammonia which caused high nitrogen concentrations in Scots pine needles and shoots of *D. flexuosa*. Phenolic components seem to play an important role too. To address the latter field experiments were carried out for investigating the effects of manipulation of organic matter composition on ectomycorrhizal flora in Scots pine stands of different age classes (Chapter 2).

The widely-used functional classifications of ectomycorrhizal fungi cannot easily explain their occurrence in the field in relation to the sensitivity for extracts. Neither the concepts of early- and late-stage fungi (Mason et al., 1982; 1983) nor the C-S-R classification (Grime, 1985) nor the classification based on epidemiological characteristics recently proposed by Newton (1992) explain the results of the present study with the five ectomycorrhizal fungi investigated.

PERFORMANCE OF ECTOMYCORRHIZAL FUNGI ON ORGANIC AND INORGANIC NITROGEN SOURCES

SUMMARY

The growth of isolates of the ectomycorrhizal fungi Coltricia perennis, Laccaria bicolor, Lactarius hepaticus *and* Paxillus involutus *originating from Scots pine stands were studied on solid media with different glucose concentrations as carbon source, with different concentrations of ammonium as inorganic nitrogen source and different concentrations of glycine as organic nitrogen source. Biomass of the isolates of the four fungi generally increased with increasing glucose concentration whereas growth rate decreased or was not affected. The biomass of* L. bicolor *and* P. involutus *increased with increasing ammonium concentration while their growth rate decreased or was not affected.* Laccaria bicolor *was able to grow on media with only glycine, too. Biomass and growth rate of* L. hepaticus *and* P. involutus *on media with ammonium were generally higher than on media with glycine as nitrogen source.* Coltricia perennis *did not clearly show preference for either ammonium or glycine. Fractal dimensions, calculated from the biomasses and the radii of the fungi, were in the range from 1 to 3. Fractal dimensions provided extra information about the foraging strategy of mycelia not obtained from growth rate and biomass production measurements, but cannot replace those because the use of fractal dimensions alone may lead to misinterpretation.*

INTRODUCTION

Several investigators noted that ammonium as nitrogen source is used by ectomycorrhizal fungi (Laiho, 1970; Lundeberg, 1970; Finlay et al., 1988). Nutrient media such as Modess and MMN for ectomycorrhizal fungi usually contain ammonium (Modess, 1941; Marx, 1969). In most forests ammonium is the dominant form of inorganic nitrogen, usually present in low concentrations (Cole, 1981). However, several investigators reported high ammonium concentrations in the ectorganic layers in Scots pine stands of different age in The Netherlands associated with high nitrogen input due to air pollution (Arnold, 1993; Van Dijk, 1993; Chapter 2). Average immission values of ammonia range from 40 to 80 kg ha^{-1} yr^{-1} with local extremes near sources around 100 kg ha^{-1} yr^{-1} which is comparable to the amounts of nitrogen applied to agricultural systems as fertilizer (Draaijers et al., 1989; Van Dijk et al., 1989; Pearson and Stewart, 1993). The numbers of ectomycorrhizal species in these stands were negatively correlated with inorganic

nitrogen concentrations in the ectorganic layers (Chapter 2). However, sporocarps of *Lactarius hepaticus* Plowr. and *Paxillus involutus* (Batsch: Fr.) Fr. were mainly observed in middle-aged and old Scots pine stands with inorganic nitrogen-rich thick litter and humus layers (Baar and Kuyper, 1993). Removal of litter and humus layers and herb vegetation ("sod-cutting") caused removal of considerable amounts of inorganic nitrogen and enhanced numbers of species and sporocarps of ectomycorrhizal fungi (Baar and Kuyper, 1993).

Besides high amounts of inorganic nitrogen the ectorganic layers and podzolic sandy soils in Scots pine stands in The Netherlands contain a significant pool of organic nitrogen (Chapters 2 and 3). Abuzinadah and Read (1986a,b; 1989) and Finlay et al. (1992) showed that several ectomycorrhizal fungi among which *P. involutus* and *Suillus bovinus* (L.: Fr.) Kuntze had the ability to utilize protein as sole nitrogen source in pure culture and in ectomycorhizal association with *Betula* and *Pinus* seedlings. This capability varied with species.

For uptake of nitrogen from the soil ectomycorrhizal fungi explore the soil finding new nutrient sources and subsequently exploiting them by changing their mycelial growth. Mycelial development of ectomycorrhizal fungi *in vitro* has been described by radial growth and biomass production (e.g. Littke et al., 1984; Gibson and Deacon, 1990; Jongbloed and Borst-Pauwels, 1990; Chapter 7). Radial growth reflects expansion of the exploitation area in the soil, whereas biomass production is a measure of accumulation of nutrients (Jongbloed and Borst-Pauwels, 1990). Fractal geometry can also be used to describe mycelial development as has been done for the saprotrophic fungi *Trichoderma viride* Pers., *Phanerochaete velutina* (DC.: Fr.) P. Karst., *Hypholoma fasciculare* (Huds.: Fr.) Kummer and *Armillaria* spp. by several investigators (Ritz and Crawford, 1990; Bolton and Boddy, 1993; Mihail et al., 1995). Fractal dimensions, expressing self-similarity of structure, reflect the compromise between explorative and exploitative growth forms and thus can be used to describe foraging strategies of fungi (Ritz and Crawford, 1990; Sugihara and May, 1990; Bolton and Boddy, 1993; Crawford et al., 1993; Mihail et al., 1995).

The objectives of the present study were to investigate whether 1. ectomycorrhizal species occurring in Scots pine stands with thick litter and humus layers containing high concentrations of inorganic nitrogen, have a good performance on media with ammonium; 2. ectomycorrhizal species occurring in sod-cut plots in Scots pine stands with thin litter and humus layers are negatively affected by high ammonium concentrations; 3. the capability of ectomycorrhizal species to utilize ammonium is negatively related to the capability to utilize glycine and 4. fractal geometry is appropiate to describe mycelial development of ectomycorrhizal fungi providing information about explorative and exploitative growth.

MATERIALS AND METHODS

Ectomycorrhizal fungi

Coltricia perennis (L.: Fr.) Murr. (F63) and *P. involutus* (F8) were collected in a middle-aged stand (planted in 1963) of *Pinus sylvestris* L. (S4), *Laccaria bicolor* (Maire) P.D. Orton (IS 7) and *Lactarius hepaticus* Plowr. (F 13) in an old stand (planted in 1940; S5), both secondary stands located in the province of Drenthe, in the northern part of The Netherlands. Isolates of these fungi were used because field observations showed that sporocarps of these fungi occurred on soils with different chemical composition. Sporocarps of *C. perennis* were observed in Scots pine stands with thin litter and humus layers on nutrient-poor non-podzolic sandy soils (Termorshuizen, 1991; Chapters 2 and 3). High numbers of sporocarps of *L. bicolor* were found in Scots pine stands on nutrient-poor non-podzolic sandy soils and on nutrient-richer podzolic sandy soils where litter and humus layers had been removed. Sporocarps of *L. hepaticus* and *P. involutus* were observed mainly in Scots pine stands with nitrogen-rich thick litter and humus layers (Baar and Kuyper, 1993). These fungi can easily be cultured and grow relatively fast (about 0.3-1 mm day^{-1}).

Inorganic nitrogen experiment

MMN media with agar (15 g l^{-1}) were used for growth of *C. perennis*, *L. bicolor* and *P. involutus*. Basal MMN medium was used as control because many ectomycorrhizal fungi grow well on this medium (Marx, 1969). Basal MMN medium contained (in g l^{-1}): malt extract (3), glucose.H_20 (10), $(NH_4)_2HPO4$ (0.25), KH_2PO4 (0.5), $MgSO_4.7H_2O$ (0.15), $CaCl_2.H_2O$ (0.067), NaCl (0.025), $FeCl_3$ (1%) and thiamine.HCl (100 μg l^{-1}) (Marx, 1969). Basal MMN medium was modified by preparing media with different concentrations of $(NH_4)_2HPO_4$ as inorganic nitrogen source in combination with different concentrations of glucose as carbon source (Table 1). The pH of the MMN media was adjusted to 5.6. *Lactarius hepaticus* was grown on basal Modess with agar (15 g l^{-1}) because *Lactarius* spp. grow well on this medium (Modess, 1941). Basal Modess medium contained (in g l^{-1}): malt extract (5), glucose.H_2O (5), KH_2PO4 (0.5), $MgSO_4.7H_2O$ (0.5), NH_4Cl (0.5), and (in ml l^{-1}) $FeCl_3$ (1%) (Modess, 1941). Basal Modess medium was modified by preparing media with different concentrations of NH_4Cl as inorganic nitrogen source in combination with different concentrations of glucose as carbon source (Table 1). The pH was adjusted to 4.7.

For inoculation, mycelial plugs of 6 mm diameter were cut from the edge of fungal colonies on basal MMN and Modess media and transferred to the different MMN and Modess media. Each treatment was replicated four times. The agar plates were kept at 22°C. The radial growth was determined by measuring the colony diameter every 3 days as long as the growth of the controls was linear or until the fungi reached the edge of the

Table 1. Modified MMN and Modess media with different concentrations of ammonium (IN, g l^{-1}) as inorganic nitrogen source, different concentrations of glycine (N, g l^{-1}) as organic nitrogen source and different concentrations of glucose (GLU, g l^{-1}) and initial pH. INGLU is similar to the basal MMN and Modess media (control media).

	MMN			Modess		
Media	Ammonium	Glucose	pH	Ammonium	Glucose	pH
½IN¼GLU	0.034	2.5	5.6	0.084	1.25	4.7
½IN½GLU	0.034	5.0	5.6	0.084	2.50	4.7
½INGLU	0.034	10.0	5.6	0.084	5.00	4.7
IN¼GLU	0.069	2.5	5.6	0.168	1.25	4.7
IN½GLU	0.069	5.0	5.6	0.168	2.50	4.7
2IN¼GLU	0.137	2.5	5.6	0.336	1.25	4.7
2IN½GLU	0.137	5.0	5.6	0.336	2.50	4.7
2INGLU	0.137	10.0	5.6	0.336	5.00	4.7
INGLU	0.069	10.0	5.6	0.168	5.00	4.7

	MMN			Modess		
Media	Glycine	Glucose	pH	Glycine	Glucose	pH
½N¼GLU	0.142	2.5	5.6	0.351	1.25	4.7
½N½GLU	0.142	5.0	5.6	0.351	2.50	4.7
½NGLU	0.142	10.0	5.6	0.351	5.00	4.7
N¼GLU	0.283	2.5	5.6	0.701	1.25	4.7
N½GLU	0.283	5.0	5.6	0.701	2.50	4.7
NGLU	0.283	10.0	5.6	0.701	5.00	4.7
2N¼GLU	0.566	2.5	5.6	1.402	1.25	4.7
2N½GLU	0.566	5.0	5.6	1.402	2.50	4.7
2NGLU	0.566	10.0	5.6	1.402	5.00	4.7

Petri dish. The colony diameters of *C. perennis* were measured for 43 days, of *L. bicolor* for 41 days, of *L. hepaticus* for 48 days and of *P. involutus* for 36 days.

When the measurements of the radial growth had stopped, mycelial biomass of the fungi was determined. Media with fungi (three replicates per treatment) were dissolved in hot water and the solutions were filtered and the filtrates were dried (100°C, 24 hr) and weighed (Jongbloed and Borst-Pauwels, 1990).

The pH of the remaining replicate per treatment was determined.

Organic nitrogen experiment

Subsequently, the same isolates were grown on modified MMN and Modess media with glycine as sole organic nitrogen source. Grov (1963) noted that glycine was present in the podzolic sandy soil in a pine forest in Norway. MMN media with different organic nitrogen concentrations were prepared by replacing $(NH_4)_2HPO_4$ with glycine ($C_2H_5NO_2$) and Modess media by replacing NH_4Cl with glycine ($C_2H_5NO_2$) (Table 1). KH_2PO_4 (0.25 g l^{-1}) was added to the MMN media with glycine to achieve a similar concentration of PO_4^{3-} as in basal MMN. Basal MMN and Modess media were used as controls. This experiment with media containing organic nitrogen was carried out in the same way as the experiment with media with inorganic nitrogen. The colony diameters of *C. perennis* and *L. bicolor* were measured for 30 days, of *L. hepaticus* for 38 days and of *P. involutus* for 39 days.

Fractal dimensions

Fractal dimensions were calculated using the method of Ritz and Crawford (1990). The two properties of large-scale correlation and self-similarity may be represented mathematically as

$$M(r) = Kr^D$$

M(r) is the mass contained within radius r, K is a constant and D is the fractal dimension. The exponent D ranges from 1 to 3 and describes how the mass of the fungus is distributed over the growth medium. If $D < 2$, the mycelium has a relatively open structure implying explorative growth (Bolton and Boddy, 1993) compared to the control medium ($D = 2$). If $D > 2$, the mycelium has a clustered, densely branched structure, implying exploitative growth compared to the control medium.

The biomass of the mycelial plug at the beginning of the experiments on basal MMN and Modess was estimated from the biomass of the mycelia on basal MMN and Modess at harvest time. Determining biomass of a mycelial plug at the beginning of the experiments according to the method described was not possible because of the small amount of biomass. Fractal dimension per Petri dish was assessed from a linear regression of the natural logarithm of the biomass of the mycelial plug at the beginning of the experiment and that at harvest time against the natural logarithm of the radius of the mycelial plug at the beginning of the experiment and that at harvest time, i.e. from the observations spanning the period of linear growth.

Statistical analysis

The growth rates, biomasses and fractal dimensions were analysed for homogeneity of variance by Bartlett's test. Normally distributed data were analysed by one-way ANOVA. Differences among means were evaluated with the LSD-test (Sokal and Rohlf, 1981). Data

not normally distributed were tested with the Kruskal-Wallis and Mann-Whitney U-tests (Siegel and Castellan, 1988).

RESULTS

Inorganic nitrogen experiment

The growth rates of *C. perennis* were significantly enhanced on all media with 2.5 and 5 g l^{-1} glucose (Table 2). The biomass of the fungus on the medium with ½INGLU was significantly larger than on basal MMN. The fractal dimensions of *C. perennis* on the media with 2.5 g l^{-1} glucose and on the 2IN½GLU-medium were significantly smaller than on basal MMN and below 2, implying explorative growth. The fractal dimension of this fungus on the ½INGLU-medium was higher than 2, suggesting exploitative growth.

The growth rates of *L. bicolor* decreased with increasing ammonium concentration. The biomass production of the fungus on all media was generally smaller than on basal MMN except for the medium with the highest concentrations of ammonium and glucose. The smallest biomass was determined on media with 2.5 g l^{-1} glucose. The fractal dimensions of *L. bicolor* were significantly enhanced on the 2IN½GLU- and 2INGLU-media.

The growth rates of *L. hepaticus* significantly increased on all media except for the 2INGLU-medium (Table 2). The biomass production and fractal dimensions of *L. hepaticus* on all media were significantly smaller than on basal Modess. The biomass and fractal dimension increased with increasing concentration of glucose.

The biomass production and fractal dimensions of *P. involutus* on the medium with the highest concentrations of ammonium and glucose were significantly larger than on basal MMN. The biomass production and fractal dimensions generally increased with increasing glucose concentration. The fractal dimension of the fungus on the medium with the highest concentrations ammonium and glucose was higher than 2 implying exploitative growth.

The pH of the MMN media was reduced from 5.6 to between 3.5 and 4.4. The pH of the Modess media was lowered from 4.7 to between 3.5 and 3.8. The reductions of the pH were the strongest on media with the highest concentration of ammonium.

Organic nitrogen experiment

The biomass production and fractal dimensions of *C. perennis* were significantly smaller on the media with 2.5 g l^{-1} glucose and on the ½N½GLU-medium than on basal MMN (Table 3). The biomass increased with increasing concentrations of glucose. The fractal dimensions on the media with glycine were lower than 2 suggesting explorative growth.

The growth rates of *L. bicolor* were significantly enhanced on all media with glycine. The biomass production and fractal dimensions (lower than 2) of this fungus on all media with glycine were significantly smaller than on basal MMN medium. The biomass generally increased with increasing organic nitrogen and glucose concentration.

Table 2. Growth rate (mm day^{-1}), biomass (mg), D (fractal dimension) and pH of *C. perennis*, *L. bicolor*, *L. hepaticus* and *P. involutus* on media with ammonium (IN) and glucose (GLU) in different ratios. Significant differences compared to the control (INGLU-medium) are indicated with * ($p < 0.05$, LSD or MWU).

Species	media	growth rate	biomass	D	pH
C. perennis	½IN¼GLU	0.34 *	11.2	1.63 *	4.4
	½IN½GLU	0.30 *	12.2	1.77	4.3
	½INGLU	0.27	17.9 *	2.12	4.3
	IN¼GLU	0.37 *	9.3	1.49 *	4.1
	IN½GLU	0.28 *	10.7	1.75	3.9
	2IN¼GLU	0.44 *	11.0	1.47 *	4.0
	2IN½GLU	0.37 *	11.1	1.57 *	3.8
	2INGLU	0.24	11.5	2.00	3.6
	INGLU (basal MMN)	0.25	11.7	1.97	3.9
L. bicolor	½IN¼GLU	0.72 *	19.7 *	1.64 *	4.2
	½IN½GLU	0.67 *	28.7	1.83 *	4.3
	½INGLU	0.70 *	21.3 *	1.68 *	4.3
	IN¼GLU	0.51 *	18.0 *	1.79 *	3.9
	IN½GLU	0.50 *	23.9 *	1.91	3.9
	2IN¼GLU	0.50 *	18.0 *	1.75 *	3.8
	2IN½GLU	0.35 *	27.8	2.25 *	3.6
	2INGLU	0.32 *	36.4	2.60 *	3.5
	INGLU (basal MMN)	0.56	32.8	2.00	3.9
L. hepaticus	½IN¼GLU	0.57 *	15.8 *	1.20 *	3.8
	½IN½GLU	0.53 *	20.9 *	1.34 *	3.6
	½INGLU	0.46 *	23.5 *	1.52 *	3.8
	IN¼GLU	0.53 *	16.9 *	1.29 *	3.8
	IN½GLU	0.48 *	23.0 *	1.50 *	3.6
	2IN¼GLU	0.51 *	16.4 *	1.33 *	3.7
	2IN½GLU	0.47 *	23.6 *	1.51 *	3.6
	2INGLU	0.33	25.0 *	1.74 *	3.5
	INGLU (basal Modess)	0.35	30.2	1.79	3.5
P. involutus	½IN¼GLU	1.04	13.9	1.79	4.1
	½IN½GLU	1.00	16.2	1.84	4.0
	½INGLU	0.96	14.8 *	1.86 *	4.0
	IN¼GLU	0.75	11.1 *	1.93	3.9
	IN½GLU	1.15	17.9	1.82	3.7
	2IN¼GLU	0.98	11.3 *	1.70 *	4.0
	2IN½GLU	1.05	27.8	2.00	3.7
	2INGLU	1.10	42.0 *	2.18 *	3.4
	INGLU (basal MMN)	1.03	24.4	1.99	3.7

Table 3. Growth rate (mm day^{-1}), biomass (mg), D (fractal dimension) and pH of *C. perennis*, *L. bicolor*, *L. hepaticus* and *P. involutus* on media with glycine (N) and glucose (GLU) in different ratios. Significant differences compared to the control (INGLU-medium) are indicated with * ($p < 0.05$, LSD or MWU).

Species	media	growth rate	biomass	D	pH
C. perennis	½N¼GLU	0.33	8.3 *	1.62 *	5.3
	½N½GLU	0.33	9.4 *	1.81 *	5.0
	½NGLU	0.34	10.9	1.97	5.0
	N¼GLU	0.33	8.9 *	1.85 *	5.4
	N½GLU	0.34	11.3	1.96	5.2
	NGLU	0.34	10.7	1.89	5.0
	2N¼GLU	0.33	8.1 *	1.76 *	5.5
	2N½GLU	0.34	10.3	1.88 *	5.3
	2NGLU	0.36	11.6	1.90	4.9
	INGLU (basal MMN)	0.35	12.2	2.00	4.1
L. bicolor	½N¼GLU	0.90 *	14.0 *	1.27 *	5.3
	½N½GLU	0.89 *	19.0 *	1.43 *	5.4
	½NGLU	0.93 *	14.5 *	1.30 *	5.3
	N¼GLU	0.90 *	13.8 *	1.27 *	5.4
	N½GLU	1.00 *	25.4 *	1.50 *	5.4
	NGLU	1.01 *	21.3 *	1.43 *	5.2
	2N¼GLU	1.17 *	17.6 *	1.26 *	5.3
	2N½GLU	1.19 *	28.8 *	1.46 *	5.3
	2NGLU	1.02 *	34.8 *	1.63 *	5.2
	INGLU (basal MMN)	0.59	35.1	2.00	3.7
L. hepaticus	½N¼GLU	0.42	3.6 *	0.97 *	5.0
	½N½GLU	0.34 *	3.5 *	1.09 *	4.9
	½NGLU	0.38 *	3.3 *	1.01 *	4.9
	N¼GLU	0.38 *	3.9 *	1.05 *	5.0
	N½GLU	0.28 *	4.1 *	1.33 *	5.0
	NGLU	0.46	3.9 *	1.01 *	4.9
	2N¼GLU	0.35	4.3 *	1.21 *	4.9
	2N½GLU	0.39	3.7 *	1.07 *	5.0
	2NGLU	0.36 *	4.6 *	1.22 *	4.9
	INGLU (basal Modess)	0.44	21.4	2.00	3.4
P. involutus	½N¼GLU	0.65 *	8.5 *	1.81	4.9
	½N½GLU	0.58 *	13.9 *	2.08	4.9
	½NGLU	0.62 *	15.4 *	2.08	4.9
	N¼GLU	0.30 *	7.9 *	2.62 *	5.4
	N½GLU	0.49 *	14.1 *	2.35 *	4.9
	NGLU	0.77 *	22.4	2.06	4.7
	2N¼GLU	0.34 *	7.7 *	2.41 *	6.1
	2N½GLU	0.34 *	12.7 *	2.52 *	5.9
	2NGLU	0.61 *	21.5	2.27	4.9
	INGLU (basal MMN)	0.92	21.1	1.99	3.7

The growth rates of *L. hepaticus* on the media with glycine were generally reduced or not affected compared to basal Modess. The biomasses of this fungus on the media with glycine were smaller than on basal Modess and on media with ammonium (Table 2). The fractal dimensions of the fungus on the media with glycine were in the range from 0.97 to 1.33 implying explorative growth. The fractal dimension with value 0.97 was calculated for the medium with the smallest concentrations glycine and glucose.

The growth rates of *P. involutus* were significantly inhibited on the media with glycine. The biomasses were significantly smaller on the media with glycine than on basal MMN except for NGLU- and 2NGLU-media. The biomass increased with increasing concentrations of glucose. The fractal dimensions significantly larger on all media than on basal MMN-medium were ranging from 2.35 to 2.52 although growth rate and biomass production were reduced.

The pH of the MMN media with glycine was changed from 5.6 to between 4.7 and 6.1. The pH of the Modess media with glycine was increased from 4.7 to 4.9 and 5.0. The pH of basal MMN and Modess media were more reduced than the pH of the media with glycine from 5.6 to between 3.7 and 4.1 and from 4.7 to 3.4, respectively.

DISCUSSION

The similarity in fractal dimensions suggests that *C. perennis* did not show a clear preference for either ammonium or glycine. The small amounts of biomass on the glycine media with 2.5 g l^{-1} glucose suggest that *C. perennis* was glucose limited.

The biomass production of *L. bicolor* on media with 0.566 g l^{-1} glycine was similar to that on media with 0.137 g l^{-1} ammonium indicating ability to utilize glycine. Ahmad et al. (1990) noted that *L. bicolor* utilized different amino acids as organic nitrogen and likely as carbon source on liquid culture.

The reduced growth rate, biomass and fractal dimensions of *L. hepaticus* on all media with glycine indicate that *L. hepaticus* is virtually unable to utilize glycine.

The reduction of the pH of the media with ammonium suggests uptake of ammonium by *L. bicolor* and *L. hepaticus*. The biomass of *L. bicolor* increased and the growth rate reduced with increasing ammonium concentrations. Jongbloed and Borst-Pauwels (1990) noted that *L. bicolor* responded to an increase of the ammonium concentration from 1 to 10 mM in MMN media with an increase in biomass production and a decrease in radial growth. *Laccaria bicolor* and *L. hepaticus* were carbon limited as shown by the small biomasses on the media with 2.5 g l^{-1} glucose.

The increased biomass of *P. involutus* in combination with unaffected growth rate on the medium with 0.137 g l^{-1} ammonium and 10 g l^{-1} glucose and the strongly reduced pH indicate that this fungus is capable to utilize ammonium as nitrogen source when sufficient

glucose is present. Utilization of ammonium by *P. involutus* was shown before in several *in vitro* studies (Lundeberg, 1970; Finlay et al., 1988; Finlay et al., 1992; Kieliszewska-Rokicka, 1992). The biomass of *P. involutus* on the NGLU- and 2NGLU-media of similar size as that on the basal MMN-medium indicates that this fungus is able to utilize glycine as nitrogen source when sufficient glucose is available. Lundeberg (1970) noted the ability of *P. involutus* to exploit glycine on liquid culture with 0.152 g l^{-1} glycine and 5 g l^{-1} glucose. The results of the inorganic and organic nitrogen experiments suggest that *P. involutus* was carbon limited.

As shown *C. perennis*, *L. bicolor*, *L. hepaticus* and *P. involutus* were carbon limited. The biomass of *L. bicolor* and *P. involutus* increased with increasing ammonium concentration suggesting that these species are nitrogen limited too. *Laccaria bicolor* and *P. involutus* possibly allocate more nitrogen to cell walls preventing catabolite repression in a way similar to saprotrophic fungi (Jennings, 1989). The biomass of *L. hepaticus* did not increase with increasing nitrogen concentration on Modess media. However, Jongbloed and Borst-Pauwels (1990) noted that the biomass of *L. hepaticus* increased with increasing ammonium concentration on MMN media suggesting nitrogen limitation. The unaffected biomass on Modess cannot be explained. The high biomass of *C. perennis* on the medium with 0.125 g l^{-1} ammonium and 10 g l^{-1} glucose suggests that this fungus is nitrophobic. Sporocarps of *C. perennis* were observed in sod-cut plots with thin ectorganic layers containing small amounts of nitrogen (Chapters 2 and 3). Termorshuizen (1991) noted that sporocarps of *C. perennis* were recorded in 6- to 11-year-old Scots pine stands with thin litter and humus layers in The Netherlands. Hintikka (1988) observed sporocarps of *C. perennis* in 30- to 50-year-old Scots pine forests with thin litter and litter humus layers in Finland.

The capability of *L. hepaticus* and *P. involutus* to utilize ammonium suggests that these species are nitrotolerant which is in accordance with field observations by Baar and Kuyper (1993). Arnolds and Jansen (1992) noted that during the last decades *L. hepaticus* increased and *P. involutus* remained approximately constant in forests in The Netherlands with high input of nitrogen from air pollution. Numbers of sporocarps of *P. involutus* increased as a result of fertilization with inorganic nitrogen (Laiho, 1970; Ohenoja, 1988). *Laccaria bicolor* was able to utilize ammonium and glycine. Numbers of sporocarps of *L. bicolor* were enhanced by fertilization of inorganic nitrogen (Ohenoja, 1988) and during the last decades *L. bicolor* has increased in The Netherlands (unpubl. results) suggesting that this species is nitrotolerant. In contrast, sporocarps of *L. bicolor* were hardly observed in grassy Scots pine stands whereas high numbers were observed in sod-cut plots in middle-aged and old Scots pine stands with thin litter and humus layers. Possible causes for this are discussed in chapter 2.

As forests age litter and humus accumulate and the organic matter content in the soil increases. Selection for proteolytic capability would be expected during ectomycorrhizal

succession (Read, 1991; Finlay et al., 1992), especially for fungi such as *L. hepaticus* and *P. involutus*, mainly observed in older stands. However, in the present study *L. hepaticus* and *P. involutus* developed much better on media with inorganic than organic nitrogen. Mycelia of these fungi might have been adapted to the nitrogen-rich conditions in The Netherlands. The ammonium tolerance of the ectomycorrhizal investigated in the present study was negatively related to the capability to utilize glycine. The proteinase production was likely repressed in the presence of ammonium as shown for *Hebeloma crustuliniforme* (Bull.) Quél. by Zhu et al. (1994).

The biomass production of *C. perennis*, *L. bicolor*, *L. hepaticus* and *P. involutus* generally increased with increasing glucose concentrations whereas growth rates were unaffected or decreased. This is in accordance with a trade-off between radial growth and biomass production as noted by Littke et al. (1984) and Paustian and Schnürer (1987). Biomass reflects carbon drain and radial growth can be translated to absorbing area of trees in the soil as suggested in chapter 7. Increasing biomass production of ectomycorrhizal fungi with increasing glucose concentrations suggests carbon limitation of ectomycorrhizal fungi as was indeed found by Gibson and Deacon (1990). They noted that so called late stage ectomycorrhizal fungi, i.c. fungi associated with maturing trees (Fleming et al., 1984), showed a large carbon demand.

The pH of the media with ammonium decreased with increasing ammonium concentration. This was also found for *L. bicolor* and *P. involutus* on liquid media by Finlay et al. (1992) and for *L. bicolor*, *L. hepaticus* and *L. rufus* on solid media by Jongbloed and Borst-Pauwels (1990). In unbuffered media acidification occurs due to ammonium uptake and proton efflux by the fungi (St John et al., 1985; Finlay et al., 1992). In unbuffered media the pH can decrease far below optimal pH for the fungi and might have affected the development of the fungi (Jongbloed and Borst-Pauwels, 1990). Jongbloed (1992) noted that the growth optimum of *L. bicolor* was pH 5.0 which is higher than the pH of the media with ammonium in the present study (Table 2).

In the present study only one isolate per species was tested and the results may not hold in general. Kieliszewska-Rokicka (1992) and Arnebrant (1994) noted that different isolates of one species reacted differently to the same treatments.

The fractal dimensions of the four ectomycorrhizal fungi were within the range from 1 to 3, except for the fractal dimension of *L. hepaticus* on the medium with the smallest concentrations of ammonium and glucose, which was 0.97.

The fractal dimensions generally increased with increasing concentrations of glucose and ammonium. This suggests that fractal dimensions based on biomass depend upon the potential of the nitrogen and carbon source from which the mycelium is extending. A possible reason for this is that low concentrations of nutrients are unable to sustain complex mycelia with a high degree of branching, whereas clustered and branched mycelia are formed when high amounts of nutrients are available (Bolton and Boddy,

1993).

The fractal dimensions of *L. hepaticus* on the media with glycine were about 1.0 to 1.3 with very low biomass production and reduced or unaffected growth rates. Nutrients were apparently limiting for the fungus (Bolton and Boddy, 1993).

Fractal dimensions of *L. bicolor* and *P. involutus* grown on the 2IN½GLU and 2INGLU-media were within the range from 2 to 3 indicating investment in the biomass by the fungi. The high fractal dimensions of *P. involutus* on the media with glycine are in contrast with the reduced growth rate and biomass production. However, the fractal dimensions show that the mycelia had dense distributions and efficiently occupied the area that had been explored.

The results of the present study show that fractal dimensions can provide information about growth strategy of mycelia which cannot be obtained from growth rate and biomass measurements alone as was also noted by Bolton and Boddy (1993). However, misinterpretation is possible when growth rate and biomass measurements are ignored. E.g. fractal dimensions in the range from 2 to 3 suggest exploitative growth and high mycelial biomass as shown for *L. bicolor* and *P. involutus* on 2INGLU-medium (Table 2), but mycelial biomass can also be low as shown for *P. involutus* on the 2N¼GLU-medium (Table 3).

In conclusion, the ectomycorrhizal fungi differed in their ability to grow on media with inorganic and organic nitrogen in combination with different concentrations of glucose. *Laccaria bicolor* was capable of utilizing ammonium and glycine. The high biomass of *C. perennis* on the medium with the smallest concentration of ammonium suggests that this fungus is nitrophobic. Sporocarps of *C. perennis* were observed in sod-cut plots in a Scots pine stand with thin ectorganic layers containing low concentrations of nitrogen (Chapters 2 and 3). The capability of *L. hepaticus* and *P. involutus* to utilize ammonium is in accordance with the occurrence of sporocarps of these nitrotolerant species mainly observed in the control and sod-added plots with thick litter and humus layers containing high ammonium concentrations (Baar and Kuyper, 1993; Chapter 2). The ammonium tolerance of the ectomycorrhizal fungi was negatively related with the capability to utilize glycine.

The use of fractal geometry provides some information about the foraging strategy of mycelia which cannot be obtained from growth rate and biomass measurements. However, fractal dimensions cannot replace growth rate and biomass measurements.

SPATIAL DISTRIBUTION OF LACCARIA BICOLOR GENETS REFLECTED BY SPOROCARPS AFTER REMOVAL OF LITTER AND HUMUS LAYERS IN A PINUS SYLVESTRIS FOREST

SUMMARY

The spatial distribution of the genets of the ectomycorrhizal fungus Laccaria bicolor *was studied by somatic incompatibility, two years after removal of the litter and humus layers in two plots of 225 m^2 in a Scots pine (*Pinus sylvestris*) stand (planted in 1974) in The Netherlands. Pairings of isolates revealed the presence of three and four genets on the two sites. Genets were up to 12.5 m in size, indicating that mycelia are perennial and are of importance for mycelial spread. Age of the genets was estimated at 13 to 26 and 16 to 31 years, for the two sites. The smallest genet was represented by two sporocarps suggesting recent colonization by spores.*

INTRODUCTION

The number of species and sporocarps of ectomycorrhizal fungi in forests of *Pinus sylvestris* L. (Scots pine) in The Netherlands has decreased during the last few decades (De Vries et al., 1985; Termorshuizen and Schaffers, 1991). At the same time litter accumulation and development of the humus profile have accelerated due to atmospheric deposition of nitrogen, and the grass *Deschampsia flexuosa* (L.) Trin. has increased in these forests (Klap and Schmidt, 1992). Removal of litter, humus and the herbaceous vegetation by sod-cutting (removal of the organic top soil and vegetation) resulted in one and a half times as many species and about three times as many sporocarps of ectomycorrhizal fungi than in the control plots after 18 months, in 15 - 50 years old Scots pine stands (Baar and Kuyper, 1993). *Laccaria bicolor* (Maire) P.D. Orton dominated the ectomycorrhizal flora of the sod-cut plots (Baar & Kuyper, 1993). Whether this increase resulted from enhanced sporocarp production of mycelia that were already present or by new establishment of mycelia by spores or by a combination is unknown. Information on the size of genets, which can be determined by somatic incompatibility reactions in culture (Rayner et al., 1984; Rayner, 1991) would aid interpretation.

Somatic incompatibility (i.e. rejection of non-self) operates in both hetero- and homothallic species. However, the interpretation of obtained results differs since heterothallic fungi generate genetically variable progeny while homothallic fungi generate clonal progeny (Rayner and Boddy, 1988).

The phenomenon has been used successfully to determine genet size in saprotrophic and pathogenic wood fungi and also in several species of the ectomycorrhizal genus *Suillus* Gray and *Laccaria proxima* (Boud.) Pat. (Fries, 1987; Dahlberg and Stenlid, 1990; Sen, 1990).

The aim of the present investigation was to map the spatial distribution of genets of *L. bicolor* in order to determine the roles of mycelial spread and establishment by spores, and to investigate whether the increased production of sporocarps resulted from mycelia present before sod-cutting or from newly established genets.

MATERIALS AND METHODS

Study sites

In 1991, a secondary middle-aged stand of *Pinus sylvestris* (S3) planted in 1974 and located in northeastern Netherlands (forestry Dwingeloo; 52° 50' N, 6° 25' E) was selected. Until 1920 the site was heathland, then *Pinus sylvestris* mixed with *Quercus rubra* L. and *Picea abies* (L.) Karsten was planted. In 1974, the stand was felled and replanted with *P. sylvestris*. In June 1990, the litter and humus layers and the herb vegetation of four plots (15 * 15 m) were removed, four untreated plots were used as controls, all plots being situated in a homogeneous area. The few naturally regenerated deciduous trees were cut down. Two plots where the highest number of sporocarps of *L. bicolor* were found were selected for studying the population structure of this species.

Field work

All sporocarps of *L. bicolor* and trees were mapped in both plots ($\pm$10 cm). A representative sample of sporocarps of *L. bicolor* was collected from both plots.

Isolations and pairings

Isolates were made by plating internal tissue from sporocarps onto Modified Melin-Norkrans medium (MMN) with 25 ppm oxytetracycline and incubated at 22 °C. Twenty-three isolates from plot one and 13 from plot two were cultured successfully. Pairings were made by confronting two isolates on MMN at a distance of 0.5 cm. In all, 49 combinations were tested for plot one and 26 for plot two. Pairings were repeated twice. Self-pairings were made of all tested isolates. After 6 weeks, intermingling isolates were considered to belong to the same genet, and those which did not intermingle to different genets. Demarcation zones of pairings which did not intermingle were inspected using a binocular microscope. The position of somatically compatible isolates was mapped to determine the spatial distribution of genets.

Estimation of the age of the genets was based on estimated growth of mycelium ranging from 20 to 40 cm yr^{-1} (Last et al., 1983; Dahlberg and Stenlid, 1990) and on

measurements by De la Bastide (pers. comm.) who measured an annual increase of distance of *L. bicolor* sporocarps around isolated *Picea abies* trees of 20 cm yr^{-1}.

RESULTS

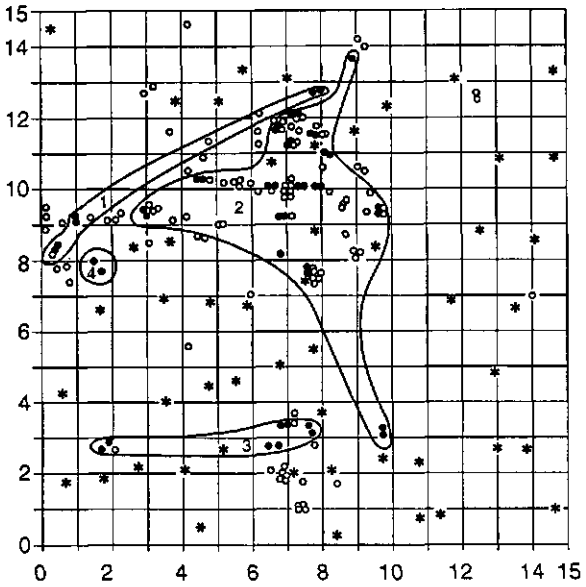
During the six weeks season of 1991, 340 sporocarps of *L. bicolor* were produced in plot one and 166 in plot two, and 117 and 32 sporocarps in the two unsampled sod-cut plots. No sporocarps of *L. bicolor* were found in the control plots. During one visit in Nov. 1991, 66 of the 138 sporocarps were collected in plot one and 46 of 153 sporocarps in plot two.

Table 1. Stand characteristics, treatment, number of sporocarps and genet characteristics of *L. bicolor*.

	plot 1	plot 2
Year of planting	1974	1974
Treatment	sod-cut	sod-cut
Number of trees per plot	50	44
Number of sporocarps observed	138	153
Number of sporocarps tested	23	13
Number of genets	4	3
Number of sporocarps belonging to largest genet	82	57
Largest diameter of genet (m)	10.5	12.5
Range of estimated age (yr)	13-26	16-31

In all, self pairings and in pairings between a few isolates the mycelia intermingled. When isolates did not intermingle demarcation zones developed which consisted of a clear purple band or thin mycelium with a loose structure. The reactions of somatic incompatibility were consistent with both replicates. An indistinct demarcation zone developed once. The isolate which developed an indistinct zone intermingled with several other isolates of genet three (Fig. 1b) indicating that this isolate also belonged to this genet. In plot one, 4 genets were detected among 23 tested sporocarps and in plot two 3 genets among 13 tested sporocarps (Fig. 1). The largest genet in plot one had a diameter of 10.5 m and the largest genet in plot two a length of 12.5 m (Table 1). The smallest genet produced two sporocarps. The age of the largest genets in plot one and two was estimated as 13 to 26 and 16 to 31 years, respectively (Table 1).

a



b

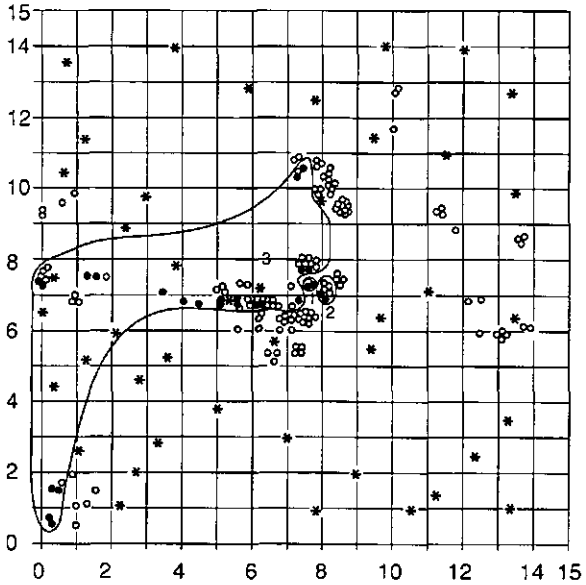


Fig 1. Spatial distribution of genets of *Laccaria bicolor* in plot one (a) and plot two (b). Lines encompass sporocarps belonging to the same genet. ● = sporocarps tested for compatibility; ○ = non-tested sporocarps; * = *Pinus sylvestris* trees.

DISCUSSION

The method of somatic incompatibility is useful for identification of *L. bicolor* genets although the demarcation zones are not as distinct as described for *Suillus luteus* (L.: Fr.) Gray and *S. bovinus* (L.: Fr.) Kuntze (Fries, 1987; Dahlberg, 1991).

The presence of four large genets indicated that most sporocarps originated from mycelia which were probably present in the soil before sod-cutting. After sod-cutting the large genets of *L. bicolor* might have survived as mycelia or remains of ectomycorrhizal roots deep in the mineral soil layer; in old rotten stumps of trees, which were not removed and possibly contained clusters of ectomycorrhizal root tips; or by saprotrophic survival on rotten wood and dead roots. The small genets may indicate recent establishment by spores (Rayner and Todd, 1979; Stenlid, 1985) after removing the ectorganic layers, especially genet one in plot two surrounded by genet three. This is consistent with observations made by Dahlberg and Stenlid (1990) who noted a high number of genets at disturbed sites. However, the small genets may also reflect remains of old genets or larger genets of which only a sector was triggered to form sporocarps due to sod-cutting.

The number of genets in the plots might have been underestimated, since isolates were not made from all sporocarps, because some were too old for isolation and some were not cultured successfully. Also, individual mycelia may not produce sporocarps every year (Dahlberg, 1991).

There was apparently no overlap of genets, indicating intraspecific competition in accordance with findings by Thompson & Rayner (1982), Dahlberg & Stenlid (1990) and Holmer and Stenlid (1991) for other basidiomycetes. The large genets might have been fragmented in ramets. Thus the mycelia may not have interconnected all sporocarps plotted within the lines encompassing genets shown in Fig. 1. Although the method may overestimate area occupied by individual mycelia, age of genets is not overestimated. At an estimated age of 13-31 years, the largest genet was much younger than the largest genet of *S. bovinus* estimated at 75 years (Dahlberg and Stenlid, 1990). The location and size of the genets indicate that single trees are sometimes connected with several genets and that several trees are in symbiosis with the same genet, although as indicated above direct evidence of mycelial interconnectedness has not been obtained. The former was also suggested by Fries (1987) and Dahlberg (1991), and ectomycorrhizal connections between plants have also been observed (Finlay and Söderström, 1989).

The control plots presumably reflect the situation before sod-cutting, and ectomycorrhizal species composition of all plots was similar before the treatment. In the control plots of this stand hardly any sporocarps of *L. bicolor* were observed (4 sporocarps in 1990 and none in 1991; pers. obs.). Isolates of *L. bicolor* are not sensitive to realistic concentrations of phytotoxic components of extracts of Scots pine needles and

shoots and roots of *D. flexuosa in vitro* (Baar et al., 1993; Chapter 7). Hence, it is unlikely that the fructification of *L. bicolor* was affected by needle or grass components in nature. The absence of sporocarps in the control plots is possibly explained by a mechanical effect in the Scots pine forest studied: in most Scots pine forests in the Netherlands the grass *D. flexuosa* forms a tight mat with a dense root system (Arnolds, 1991; Nabuurs, 1991). Removal of this mat might hence favour sporocarp formation by ectomycorrhizal fungi. The estimate of the minimum age (13 years) of the largest genet indicates that mycelia of *L. bicolor* were present in the soil underneath the vegetation dominated by *D. flexuosa* before sod-cutting, without forming sporocarps. Sod-cutting apparently enhanced formation of sporocarps of *L. bicolor* by mycelia that were already present.

Sporocarps of *L. bicolor*.

GENERAL DISCUSSION

INTRODUCTION

Field observations led to hypotheses on the role of ectorganic layers and tree vitality in the decline of ectomycorrhizal fungi in Scots pine stands in The Netherlands (Chapter 1). These hypotheses were tested in field experiments. The effects of manipulation of litter and humus layers on sporocarps and mycelia of ectomycorrhizal fungi and ectomycorrhizal root tips were studied in Scots pine stands of different age (Chapters 2, 3, 4, 5 and 9). Laboratory experiments were set up for a lower-level explanation of the results of field experiments. Laboratory experiments with ectomycorrhizal fungi associated with Scots pine seedlings were carried out for studying the effects of different soils on ectomycorrhizal development (Chapter 6). In pure culture the effects of components of litter and humus in Scots pine stands on ectomycorrhizal performance were investigated (Chapters 7 and 8).

ECTOMYCORRHIZAL FUNGI AS AFFECTED BY LITTER AND HUMUS

The decline of ectomycorrhizal fungi above and below ground in Scots pine stands can be largely explained by the chemical composition of litter and humus layers (Chapters 2, 3, 5 and 7). Evidence was obtained that nitrogen originating from air pollution, pH and phenolic compounds affect the occurrence of ectomycorrhizal species. The effects of litter and humus were most pronounced in middle-aged and old stands (Chapters 2 and 3).

Inorganic nitrogen concentrations increase with increasing thickness of litter and humus (Chapter 2). Negative correlations between high nitrogen concentrations of the litter and humus layers and numbers of ectomycorrhizal species above ground were found (Chapter 2). Below ground, the numbers of ectomycorrhizal root tips in the upper mineral soil in the present study (Chapter 4) were of similar extent as in humus and in upper mineral soil in other Scots pine stands in The Netherlands (Kuyper, 1990; Termorshuizen, 1991), but were much lower than in humus in Scots pine stands in Norway and Finland (Ohtonen et al., 1990; Timmermann, 1994). Earlier investigations showed that ectomycorrhizal development was negatively related to increasing nitrogen content of the soils (Ohtonen et al., 1990; Termorshuizen and Ket, 1993).

Sporocarps of *Lactarius hepaticus* and *Paxillus involutus* were mainly observed in middle-aged and old Scots pine stands with inorganic nitrogen-rich litter and humus layers (Baar and Kuyper, 1993; De Vries et al., 1995; Chapter 2). *Lactarius hepaticus* and *P. involutus* were capable to utilize high amounts of ammonium *in vitro* (Chapter 8)

indicating that these species are nitrotolerant. Sporocarps of *C. perennis* and *Suillus bovinus* were observed in sod-cut plots with thin litter and humus layers containing low amounts of inorganic nitrogen (Chapter 2). *Coltricia perennis* had the highest biomass with the lowest amount of ammonium *in vitro* which suggests that this species is nitrophobic (Chapter 8). In a laboratory experiment ectomycorrhizal development of *S. bovinus* associated with seedlings on humus with high ammonium concentrations was poor (Chapter 6). Sensitivity of *S. bovinus* to excess nitrogen was reported by Wallander and Nylund (1991) and Arnebrant (1994).

In addition, positive correlations between pH of litter and humus layers and numbers of ectomycorrhizal species above ground were recorded (Chapter 2). Shaw and Lankey (1994) also noted a positive correlation between ectomycorrhizal species above ground and soil pH in Scots pine stands. Ectomyorrhizal development of *L. bicolor* was poor on humus from a middle-aged secondary stand with $pH(CaCl_2)$ 2.8 *in vitro* (Chapter 6). Kamminga-Van Wijk (1991) reported acid sensitivity of *L. bicolor* and optimal growth of this fungus associated with Douglas fir seedlings in hydroculture at pH 4.0. Jansen and Van Dobben (1987) noted that *Cantharellus cibarius* was hardly observed in stands with $pH(H_2O) < 4.2$ and suggested that this species was acid sensitive.

In chapter 2, evidence was obtained that the numbers of ectomycorrhizal species were affected by K^+ concentrations. However, potassium likely played a minor role compared to nitrogen. Weak positive or no effects on the numbers of ectomycorrhizal fungi were found in fertilization experiments (Wästerlund, 1982a; Shubin, 1988). The high K^+ concentrations in the ectorganic layers more likely reflect the presence of *D. flexuosa*.

Low numbers of species and sporocarps of ectomycorrhizal fungi above ground were observed in the control and sod-added plots in Scots pine stands with thick litter and humus layers and a herb vegetation dominated by the grass *Deschampsia flexuosa* (Chapters 2 and 3). The ectomycorrhizal colonization potential below ground was reduced by sod-adding too (Chapter 5). Isolates of several ectomycorrhizal fungi, among which *Laccaria proxima* and *Rhizopogon luteolus*, were sensitive to phenolic compounds in extracts of Scots pine needles and the grass *D. flexuosa* (Chapter 7). Phenolic compounds produced by *Picea abies* and present in humic solutions reduced the respiration of *Cenococcum geophilum* and *Laccaria laccata* (Pellisier, 1993).

The isolate of *Laccaria bicolor* used in our study was insensitive to litter and grass extracts. The observed inhibition of fructification of this fungus may still be caused by *D. flexuosa* which forms a tight mat with a dense root system (Chapters 7 and 9). In addition, competition for phosphorus between ectomycorrhizal fungi of Scots pine and arbuscular mycorrhizal fungi (AM-fungi) of *D. flexuosa* may occur. With excess nitrogen AM-fungi take up phosphorus more efficiently than ectomycorrhizal fungi (Eason et al., 1991).

Sod-adding changed the structure of the humus layers to compact and moist humus layers (unpubl. results). Such layers are probably less aerated than the humus layers in the control plots which might have hampered the growth of ectomycorrhizal mycelia. Less

aerated humus could lead to oxygen deficiency or high carbon dioxide concentrations. *In vitro* experiments showed that increasing CO_2 concentrations reduce the growth rate of *S. bovinus* (Hintikka and Korhonen, 1970).

OCCURRENCE OF ECTOMYCORRHIZAL FUNGI IN RELATION TO TREE VITALITY

Several investigators have related tree vitality to the occurrence of ectomycorrhizal fungi (Meyer, 1988; Termorshuizen and Schaffers, 1987; Jansen and De Vries, 1988). However, a proper definition of tree vitality is hard to give. An intuitive concept of tree vitality would include ability of recovery after stress, normal growth and functioning of leaves, roots, etc. Several methods to describe tree vitality on the basis of external characters and chemical analyses of needles have been developed, but there is no method to describe the specific effects of different air pollution components (Van den Ancker et al., 1987). The relation between tree vitality and measured parameters has been supposed to be linear. However, it is more likely that there is an optimum relationship between tree vitality and measured parameters. Termorshuizen and Schaffers (1987) described tree vitality by a.o. tree girth, crown density and needle occupation, following the method of the Dutch State Forestry Service (Anonymous, 1987). They noted positive correlations between numbers of species and sporocarps of ectomycorrhizal fungi in old Scots pine stands and tree vitality. In the present study, tree vitality was measured by external characteristics of the trees similar to Termorshuizen and Schaffers (1987) and by chemical analyses of the needles. The external characteristics describing tree vitality did not differ between the treatments in all stands (unpubl. results) and did not explain the occurrence of ectomycorrhizal fungi. The nitrogen concentrations in the needles were negatively correlated with the numbers of ectomycorrhizal species (Chapter 2). High ammonia input may cause leaf damage (Van der Eerden et al, 1992; Van Dijk, 1993), leaching of cations such as K^+ and Mg^{2+} (Nihlgård, 1985; Roelofs et al., 1985; Schulze, 1989) and reduction of photosynthesis. Reduced photosynthesis may lead to diminished production and transport of photosynthates (Lorenc-Plucinska, 1984). High nitrogen input may induce increased uptake of nitrogen by trees causing conversion of nitrogen in amino acids (Van der Eerden, 1982; Näsholm and Ericsson, 1990; Van Dijk et al., 1992) which requires carbohydrates. Björkman (1942) suggested that decreased ectomycorrhizal development at high nitrogen levels was a result of reduced carbohydrates available in the roots. In contrast, Wallander (1992) noted that reduced extramatrical mycelium of ectomycorrhizal fungi at high nitrogen levels is a result of a shift in the carbon allocation from fungal growth to the process of nitrogen assimilation in the fungus or root.

SUCCESSION OF ECTOMYCORRHIZAL FUNGI IN SCOTS PINE STANDS

The observed occurrence and development of the ectomycorrhizal fungi in Scots pine stands on different soils (Chapter 2, 3, 5 and 6), their sensivity for extracts of Scots pine needles and the grass *D. flexuosa* (Chapter 7), and their utilization of organic and inorganic nitrogen sources (Chapters 6 and 8) are not concordant with the "early-" and "late-stage" concept (Mason et al., 1982; Deacon et al., 1983), the C-S-R-concept (Grime, 1985) and the classification based on epidemiological characteristics (Newton, 1992).

Tricholoma species form abundant rhizomorphs (Godbout and Fortin, 1985) which is considered to be a characteristic of late-stage fungi (Dighton and Mason, 1985). Sporocarps of *Tricholoma* species were, however, observed in young Scots pine stands by Termorshuizen (1991) and mainly in 15- to 20-year-old stands in the present study (Chapter 3).

Mycelia of *L. bicolor* established in the sod-cut plots both by mycelia present for at least 13 years in the mineral soil and by spores (Chapter 9). This indicates that in the functional classification based on epidemiological characteristics (mycelial spread vs. spores) (Newton, 1992), *L. bicolor* has attributes of both groups. Dahlberg and Stenlid (1990) noted that the spatiotemporal distribution of *S. bovinus* indicates that the C-S-R-concept (Grime, 1985) does not hold for this species.

The "early-" and "late-stage" concept is based on the r-K concept or similar to the R-selection and S-selection in the C-S-R-concept (Last et al., 1987). During canopy closure the strongest selection for C-strategies may occur (Dahlberg and Stenlid, 1990). However, in the present study evidence was obtained that succession of ectomycorrhizal fungi is largely determined by age of the humus profile (Chapters 2 and 3) and not by the phase of canopy closure.

Selection for proteolytic capability and insensitivity for humus compounds like phenolics would be expected during succession, especially for late-stage fungi such as *L. hepaticus*, *P. involutus* and *X. badius* (Perry and Choquette, 1987). However, isolates of *L. hepaticus* and *P. involutus* developed much better on media with inorganic nitrogen than with organic nitrogen (Chapter 8), while isolates of *P. involutus* and *X. badius* were sensitive to needles extracts (Chapter 9).

The ectomycorrhizal species composition also differed between the control plots in primary and secondary middle-aged Scots pine stands, indicating that primary succession differs from secondary succession (Chapter 3). The numbers of ectomycorrhizal species in secondary Scots pine stands were furthermore reduced by accumulation of nitrogen-rich litter or humus and dominance of the grass *D. flexuosa* (Chapter 3). Shaw and Lankey (1994) noted that organic matter content of the soil was negatively correlated with ectomycorrhizal species richness.

In addition, many ectomycorrhizal fungi have declined during the last few decades. The remaining species such as *L. hepaticus* and *P. involutus*, which dominate the

ectomycorrhizal flora in Scots pine stands with thick litter and humus layers and herb vegetation dominated by *D. flexuosa*, seem to be nitrotolerant and might therefore be adapted to the nitrogen-rich conditions in The Netherlands (Chapter 8).

ECTOMYCORRHIZAL FUNGI ABOVE AND BELOW GROUND

The occurrence of ectomycorrhizal fungi above ground was compared with that below ground. In Douglas fir stands sporocarps of ectomycorrhizal fungi were positively correlated with numbers of ectomycorrhizal root tips (Jansen and De Nie, 1988; Jansen, 1991). Termorshuizen and Schaffers (1991) did not find such correlations in Scots pine stands.

Plots in Scots pine stands of different age were systematically searched from 1990 to 1993 for sporocarps of ectomycorrhizal fungi (Chapters 2 and 3). This approach was necessary because numbers of species and sporocarps varied over the four years of investigation likely due to weather conditions (Eveling et al., 1990; Gulden et al., 1992; Såstad and Jenssen, 1993).

In 1992, the results of the sporocarp surveys in the Scots pine stands planted in 1987 (S1) and planted in 1974 (S3) were related to ectomycorrhizal types (more or less corresponding to genera) occurring on Scots pine seedlings five months after outplanting (Chapter 5). Correlations between the occurrence of ectomycorrhizal genera above and below ground were poor.

In November 1992, the numbers of ectomycorrhizal root tips in the upper 10 cm of the mineral soil of the plots in the stands planted in 1974 and in 1924 (S3 and S6) did not significantly differ between the treatments (Chapter 4). In contrast, the numbers of species and sporocarps of ectomycorrhizal fungi were significantly higher in the sod-cut plots (Chapters 2 and 3). Termorshuizen (1990) noted that the numbers of ectomycorrhizal root tips in the humus layers and upper mineral soil in young and old Scots pine stands did not significantly differ, although the numbers of species and sporocarps of ectomycorrhizal fungi had decreased. He suggested that ectomycorrhizal species were affected in their ability to fructify or that nitrotolerant species had taken their places on roots. This indicates that fructification of ectomycorrhizal fungi does not necessarily reflect the amount and activity of ectomycorrhizal root tips as noted by Dahlberg (1991) and Wallander (1992).

REMOVAL OF LITTER AND HUMUS LAYERS AS A MEANS OF RESTORATION OF ECTOMYCORRHIZAL FLORA

In Scots pine stands thick litter and humus layers and herb vegetation dominated by the

grass *D. flexuosa* negatively affected development and fructification of many ectomycorrhizal fungi (Chapters 2, 3, 5, and 9). Sod-cutting in Scots pine stands enhanced numbers of ectomycorrhizal species and sporocarps, particularly in middle-aged and old stands (Chapter 2). The humus profile development was set back. The reduction of nitrogen as ammonia originating from air pollution, and complex organic components such as phenolics positively affected the fructification of several ectomycorrhizal fungi (Chapters 2, 3 and 7). The increased pH of the litter layers in the sod-cut plots may also have positively affected the ectomycorrhizal fungi (Chapter 2).

Sod-cutting in the Scots pine stand planted in 1987 (S1) did not positively affect numbers of species and sporocarps of ectomycorrhizal fungi, because the difference between before and after treatment was too small (Chapter 2).

Sod-cutting in Scots pine stands resulted in an increase of numbers of ectomycorrhizal species and sporocarps of ectomycorrhizal fungi, including species that are threatened in The Netherlands, such as *C. cibarius*, *Coltricia perennis*, *R. luteolus*, *Russula adusta* and *Tricholoma albobrunneum* (Chapters 2 and 3). This indicates that sod-cutting can be used as a means of restoration of the ectomycorrhizal flora.

After sod-cutting the Scots pine trees did not die and their roots recovered. Vitality of the trees described by external characteristics did not differ between sod-cut and control plots three and a half years after the treatment (unpubl. results). The ectomycorrhizal root tips in the sod-cut plots in the Scots pine stand planted in 1974 (S3) increased until two years after the treatment to the same extent as in the control plots. The roots in the sod-cut plots in the Scots pine stands planted in 1924 (S6) increased so much that two and a half years after the treatment the numbers of ectomycorrhizal root tips were higher than in the control plots (Chapter 4). Sod-cutting also increased the ectomycorrhizal colonization potential (Chapter 5).

Although sod-cutting enhanced numbers of ectomycorrhizal species and sporocarps, strongly threatened species such as *Hygrophorus hypotheius*, *Tricholoma portentosum* and *T. focale* (Arnolds, 1989), observed in young primary Scots pine stands (Termorshuizen, 1991; Chapter 3), were not (yet?) found in the sod-cut plots. The absence of these ectomycorrhizal species in the sod-cut plots can partly be explained by the high nitrogen concentrations and organic matter content of the mineral soil, particularly of podzolic sandy soil in the Scots stands planted in 1980 and 1974 (S2 and S3), that were not affected by sod-cutting (Chapter 2). Another explanation is that, although many spores are dispersed by wind (Lamb, 1979), chances are small for spores of species, which do not occur in the neighbourhood of the sod-cut plots, to fall down in the relatively small sod-cut plots and establish successfully (Chapter 3).

Sod-cutting is a means to restore a (large) part of the ectomycorrhizal flora in Scots pine stands, particularly in middle-aged and old stands (Chapters 2 and 3). However, the most necessary measure of restoration of ectomycorrhizal flora is to reduce nitrogen input from the air, especially ammonia (Termorshuizen, 1990; Van Dijk, 1993). This requires

fundamental changes in agricultural management and involves great financial efforts (Van Dijk, 1993).

IMPLICATIONS OF REMOVAL OF LITTER AND HUMUS LAYERS FOR OTHER ORGANISMS

Removal of litter and humus layers and herb vegetation in Scots pine stands has consequences for other organisms such as saprotrophic fungi, herbs and soil fauna.

The mycoflora of Scots pine stands not only consists of ectomycorrhizal fungi, but also of saprotrophic fungi. Most saprotrophic fungi in Scots pine stands did not show a decline during the last few decades and are not threatened (Arnolds and Jansen, 1992). Sporocarps of saprotrophic fungi were not found half a year after sod-cutting in the stands investigated. Three and a half years after sod-cutting hardly any sporocarps of saprotrophic fungi were observed in the sod-cut plots of all stands (pers. obs.). At that time the litter and humus layers were still thinner than in the control plots (Chapters 2 and 3).

The herb vegetation was completely removed in the treated plots (Chapters 2 and 3). Three and a half years after the treatment, sod-cutting had positively affected *Calluna vulgaris*, *Carex pilulifera*, *Juncus squarrosus* and *Rumex acetosella* in the different stands (unpubl. results). The occurrence of most plant species in the sod-cut plots was independent of the age of the stands. The same species were found to increase in a similar study by De Vries et al. (1995).

Verstraten et al. (1989) suggested that sod-cutting removes an important part of the soil fauna. In addition, the habitat conditions after sod-cutting will be more subject to large fluctuations of temperature and moisture, which might adversely affect the surviving organisms. Other species less dependent on the protection of litter and humus layers and herb vegetation may recover soon after the disturbance (Klap and Schmidt, 1992).

Each functional group reacts on sod-cutting in a specific way as shown by research on nematodes (De Goede, pers. comm.). Sod-cutting in a Scots pine stand planted in 1924 (S6) reduced the total number of nematodes. One year after sod-cutting the nematode fauna of the newly developed ectorganic layers comprised fungal, bacterial and algal feeding nematodes, whereas plant feeders, predators and omnivores were not detected. In the mineral soil only insect parasites were negatively affected by sod-cutting (De Goede, pers. comm.).

SUGGESTIONS FOR FURTHER RESEARCH

The present study indicates that development of litter and humus layers with high nitrogen and phenolic concentrations causes a decline of ectomycorrhizal fungi in Scots pine stands, and that removal of these layers could lead to a (partial) restoration of ectomycorrhizal species richness. Based on these results new questions arise that warrant further research.

It was shown that extracts of Scots pine litter and *D. flexuosa* inhibited the growth of several ectomycorrhizal fungi. These extracts contained phenolics which were responsible for the growth reduction of the ectomycorrhizal fungi (Chapter 7). Münzenberger et al. (1990) noted that amounts of phenolics were reduced in ectomycorrhizal roots of *Lactarius deterrimus* and *Laccaria amethystina* associated with *P. abies*, except for the hydroxystilbenes piceatannol, its glucoside and *iso*rhapontin. This suggests that sensitivity of an ectomycorrhizal species is phenolic specific which warrants additional research.

Reduction of ectomycorhizal species above ground is not clearly correlated to the reduction of ectomycorrhizal biomass below ground (Termorshuizen and Schaffers, 1991; Dahlberg, 1991; Wallander, 1992; Chapter 5). Mycelia may persist in the soil but sporocarp formation may be inhibited as shown for *L. bicolor* (Chapter 9). The fundamental question arises under what conditions do mycelia form sporocarps? Do mycelia form sporocarps under stress conditions or when sufficient nutrients (nitrogen, carbon) are available to invest in biomass? To what extent do direct effects of external factors such as high nitrogen availability and mechanical effects of the herb vegetation inhibit sporocarp formation?

Low numbers of species and sporocarps of ectomycorrhizal fungi and rather high numbers of species and sporocarps of saprotrophic fungi (pers. obs.) were observed in the control and sod-added plots with thick litter and humus layers. These layers contain a considerable amount of organic nutrients. Part of the organic nutrients might be available to ectomycorrhizal fungi. Isolates of *L. bicolor* and *P. involutus* could utilize glycine as a nitrogen source (Ahmad et al., 1990; Lundeberg, 1970; Chapter 8). Hudson (1986) noted that several ectomycorrhizal fungi among which *P. involutus* and *T. terrestris* are capable of breaking down litter *in vitro*. However, it is unclear to what extent competition between ectomycorrhizal and saprotrophic fungi for organic nutrients occurs and in which way this might affect organic matter decomposition and nutrient cycling (Gadgil and Gadgil, 1975).

Compact and moist humus layers were observed in the sod-added plots (unpubl. results). These layers are probably less aerated than the humus layers in sod-cut plots which might have inhibited the growth of ectomycorrhizal mycelia. The structure of the newly developed ectorganic layers in the sod-cut plots was similar to that in the spontaneously grown stands (P1 and P2) with a rich ectomycorrhizal flora (pers. obs.). Sod-cutting on non-podzolic sandy soil enhanced numbers of ectomycorrhizal species and sporocarps more than in stands on podzolic soils. Besides high nutrient concentrations, organic matter

content and low pH, compaction of the podzolic soil might have reduced ectomycorrhizal development. Skinner and Bowen (1974) noted that mycelial growth of *R. luteolus* was reduced by compaction of podzolized forest soil. Measurements of compaction and aeration of ectorganic layers and mineral soil may provide information about development of ectomycorrhizal fungi.

The herb vegetation of control and sod-added plots was dominated by *D. flexuosa*. In these plots *L. hepaticus* was a common species as shown by sporocarp surveys (Chapter 2). In a further study complementary data could be collected on the possible competition for phosphorus between *L. hepaticus* and AM-fungi of *D. flexuosa*.

Sod-cutting did not (yet?) affect the chemical composition of the needles of the Scots pine trees compared to those in the control plots (Chapter 2). The ectomycorrhizal flora of the sod-cut plots differed in species richness and composition from the control plots. This suggests that the uptake of nutrients and water by the ectomycorrhizal fungi in the sod-cut plots is comparable to that in the control plots. In further study complementary data could be collected on the role of *L. bicolor*, the dominant species in the sod-cut plots, and *L. hepaticus*, common in the control plots, in nutrient uptake.

In addition, more research on the physiology of ectomycorrhizal fungi is necessary. In the spontaneously grown 15- to 20-year-old stands (P1 and P2) in the drift sand area most ectomycorrhizal species were found in plots with ectorganic layers with low nitrogen, phosphate and potassium concentrations and somewhat higher pH (around 4) as indicated in the Canonical Correspondence Analysis (CCA). Remarkably, a few species observed in P1 and P2 such as *Lactarius glyciosmus*, *T. focale* and *T. portentosum* were found at sites with relatively high phosphorus and potassium concentrations and relatively low pH in the ectorganic layers as indicated in the CCA (Chapter 3). An explanation has not yet been found.

In the *in vitro* experiments only one isolate per species was tested (Chapters 6, 7 and 8) and the results may not hold in general. Experiments should be repeated with more isolates. In addition, in the *in vitro* experiments ectomycorrhizal species were selected on the basis of their occurrence in the field, but only species which could be easily cultured were used. It would be very interesting to use other species like *Inocybe lacera*, *Sarcodon imbricatus* and species of *Tricholoma*. Methods should be developed to culture these species.

Removal of litter and humus layers and herb vegetation can be used as a means of restoration of ectomycorrhizal species richness and diversity. The Dutch nature conservation agencies Staatsbosbeheer and Vereniging tot Behoud van Natuurmonumenten have recently removed litter and humus layers and herb vegetation on sites in four Scots pine and two Oak stands in order to restore the ectomycorrhizal flora (Klap and Schmidt, in press). However, further research on the consequences of sod-cutting for other organisms than ectomycorrhizal fungi is necessary.

Strongly threatened species were not observed in the sod-cut plots in the Scots pine stands. Spores of the nitrogen-sensitive species should be sown in the sod-cut plots in stands on non-podzolic and podzolic sandy soil in order to investigate whether high nitrogen concentrations and organic matter content of the mineral soil affect germination of the spores and development of the mycelia.

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SUMMARY

INTRODUCTION

The main objectives of the present study, which was started in June 1990, were to investigate whether ectomycorrhizal fungi in Scots pine stands are negatively affected by litter and humus layers or whether ectomycorrhizal fungi decrease as a result of reduced tree vitality. If indeed litter and humus layers negatively influence ectomycorrhizal fungi, removal of these layers might be used as a way to restore the ectomycorrhizal flora in Scots pine stands. The project included both field and laboratory experiments.

FIELD EXPERIMENTS

In a field study litter and humus layers and herb vegetation were removed ("sod-cutting") in young (planted in 1987 and 1980), middle-aged (planted in 1974 and 1963) and old (planted in 1940 and 1924) Scots pine stands situated in the northeastern part of The Netherlands (Chapter 2). Removed litter and humus were added to existing ectorganic layers ("sod-addition") in young and middle-aged stands simulating thick litter and humus layers in old stands. Both treatments were carried out in 1990. Control treatments were present. Surveys carried out in 1993 showed that sod-cutting enhanced numbers of species and sporocarps of ectomycorrhizal fungi, mainly in middle-aged and old stands. Sod-addition did not affect numbers of ectomycorrhizal species and sporocarps. Sod-cutting reduced nitrogen concentrations of ectorganic layers and raised the pH. Nutrient concentrations and pH of ectorganic layers were not significantly affected by sod-adding. The number of ectomycorrhizal species was negatively correlated with nitrogen concentration in the needles.

The species composition and sporocarp abundance of ectomycorrhizal fungi in the two middle-aged secondary Scots pine stands were compared with those in two spontaneously established 15- to 20-year-old Scots pine stands on nutrient-poor sandy soil in a drift sand area in the central part of The Netherlands (Chapter 3). Species richness and diversity in the spontaneously grown stands were higher than in the control plots in the secondary stands. Sod-cutting in the secondary stand planted in 1963 on nutrient-poor non-podzolic sandy soil changed soil conditions towards those in the spontaneously grown stands. Species richness and diversity of ectomycorrhizal fungi became more similar to those in the spontaneously grown stands. Sod-cutting in the secondary stand planted in 1974 on podzolic sandy soil was far less effective due to relatively high nutrient concentrations, organic matter content and low pH in the mineral soil.

The spatial distribution of mycelia of *Laccaria bicolor* in two sod-cut plots in the secondary stand planted in 1974 was studied by somatic incompatibility tests two years after the treatment. The aim was to investigate whether the increase of *L. bicolor* in the sod-cut plots resulted from enhanced sporocarp production of mycelia that were already present or by new establishment of mycelia by spores or by a combination (Chapter 9). The results demonstrated that mycelia of *L. bicolor* were present in the mineral soil underneath the vegetation dominated by *Deschampsia flexuosa* before sod-cutting for at least 13 years. Other mycelia recently established in the sod-cut plots by spores, but numerically this was rather unimportant.

Not only the effects of manipulation of litter and humus layers on ectomycorrhizal fungi above ground were studied, but also below ground.

In 1991 and 1992, root growth and ectomycorrhizal development in the mineral soil in one of the middle-aged spontaneously grown stands on non-podzolic sandy soil was compared with those in the Scots pine stands planted in 1974 on podzolic sandy soil and planted in 1924 on non-podzolic sandy soil (Chapter 4). Ectomycorrhizal root tips were found in all plots to a soil depth up to at least 60 cm. After sod-cutting the Scots pine roots recovered in the stands planted in 1974 and 1924. Sod-addition did not affect root length and ectomycorrhizal development. Root length and numbers of ectomycorrhizal root tips in the middle-aged spontaneously grown stand plots on non-podzolic sandy soil were larger than in the middle-aged secondary stand plots on podzolic sandy soil. Small root length and low numbers of ectomycorrhizal root tips were associated with high nitrogen concentrations in the upper mineral soil.

Five-month-old, sterile-grown Scots pine seedlings, inoculated with *L. bicolor, Paxillus involutus* or *Rhizopogon luteolus* and non-inoculated seedlings were used as baits in order to investigate the colonization potential of ectomycorrhizal fungi in the stands planted in 1987 and in 1974 (Chapter 5). The seedlings were harvested after one growing season. Ectomycorrhizal composition (at the genus level) on the seedlings was independent of initial inoculum, but determined by both treatment and age of the stands. In both stands, removal of litter and humus layers increased, and addition of organic material decreased the numbers of ectomycorrhizal types on the seedlings. No clear correlation between ectomycorrhizal species above and below ground was found.

LABORATORY EXPERIMENTS

Studies of ectomycorrhizal fungi associated with Scots pine seedlings and in pure culture were carried out to provide a lower-level explanation of the results of the field experiments. The ectomycorrhizal fungi used in the laboratory experiments were chosen for their differential behaviour in the field.

In perspex growth chambers, Scots pine seedlings inoculated with *L. bicolor, R. luteolus*

and *Suillus bovinus* were grown on peat and on different forest soils in order to compare ectomycorrhizal development (Chapter 6). Ectomycorrhizal development of *S. bovinus* was reduced on humus and podzolic soil from the middle-aged, secondary stand planted in 1974. Ectomycorrhizal development of *L. bicolor* on podzolic soil was significantly more extensive than on peat in contrast to that of *R. luteolus*. Ectomycorrhizal development of these three fungi on the different substrates was largely in accordance with field observations. Sporocarps of *R. luteolus* and *S. bovinus* were only observed in the spontaneously grown stands on non-podzolic soil and in the sod-cut plots on non-podzolic soil. The ectomycorrhizal flora of the sod-cut plots in middle-aged and old stands was dominated by *L. bicolor*, particularly on podzolic sandy soil.

In a pure culture study, aqueous extracts of Scots pine needles of different provenance and shoots and roots of the grass *D. flexuosa* were investigated for their effects on the growth of isolates of ectomycorrhizal fungi (Chapter 7). Highest concentrations of phenolics and nitrogen were found in the extracts of shoots of *D. flexuosa*. Concentrations in needle extracts were much lower. *Laccaria proxima* and *R. luteolus* were more sensitive to needle and grass extracts than *P. involutus* and *Xerocomus badius*. The sensitivity of *L. proxima* and *R. luteolus* agreed with field observations. Sporocarps of *L. proxima* were observed in young stands. Sporocarps of *R. luteolus* were not recorded in grassy Scots pine stands. The growth reduction of *P. involutus* and *X. badius* by the needle and grass extracts is not in accordance with field observations. Sporocarps of these fungi were mainly found in older stands. *Laccaria bicolor*, which was insensitive to needle and grass extracts, could persist as mycelium in grassy Scots pine stands, but tremendously increased sporocarp production after sod-cutting.

In a pure culture study, the capability of ectomycorrhizal fungi to utilize organic and inorganic nitrogen sources in a range of different glucose and nitrogen availabilities was studied (Chapter 8). *Laccaria bicolor* was capable of growing on media with ammonium and on media with glycine as nitrogen source. *Lactarius hepaticus* and *P. involutus* generally developed better on media with ammonium than on media with glycine. *Coltricia perennis* did not clearly show preference for either ammonium or glycine. Sporocarps of *L. hepaticus* and *P. involutus* were mainly found on thick litter and humus layers with high concentrations of ammonium. Sporocarps of *C. perennis* occurred in sod-cut plots on nutrient-poor non-podzolic sandy soil. Ammonium-tolerant species showed a worse performance on media with glycine than *C. perennis*.

RESTORATION OF THE ECTOMYCORRHIZAL FLORA

Litter and humus negatively affected development and sporocarp formation of many ectomycorrhizal fungi. Removal of litter and humus layers and herb vegetation dominated

by *D. flexuosa* enhanced numbers of ectomycorrhizal species and sporocarps, and ectomycorrhizal colonization potential. The enhancement was largest in the sod-cut plots in middle-aged and old stands on non-podzolic sandy soil. After sod-cutting sporocarps of rare and threatened ectomycorrhizal species among which *Cantharellus cibarius*, *Coltricia perennis* and *Tricholoma albobrunneum*, occurred which were not present in the control plots. Considering the results of the present study Dutch nature conservation agencies (Staatsbosbeheer and Vereniging tot Behoud van Natuurmonumenten) have recently removed litter and humus layers and herb vegetation in four Scots pine and two Oak stands in different parts of The Netherlands to restore the ectomycorrhizal flora. Sod-cutting will, however, only temporarily restore the ectomycorrhizal flora in Scots pine stands as long as the excess nitrogen input from the air, mainly ammonia originating from intensive livestock industry, is not reduced.

SAMENVATTING

INLEIDING

Ectomycorrhizaschimmels leven samen met wortels van bomen (symbiose) en zijn onmisbaar in bosecosystemen. Ectomycorrhizaschimmels vormen in de bodem mycelia (zwamvlokken), van waaruit hyfen (schimmeldraden) om worteltopjes groeien. Een wortel samengegroeid met een ectomycorrhizaschimmel wordt een ectomycorrhiza genoemd. De bomen leveren suikers aan de schimmels, die op hun beurt nutriënten (voedingsstoffen) en water uit de bodem aan de bomen leveren. Andere functies van ectomycorrhizaschimmels betreffen het verhogen van de weerstand van bomen tegen droogte, ziekteverwekkers en zware metalen. In de herfst vormen de mycelia vruchtlichamen (paddestoelen), waarvan Cantharel, Eekhoorntjesbrood en Vliegenzwam bekende voorbeelden zijn.

In Nederland zijn gedurende de laatste decennia de vruchtlichamen van ectomycorrhizaschimmels van Grove den achteruitgegaan. Deze afname van vruchtlichamen in oude (> 50 jaar) Grove-dennenbossen in Nederland is het grootst in gebieden met veel luchtverontreiniging van stikstof, veroorzaakt door de bio-industrie. In oude dennenbossen hebben dennenaalden zich opgehoopt tot dikke strooisel- en humuslagen en domineert het gras Bochtige smele. In de strooisel- en humuslagen is veel stikstof vastgelegd. Uit voorgaand onderzoek bleek dat er een negatief verband bestond tussen het aantal soorten ectomycorrhizaschimmels bovengronds (paddestoelen) en de dikte van de strooisel- en humuslagen. In Grove-dennenbossen met veel Bochtige smele werden nauwelijks vruchtlichamen van ectomycorrhizaschimmels waargenomen. Ook een verminderde vitaliteit (gezondheidstoestand) van de Grove dennen ten gevolge van luchtverontreiniging kan een oorzaak zijn van de achteruitgang van ectomycorrhizaschimmels.

Het doel van dit onderzoek, gestart in 1990, was vast te stellen of ectomycorrhizaschimmels van Grove den negatief beïnvloed worden door ophoping van strooisel en humus of afnemen door verminderde vitaliteit van de bomen. Als uit de resultaten zou blijken dat strooisel en humus ectomycorrhizaschimmels negatief beïnvloeden dan kan het verwijderen van strooisel en humus (de ouderwetse "strooiselroof") als beheersmaatregel worden toegepast om verdwenen ectomycorrhizaschimmels terug te krijgen.

VELDEXPERIMENTEN

Om de achteruitgang van ectomycorrhizaschimmels nader te onderzoeken zijn in Drenthe in het voorjaar van 1990 in proefvlakken van jonge (aangeplant in 1987 en 1980), middeloude (aangeplant in 1974 en 1963) en oude (aangeplant in 1940 en 1924) Grove-dennenbossen strooisel- en humuslagen en het gras Bochtige smele verwijderd (plaggen) (Hoofdstuk 2). Deze bossen zijn aangeplant op plaatsen waar in het verleden al bossen zijn aangeplant en worden aangeduid met secundaire bossen. Elders in de jonge en middeloude secundaire bossen zijn strooisel- en humuslagen op de bestaande gebracht om dikke lagen na te bootsen. Onbehandelde proefvlakken werden als controle beschouwd.

Uit waarnemingen gedaan in 1993 bleek dat in alle bossen na plaggen de aantallen vruchtlichamen van ectomycorrhizaschimmels waren toegenomen. Ook verschenen soorten die uit deze bossen verdwenen waren, zoals Cantharel, Narcisamaniet, Tolzwam en Rookrussula. Door in dennenbossen te plaggen werd met het weghalen van de strooisel- en humuslagen en het gras Bochtige smele een groot deel van de stikstof verwijderd. Ook werden humuscomponenten, waaronder fenolen (moeilijk afbreekbare stoffen) verwijderd, hetgeen een positief effect had op veel soorten ectomycorrhizaschimmels. Het opbrengen van strooisel en humus had geen effect op het aantal vruchtlichamen. De volgens uiterlijke kenmerken beschreven vitaliteit van de bomen veranderde niet ten gevolge van plaggen en opbrengen.

Twee middeloude (15-20 jaar) Grove-dennenbossen op het Hulshorsterzand zijn ter vergelijking geselecteerd als referentie. Deze bossen zijn primaire bossen, dat wil zeggen dat ze spontaan zijn opgekomen op voormalige zandverstuiving. De bodem is arm aan nutriënten en er vindt nauwelijks ophoping van dennenaalden plaats.

Het aantal soorten ectomycorrhizaschimmels en de soortensamenstelling in de twee middeloude secundaire bossen in Drenthe is vergeleken met die in de primaire bossen (Hoofdstuk 3). Drie en een half jaar na plaggen vertoonden de bodems gelijkenis met die in het primaire bos. Ook de rijkdom aan en de samenstelling van soorten ectomycorrhizaschimmels in de geplagde proefvlakken begon te lijken op die in het primaire bos. De soortenrijkdom in de primaire bossen was echter (nog) groter dan in de geplagde proefvlakken.

In het Grove-dennenbos, aangeplant in 1974, werd twee jaar na plaggen onderzocht hoe de Tweekleurige fopzwam geplagde proefvlakken had gekoloniseerd (Hoofdstuk 9). Uit de resultaten bleek dat mycelia van de Tweekleurige fopzwam in de minerale bodem onder de grasmat van Bochtige smele meer dan 10 jaar aanwezig waren geweest zonder vruchtlichamen te vormen. Slechts enkele mycelia hebben zich in de geplagde proefvlakken nieuw gevestigd door inwaaien van sporen. Dit betekent dat sommige soorten nog enige tijd kunnen overleven zonder vruchtlichamen te vormen. In zulke gevallen kan plaggen snel tot gunstige resultaten leiden.

In 1991 en 1992 werden wortelgroei en ectomycorrhizavorming in de nutriëntarme

bodem in één van de Grove-dennenbossen op het Hulshorsterzand vergeleken met een middeloud bos op nutriëntrijke podzolbodem en een oud bos op nutriëntarme zandbodem (Hoofdstuk 4). Ectomycorrhizas werden in de drie bossen gevonden tot op een diepte van ten minste 60 cm. Na het plaggen trad herstel van de Grove-dennewortels op in de middeloude en oude Drentse bossen. De wortellengte en het aantal ectomycorrhizas in het primaire bos op het Hulshorsterzand waren echter groter dan in het middeloude Drentse bos op podzolbodem. De geringere wortellengte en het lage aantal worteltopjes gingen samen met hoge stikstofgehaltes in de bovenste laag van de podzolbodem en hoge leeftijd van de bomen. Het opbrengen van strooisel en humus had geen effect op de wortellengte en ectomycorrhizavorming.

Vijf maanden oude, steriel gekweekte zaailingen van Grove den, met ectomycorrhizas van Tweekleurige fopzwam, Krulzoom en Okerkleurige vezeltruffel en zonder ectomycorrhizas, werden in mei 1992 geplant in twee secundaire Drentse bossen (aangeplant in 1987 en in 1974). Onderzocht werd of de ectomycorrhizaschimmels op de zaailingen zich konden handhaven en zo niet, door welke ectomycorrhizaschimmels uit de bosbodem ze vervangen werden (Hoofdstuk 5). Ook werd gekeken welke ectomycorrhizaschimmels uit de bosbodem zich vestigden op de zaailingen die nog geen ectomycorrhizas hadden op het moment van uitplanten. Na één groeiseizoen werden de zaailingen uitgegraven en meegenomen naar het laboratorium. Op de wortels van de zaailingen werden andere ectomycorrhizaschimmels aangetroffen dan op het moment van uitplanten. De soortsamenstelling varieerde met de behandeling. Verwijderen van strooisel en humus verhoogde in beide bossen het aantal soorten ectomycorrhizaschimmels op de zaailingen, opbrengen van strooisel en humus daarentegen verlaagde het aantal soorten.

LABORATORIUMEXPERIMENTEN

In het laboratorium werden experimenten gedaan met ectomycorrhizaschimmels in symbiose met zaailingen van Grove den om de resultaten van de veldexperimenten beter te kunnen begrijpen. Ook werden experimenten uitgevoerd met alleen mycelia in petrischalen. De ectomycorrhizaschimmels in de laboratoriumexperimenten werden gekozen op grond van hun aanwezigheid in Grove-dennenbossen.

In perspex groeikamers werd de ectomycorrhizavorming van de Tweekleurige fopzwam, de Okerkleurige vezeltruffel en de Koeieboleet in symbiose met zaailingen van Grove den op turf (controle) en verschillende bosbodems (humus uit een primair bos en humus en podzolbodem uit een secundair bos) onderzocht (Hoofdstuk 6). De ectomycorrhizavorming van de Koeieboleet op humus en podzolbodem uit het secundaire bos werd geremd. De ectomycorrhizavorming van de Tweekleurige fopzwam was op podzolbodem groter dan op turf in tegenstelling tot die van de Okerkleurige vezeltruffel. De mate van ectomycorrhizavorming van deze drie schimmels op verschillende substraten was

grotendeels overeenkomstig veldwaarnemingen. Vruchtlichamen van de Okerkleurige vezeltruffel en de Koeieboleet werden alleen waargenomen in de primaire bossen op het Hulshorsterzand en in de geplagde proefvlakken op nutriëntarme zandbodems. De meest voorkomende soort op de geplagde proefvlakken was de Tweekleurige fopzwam, met name op de nutriëntrijke podzolbodems.

De effekten van waterextrakten van naalden van Grove den van verschillende herkomst, van de spruiten (bovengrondse delen) en wortels van Bochtige smele op de groei van mycelia van ectomycorrhizaschimmels op petrischalen zijn onderzocht (Hoofdstuk 7). De hoogste concentraties van fenolen en stikstof werden gevonden in de extrakten van de spruiten van Bochtige smele. De concentraties in de naaldextrakten waren veel lager. De mycelia van de Heidefopzwam en van de Okerkleurige vezeltruffel waren gevoeliger voor de naald- en grasextrakten dan die van de Krulzoom en de Kastanjeboleet. De gevoeligheid van de Heidefopzwam en de Okerkleurige vezeltruffel kwam overeen met de veldwaarnemingen. Vruchtlichamen van de Heidefopzwam werden waargenomen in de jonge bossen, terwijl in de vergraste Grove-dennenbossen geen vruchtlichamen van de Okerkleurige vezeltruffel werden aangetroffen. De groeivermindering van de mycelia van de Krulzoom en de Kastanjeboleet door de naald- en grasextrakten kwam niet overeen met veldwaarnemingen. Vruchtlichamen van deze soorten werden vooral gevonden in oudere bossen met dikke strooisel- en humuslagen. De Tweekleurige fopzwam, die ongevoelig was voor naald- en grasextrakten, kon als mycelium in de vergraste bossen overleven. Na plaggen nam het aantal vruchtlichamen van de Tweekleurige fopzwam enorm toe.

Het vermogen van ectomycorrhizaschimmels om organische en anorganische stikstof te benutten werd bestudeerd met behulp van mycelia in petrischalen (Hoofdstuk 8). De Tweekleurige fopzwam was in staat te groeien op voedingsbodems met ammonium als anorganische en glycine als organische stikstofbron. Mycelia van de Levermelkzwam en de Krulzoom groeiden beter op voedingsbodems met ammonium dan op voedingsbodems met glycine. De Tolzwam had geen duidelijke voorkeur voor ammonium of glycine. Vruchtlichamen van de Levermelkzwam en de Krulzoom werden hoofdzakelijk gevonden op dikke strooisel- en humuslagen met hoge concentraties ammonium. Vruchtlichamen van de Tolzwam werden aangetroffen in de geplagde proefvlakken bos op nutriëntarme zandgrond.

HERSTEL VAN DE ECTOMYCORRHIZASCHIMMELS

Strooisel en humus beïnvloedden de groei en vruchtlichaamvorming van veel ectomycorrhizaschimmels negatief. Het verwijderen van de strooisel- en humuslagen en de kruidlaag gedomineerd door Bochtige smele verhoogden het aantal soorten ectomycorrhizaschimmels en het aantal vruchtlichamen van deze schimmels. De toename was het grootst in de geplagde proefvlakken in middeloude en oude Grove-dennenbossen

op nutriëntarme zandgrond. Na plaggen verschenen vruchtlichamen van in Nederland zeldzame en bedreigde soorten ectomycorrhizaschimmels, waaronder Cantharel, Tolzwam en Witbruine ridderzwam. Deze soorten werden niet aangetroffen in vergraste Grove-dennenbossen. De gezondheidstoestand van de bomen verslechterde niet door het plaggen. De gunstige resultaten van dit onderzoek heeft er mede toe geleid dat Staatsbosbeheer en de Vereniging tot Behoud van Natuurmonumenten recent strooisel, humus en kruidlaag hebben verwijderd in vier Grove-dennenbossen (Hulshorst, Kootwijk, Loonse and Drunense duinen, Terschelling) en in twee Eikenbossen (Schoorl en Steenwijk) om de ectomycorrhizaflora te herstellen. Plaggen is echter een tijdelijke maatregel en het is noodzakelijk om de luchtverontreiniging van stikstof te verminderen, met name ammoniak afkomstig van de bio-industrie. Dit voorkomt dat het resultaat van plaggen te niet wordt gedaan door hernieuwde ophoping van strooisel en humus waarin veel stikstof aanwezig is.

CURRICULUM VITAE

Jacqueline Baar werd geboren op 31 maart 1963 te Putten. Na het behalen van het diploma Gymnasium-ß aan het Johan van Oldenbarneveltgymnasium te Amersfoort, ging zij in 1981 biologie studeren aan de toenmalige Landbouwhogeschool te Wageningen. Na het behalen van het kandidaatsexamen werden twee stages gelopen, één in Schotland en één in de Verenigde Staten. De doctoraalstudie bestond uit het hoofdvak fytotoxicologie van de luchtverontreinging en de bijvakken vegetatiekunde en fytopathologie. Zij verrichtte onderzoek naar effecten van luchtverontreinging van ammoniak op heideplanten en naar het voorkomen van fyllosfeerorganismen bij verschillende ozonconcentraties. Tijdens haar studie werkte zij in 1988 vijf maanden als vegetatiekarteerder bij de provincie Noord-Brabant waaruit de invulling van het bijvak vegetatiekunde, onderzoek naar verdroging van elzenbossen, voortkwam. Aansluitend aan haar afstuderen in maart 1989 werkte zij nog een zomer als vegetatiekarteerder bij de provincie Noord-Brabant. Daarnaast verzorgde zij determinatie- en ecologiecursussen voor de Stichting River Research. In juni 1990 werd zij aangesteld als Onderzoeker in Opleiding bij het Biologisch Station Wijster van de Landbouwuniversiteit Wageningen. Het project werd gefinancieerd door de toenmalige Stichting BION-NWO (thans SLW-NWO). Het onderzoek dat in dat kader werd verricht resulteerde in dit proefschrift.