Acta Universitatis Agriculturae Sueciae

Silvestria 33

Effects of Air Pollution and Forest Regeneration Methods on the Community Structure of Ectomycorrhizal Fungi

Ola Kårén

Swedish University of Agricultural Sciences

Effects of Air Pollution and Forest Regeneration Methods on the Community Structure of Ectomycorrhizal Fungi

Ola Kårén
Department of Forest Mycology and Pathology
Uppsala

Doctoral thesis
Swedish University of Agricultural Sciences
Uppsala 1997

Acta Universitatis Agriculturae Sueciae
Silvestria 33

ISSN 1401-6230
ISBN 91-576-5317-8
© 1997 Ola Kårén, Uppsala
Tryck: SLU Service/Repro, Uppsala 1997

Abstract

Kårén, O. *Effects of air pollution and forest regeneration methods on the community structure of ectomycorrhizal fungi.* Doctor's dissertation.
ISSN 1401-6230, ISBN-91-576-5317-8

This thesis describes the ectomycorrhizal community and how it is affected by nitrogen fertilization and forest regeneration methods. Fertilizer treatments included ammonium sulphate (NS), lime +P+K+Ca+Mg+S (N-free) and ammonium nitrate, applied in spruce (*Picea abies*) stands in south Sweden and a pine (*Pinus sylvestris*) stand in north Sweden. None of the fertilizers decreased the proportion of short-roots colonized by ectomycorrhizal fungi. Estimations of fungal biomass of short-roots in NS and N-free treatments confirmed this. These results contrast with the 60-100% decreases in sporocarp production of ectomycorrhizal fungi reported by other investigators. Molecular methods were used to identify ectomycorrhizal fungi on short-roots after testing their applicability in a separate study. N fertilization had a pronounced effect on the species composition on mycorrhizal roots. The decreased sporocarp production of ectomycorrhizal fungi was suggested to depend on a decreased abundance of species sensitive to N, decreased total allocation of carbohydrates to the fungi, and a changed carbohydrate allocation within the fungi.

A second study investigated the community structure of ectomycorrhizal fungi in 11 pine stands in central Sweden. Three stands ("O") consisted of 150-year-old, unmanaged forests, and the remaining were 30-40 years old. Of the latter, four were the result of regeneration by planting on clear-cuts ("P"), and four developed under shelterwood trees that were successively removed ("S"). Mycorrhizal roots, sampled in 1995 and sporocarps (in 1995-96) were identified. The number of species found as mycorrhizas or as sporocarps was lowest in P, whereas species richness was similar in S and O. The species compositions of mycorrhizas or sporocarps were least similar between P and O, but more similar between S and O. Between 45-90% of the mycorrhizas were formed by species not observed in the sporocarp inventory. Multivariate analyses indicated that species composition of ectomycorrhizal fungi was correlated with the age of the forest, soil factors which were partly correlated with age and to some extent also type of regeneration method.

It is suggested that nitrogen deposition and N-fertilization reduce the diversity of ectomycorrhizal fungi. Effects of forest regeneration methods appear less pronounced, but fungi restricted to old forests may need protection.

Key words: ectomycorrhiza, diversity, nitrogen, pollution, management, regeneration, ribosomal DNA, PCR, RFLP, identification

Authors's adress: Ola Kårén, SLU, Department of Forest Mycology and Pathology, Box 7026, S-750 07 Uppsala, Sweden

Contents

Introduction, 7
What is a mycorrhiza and what is its function?, 7
The ectomycorrhizal symbiosis, 8
A definition of the ectomycorrhizal community, 9
Methods used to study ectomycorrhizal communities, 10
Sporocarp studies, 10
Classification and identification of mycorrhizal roots, 11
Diversity of ectomycorrhizal communities, 12
Impacts of air pollution on ectomycorrhizal communities, 13
Effects of forest regeneration methods on ectomycorrhizal communities, 15
Aims, 15

Materials and Methods, 17
Molecular biological methods, 17
Analyses of fungal species on mycorrhizal short roots, 18
Ergosterol assay for fungal biomass estimations of fine-roots, 19
Experimental approaches, 20
I, 20
II & III, 20
IV, 21
The Norrliden N-fertilization experiment, 22

Results, 23
Paper I, 23
Paper II, 24
Paper III, 25
Paper IV, 25
The Norrliden N-fertilization experiment, 28

Discussion, 30

Effects of NS and N-free fertilization, 30
Dramatic effects on sporocarp production but only minor effects on mycorrhizal colonization of fine-roots, 30
Decreased allocation of carbohydrates from the host to the fungus, 31
Changes in the community structure, 31
Changes in carbohydrate allocation within the fungus, 33
Several contributing factors affected the mycorrhizal fungi, 34
Effects of forest regeneration methods, 34
Community structure of ectomycorrhizal fungi in forests regenerated after clear-cutting and planting or under shelterwoods, 35

Persistence of ectomycorrhizal fungi throughout the regeneration phase, 36
Colonization of ectomycorrhizal fungi from surrounding stands, 37
Succession of mycorrhizal fungi in forests, 39
Introduction - Primary succession, 39
Secondary succession, 39
Secondary succession in ectomycorrhizal communities, 39
Is there a secondary succession of mycorrhizal fungi as forests grow older?, 40
Relations between mycorrhizas and sporocarps, 41
Concluding remarks, 43
References, 44
Acknowledgements, 53

Appendix

Papers I-IV

This thesis is based on the following papers, which will be referred to by their Roman numerals:

I Kårén O., Högberg N., Dahlberg A., Jonsson L. and Nylund J.-E. 1997. Inter- and intraspecific variation in the ITS region of rDNA of ectomycorrhizal fungi in Fennoscandia as detected by endonuclease analysis. *The New Phytologist* **136** (**2**): 000-000.

II Kårén, O., and Nylund, J.E. 1996. Effects of N-free fertilization on ectomycorrhiza community structure in Norway spruce stands in southern Sweden. *Plant and Soil*. **181**: 295-305.

III Kårén O. and Nylund J.-E. Effects of ammonium sulphate on the community structure and biomass of ectomycorrhizal fungi in a Norway spruce stand in SouthWest Sweden. *Canadian Journal of Botany*, in press.

IV Kårén O., Jonsson M., and Nylund J.-E. Community structure of ectomycorrhizal fungi in young, managed and old, unmanaged *Pinus sylvestris* forests. Manuscript.

Papers I-III are reprinted with kind permission from the publishers.

Introduction

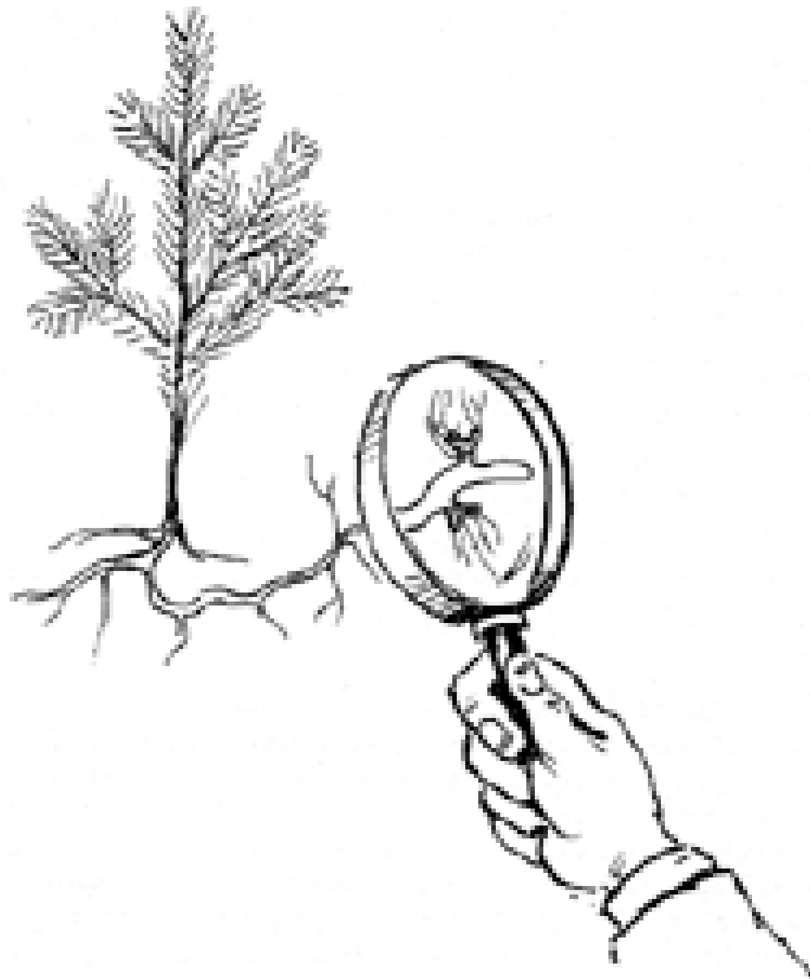
The work described in this thesis has focused on the *ectomycorrhizal community* and how it is affected by nitrogen deposition and forest regeneration methods. The following questions were addressed:

- How can communities of mycorrhizal fungi in coniferous forests be described?
- Do the frequently observed decreases of sporocarps following N-fertilization or increased levels of N-deposition by air pollution indicate corresponding reductions in the below-ground community (fungal species on roots or hyphae in the soil)?
- What are the effects of nitrogen-free fertilizers which intend to counteract nutrient imbalances in forests exposed to air pollution?
- Are modern forest regeneration methods affecting the abundance and species richness of mycorrhizal fungi?
- Can sporocarp inventories alone accurately estimate the total number of species and their abundance, or is it also necessary to investigate the fungi present on the short-roots of the trees?

What is a mycorrhiza and what is its function?

“Mycorrhiza” is the term used to describe the symbiosis formed between fungi and roots of plants. Literally, the word means “fungus-root” (from the Greek words *mykes* and *rhiza*). It was first used by Frank (1885), who discovered the structure and hypothesized that it represented a mutual symbiotic relationship in which the fungus obtains carbohydrates (sugars) from the plant, and, in return, supplies the plant with minerals. Since then, several different forms of mycorrhizal associations between fungi and plants have been discovered, the most common of which are vesicular-arbuscular mycorrhiza, ectomycorrhiza, ericoid mycorrhiza, monotropoid mycorrhiza , and orchid mycorrhiza (Smith & Read; 1997). Vesicular-arbuscular mycorrhiza, the most widespread type (in terms of number of hosts and their distribution), is mainly formed between vascular plants (e.g herbs and grasses, but also some shrubs and trees) and Zygomycetes. Ectomycorrhiza is formed between woody plants (most conifers and many broad-leaved trees, including several tropical trees, many woody shrubs, and even some herbaceous plants) and fungi, mainly in the subdivisions Basidomycetes and Ascomycetes. Ericoid mycorrhiza is formed by plants of the Ericaceae (e.g. *Calluna* and *Vaccinium*) and fungi from the Ascomycetes. Monotropoid mycorrhiza is formed by achlorophyllous (Monotropideae) plants which gain carbon from the ectomycorrhizal fungus (Basidiomycetes), which in

turn derives its carbon from the host trees. Orchid mycorrhiza is a highly specialized form of symbiosis between orchids and various Basidiomycetes.



The ectomycorrhizal symbiosis

To understand the functioning of the ectomycorrhizal symbiosis, three anatomical structures need to be described: the mantle, the Hartig net and the external mycelium. In contrast to the other forms of mycorrhizal symbioses, short-roots of ectomycorrhizal plants are always covered by a *mantle* (or ”sheath”) of hyphae. The structure and thickness of the mantle vary greatly depending on the species forming the symbiosis (Agerer, 1987-97) and environmental conditions. The mantle is the interface between the plant and the soil. For the fungus, the mantle can serve as a nutrient and carbon depot, and in forest ecosystems considerable amounts of N and P can be stored in it (Vogt *et al*., 1982). The second character distinctive of ectomycorrhizal symbioses is the

Hartig net (from Hartig [1840] who was the first to describe the structure of an ectomycorrhizal root), a network of hyphae growing between cells in the outer part of the root (the cortex). This is the interface between the fungus and the host plant, where carbohydrates are exchanged for minerals. In contrast to vesicular-arbuscular, ericoid and orchid mycorrhiza, the fungus does not penetrate the host cell walls, except from associations between certain host or fungal species, or in senescent tissue. A third anatomical character that characterizes the ectomycorrhizal symbiosis is the formation of an *external mycelium,* consisting of hyphae extending from the fungal mantle out into the soil. Thus, for plants that are normally mycorrhizal, the term "mycorrhizosphere" (e.g. the mycorrhizal mantle and the external mycelium) is probably a better description of the interface between the plant and the soil environment than just "rhizosphere" (Linderman, 1988). The soil mycelium is important in that it provides a large surface area for nutrient uptake (Rousseau *et al*., 1994), mobilization of mineral nutrients (e.g. from proteins: Melin, 1925; Abuzinadah & Read, 1986), water uptake (Boyd *et al*., 1986) and interactions with other soil organisms (Garbaye, 1994). For many fungal species, the soil mycelium is also an important means of vegetative dispersal to uncolonized roots (Read, 1992).

A definition of the ectomycorrhizal community

A community can been defined in different ways. Early definitions emphasized how organisms were arranged spatially and how different communities could be linked to environmental variables (Cowles, 1901; Clements, 1905). Gleason (1939) also defined the community in a temporal aspect "...a piece of vegetation which maintains ... a reasonable permanence over a considerable time". Whittaker (1970) focused on interactions between organisms in the community and expressed the view that the community is a type of organism: "An assemblage of populations of plants, animals, bacteria and fungi that live in an environment and interact with one another, forming together a distinctive living system with its own composition, structure, environmental relations, development, and function". Attention to the interaction between organisms was emphasized even more by MacMahon *et al.* (1981) who stated that a community is: "...the organisms which affect, directly or indirectly, the expected reproductive success of a reference organism". When applied to mutualistic symbiotic associations (mycorrhizal fungi and their host plants, N-fixing bacteria and nodulating host-plants etc.) this definition implies that the reproductive success of an organism (fitness) needs to be studied together with its partner. Thus, ideally, mycorrhizal communities should be studied not only with respect to the interactions between the fungal organisms or between the hosts, but also between the hosts and the fungi (Allen *et al*., 1995). However, this has rarely been done, and most theories on community structure and interactions between organisms (whether they are known to form mutualistic symbiotic associations or not) have almost exclusively originated from a plant or animal perspective (e.g.

competition, Connell, 1983; life history strategies, Mac Arthur & Wilson, 1967; Grime, 1974; differential resource utilization, Tilman, 1982).

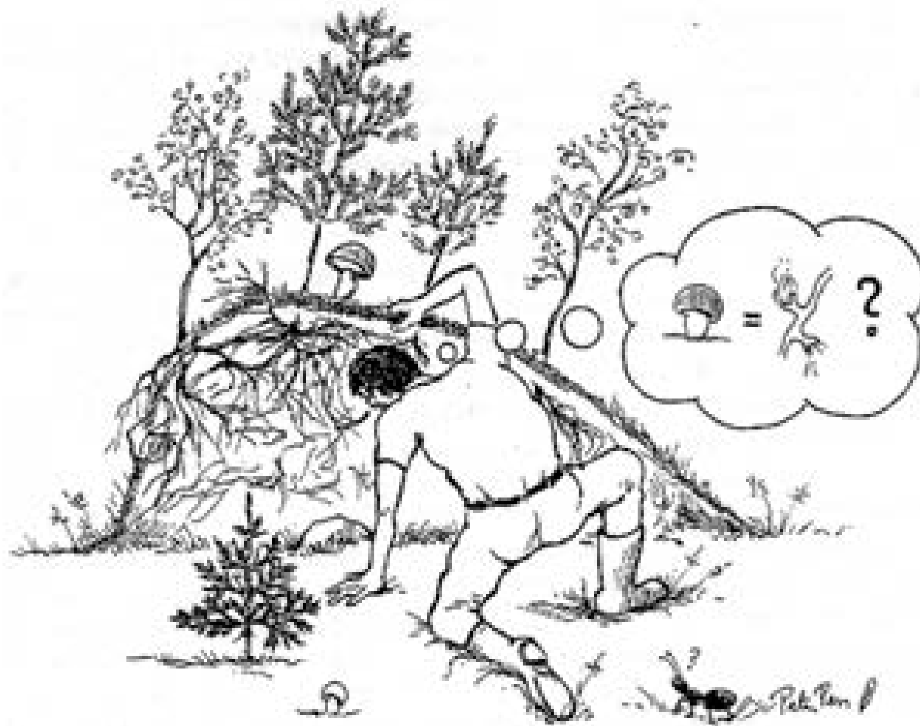
The diversity of a community consists of two components: *species richness*, which is a measure of the number of species present at a site and *evenness*, which describes how individuals in the community are distributed among the species (Begon *et al*., 1986; Magurran, 1988). However, in plant and fungal communities, where individuals are difficult to define, the biomass (Pielou, 1966) or percent cover (Whittaker, 1965) of a species is the usual unit of measure. In this thesis *community structure* has been used as a general term for encompassing species composition, richness and evenness.

In work described in this thesis ectomycorrhizal communities of coniferous forests were investigated in two ways: 1) by identifying the mycorrhizal coniferous roots sampled from the organic soil horizon and 2) by making sporocarp inventories of Basidiomycetes. Thus the community of mycorrhizal fungi present on short-roots was defined as all fungal species forming a mycorrhiza with a mantle with a Hartig net (Wilcox, 1968). For the sporocarp inventory only the part of the ectomycorrhizal community that formed large, conspicuous sporocarps was monitored. Thus other potentially important species (asexual species, species forming hypogeous [below-ground] sporocarps or resupinate sporocarps hidden under logs and branches) were ignored.

Methods used to study ectomycorrhizal communities

Sporocarp studies

Knowledge of the ecology of ectomycorrhizal fungi in forest ecosystems is mainly derived from inventories of sporocarps (e.g. Haas, 1932-33; Romell, 1938, reviews by Wilkins, 1948; Cooke, 1953; Vogt *et al*., 1992). The most obvious reasons for the focus on sporocarps are that a) compared with mycorrhizal roots they can be more easily identified and that b) sporocarps of many important mycorrhizal species are commercially valuable (e.g. chanterelles [Danell, 1994; Watling, 1997] and truffles [Giovannetti *et al*., 1994]). However, the sporocarp constitutes only a small part of the biomass of the fungus. Most of the energy and nutrients are allocated to mycorrhizas and the soil mycelium (Dahlberg & Stenlid, 1994; Colpaert *et al*., 1992; Markkola *et al*., 1995). Furthermore, little is known about the degree to which allocation to sporocarps varies between and within species (Gardes & Bruns, 1996). Another disadvantage with sporocarp inventories is that production is highly dependent on weather conditions (moisture and temperature; Lange, 1978; Eveling *et al*., 1990) and thus varies considerably between years, making it necessary to monitor sites over several years in order to accurately depict the community structure of sporocarp-producing ectomycorrhizal fungi (Vogt *et al*., 1992).



Classification and identification of mycorrhizal roots
Large efforts have been made to identify mycorrhizal fungi on the short-roots. Through the detailed morphological characterization (classification of ”morphotypes”) of mycorrhizas using a microscope (Ingleby *et al*., 1990; Agerer, 1987-97; Gronbach, 1988) many species can now be identified. With this method the fungal mantle and external mycelium are classified, and ultimately the morphotype can be linked to the correct fungal species. A shortcoming of the method is that it is time-consuming and highly dependent on the skill of the investigator. Despite considerable efforts, the mycobionts in many mycorrhizas still cannot be distinguished from one another (Egli *et al*., 1993) or remain unidentified (Yamada & Katsuya, 1996; Visser, 1995; Helm *et al*., 1996).

Molecular biological methods offer another possible way to identify the fungi of mycorrhizal root tips (Mullis & Faloona, 1987; White *et al*., 1990; Gardes *et al*., 1991; Erland, 1995; Gardes & Bruns, 1996). Previous work has shown the internal transcribed spacer (ITS) region of the rRNA gene is suitable for this purpose, and primers have been designed that make it possible to isolate this region (White *et al*., 1990; Bruns *et al*., 1991; Gardes *et al*., 1991; Gardes & Bruns, 1993).

Two characteristics of the ITS region of the rDNA make it more useful than other parts of the genome when it comes to molecular biological analyses: a) It varies enough between species to allow them to be separated from another, and b) variation within species is low, so that a species can be recognized even if it is sampled from geographically distant sites. To examine this further, the degree of

within- and between-species variation of the ITS region of rDNA extracted from sporocarps was investigated in 44 ectomycorrhizal fungal species sampled from Denmark, Finland, Norway and Sweden (paper I). For this purpose and to facilitate the later identification of mycorrhizas sampled in field studies (III & IV) we chose to only include species commonly found in sporocarp inventories (Hansen & Knudsen, 1992; Dahlberg, 1991). In total, 132 vouchers from 44 different species in 17 genera were studied. To enable comparisons of samples analyzed in different gel-runs it was also necessary to know the precision of the method (e.g. how precisely the size of a fragment can be estimated when it is run on different gels, the smallest detectable size of fragments, how similar two fragments can be and still be separated from each other). To test the level of precision we assessed how precisely fragment sizes of samples that had been run on several agarose-gels could be estimated (I).

Diversity of ectomycorrhizal communities

The total number of species that form ecto- and ectendomycorrhizas has been estimated at 5000-6000 species worldwide (Molina et al., 1982). In the boreal forests of northern Europe the most important host trees are Scots pine (*Pinus sylvestris* (L.)), Norway spruce (*Picea abies* (L. Karst.)), birch (*Betula pendula* (Roth.*), B. pubescens(*L.*))* and aspen (*Populus tremula (*L.*))*. In Sweden, Hallingäck (1994) estimated that about 500 fungal species form mycorrhiza with Scots pine, Norway spruce and birch. Both of these figures are uncertain, however, since they are based on the fruiting behaviors of the fungal species (or families), which have rarely been verified by direct connections to mycorrhizas or synthesized *in vitro*. On the other hand, it is likely that these figures are underestimated since little is known about the ecology of many species with inconspicuous sporocarps or asexual fungi (e.g. Corticiaceae, Thelephoraceae and many Ascomycetes).

Data from sporocarp inventories of coniferous forest in northern Europe indicate that there are typically 50-150 species in a stand (oligotrophic spruce forests: Mehus, 1986; Gulden *et al*., 1992; Brandrud, 1995; Wiklund *et al*., 1995; Dahlberg *et al*., 1997; oligotrophic Scots pine: Hintikka, 1988; Väre *et al*., 1996). In a four-year sporocarp study of mycorrhizal fungi in 14 coniferous stands dominated by Scots pine, Väre *et al*., (1996) found a total of 115 species in the Agaricales and Boletales (90 of them supposedly associated with Scots pine). Based on the fungal literature of Hansen & Knudsen (1992), they estimated that about 130 additional species of the two families are associated with Scots pine in Finland, and when species of the Ascomycotina and Aphyllophorales were included, the estimate increased to 250-300 species. Since the total number of species found within a stand varies depending on its size and heterogeneity, historical events, the number of years that the site has been studied, weather conditions, skill of the investigator, etc., it is difficult to obtain exact measurements of species richness within individual stands. Nevertheless, despite the obvious bias in favor of species producing large sporocarps in previous

surveys, some general conclusions can be drawn: For example, the results suggest that the number of ectomycorrhizal fungi in northern Europe is considerably larger than the number of host plants forming ectomycorrhiza. Thus, it is likely that the mechanisms regulating the diversity of ectomycorrhizal fungi only to a minor degree are determined by the diversity of their hosts (Allen *et al*., 1995).

Impacts of air pollution on ectomycorrhizal communities

In forest plots in western Europe, where sporocarps have been monitored several times during the last century, there has been a drastic decrease in the total number of mycorrhizal species, whereas no clear trend has been seen for the saprophytic species (e.g. Grosse-Brauckmann & Grosse-Brauckmann, 1978; Derbsch, 1987). Since a similar response pattern had been noted in several N-fertilization experiments, Arnolds (1991) suggested that air pollution, particularly nitrogen deposition, was the main cause of the decline in sporocarp formation. Reductions in sporocarp formation by most species following N-fertilization have been reported in numerous studies (e.g. Romell, 1938; Hora, 1959; Menge & Grand, 1978; Ritter & Tölle, 1978; Rühling & Tyler, 1991; Termorzhuizen, 1993; Wiklund *et al*., 1995; Brandrud, 1995. Similar results have been obtained from air-pollution gradient studies: Termorshuizen & Schaffers, 1987; Markkola *et al*., 1995; Brandrud, 1995. Alexander & Fairley (1983) found a slightly decreased level of mycorrhization of short-roots following fertilization with 300 kg N, applied as a single dose to a *Picea sitchensis* plantation in Scotland. In the N-fertilized plots three unidentified morphotypes, not found in control plots, together accounted for 30% of the mycorrhizal root tips, while the two most common morphotypes in control plots were less common in N-plots, suggesting that changes in species composition may have occurred (Alexander & Fairley, 1983). In contrast, Termorshuizen (1993) did not find any decrease in the degree of mycorrhizal colonization of short-roots or the number of mycorrhizas per unit soil volume in a young Scots pine stand that had been fertilized with up to 30 or 60 kg N ha^{-1}. Thus, a reduction in the number and/or degree of mycorrhizal colonization of short-roots may only partly explain the negative effects of N-fertilization on sporocarp formation by mycorrhizal fungi. Changes in the community structure (Alexander & Fairley, 1983) of mycorrhizal species that produce sporocarps may be one explanation, but there is still little known about how mycorrhizal species present on short-roots are affected by N-fertilization.

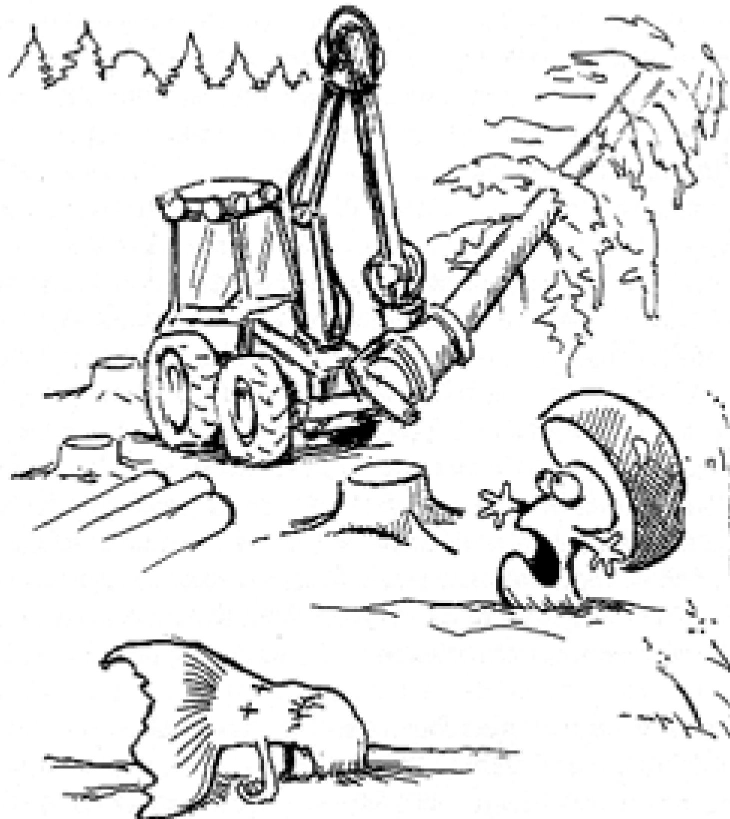
To investigate effects of N-deposition on the degree of mycorrhizal colonization of short-roots and amounts of fungal biomass in mycorrhizas, as well as on the community structure of mycorrhizas, mycorrhizal roots from an N-fertilization experiment in south Sweden were studied (paper III).

Air pollution affects several other factors such as tree vitality, soil nutrient status and acidity. Hallbäcken & Tamm (1986) reported that the pH had decreased by up to 0.9 units between 1927 and 1982-84 in a south Swedish spruce forest. Since

only part of the pH decrease could be attributed to biological acidification induced by the uptake of mineral nutrients by the trees, acidic deposition was suggested as the main cause (Hallbäcken & Tamm, 1986). Falkengren-Grerup *et al*. (1987) found that there had been a 50% reduction in base cation concentration, and up to a 0.5 unit (in acid soils) or 1.5 unit (in less acidic soils) decrease in the pH between 1949 and 1985 in beech forests in south Sweden.

”Vitality fertilization”, i.e. lime combined with all essential plant nutrients except N, has been suggested as a method for compensating losses of base cations and for counteracting increased acidity (Evers & Hüttl, 1991). Previous liming-experiments conducted both in the field and in the laboratory showed that liming could have an adverse effect on frequencies of certain mycorrhizal morphotypes (Lehto, 1984; Erland & Söderström, 1990). Erland *et al*. (1990) found that liming decreased the growth of the mycelium of some morphotypes and species when grown asymbiotically on agar. However, when grown in symbiosis the effect of liming on mycelial growth was less pronounced (Erland *et al*., 1990).

The fact that relatively little was known about the combined effects (lime and nutrients) of the vitality fertilizer on the degree of mycorrhizal colonization of short-roots, fungal biomass in mycorrhizal roots and the species composition of mycorrhizal fungi motivated further investigations (paper II).



Effects of forest regeneration methods on ectomycorrhizal communities

Only a small proportion of the productive boreal forests in northern Europe is protected from forestry or other land-use forms. Excluding high-altitude forests, the current figure for Sweden is ca 0.8% (A. Arnell, pers. comm.). In Sweden, forestry practices may affect the species composition and species richness of mycorrhizal fungi in several ways. In studies conducted in other parts of the world, some authors have noted an increase in species richness with increasing age of the trees (sporocarps: Miller, 1983; Keizer & Arnolds, 1994; mycorrhizas: Visser, 1995) and changes in community structure indicating succession (sporocarps: Last *et al*., 1984; Keizer & Arnolds, 1994; Termorshuizen & Schaffers, 1990; Baar & ter Braak, 1996; mycorrhizas: Gibson & Deacon, 1988; Visser, 1995). Thus, slow-growing species that are dependent on old forests to maintain viable populations could be negatively affected by the use of shorter rotation periods. Of the other silvicultural methods in common use today (excluding fertilization with N and other nutrients) it is likely that the type of regeneration method is the most important factor affecting the species composition and richness of mycorrhizal fungi. When trees are harvested, the mycorrhizal fungi living on roots get their energy supply cut off and have to find new partners to form a symbiosis with. Initially this could be via mycelial growth to roots of intact hosts (Robertson, 1954; Fleming, 1983), but after longer periods the only means of dispersal is via spores or other propagules (e.g. sclerotia of *Cenococum geophilum*: Vogt *et al*., 1982; *Paxillus involutus*: Laiho, 1970; conidia of *Phialophora finlandii*: Wang & Wilcox, 1985). By comparing seedlings planted on clear-cut sites with those in a nearby forest stand, Dahlberg & Stenström (1991) found that several species common on short-roots in the forest stand also colonized seedlings in the clear-cut sites. Similarly, the formation of several different mycorrhizal types was observed on seedlings planted on a 40-year-old clear-cut, where regeneration had failed (Stenström, unpublished). Thus, dispersal via spores or propagules stored in the soil seems to be an effective means of spread and site re-colonization for some species. In addition, mycelia surviving on roots are probably important, but the length of time that the colonization capacity can be maintained following clear-cutting is still not known (Dahlberg & Stenström, 1991). In forests, mycelial spread is probably more important than spore dispersal. Dahlberg & Stenlid (1990) found that as forests became older the number of clones of *S. bovinus* decreased, while their size increased. They found no evidence that new individuals became established as the forest aged, and therefore concluded that once *S. bovinus* had become established mycelial growth was probably the only means of spread within the stand. In a study of mycorrhizas of birch seedlings (*B. pendula* Roth.) that had been planted under older birch trees, Fleming (1983) found that seedlings that had been root-isolated from the birch trees lacked some mycorrhizal types found on the old trees and on non-isolated birch seedlings. Consequently, unless the disturbance is intense and alters the conditions

drastically (soil moisture, acidity, nutrient status, etc.), some fungal species may persist for several forest generations.

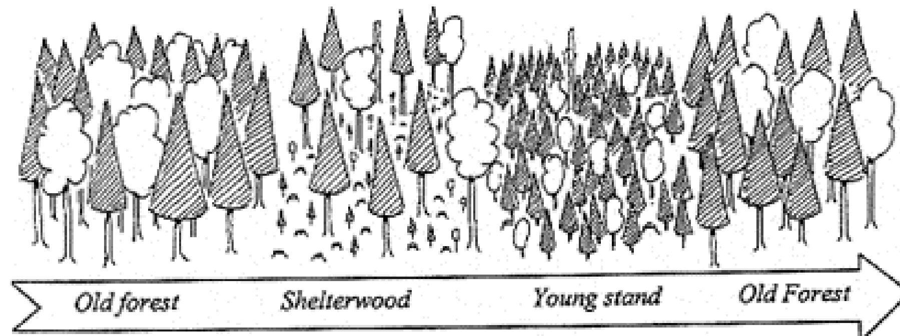


Fig. 1. Shelterwood regeneration.

Today, managed forests are usually regenerated by clear-cutting followed by planting, but recently other methods, such as shelterwood regeneration have become common. With this method the mature forest is only partially cut, and when a new generation of trees is established under the shelterwood trees, the old trees are removed (Fig. 1). For the ectomycorrhizal fungi, one potentially important consequence of shelterwood management is that hosts are continuously available for maintaining the species populations already present in the old forest. Since other factors, such as soil nutrients, acidity and moisture, may also differ between clear-cut and shelterwood sites (Hannertz & Hånell, 1997), it is also possible that the establishment of new species and maintenance of existing species populations might differ in the establishment phase, which in turn may affect any eventual secondary succession of the mycorrhizal fungi. To investigate this possibility, mycorrhizal communities of young forests that had been regenerated following clear-cutting or under a shelterwood were compared with each other and with old, unmanaged forests (paper IV). In this comparison, an attempt was made to identify species present on mycorrhizal roots as well as those producing sporocarps.

Aims

1. To investigate whether variation in the internal transcribed spacer (ITS) region of the fungal rDNA is a) high enough to distinguish between species and b) low enough within species to be of diagnostic value even for samples from geographically distant sites.
2.To develop molecular biological methods that make it possible to analyze ITS restriction profiles of fungal rDNA of mycorrhizal roots in order to separate them from one another and match them with profiles obtained from sporocarp references.

3. To determine if nitrogen-free or ammonium-sulphate fertilization changes the composition of ectomycorrhizal fungi on short-roots, the degree of mycorrhizal colonization of short-roots, and the relative and total fungal biomasses of mycorrhizal fine-roots (<1 mm in diam.).
4. To study how the properties of mycorrhizal communities (i.e. species composition, species richness and diversity) determined based on surveys of mycorrhizal roots correspond with the properties determined on the basis of sporocarp surveys.
5.To investigate how different forest regeneration methods affect the species composition of ectomycorrhizal fungi in Scots pine forests, and to what extent the species composition is related to the age of the forest.
6. To determine the degree to which soil factors and characteristics of the tree stand may influence the species composition of mycorrhizal fungi

Materials and Methods

Molecular biological methods

Previous work has shown the internal transcribed spacer (ITS) region of the rRNA gene to be suitable for identification of fungal species (White *et al.*, 1990; Bruns *et al.*, 1991; Gardes *et al.*, 1991). Primers have been designed that make it possible to isolate this region (White *et al.*, 1990; Gardes & Bruns, 1993). Using the universal primers ITS1 and ITS4, it was possible to amplify small amounts of rDNA extracted from both sporocarps (I) and mycorrhizas (III and IV) using the polymerase chain reaction (PCR, Mullis & Faloona, 1987). (This primer pair is usually called "universal" [Bruns *et al.*, 1991] since it also amplifies the ITS of certain plants [see *e.g.* Paolocci *et al.*, 1995]. However, for mycorrhizas of Scots pine and Norway spruce we have not observed any co-amplification of host rDNA [Nylund *et al.*, 1995]). The amplified DNA sequence was investigated by digesting the DNA with three enzymes (*CfoI*, *Hinf* I and *Mbo* I) yielding ITS fragments of different sizes (in the text referred to as the restriction fragment length polymorphism method, RFLP). ITS restriction fragments of individual samples were size-fractioned on agarose-gels using electrophoresis, and the DNA was visualized using ethidium bromide (White *et al.*, 1990). Restriction fragments were recorded on Polaroid™ film and scanned using a flat-bed scanner. The scanned picture was analyzed with a software program (Taxotron®, Pasteur Institute, Paris, France) with which the sizes of fragments are estimated, and restriction patterns are compared using numerical taxonomy (Sneath & Sokal, 1973). For a further explanation of the methods, see Fig. 2 and paper I. In this thesis the nomenclature of the ectomycorrhizal fungi (Agaricales) follows Hansen & Knudsen (1992), except for taxa in the Corticiaceae where that of Eriksson *et al.* (1981) and Hjortstam *et al.* (1988) is used.

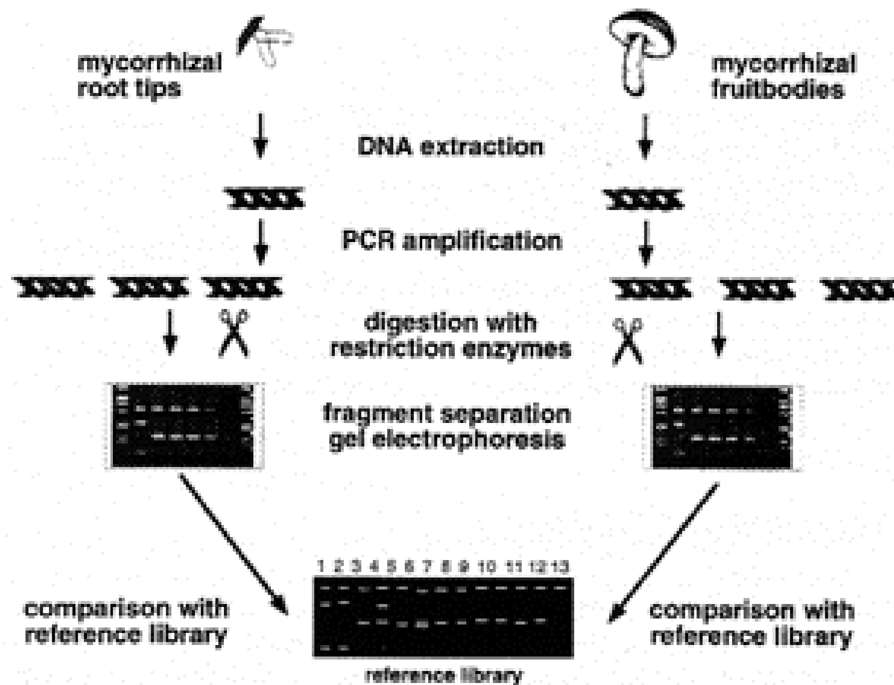


Fig. 2. Identification of mycorrhizas. Schematic overview of how ITS - restriction patterns from fungal rDNA extracted from mycorrhizal short-roots and sporocarps were matched with each other. (Paper I, III and IV).

Analyses of fungal species on mycorrhizal short roots

To identify mycorrhizal species present on roots, two different methods were used: morphological classification of mycorrhizas and a molecular classification based on PCR-RFLP of the ITS region of the rDNA. In papers II and III mycorrhizal root-tips were classified into *morphotypes*, i.e. into different classes on the basis of their root morphology and characteristics of the fungal symbiont. Ideally a morphotype describes the mycorrhizas formed by a single fungal species, but in many cases the morphotypes consist of several species. In papers II, III and IV only healthy, turgid mycorrhizas with a firm, light-colored stele and intact meristem were investigated. The mycorrhizas were classified into morphotypes on the basis of the following macroscopic characters: a) color and texture of the fungal mantle covering the root, b) presence and appearance of extramatrical hyphae and/or rhizomorphs. To determine the identity of the morphotypes described in papers II & III, PCR-RFLP analysis of the ITS region of fungal rDNA was used (White *et al*., 1990; Gardes *et al*. 1991; paper I). In the study described in paper IV, mycorrhizal tips from 11 forest stands (see below) were sampled in an objective, randomized way and subjected to PCR-RFLP

analysis of the ITS-region (as in I). The ITS-RFLP patterns were compared with ITS-RFLP patterns of reference material (mostly obtained from rDNA extracted from sporocarps, in some cases mycelial strains of known species). In addition, the ITS sequence was determined for some of the most commonly found ITS-RFLP patterns of mycorrhizas.

Ergosterol assay for fungal biomass estimations of fine-roots

To estimate the fungal biomass of fine-roots (<1 mm in diameter), the ergosterol assay (Nylund & Wallander, 1992) was used. Ergosterol (24*Beta*-methylcholesta-5,7,*trans* 22-trien-3*Beta*-ol) is a sterol found mainly in the membranes of fungi (Weete, 1980). The 5,7-double bond of ergosterol permits sensitive assay by measurements of the A_{282} of UV light and since this particular bond is rare among major sterols of vascular plants (Newell *et al*., 1988), ergosterol analysis can be used for detecting and quantifying the fungal biomasses of various biological materials, including soils (West, *et al*.,1987), seeds (Seitz *et al*., 1977) and mycorrhizas (Salmanovicz & Nylund, 1988). When it comes to detecting fungal biomass in mycorrhizas, the ergosterol essay is more sensitive than the chitin assay, another commonly used method (Martin *et al*., 1990; Johnson & McGill, 1990). Although amounts of both ergosterol and chitin are correlated with fungal biomass (Seitz *et al*., 1977; Martin *et al*., 1990; Johnson & McGill, 1990), ergosterol is probably a better measure of the active fungal biomass since it is a membrane-bound component. Chitin, by contrast, is a major component of the fungal cell wall and thus is present in both young and old, inactive hyphae. In addition, chitin is also found in other organisms (e.g. insects), and roots contain other compounds that may interfere with the detection of chitin, such as aldehydes (Plassard *et al*., 1983). Estimates of fungal biomass in mycorrhizas sampled in the field based on the ergosterol and chitin assays can only be accurate if concentrations of these compounds are correlated with the fungal biomass of the species present in the EM communities. However, the ergosterol concentration has been found to vary both between and within species (Antibus & Sinsabaugh, 1993, Martin *et al*., 1990; Bermingham et al., 1995) and depends on the physiological condition (age, growth rate) of the fungal isolate (Bermingham *et al*., 1995; Martin *et al*., 1990). Consequently, the measure gives only an approximate estimate of the fungal biomass. Similarly, chitin concentrations also vary within and between species and are influenced by physiological factors (Martin *et al*., 1990). To date, there are still no reliable estimates of ergosterol concentrations in mycorrhizal fungi growing in symbiosis. In the studies described in papers I and II we used an ergosterol conversion factor based on the median ergosterol concentration of 23 EM species (Antibus & Sinsabaugh, 1993). Although the fungi studied by Antibus & Sinsabaugh (1993) had been grown asymbiotically (on solid nutrient agar), a dual analysis of ergosterol and a plant-specific sterol (sitosterol) in field-sampled mycorrhizas indicated that this ergosterol conversion factor seemed to correlate well with the fungal biomass of mycorrhizas (Antibus & Sinsabaugh, 1993).

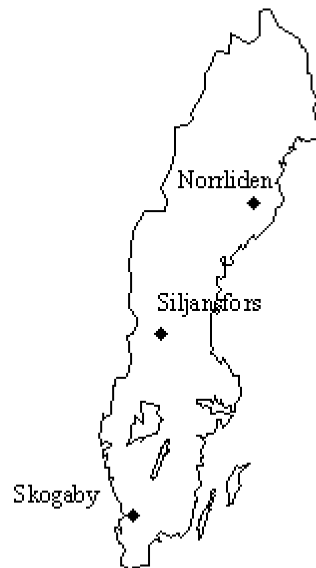


Figure 3. Location of the field experiments in Skogaby (II & III), Siljansfors (IV). Preliminary results from an N-fertilization experiment in Norrliden were included for comparisons with III & IV.

Experimental approaches

I. The RFLP study - The degree of inter- and intra-specific variation in the ITS region of fungal rDNA extracted from sporocarps and mycelia of 44 species were estimated using PCR-RFLP analysis of the ITS region of mycorrhizal fungi. The fungal material had been sampled throughout Fennoscandia. In total, 132 vouchers were studied, most of which were dried herbarium vouchers that had been collected from 1940 to 1995 and stored in fungal herbaria throughout the Nordic countries. On average, three herbarium vouchers of each species were analyzed; thus, the aim was not to precisely determine the intra-specific variation in any particular species, but rather to get a general estimate of the degree of intra-specific variation of ectomycorrhizal fungi. To further analyze differences between two species that belonged to a group of five species with identical ITS-RFLP patterns, the ITS-sequence was determined from two vouchers of each of the two species.

II & III. Both of these experiments were set up as fertilization trials. For the N-free fertilization experiment (II) we used two different field experimental areas: a) Skogaby Experimental Forest and b) an area used for a similar fertilization experiment which had been established by the Swedish Forestry Research Institute. For the NS-experiment (III) we used plots in Skogaby.

Skogaby Experimental Forest in southwestern Sweden (Fig. 3) is a homogeneous, 30-year-old stand of Norway spruce. The soil type is a haplic podzol (FAO-UNESCO, 1988), and the parent material is a sandy, loamy till. The

annual precipitation is about 1100 mm. The stand was planted in 1966 and was the second generation of coniferous forest on a former *Calluna* heathland. The site is located 30 km southeast of Halmstad, 25 km from the sea. The experiment was laid out as randomized blocks with four replicates. In the N-free fertilization experiment (also called the V-treatment by other investigators) Skog-Vital, a commercial fertilizer (P:K:Ca:Mg:S | 48:43:218:46:75 kg ha^{-1}), was spread manually on 2000 m^2 plots. One third of the dose was applied on each of two occasions in 1988, and the remaining third was applied in 1989. In N-free fine-roots were sampled once yearly (in November) during 1991-93. In addition, fine-roots were sampled in October 1992 in three 50-75 year-old Norway spruce stands in southeastern Sweden used for a similar N-free fertilization experiment (consisting of one treatment and one control plot per site). In the NS treatment in Skogaby (III) the stand was fertilized manually three times a year, starting in 1988, with $(NH_4)_2SO_3$ (N=100, S=114 kg*ha^{-1}*$year^{-1}$). Sampling of fine-roots in the LFH-layer (the organic horizon, containing the litter-, fermentation- and humus-layers) was carried out in Novemeber 1992 and 1993. Mycorrhizal colonization rates (% short roots colonized by mycorrhizal fungi), rates of colonization by different morphotypes and fungal biomass of mycorrhizas (ergosterol assay) were determined by analyzing fine-roots sampled from the upper organic soil horizon (the LFH-layer) of four treatment and four control plots. To identify mycorrhizas the ITS region of fungal ribosomal DNA was amplified using the polymerase chain reaction and digested with endonucleases (III). The resulting RFLP patterns from each mycorrhizal root tip were compared with the field-sampled material and with the sporocarp ITS-RFLP references. As the ITS-RFLP references covered the majority of the sporocarp production (biomass) in Skogaby, a comparison between species found on mycorrhizal short-roots or as sporocarps was made.

IV.

The diversity and species composition of ectomycorrhizal (EM) fungi were studied in 11 Scots pine stands in Siljansfors Forest Research Park in central Sweden (Fig. 3, 4). The experiment was designed as a survey study in which the ectomycorrhizal communities in three types of forests were compared. Three stands (”O”) consisted of 150-year-old, unmanaged forests, and the remaining stands were 30-40 years old. Of the latter, four were the result of regeneration by planting on clear-cuts (”P”), and four developed under shelterwood trees that were successively removed (”S”) (Fig. 1). Sporocarps were inventoried on seven and nine occasions in 1995 and 1996, and 25 fine-root samples of the LFH-layer in each plot were sampled once in 1995 using a soil corer. From each fine-root sample single mycorrhizal tips (between 1 and 3 mycorrhizal short-roots per sample were analyzed) were chosen in an objective, random way and subjected to PCR-RFLP analysis for species determination using three restriction enzymes (*Hinf* I, *Mbo* I and *Taq* I) in accordance with methodology described previously and in (I).

In addition, soil from the LFH-layer of all plots was added to two-month-old Scots pine seedlings grown in microcosms in a growth chamber. The aim of this experiment was to assess the mycorrhizal colonization potential of soil that had been disturbed and to determine which species were capable of colonizing seedlings. After six months in the growth chamber the seedlings were harvested, and mycorrhizal root tips were sampled, whereupon PCR-RFLP analysis was carried out as described above, and the resulting RFLP patterns from each mycorrhizal root tip were compared with the field-sampled material and with the sporocarp ITS-RFLP references.

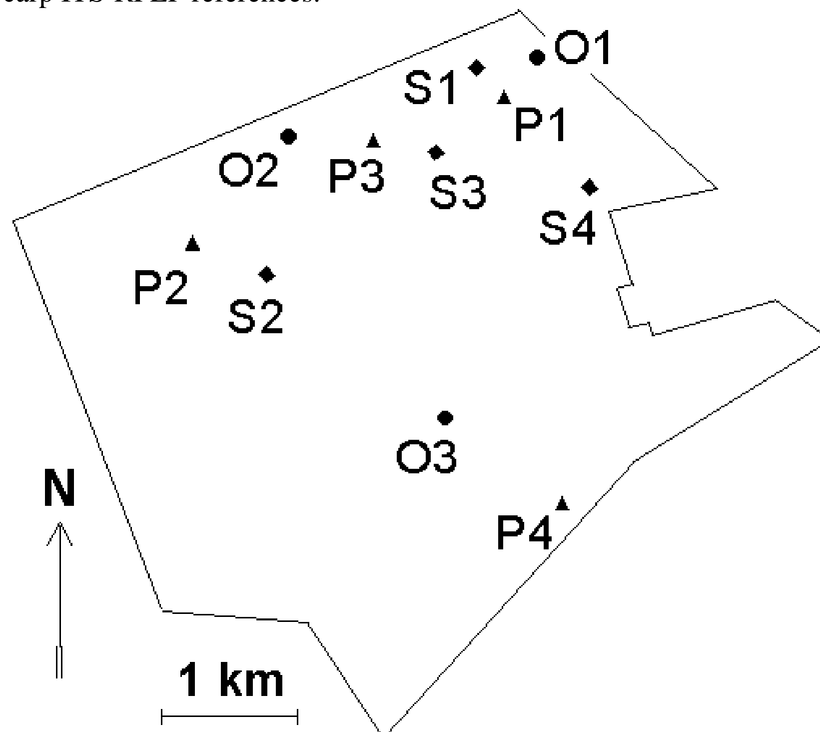


Fig 4. Location and map of plots in Siljansfors Forest Research Park. P= 40-year-old forests originating as planted seedlings on clear-cut sites, S= 40-year-old forests regenerated under a shelterwood, O= 150-year-old, unmanaged forests. Numbers denote blocks.

The Norrliden N-fertilization experiment

In addition, this thesis includes some preliminary results obtained from a long-term N-fertilization experiment in northern Sweden (Fig. 3). This study was a part of a nutrient fertilization experiment (Holmen, *et al.*, 1976) and consisted of six randomized 15*15 m plots, of which three were controls (C) and three had been fertilized with ammonium nitrate ("AN"). The AN-plots were fertilized manually with 60 kg N during 1971-73 and in 1993, 40 kg N during 1974-1976 and 30 kg N from 1977 onwards (all values per hectare, single doses with NH_4NO_3 were used, in 1991 and 1994 no fertilization was carried out). Each plot

was surrounded by a 7.5-m-wide zone that received the same treatment. The plots consisted of 45-year-old Scots pines that had been planted as 2-year-old seedlings in 1953. The former forest was an old spruce-dominated stand that had been clear-cut in 1951. A prescribed burning was carried out in 1952. In 1995 and 1996, 64 and 100 fine-root samples, respectively, were taken from the LFH-layer in each plot using a soil corer (IV). From each fine-root sample, single mycorrhizal tips (1 or 2 mycorrhizal short-roots per sample) were chosen in an objective, randomized way (IV) and subjected to PCR-RFLP analysis as described in III and IV. In total, 140 mycorrhizal tips from four plots were analyzed in 1995. Since the analyses of species composition in 1995 and 1996 gave similar results, we chose to present only the data from 1996, which contained most samples (200 and 146 single mycorrhizal tips from control and AN respectively were analyzed).

Results

Paper I

Intraspecific polymorphisms in the ITS region of the rDNA were found in seven of the 44 species. Polymorphisms were due to length mutations, ranging from 5 to 15 base-pairs in four of the seven polymorphic species, and mutations in enzyme restriction sites in six species, mostly affecting only one enzyme, but in two species two enzymes were affected. In addition, the ITS sequences of two vouchers each of *C. armillatus* and *C. traganus* were determined. No intraspecific variation was found, confirming that intraspecific variation of the ITS was low.

Using a single enzyme, a unique RFLP pattern (that could be distinguished from the other patterns) was obtained from more than half of the species, and by combining different endonucleases 34 (77%) of the species could be distinguished from one another. The remaining RFLP types occurred in one genus, *Cortinarius*, which also was the best represented of the genera, with 17 species. Thus, it is likely that if the other genera had also contained more species, a lower share of the RFLP patterns would have been considered species specific. On the other hand, the risk of considering species belonging to distantly related genera as identical is probably low as long as several enzymes are used. The sequence analysis of the two *Cortinarius* species showed that about 6% (36) of the base-pairs in the ITS region differed between the two species, suggesting that by using more enzymes (or, alternatively, by using sequence analysis to determine the optimal set of enzymes) it should be possible to also distinguish between species that could not be separated from one another in the present study.

On the basis of the low intra- but high inter-specific variation in the ITS region, it was concluded that it should be to possible to recognize most ectomycorrhizas formed by the 44 investigated species by comparing their ITS-RFLP profiles with this dataset, if the mycorrhizas are sampled from a site located in Fennoscandia. However, in datasets from even larger geographical areas encompassing a higher degree of intraspecific variation in the ITS region, or when mycorrhizas from several distant sites are compared, it may be necessary to include local reference species.

Paper II

The N-free fertilization did not decrease the mycorrhizal colonization rate (close to 100% of all short-roots were mycorrhizal) or the relative amounts of fungal biomass in the fine-roots. These results disagree with the previously reported 50% reduction in total production (biomass) in N-free fertilized plots (Wiklund *et al.*, 1995). Several possible causes for this discrepancy were discussed, of which two were considered most likely: a) a decreased allocation of C to roots and b) changes in C allocation within the ectomycorrhizal fungi, favoring the uptake of N and reducing allocation to sporocarps via indirect effects of the fertilizer on N mineralization. Analyses of morphotype frequencies indicated that no large shifts had occurred in N-free compared to control. Only one relatively uncommon morphotype showed a decrease in frequency from 3% to 0.5% of the mycorrhizal tips. However, even though this type constituted only a minor part of the

mycorrhizas in control, species forming this morphotype accounted for about 30% of the sporocarp production (biomass). Thus, a third possible explanation is that the decreased sporocarp production may have been induced by changes in the abundance of certain mycorrhizal species on the short-roots.

Paper III

The effect of NS on mycorrhizal roots was similar to that of N-free fertilizer described above; i.e. no decrease in mycorrhizal colonization or relative amount of fungal biomass in roots was found. The amount of standing fine-root biomass (and mycorrhizas) decreased by 50%. The morphotype study indicated that shifts had occurred in a brown morphotype that constituted about 50% of the mycorrhizas in control and 80% in NS. However, the RFLP analysis revealed that this morphotype consisted of several species; thus it was difficult to determine the effect of NS fertilization on the frequencies of individual species. The most common RFLP-type matched the restriction patterns of *Tylospora fibrillosa*. *Tylospora fibrillosa* was found in both control and NS, and was the most common RFLP-type of the brown type. The effect of NS on the production of sporocarps by mycorrhizal fungi was drastic - nearly all species ceased to fruit within a year of the fertilizer application, and from then on, sporocarp production was almost completely inhibited (Wiklund *et al*., 1995). It was suggested that NS affected the mycorrhizal fungi in a way similar to that of the N-free fertilizer, although the former had stronger effects on the standing biomass of fine-roots and community structure. Of the 58 samples analysed, 21 different restriction profiles could be distinguished. Only four of the restriction profiles matched the restriction patterns of the dominant sporocarps on the site.

Paper IV

In the study conducted in Siljansfors (Fig. 3, 4) we examined the hypotheses that 1) the species composition of mycorrhizal fungi in a secondary stand is determined by whether a continuous tree layer is present (shelterwood-regenerated forests, ”S”) or absent (forests established after clear-cutting and planting ”P”) during the establishment phase and 2) that as the forest grows older mycorrhizal fungi communities undergo secondary succession.

This was based on a comparison of the diversity and species composition of ectomycorrhizal (EM) fungi in 11 *P. sylvestris* stands. Three stands (”O”) consisted of 150-year-old, unmanaged forests, and the remaining were 30-40 years old. Of the latter, four were the result of regeneration by planting on clear-cuts (P), and four developed under shelterwood trees that had been successively removed (S). Sporocarps were inventoried on seven occasions in 1995 and on nine occasions in 1996, and mycorrhizal root tips were sampled once in 1995 and identified using molecular biological methods.

Both the inventory of mycorrhizal fungi present on short-roots and the sporocarp study showed that most species occurred in similar frequencies in both P, S and O. On mycorrhizal roots, only one species, RFLP type 1, was affected

by the type of regeneration method. This RFLP-type occurred in significantly higher frequencies in P (on average in 40% of the short-roots) compared to 20% in 12 % in S and O respectively (p<0.05). In addition, *Russula decolorans* tended to be more common (p<0.09) in S and O (6% and 14% respectively) than in P, where no mycorrhizal short-roots matching the ITS-RFLP pattern of *R. decolorans* were found.

The surveys of sporocarp production showed that three species were affected by the presence of continuous tree layer during the regeneration phase*: Russula decolorans*, *Cortinarius gentilis* and *C. duracinus,* whose production of sporocarps were higher in S and O than in P (p<0.03).

To further analyse whether the species composition differed between the three types of forests, an index of similarity, taking the species frequency distributions of the whole communities (mycorrhizal roots or sporocarps) into consideration, was calculated. The fungal community, characterized based on surveys of mycorrhizal roots or sporocarps, differed most between P and O, while the fungal community of S had intermediate characteristics. Species richness (number of EM species in sporocarp and mycorrhiza communities taken together) was lowest in P (Fig. 5). The high diversity of the mycorrhizal communities in shelterwood-regenerated forests and the large degree of similarity between them and mycorrhizal communities in both the planted and old forests suggest that shelterwood regeneration favored establishment of species with a high dispersal ability while allowing fungi present in the previous stand to persist.

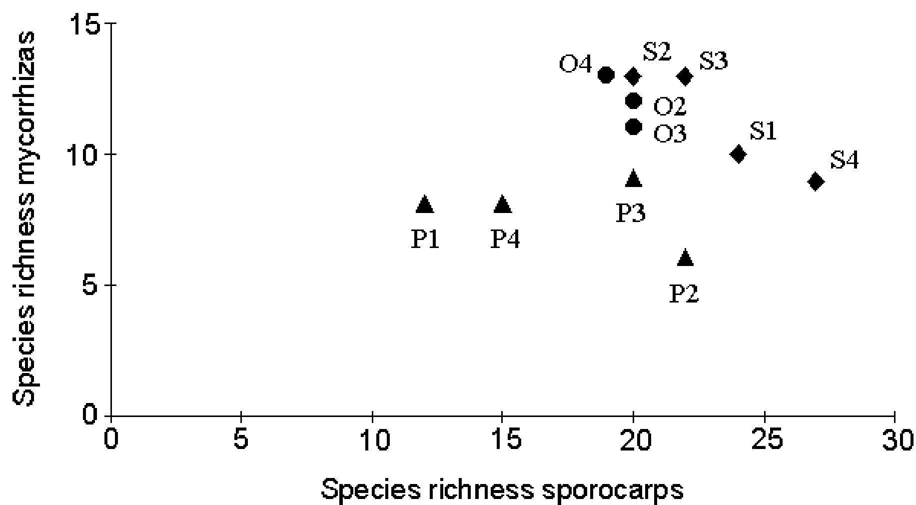


Fig 5. Species richness (=number of species) of sporocarps and species found on mycorrhizal root tips in plots at Siljansfors Forest Research Park (paper IV). P= forests that developed on planted clear-cut sites, S=shelterwoods, O=old, unmanaged forests. Numbers denotes blocks.

In the bioassay study where soil sampled from the field was used for inoculation of non-mycorrhizal seedlings, 22 different RFLP-types were found in the 62

mycorrhizal tips analyzed. Thirteen of these RFLP-types were also found in field-sampled mycorrhizas and accounted for about 50% of the total number of tips sampled from the seedlings. None of the RFLP-types matched *Piloderma croceum* or *Tylospora fibrillosa*, which were two of the most common species on the field-sampled mycorrhizal roots (paper IV and Fig. 6). Observation of morphotypes suggested that <0.1% of the short-roots of the bioassay seedlings were colonized by *P. croceum*, which was considerably lower than in the field.

Multivariate statistical analyses showed that the abundances of most species were affected by forest age and by the Mg^{2+}concentration, pH and cation exchange capacity of the soil, while effects of the shelterwood were less pronounced.

In each plot between 45 and 90 % of the mycorrhizal tips were formed by species not represented in the sporocarp inventory. Thus the community structure, as reflected by the results of inventory of species present on mycorrhizal short-roots differed substantially from that reflected by the sporocarp inventory. This is illustrated in Fig. 6, where the mycorrhizal-tip colonization frequencies for the most common species in all P, S and O plots are compared with their corresponding abundances measured on the basis of sporocarp surveys (recalculated from Table 4 and 5 in paper IV).

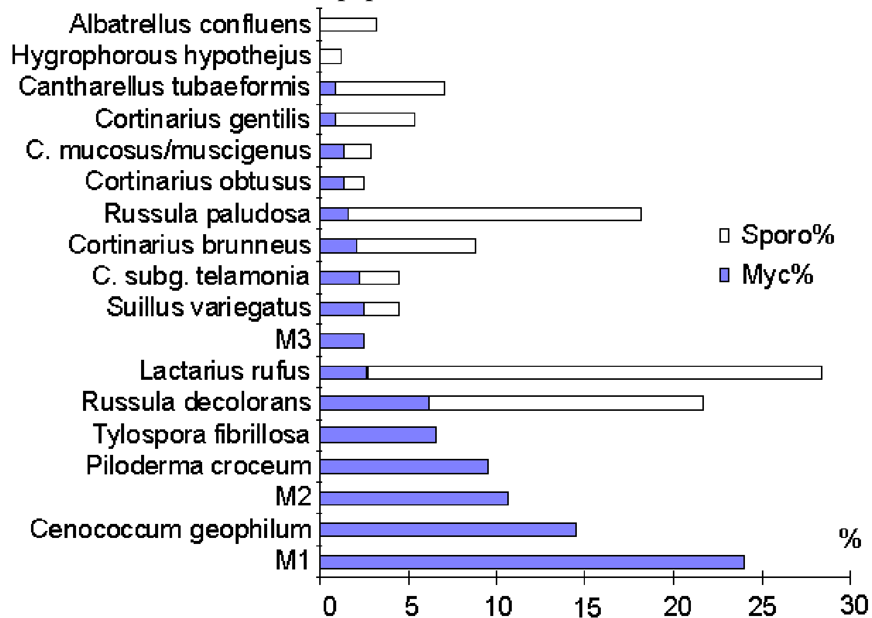


Fig. 6. Species composition as reflected by inventories of mycorrhizal root tips (% of the total number of mycorrhizal tips; Myc%) or of sporocarps (% dwt of total production; Sporo%) in 11 *Pinus sylvestris* dominated stands in central Sweden (paper IV). The ten most abundant species present as mycorrhizas or as sporocarps were included. M1, M2 and M3 are unidentified fungi, C. = *Cortinarius.* C. subgenus *telamonia* includes the sporocarp production of *C. biformis*, C. *malachius* and *C. paleaceus* (see papers I and IV).

The Norrliden N-fertilization experiment

The aim with this study was to determine how ammonium nitrate fertilization (”AN”) affected the species composition and richness of ectomycorrhizal fungi in a Scots pine forest. Although the results are somewhat preliminary (e.g. further analyses will be made to determine unidentified mycorrhizas to species or genus), they were included to allow a comparison of the community structure in studies III and IV.

The PCR-RFLP analyses of mycorrhizal root tips showed that the frequencies (% of mycorrhizal short-roots) of several of the most common restriction patterns obtained from mycorrhizas differed between the control and AN plots (Figs. 7a and 7b). Frequencies of *Lactarius rufus* and *Tylospora fibrillosa* were higher in the AN plots than in the controls ($p<0.05$). In addition, an unidentified RFLP-type ”x30” tended to be more common in AN ($p<0.07$). Several other RFLP-types that occurred in all three control plots were less frequent or totally absent in AN: *Cortinarius semisanguineus*, *C. brunneus*, *C. fervidus*, *Piloderma croceum, Suillus variegatus*, an unidentified RFLP-type (”M2”) and an RFLP-type matching the restriction patterns of several species in *Cortinarius* subgenus *telamonia* ($p<0.05$). RFLP-type M2 matched a common RFLP-type in study IV, in which we suggested that it was closely related to *Piloderma croceum* (93% similarity in terms of ITS-sequences). *S. variegatus* and RFLP-type M2 were the only RFLP-types matching these species that were found in any of the AN plots. The frequency of the most common restriction pattern (RFLP-type ”M1”) did not differ between mycorrhizas in control plots and those in the AN plots. This was also the most common RFLP-type in the young forests (P and S) in Siljansfors (IV). Evenness tended to be higher in control plots (Simpson’s 1/D was 6.4-11.6 in control and 5.0-6.5 in AN, $p<0.12$).

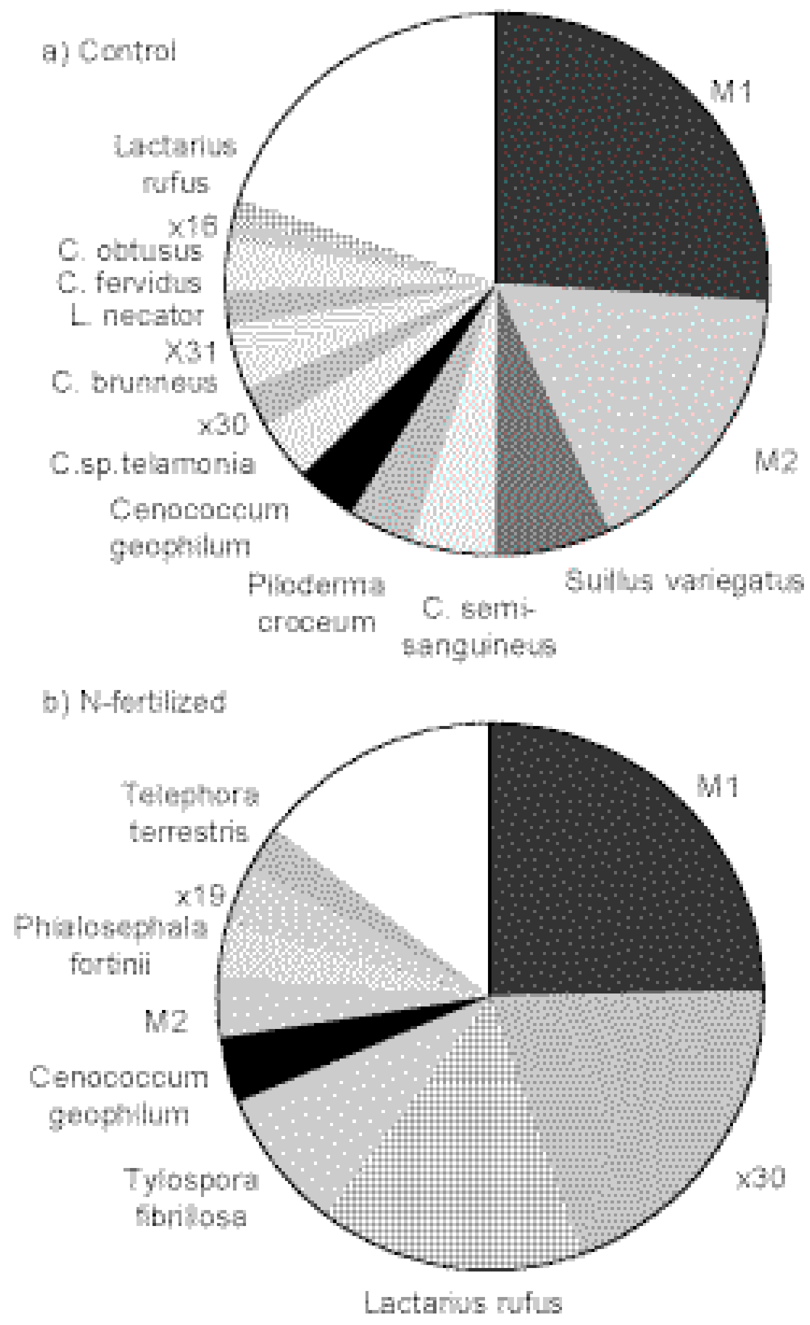


Fig 7. Mycorrhizal species frequencies (% of mycorrhizal short-roots) in a) control and b) ammonium nitrate fertilized in Norrliden. M1-M2 are identical to M1-M2 in paper IV. X16, X19, X30 & X31 are unidentified RFLP patterns. (ITS-RFLP analyses on mycorrhizas sampled in Sep. 1996) C. = Cortinarius; L. = Lactarius

Discussion

Effects of NS and N-free fertilization

Dramatic effects on sporocarp production but only minor effects on mycorrhizal colonization of fine-roots
The investigations of mycorrhizal fungi in NS- and N-free fertilized plots in Skogaby showed that the degree of colonization of short-roots by mycorrhizal fungi was unaffected by fertilizer treatments (close to 100% colonization). These results also agree with the estimations of fungal biomass (ergosterol) in fine-roots, which did not reveal any differences between the control, NS and N-free plots.

In contrast to these results, the sporocarp production of ectomycorrhizal fungi and the number of species producing sporocarps were negatively affected by both NS and N-free fertilization (Wiklund *et al.* 1995). A similar decrease in sporocarp production was also observed in the irrigation-fertilization (IF) plots in another experiment in Skogaby. The IF plots received the same total amounts of N as the NS treatment, but the fertilizer in IF was sprayed below the canopy during the growing season (NH_4-N:NO_3-N:P:K:Ca:Mg:S | 100:17:48:6:6:9 kg ha^{-1} year^{-1}, N supplied as NH_4NO_3). During 1991-93, total production (dry weight) was close to zero in the NS and IF plots, and the four species recorded were only represented by one to two sporocarps each. By contrast, in the control plots 2000-4000 sporocarps were produced each year in the same period. In N-free fertilized plots the total production (dry weight) decreased by about 50% during the same period, and several species ceased to fruit (Wiklund *et al.*, 1995).

Before discussing possible reasons for the drastic reduction in sporocarp production caused by the fertilization treatments, I want to briefly compare the NS- N-free- and IF-treatment in terms of their effects on trees and on the nutrient status and acidic properties of the soil. The amounts of N taken up by the trees increased in NS, IF and N-free (above-ground uptake of N during 1988-90 was 240, 295 and 154 kg N ha^{-1}, respectively, compared to 96 kg in control, Nilsson & Wiklund, 1994). In NS- and IF-treatments concentrations of inorganic N in the soil increased. For instance, the concentration of ammonium ions increased ≥5-fold in water extracts of LFH-soil in NS and IF in 1992 (Majdi & Bergholm, 1995; Bergholm, pers. comm.). The effects on other factors that may influence the mycorrhizal fungi, e.g. base saturation and pH (Tyler, 1985; Erland, 1990), were not consistent across all three treatments. For instance, there was a decrease in base saturation levels and a slight increase in the pH of water extracts of soil samples in the LFH-layer in NS, whereas in N-free plots both of these variables increased slightly, and in the IF plots the pH decreased while the base saturation level increased (Majdi & Bergholm, 1995; Bergholm, pers. comm.). Because the effects on the acidic properties and base saturation of the soil were small and showed no consistent differences between treatments, it would appear that nitrogen was the main factor influencing sporocarp formation by mycorrhizal fungi (Wiklund *et al.*, 1995) as

well as the species composition and abundance of mycorrhizal fungi on short-roots (II, III). The fact that the effect on sporocarp formation was similar to that reported in other N-fertilization studies (e.g. Menge & Grand, 1978; Rühling & Tyler, 1991; Termorzhuizen, 1993; Wiklund *et al.*, 1995; Brandrud, 1995) and much more pronounced than in experiments with acidic deposition (Wästerlund, 1982; Såstad & Jenssen, 1993) suggests that nitrogen was the main factor influencing the mycorrhizal fungi in the fertilizer treatments in Skogaby.

There are several possible reasons for why fertilization suppressed sporocarp production by the mycorrhizal fungi (II & III): Fertilization may have a) induced trees to allocate less carbohydrates to the mycorrhizal fungi, b) altered the species composition of the mycorrhizal fungi community in favor of species that do not produce sporocarps or c) caused internal changes in carbohydrate allocation patterns within mycobionts leading to a reduction in the relative amounts of carbohydrates allocated to sporocarps.

Decreased allocation of carbohydrates from the host to the fungus

Could fertilization have reduced the allocation of tree carbohydrates to the mycorrhizal fungi? Inventories of mycorrhizas showed that neither the degree of colonization of short-roots by mycorrhizal fungi nor the fungal biomass proportion of mycorrhizal roots was affected by the NS or N-free treatment. However, the total standing biomass of fine-roots and number of mycorrhizal short-roots in the NS and N-free plots had decreased by about 50% and 20-30% respectively (III & II, Clemensson-Lindell & Persson, 1995). Direct observation using rhizotrons (video recordings of root growth) confirmed that fine-root production decreased in the NS- and N-free plots (Majdi & Persson, 1995; Majdi & Kangas, 1997). Thus, a reduced allocation to roots may at least partly explain the decrease in sporocarp production.

Changes in the community structure

Could fertilization have altered the species composition or relative abundances of the various species in the mycorrhizal fungi community? The classification of mycorrhizal morphotypes indicated that the most common morphotype, a smooth, brownish mycorrhiza increased in frequency in NS (80 % of the short- roots were colonized by this type compared with 50% in control plots) while several others decreased in frequency. However, the PCR-RFLP analyses of the ITS region of fungal rDNA extracted from mycorrhizas in Skogaby showed that the brownish mycorrhiza was composed of several species. The most common restriction pattern from mycorrhizas matched *Tylospora fibrillosa* and was found in both NS and C. The second most common RFLP-type was only found in mycorrhizal roots from NS and did not match any of the RFLP-types of the ITS of fungal rDNA of sporocarps belonging to the most common species in control plots in Skogaby (I, III). Thus, it is possible that an increased frequency of some species that did not produce sporocarps may have contributed to the decreased sporocarp production in NS. However, only a small number of mycorrhizas were analyzed using PCR-

RFLP, thus preventing any firm conclusions from being drawn as to whether the frequency of certain species was affected by the NS treatment.

Analyses from the Norrliden experiment showed that the species composition of mycorrhizal fungi present on mycorrhizal roots was changed drastically by N-fertilization (Fig. 7). The changes were most pronounced for *Suillus variegatus*, *Piloderma croceum* and several *Cortinarius* species, the frequencies of which were reduced in the AN plots (in some cases they were totally absent), while occurring in two or more control plots. *Lactarius rufus* and a few other species increased in frequency in AN (Fig. 7). The effects of fertilization on sporocarp production by mycorrhizal fungi in Norrliden were similar to those reported in the Skogaby trial and in other cited N-fertilization experiments (observations in Norrliden were based on one inventory per year in 1988 and 1991, T. Persson, pers. comm.; and three times per year in 1994 and 1995, personal obs.). In AN plots the number of species found in the sporocarp inventory was decreased (mainly *Cortinarius* spp.), as was the number of sporocarps found (e.g. *L. rufus* which was the most common in C and AN), compared with controls. Thus, the decreased number of species producing sporocarps in AN may in part be explained by the reduction in the number of *Cortinarius* species on mycorrhizal roots (or to the fact that they decreased in frequency on the short-roots, in turn reducing the chance of being detected in the sporocarp inventories).

In Skogaby, it is likely that a relatively small change in the colonization capacity of the mycorrhizal fungi would have caused a marked change in community structure. Using rhizotrons, the average life-span of fine-roots (<2 mm) was estimated at 280 and 250 days in control and NS plots, respectively, based on observations made from August 1992 to August 1993 (Majdi and Kangas, 1997). If one assumes that the mycorrhizas and fine-roots have the same longevity, then for a species (or morphotype) with an initial frequency of 50% (e.g. the smooth, brownish morphotype in control plots in paper III) at the start of the NS-fertilization (1988) a 7% increase in the ability to colonize a short-root would result in its frequency increasing to >80% five years later (e.g. the most common morphotype in NS plots in paper III). However, since the life-span of mycorrhizas may be considerably shorter (Downes *et al.*, 1992), even a smaller change in the colonization potential might cause a change in community structure. Given the large, but variable, responses that different fungal species show to increased levels of inorganic nitrogen in the soil medium regarding their mycelial growth, fungal biomass of ectomycorrhizal short-roots (Arnebrant, 1994; Wallander and Nylund, 1992) and ability to colonize short-roots (Arnebrant, 1996), it seems reasonable to assume that an increased concentration of nitrogen changed the ability of mycorrhizal fungi to colonize the short-roots of the trees in the fertilized plots in Skogaby. Similarly, for the mycorrhizal communities on short-roots in Norrliden, which were exposed to elevated levels of nitrogen over a longer period (25 years), even minor changes in the ability to colonize short roots of the trees would have been sufficient to change the species composition on roots.

However, this is probably not a good explanation for the overall decrease in sporocarp production following N-fertilization. For instance, in the NH_4NO_3-fertilized plots in Norrliden, *Lactarius rufus* produced fewer sporocarps (<10% of control, T. Persson, pers. comm. and pers. obs.), despite its 5-fold increase in frequency on mycorrhizal short-roots compared with the control. Analyses of mycorrhizas sampled the previous year confirmed that *L. rufus* was more common in N-fertilized plots that in control plots; the difference in *L. rufus* frequencies between the control and AN was even larger that year (Kårén & Nylund, unpublished).

Changes in carbohydrate allocation within the fungus

Could fertilization have caused internal changes in carbohydrate allocation patterns within mycobionts, leading to a reduction in the relative amounts of carbohydrates allocated to sporocarps? Björkman (1942) proposed that nitrogen was assimilated by the host rather than by the fungus. However work by France & Reid (1983) and by Finlay *et al*. (1988) has demonstrated that ammonium is assimilated in the hyphae of ectomycorrhizal fungi, and then transported to the host. Wallander (1995) proposed that under conditions of increased N availability, a fungus will tend to allocate more carbohydrates to the energy-demanding process of nitrogen assimilation than to vegetative growth, which in turn may reduce the energy spent on sporocarp formation. Thus ectomycorrhizal fungi that have a low nitrogen assimilation rate may be more tolerant of increased levels of N (Wallander, 1995). In unpolluted temperate forests, nitrogen is the main nutrient limiting plant growth (Tamm, 1991). It is thus reasonable to believe that the ectomycorrhizal fungi are adapted to an environment with low availability of nitrogen. More than 90% of the nitrogen in the surface layer of most soils occurs in organic forms (Kelley & Stevenson, 1996) and is unavailable for direct uptake by the plants (Read, 1987). However, proteolytic capability (Ramstedt & Söderhäll, 1983) and the ability to utilize proteins and amino acids and to transfer nitrogen to host trees have been demonstrated for several ectomycorrhizal fungi (Abuzinadah & Read, 1986; Finlay *et al*., 1989; Botton & Chalot, 1995). Most fungi are also capable of utilizing NH_4 and/or NO_3, although the inter- and intra-specific variation in their utilization efficiencies is high (e.g. Ek, 1993; Keller, 1996 and reviews by Botton & Chalot, 1995; Martin & Botton, 1997). The form of available nitrogen is also important in regulating uptake and assimilation of N by the fungus (Lorillou *et al*., 1996; Botton & Chalot, 1995). Thus, given the high diversity of physiological mechanisms used to metabolize different N-forms among ectomycorrhizal fungi, it is likely that their ability to compete for nitrogen and colonize the short-roots of the trees was altered by N-additions in Skogaby and Norrliden.

In fact, changes in carbohydrate allocation patterns due to the increased availability of nitrogen may partly explain why following the N-fertilization in Norrliden some species seem to have persisted or even increased in abundance on mycorrhizal roots while their sporocarp production decreased (e.g. *L. rufus*).

Several contributing factors affected the mycorrhizal fungi
In conclusion, several factors seems to be involved in the fertilization-induced reduction in sporocarp production by mycorrhizal fungi in the N-free and the NS-fertilized plots in Skogaby and the NH_4NO_3-fertilized plots in Norrliden.

The degree to which the decrease in sporocarp production by ectomycorrhizal fungi in N-free plots is related to indirect N-effects induced by the fertilizer has yet to be determined (Wiklund *et al*., 1995; paper II). If N-free fertilization increases the N-mineralization rate in the upper organic horizons, where most fine-roots were located (Clemensson-Lindell & Persson, 1995), it is possible that the ectomycorrhizal fungi would be affected negatively, especially in cases where the pool of N in the soil has been increased by N-deposition. However, in N-free plots in 1993, four years after the fertilization application, pools of NH_4-N and net N-mineralization rates in incubation studies were unchanged in all horizons to 50 cm depth; no NO_3-N was found in the LFH-layer (Persson T., pers.comm.). Thus, it seems unlikely that the reduction in sporocarp production (total biomass) of ectomycorrhizal fungi, which did not start until 1991, several years after the fertilization (Wiklund *et al*., 1995), can be explained by changes in carbon allocation within the fungus due to the mechanisms proposed by Wallander (1995). A change in species composition in the N-free fertilized plots, which was proven to have taken place in Norrliden, perhaps together with a decreased allocation of carbohydrates to the roots, appears to be a more likely explanation.

However, to reveal the mechanisms behind the observed effects of N-fertilization on the species composition of mycorrhizal fungi and their production of sporocarps and to discuss the implications of our findings in relation to atmospheric N-deposition, it will be necessary to carry out experiments in both the field and laboratory in order to study the effects of different N-forms on C- and N-metabolism within the mycorrhizal fungi, and to determine how interactions between fungi and between the fungi and the host are altered by N-deposition. Moreover, it is also necessary to find out whether the functioning of mycorrhizal fungi changes when the species composition is altered. In line with Wallander (1995) it is possible that the decreased production of sporocarps may indicate that more energy is being expended on energy-demanding N-assimilation, which may have adverse effects on the soil mycelia and its uptake of other mineral nutrients.

Effects of forest regeneration methods

In the study conducted in Siljansfors we examined the hypothesis that 1) the species composition of mycorrhizal fungi in a secondary stand is to some extent determined by whether a continuous tree layer is present (shelterwood-regenerated forests, ”S”) or absent (forests established after clear-cutting and planting ”P”) during the establishment phase.

Community structure of ectomycorrhizal fungi in forests regenerated after clear-cutting and planting or under shelterwoods

Of the species found either as sporocarps or as mycorrhizal roots in P and S, few seemed to have been affected by the type of regeneration method. In the sporocarp inventory only three of the 62 species found in P and S (*R. decolorans*, *C. gentilis* and C. *duracinus*) seemed to have been favored by the shelterwood. The analyses of mycorrhizas suggested that only one species, an unidentified mycorrhiza ("M1"), possibly belonging to the Corticiaceae (IV), was affected by the type of regeneration method, being more common in P than in S and O. In addition, *R. decolorans* tended to be more frequent in S and O ($p<0.09$), which seems to confirm the results of the sporocarp inventory. In total, 33 different RFLP-types were found in P and S.

To further investigate the possibility that the species composition of ectomycorrhizal fungi (mycorrhizas or as sporocarps) was affected by regeneration method, the degree of similarity between ectomycorrhizal communities in different forest stands was calculated using the Steinhaus index of similarity. Both the comparisons based on species frequency distributions of sporocarps and those based on mycorrhizal roots suggested that mycorrhizal communities in the shelterwood plots were more similar to those in the old stands than to those in the stands established on clear-cuts. The largest difference in species composition was found between P and O stands. This suggests that conditions in the shelterwood (symbiosis on short-roots of old trees could be maintained, soil-acidity, -moisture and -nutrients, etc.) may have differed compared to clear-cut sites. This may have enhanced the survival of species that were present before the P and S stands were regenerated, so that more species could persist under the shelterwood. In addition, it is also possible that the disturbance was larger in the clear-cut and planted forests, which may have favored immigration and establishment of species from surrounding stands to a larger extent than in S stands.

Similarly, the higher species richness in S compared to P (Fig. 5) may be due to that the partial cut not only favored establishment of fungi that depend on disturbance, but also allowed species dependent on a living host for their vegetative spread to survive during the establishment phase of the young forest

The multivariate analyses (ordination) suggested that several *Russula* species and some *Cortinarius* species were positively affected by shelterwood management, since they were more common in S than in P. These species were also common in the old forests. Variation in species abundances could partly be explained by soil chemical factors (pH, cation exchange capacity [CEC] and Mg^{2+} concentration of the organic horizon). However, several other variables (e.g. pH, CEC and stand productivity, but not Mg^{2+}) were highly correlated with forest age and type of regeneration method, making it difficult to quantify the effect of any of the soil variables alone.

It is also possible that the abiotic environmental conditions in the shelterwood-regenerated forests differed from those in the planted forests, affecting the establishment of ectomycorrhizal fungi in the early or later phases of the regeneration. However, analyses of soil samples from the organic horizon (e.g. mineral nutrient status, acidic properties, organic matter and moisture contents) showed that only the pH differed. The pH was higher in planted forests than in S and O, which did not differ from each other.

In a study of vegetation changes up to eight years following shelterwood regeneration of Norway spruce, Hannertz & Hånell (1997) also found that species richness was higher in shelterwoods than in clear-cuts. Species adapted to shaded and moist conditions were favored by the shelterwood, whereas nitrophilous (*sensu* Ellenberg *et al.,* 1991) species were more common in clear-cut sites. It is possible that differences in soil factors during the early phases of establishment may have changed the competitive ability of species already present in the stand or altered conditions affecting the establishment of mycorrhizal fungi moving in from surrounding stands.

In conclusion, for most common species studied, factors other than the type of regeneration method seem to largely determine their ability to persist at the site during the regeneration phase or their ability to colonize from surrounding stands.

Persistence of ectomycorrhizal fungi throughout the regeneration phase

One way for ectomycorrhizal fungi to persist during the period between a clear-cutting and establishment of the new stand could be to survive on the roots of seedlings or roots of other potential hosts that were left in the stand, or to temporarily inhabit the short-roots of the harvested trees. However, since small trees were cleared from all P plots before harvesting the old forest, this factor was probably of minor importance. That ectomycorrhizal fungi can colonize other hosts has been shown for species such as *Piloderma sp.* which also forms arbutoid mycorrhizas with *Arbutus menziesii* and *Arctostaphylos uva-ursi* (Zak 1976; Molina & Trappe, 1982). However, so far, the only host species in Scandinavian forests that has been shown to form arbutoid mycorrhiza is *Arctostaphylos uva-ursi*, which was absent in most plots and uncommon where it occurred. The extent to which ectomycorrhizal fungi can live in symbiosis with species such as *V. vitis-idae* or *V. myrtillus*, which were common in all stands in Siljansfors, is not known. Linderman & Call (1977) found that *Thelephora terrestris* formed ectomycorrhiza with *Vaccinium ovatum*. Interestingly, all five of the most frequent species found on mycorrhizal short-roots in Siljansfors (*Piloderma croceum,* the other Corticiaceae -like taxa, and the ascomycete *Cenococcum geophilum*) belong to taxa that form mycorrhizas with Ericaeae (Zak, 1976, Massicotte *et al*., 1993).

When a tree is cut, the mycelium of ectomycorrhizal fungi associated with its roots gradually die-off, reducing their ability to colonize seedlings by mycelial

growth. Furthermore, after dry periods in the summer or after freezing-thawing during winter, the viability of the mycelia is generally reduced. One way of prolonging survival would be to shift over to saprophytism, enabling them to, at least temporarily, utilize the short-roots of the harvested trees. Some ectomycorrhizal fungi can produce cellulases (e.g. Norkrans, 1950; Trojanowski *et al*., 1984) lignases (Trojanowski *et al*., 1984; Bending & Read, 1997) and polyphenol oxidases (Lindeberg, 1948). However, little is known about the extent to which ectomycorrhizal fungi can compete with saprophytic fungi in the early stages of decomposition of detached short-roots. On the other hand, although mycorrhizal fungi have lower enzyme activities than the more specialized saprophytic fungi (Lindeberg, 1948), short-roots colonized by mycorrhizal fungi with saprophytic capabilities could, perhaps, provide refuge until a new host is found.

Another important means of persistence on a site is in the form of soil sclerotia, e.g. *Cenococcum geophilum*. Mycorrhizal short-roots of *C. geophilum* were found in nearly all plots, as well as on short-roots of the seedlings in the bioassay study. In addition, sclerotia were observed in several of the soil cores from which short-root tips were sampled for the PCR-RFLP analyses. Dahlberg *et al*. (1997) estimated the standing biomass of *C. geophilum* sclerotia at 440 kg per hectare in a 100-year-old Norway spruce forest in south Sweden, which equaled the standing fungal biomass of mycorrhizal short-roots. Stenström (unpublished) found that *C. geophilum* formed mycorrhizas on seedlings that were planted on up to 20-year-old clear-cuts where regeneration had failed, suggesting that sclerotia, or perhaps other hosts (see above), may facilitate its survival on a site.

In the absence of suitable hosts, fungi can also survive in the form of spores or hyphae. Torres & Honrubia (1994), who tested the viability of spores obtained from a sporocarp suspension of 14 species of ectomycorrhizal fungi, found that storage at 3-4 ^{o}C or freezing at -15 ^{o}C caused an almost complete loss of viability for all but a few species. It seems reasonable to believe that for most species spore viability should decrease with time since the regeneration cutting. Spores produced by populations in surrounding stands, sclerotia, and the maintenance of mycelial growth via symbioses with other hosts should be more important. One example of a species whose mycelia seemed to have low viability once the mycorrhizal short-roots had been detached was *Piloderma croceum* (IV, see also Danielson & Visser, 1989). Even though the soil inocula used for inoculating the bioassay seedlings (IV) contained *Piloderma* rhizomorphs and *Piloderma* short-roots, almost no (<0.1%) *Piloderma* mycorrhizas were found on the short-roots of the bioassay seedlings, and none of the RFLP-types of the bioassay seedlings matched the RFLP patterns of *Piloderma croceum*. Since *P. croceum* occurred in all three stand types (P, S and O) it is likely that other factors largely determined its ability to persist on the site or to immigrate from surrounding stands.

Colonization of ectomycorrhizal fungi from surrounding stands
In primary successions, spore dispersal seems to be the important means of spread for certain ectomycorrhizal fungi (Cazares & Trappe, 1994; Gryta *et al.*, 1997). In forest nurseries, where conditions are similar to those in primary succession in several respects, spores from species of *Laccaria*, *Hebeloma*, *Rhizopogon* and *Suillus* have been used for inoculating seedlings (Castellano & Molina, 1989). *Thelephora terrestris* appears to spontaneously establish itself on the short-roots of seedlings in forest nursery soils worldwide (Mikola, 1970). Relatively little is known about the importance of spore dispersal in secondary successions compared with that of mycelial growth or spread via propagules. Studies carried out by Fries and co-workers showed that for some species spore germination occurred spontaneously under aseptic conditions, whereas for others spore germination could only be induced by exudates from tree roots (reviewed by Fries, 1987). This suggests that at least some species are adapted to germinate in the vicinity of roots (Fries, 1987). Dispersal of spores by mammals may also be important in primary habitats (Cazares & Trappe, 1994), as well as in secondary forests (Maser *et al.*, 1978). Certain fungi may be dispersed by incects (e.g. *Trichoderma harzianum* and *Henoticus serratus,* Wikars*,* 1997), but little is known about the relationships between ectomycorrhizal fungi and insects.

How important are spores for establishment within a stand? Using somatic incompatibility pairings of isolates of *Suillus bovinus* in four Scots pine stands, Dahlberg & Stenlid (1990) found that older forests contained fewer, but larger (up to 30 m in diameter), clones of *S. bovinus* compared with younger stands. They found no evidence that new individuals became established as the forest aged and therefore concluded that once *S. bovinus* had become established mycelial growth was probably the only means of spread within the stand. Similarly, Baar *et al*. (1994) found that genets of *L. bicolor* in a secondary, 17-year-old Scots pine stand in the Netherlands were up to 12 m in diameter. These studies suggest that mycelial growth in established forests may be more important than spore dispersal for the colonization of short-roots. Although few species have been investigated so far, making it difficult to generalize, the findings suggest that initial colonization during the early phases of the disturbance (e.g. type forest regeneration method) may determine whether a species will also occur in the older forest.

In conclusion, the large variety of survival and dispersal strategies found among ectomycorrhizal fungi might explain why so many species seemed to be unaffected by the type of forest regeneration method used. However, for those species that disperse mainly through mycelial growth and are negatively affected by large-scale disturbances that eliminate their hosts, the type of forest regeneration methods may be of importance. The study in Siljansfors suggests that some species may have been favored by shelterwood management (e.g. certain *Russula* spp. and *C. gentilis*). However, on the basis of sporocarp observations, none of these are considered as threatened in Sweden (Hallingbäck,

1994). The study in Siljansfors is one of very few studies made thus far where inventories of both mycorrhizal shorts-roots and sporocarp inventories have been carried out. However, it would be premature to conclude that the lack of response of most species to the forest regeneration methods studied in Siljansfors indicates that such methods have little influence on the species richness of mycorrhizal fungi. It is also possible that diversity will be positively affected by increasing the rotation period in managed forests, which will be discussed in the following section.

Succession of mycorrhizal fungi in forests

Introduction - Primary succession

Succession can be defined as "*the non-seasonal, directional and continuous pattern of colonization and extinction on a site by species populations*" (Begon *et al*., 1986). Thus community succession, i.e. the successive replacement of a species or group of species by other species, should be predictable. Primary succession takes place when a site has not previously been influenced by a community. Communities developing on newly exposed soils formed by retreating glaciers (Cooper, 1923; Chapin *et al*., 1994; Helm *et al*., 1996) and soils originating from volcanic eruptions (Allen, 1987) are typical examples of primary succession. Often, early successional species improve the originally harsh conditions. For instance, the N-status of the soil is improved by N-fixing plants; the organic matter content of the soil increases, and moisture conditions becomes more favorable, thereby enabling later successional species to become established (the facilitation model, Connell & Slatyer, 1977). However, early species can also partly or completely prevent the recruitment of late-arriving species (Sousa, 1979).

Secondary succession

Although primary succession is important, secondary succession, which occurs on sites that have already been influenced by a community, is the dominating pattern in most ecosystems. In contrast to the case of primary succession, the site may already be occupied by plants (and fungi) and contain propagules (seeds, spores and resting structures such as thick-walled hyphae and fungal sclerotia). In addition, conditions on such a site may be conducive to the establishment of late successional species. As a result, succession is much more unpredictable, and the distinction between early and late successional species is less apparent than in primary succession.

Secondary succession in ectomycorrhizal communities

One of the few studies that has attempted to identify both mycorrhizas and sporocarps in secondary forests of different ages was made by Visser (1995), who investigated ectomycorrhizal fungi in four *Pinus banksiana* [Lamb.] stands

(6, 41, 65 and 122 years old) that had regenerated after wildfire. She found that six unidentified Russula-like mycorrhizas were absent in the youngest stand, but accounted for about 20% of the number of mycorrhizas in all older stands, in agreement with sporocarp observations. Very few species present in the youngest stand were completely replaced in the older stands; instead, most species occurred in all four forests, and she concluded that succession defined as species replacement did not seem to have applied. Despite this, she maintained that both inventories "revealed a distinct sequence of mycorrhizal fungi with stand age consisting of early-stage,... multi-stage... and late-stage-fungi". However, no attempt was made to statistically analyze the mycorrhizal morphotype and sporocarp species frequencies owing to the high degree of variation in abundances of individual species (Visser, 1995).

The concept of "early-stage" versus "late-stage" fungi was first used by Mason *et al*. (1982) and Deacon *et al*. (1983), who found a progressive change in morphotypes and sporocarp formation with distance from the tree bases of birches planted in an agricultural brown-earth soil. This concept was criticized by Danielson (1984), who found that many fungi could be referred to as multi-stage, since they occurred in both stages. Since then, the concept has been applied in several studies of primary and secondary succession of mycorrhizal communities where fungal species have been placed in any of the three categories (*e.g*. Fox, 1986; Fleming, 1985; Gibson & Deacon, 1988; Visser, 1995; Helm *et al*., 1996). However, since the concept was mainly derived from observations made in connection with experiments under conditions typical of primary succession, its validity for secondary successions remains to be tested (Deacon & Fleming, 1992). Based on their study of the temporal sequence of ectomycorrhizal communities (sporocarps) in oak forests in the Netherlands, Keizer & Arnolds (1994) concluded that the "early-/ late-stage" classification was inappropriate. Instead, they suggested no fewer than five classes depending on age (plus one group with fungi whose sporocarp production seemed unaffected by age). Thus, there seems to be little consensus as to whether secondary succession of mycorrhizal fungi can be viewed as a species-replacement process, where the abundances of some species can be accurately predicted based on the successional phase.

Is there a secondary succession of mycorrhizal fungi as forests grow older?

In the study conducted in Siljansfors we examined a second hypothesis, i.e. that as the forest grows older mycorrhizal fungi communities undergo secondary succession. Was there a "*non-seasonal, directional and continuous pattern of colonization and extinction on a site by species populations*" (Begon *et al*., 1986)?

As described in previous sections, the surveys of mycorrhizas and sporocarps showed that most species were present in young as well as old forests. These results were in agreement with previous comparisons of ectomycorrhizal

communities (sporocarps and/or mycorrhizas) in secondary forests of different ages (Danielson, 1984; Hintikka, 1988; Visser, 1995). Further evidence suggesting that many species were able to colonize seedlings becoming established under the shelterwood or in the clear-cut stands was provided by the bioassay study (IV), where several species found on field-sampled mycorrhizal short-roots also formed mycorrhizas on the young seedlings. Thus, few species were completely replaced in the mycorrhizal communities, characterized on the basis of inventories of mycorrhizal short-roots or sporocarps. However, the multivariate analyses (ordination and partial redundancy analyses) suggested that there was a shift in the species composition of ectomycorrhizal fungi as the forest grew older. This indicates that there was a 'directional continuous pattern of colonization', but without any species being replaced. Visser (1995) came to the same conclusion in her study of ectomycorrhizal communities in the *P. banksiana* stands that had regenerated after wildfires. She suggested that this pattern may be a result of differences in growth rates between species and the competition exerted by other fungal species.

The study in Siljansfors suggests that factors influencing the regeneration and establishment of ectomycorrhizal species on a site may also be important. For instance, the ordination suggested that the abundance of some species in the genus *Russula* did not differ between shelterwood- regenerated stands and the old stands, whereas they were less common in the forests that were regenerated by planting on clear-cut sites. *P. croceum*, on the other hand, was abundant in all forest types, but colonized only a few (<0.1%) short-roots of the seedlings in the bioassay study. In contrast to *Piloderma croceum*, *Cenococcum geophilum* was abundant in stands of all ages and colonized short-roots readily in the bioassay study. The degree to which immigration from surrounding stands contributed to the establishment of the studied taxa is not known. However, the variety of responses of for instance *Russula*, *Piloderma* and *Cenococcum* to cutting intensity and soil treatments indicates that there are considerable differences among ectomycorrhizal fungi in life-history strategies, affecting their persistence on a site and their dispersal between sites.

Relations between mycorrhizas and sporocarps

Can sporocarp inventories alone accurately estimate the total number of species and their abundance, or is it also necessary to inventory the fungi present on the short-roots of the trees? To answer these questions it is necessary to return to the definition of the ectomycorrhizal community. If the aim is to determine the number of *all* species present on the mycorrhizal short-roots and their relative abundances on the short-roots, the answer for the ectomycorrhizal communities in Siljansfors and Norrliden is quite simply no (Fig. 5, 6, 7). The most important reason seems to be the large abundance of species that are easily overlooked in ”traditional” sporocarp inventories, i.e. where only species fruiting above ground and producing large, stipitate sporocarps are included. Such (over-looked) species can be found in the Corticiaceae, the members of which seemed to

dominate the mycorrhizal flora of short-roots in Siljansfors and in Norrliden. This is in agreement with Gardes and Bruns (1996), where observed discrepancies between the above- and below-ground occurrence of mycorrhizal fungi in a *Pinus muricata* forest were suggested to be related to the high abundance of thelephoroid mycorrhizas (e.g. *Tomentella sublilacina*) as well as to species-related differencies in the pattern of resource allocation to mycorrhizas and sporocarps, differences in carbon transfer efficiency and to the possibility that some abundant fruiters also had access to saprotrophic sources of carbohydrates. Similar results are illustrated in Fig. 6, where the mycorrhizal-tip colonization frequencies for the most common species in all P and S plots are compared with their corresponding abundances measured on the basis of sporocarp surveys. There is considerable variation between species in the relation between the relative abundance of their mycorrhizas and that of their sporocarps. For instance, *L. rufus* accounted for a much larger proportion of the total sporocarp production (dry weights) than *S. variegatus*, even though the two species seemed to occur in equal frequencies on the short-roots. Possible explanations for this discrepancy could be that a) temporal variation in the production of sporocarps or mycorrhizas combined with low sampling frequencies (mycorrhizas were only sampled once in one year, while sporocarps were monitored during two seasons) resulted in sampling-related bias; b) the depth distributions differed between species (mycorrhizas were only sampled from the organic horizon, while sporocarps may have been produced from mycorrhizas in the organic or mineral horizons); c) sporocarp production may be more dependent on a ”critical mass” of *mycelia* than on the number of mycorrhizas; d) physiological factors affecting carbon allocation (e.g. age of the fungal individual, Di Battista, et al., 1996, or age of the host) or e) the number of mycorrhizas analyzed in Siljansfors was low, which may have prevented any reliable observations from being made (owing to high spatial or temporal variation). For a further discussion, see e.g. Gardes & Bruns (1996).

The findings from the studies in Siljansfors and Norrliden suggest that the results of sporocarp inventories poorly reflect the species frequencies of mycorrhizal fungi on short-roots, and may, in fact, only reveal a small proportion of species present. However, since even in the most ambitious study, only a minute fraction of the total number of short-roots can be ever analyzed, sporocarp inventories can supplement mycorrhizal surveys. For instance, in control plots in Skogaby (III), the number of mycorrhizal short-roots per hectare in the organic horizon was estimated at $26.6\ 10^{9}$! This disproportion suggests that well-designed field studies (e.g. the use of sampling designs that take spatial and temporal variation in account) are a prerequisite in any attempts to investigate ectomycorrhizal communities.

Concluding remarks

The application of ammonium nitrate fertilizer seemed to affect the community structure in Norrliden much more radically compared with the two types of forest regeneration methods applied at Siljansfors. The control plots in Norrliden resembled the plots in Siljansfors (e.g. having *Piloderma* sp. and several *Cortinarius* species in common) much more closely than they resembled the N-treated plots in Norrliden. It is difficult to say to what extent the results obtained from the N-fertilization experiments can be used to predict the impacts of the ongoing atmospheric deposition of nitrogen on ectomycorrhizal fungi. However, the drastic changes that occurred in the species composition on mycorrhizal roots in the fertilized plots at Norrliden and the great impact that N-fertilizers have on the sporocarp formation of ectomycorrhizal fungi (e.g. Arnolds, 1991; Wiklund et al., 1995) are alarming.

Shelterwood regeneration seemed to favor total species richness, presumably by allowing new species to colonize or increase in abundance, while also permitting species from the older forest to persist. This suggests that it should be possible to maintain important groups of species, and perhaps even create diverse ectomycorrhizal communities, by using forest regeneration methods that mimic more natural disturbances.

However, more work is needed to reveal the underlying mechanisms shaping the structure of mycorrhizal communities and the extent to which the changes effected by forest management practices resemble the changes caused by natural disturbances.

Although the shelterwood regenerated and old forests appeared to have similar communities of ectomycorrhizal fungi, it is worth noting that many species occur at a low frequency. Since the number of forests and samples in this study were restricted, it is possible that functionally important species may have been missed. Since successional changes may also occur, the apparent similarities between the shelterwood regenerated and old forests may not be maintained with time. The value of mature forests as reservoirs for species that are important in later successional stages of the forest should therefore not be discounted.

We are only just beginning to get an idea of which species are most common in our forests and what their ecological functions are. It has become apparent that only a few species dominate, while the rarest species, each occuping only a small fraction of the total population of mycorrhizas, account for most of the species richness. Most physiological experiments have so far been conducted using ectomycorrhizal fungi that are easily cultured (e.g. *Laccaria*, *Hebeloma* and *Paxillus*), but only seldomly found in the field. More efforts should therefore be made to study the ecologically important species in our forests.

References:

Abuzinadah, R.A. and Read, D.J. 1986. The role of proteins in the nitrogen nutrition of ectomycorrhizal plants. I. Utilization of peptides and proteins by ectomycorrhizal fungi. *New Phytologist* **103**, 481-493.

Agerer R. 1987-1997. Colour Atlas of Ectomycorrhizae. 1st-8th edn. Schwäbisch Gmünd, Germany: Einhorn-Verlag.

Alexander, I.J., and Fairley, R.I. 1983. Effects of N fertilisation on populations of fine-roots and mycorrhizas in spruce humus. *Plant and Soil*. **71**: 49-53.

Allen, M.F. 1987. Re-establishment of mycorrhizas on Mount St. Helens: migration vectors. *Trans. Brit. Mycol. Soc*. **88**: 413-417

Allen, E.B., Allen, M.A., Helm, D.J., Trappe, J.M., Molina, R.M., Rincon, E. 1995. Patterns and regulation of mycorrhizal plant and fungal diversity. *Plant and Soil*: **170**: 47-62.

Antibus, R., K. and Sinsabaugh, R. L, 1993. The extraction and quantification of ergosterol from ectomycorrhizal fungi and roots. *Mycorrhiza*. **3**: 137-144.

Arnebrant, K. 1994. Nitrogen amendments reduce the growth of extramatrical ectomycorrhizal mycelium. *Mycorrhiza*, **5**: 7-15.

Arnebrant, K. 1996. Effects of nitrogen amendments on the colonization potential of some different ectomycorrhizal fungi grown in symbiosis with a host plant. *In*: Mycorrhiza in integrated systems. From genes to plant development. *Edited by* C. Azcon-Aguilar and J.M Barea. Commission of the European Union (COST 8.21). Luxembourg. pp. 71-74.

Arnolds, E. 1991. Decline of ectomycorrhizal fungi in Europe. *Agr. Ecosys. Environ.* **35**, 209-244.

Baar, J., Ozinga, W.A., & Kuyper, T. 1994. Spatial distribution of *Laccaria bicolor* genets reflected by sporocarps after removal of litter and humus layers in a *Pinus sylvestris* forest. *Mycol. Res*. **98**: 726-728.

Baar, J. and ter Braak, C.J.F. 1996. Ectomycorrhizal sporocarp occurence as affected by manipulation of litter and humus layers in Scots pine stands of different age. *Appl. Soil Ecol*. **4**: 61-73.

Begon, M., Harper, J.L. and Townsend, C.R. 1986. *Ecology: individuals, populations and communities.* Sunderland, Massachusetts: Sinauer Assoc., Inc.

Bending, G.D. & Read, D.J. 1997. Nitrogen mobilization from protein-polyphenol complexes by ericoid and ectomycorrhizal fungi. *Soil Biology and Biochemistry*. X: 000-000.

Bermingham, S., Maltby, L and Cooke, R.C. 1995. A critical assessment of the validity of ergosterol as an indicator of fungal biomass. *Mycol. Res*. **99**: 479-484

Björkman, E. 1942. Über die bedingungen der Mykorrhizabildung bei Kiefer und Fichte. Symb. Bot. Ups. 6, 2.

Botton, B., and Chalot, M. 1995. Nitrogen assimilation: enzymology in ectomycorrhizas. *In*: *Mycorrhiza- structure, function, molecular biology and biotechnology*. *Edited by* A. Varma and B. Hock. Springer-Verlag, Berlin. pp 325-363.

Boyd, R., Furbank, R. T. and Read, D.J. 1986. Ectomycorrhiza and the water relations of trees. In: *Physiological and Genetical Aspects of Mycorrhizae*. Eds: V. Gianinazzi-Pearson & S. Gianinazzi). INRA, Paris. pp. 698-693.

Brandrud, T. E. 1995. The effects of experimental nitrogen addition on the ectomycorrhizal fungus flora in an oligotrophic spruce forest at Gårdsjön, Sweden. *For. Ecol. Management* **71**, 111-122.

Bruns TD, White TJ, Taylor JW. 1991. Fungal molecular systematics. *Ann. Rev. Ecol. Syst.* **22**: 525-64.

Castellano, M. A. and Molina, R. 1989. Mycorrhizae. In: The Container Tree Nursery Manual. Vol. 5. Eds. T.D. Landis, R.W. Tinus, S.E. McDonald and J.P. Barnett. Agriculture Handbook 674. USDA Forest Service, Washington, DC, USA. pp. 101-167.

Cazares, E. and Trappe, J.M. 1994. Spore dispersal of ectomycorrhizal fungi on a glacier forefront by mammal mycophagy. *Mycologia*. **86**: 507-510.

Chapin, F.S., Walker L.R., Fastie C.L. and Sharman L.,C. 1994. Mechanisms of primary succession following deglaciation at Glacier Bay, Alaska. *Ecological Monographs*, **64**:149-175.

Clemensson-Lindell, A., and Persson, H. 1995. Fine-root vitality in a Norway spruce stand subjected to varying nutrient supplies. *Plant Soil*. **168-169**: 167-172.

Clements, F.E. 1905. (1977). Research Methods in Ecology. University Publishing Company, Lincoln, (reprinted 1977, Arno Press, New York).

Colpaert, J.V, Van Assche, J. A. and Luijtens, K. 1992. The growth of the extramatrical mycelium of ectomycorrhizal fungi and the growth response of *Pinus sylvestris* L. *New Phytol*. **120**, 127-135.

Connell, J.H. 1983. On the prevalence and relative importance of interspecific competition: evidence from field experiments. *American Naturalist*. **122**: 661-696.

Connell, J.H. and Slatyer, R.O. 1977. Mechanisms of succession in natural communities and their role in community stability and organisation. *American Naturalist*. **111**: 1119-1144.

Cooke, W.B. 1953. A survey of literature on fungus sociology and ecology - II. *Ecology*. **34**: 211-232.

Cooper, W.S. 1923. The recent ecological history of Glacier Bay, Alaska: I. The interglacial forests of Glacier Bay. *Ecology*. **4**: 93-128.

Cowles, H. C. 1901. The physiographic ecology of Chicago and vicinity; A study of the origin, development, and classification of plant societies. *Botanical Gazette*. **31**: 73-108.

Dahlberg A 1991 Ectomycorrhiza in coniferous forest: structure and dynamics of populations and communities. Ph.D. thesis, Dept. of Forest Mycology and Pathology, Sw. Univ. Agr. Sc., Uppsala, Sweden.

Dahlberg, A. and Stenlid, J. 1990. Population structure and dynamics in *Suillus bovinus* as indicated by spatial distribution of fungal clones. *New Phytologist*. **115**: 487-493.

Dahlberg, A. and Stenlid, J. 1994. Size, distribution and biomass of genets in populations of *Suillus bovinus* (L.:Fr.) Roussel revealed by somatic incompatibility. *New Phytol.* **128**, 225-234.

Dahlberg, A. and Stenström, E. 1991. Dynamic changes in nursery and indigenous mycorrhiza of *Pinus sylvestris* seedlings planted out in forests and clearcuts. *Plant and Soil*. **136**: 73:86.

Dahlberg, A., Jonsson, L. and Nylund, J.-E. 1997. Species diversity and distribution of biomasss above and below ground among ectomycorrhizal fungi in an old Norway spruce forest in south Sweden. *Can. J. Bot*. (in press).

Danell, E. 1994. *Cantharellus cibarius*: Mycorrhiza Formation and Ecology. Thesis. Uppsala University.

Danielson, R.M. 1984. Ectomycorrhizal associations in jack pine stands in northeastern Alberta. *Can. J. Bot.* **62**: 932-939.

Danielson, R.M., and Visser, S. 1989. Effects of forest soil acidification on ectomycorrhizal and vesicular-arbuscular mycorrhizal development. *New Phytol.* **112**: 41-47.

Deacon, J.W., Donaldsson, S.J., & Last, F.T. 1983. Sequences and interactions of mycorrhizal fungi on birch. *Plant and Soil*. **71**: 257-262.

Deacon, J.W. & Fleming, L.V. 1992. Interactions of ectomycorrhizal fungi. *In*: Mycorrhiza functioning. *Edited by*: M. Allen. Chapman and Hall, London, UK. pp. 134-160.

Derbsch, H. 1987. Die Blätter- und Pilzflora des Völklinger Kreuzberggebietes. In. H. Derbsch and J.A. Schmitt (Eds). *Atlas der Pilze des Saarlandes*, Teil 2.: Nachweise, Ökologie, Vorkommen und Bescreibungen: 3-22. Saarbrücken.

Di Battista, C., Selosse, M.A., Bouchard, D., Stenström, E., and Le Tacon, F. 1996. Variations in symbiotic efficiency, phenotypic characters and ploidy level among different isolates of the ectomycorrhizal basidiomycete Laccaria bicolor strain S 238. *Mycol. Res*. **100**: 1315-1324.

Downes, G.M., Alexander, I.J., and Cairney, J.W.G. 1992. A study of spruce (*Picea sitchensis* (Bong) Carr.) ectomycorrhizas. I. Morphological and cellular changes in mycorrhizas formed by *Tylospora fibrillosa* (Burt) Donk and *Paxillus involutus* (Batsch ex Fr.) Fr. *New Phytol*. **122**: 141-152.

Egli, S., Amiet, R., Zollinger, M. and Schneider, B. 1993. Characterization of *Picea abies* (L.) Karst. ectomycorrhizas: discrepancy between classification according to macroscopic versus microscopic features. *Trees*. **7**, 123-129.

Ek, H. 1993. Nitrogen acquisition, transport and metabolism in intact ectomycorrhizal associations studied by 15N stable isotope techniques. PhD-thesis. Dept. of Ecology, Lund University, Sweden.

Ellenberg, H., Weber, H.E., Düll, R., Wirth, V. Werner, W. and Paulisson, D. 1991. Zeigewerte von Pflanzen in Mitteleuropa. Scripta Geobotanica XVII. Göttingen, 248 pp. (with english summary).

Eriksson J., Hjortstam K., Ryvarden, L. 1981. *The Corticiaceae of North Europe. Vol 6. Phlebia-Sarcodontia.* Oslo: Fungiflora.

Erland, S. 1990. Effects on liming on pine ectomycorrhiza. PhD-thesis. Dept. of Ecology, Lund University, Sweden.

Erland, S. 1995. Abundance of *Tylospora fibrillosa* ectomycorrhizas in a South Swedish spruce forest measured by RFLP analysis of the PCR-amplified rDNA ITS region. *Mycol. Res*. **99**: 1425-1428.

Erland, S. and Söderström, B.1990. Effects of liming on ectomycorrhizal fungi infecting Pinus sylvestris L. I. Mycorrhizal infection in limed humus in the laboratory and isolation of fungi from mycorrhizal roots. . *New Phytol*. **115**: 675-682

Erland, S., Söderström, B., and Andersson, S. 1990. Effects of liming on ectomycorrhizal fungi infecting *Pinus sylvestris* L. II. Growth rates in pure culture at different pH values compared to growth rates in symbiosis with the host plant. *New Phytol*. **115**: 683-688.

Eveling, D.W., Wilson, R.N., Gillespie, E.S., and Bataille, A. 1990. Environmental effects on basidioma counts over fourteen years in a forest area. *Mycol. Res*. **94**: 998-1002.

Evers, F., H., and Huettl, R., F. 1991. A new fertilisation strategy in declining forests. *In* Zöttl H W and Huettl R F (eds.) *Management of nutrition in forests under stress*. Dordrecht: Kluwer Academic Publishers,. pp. 495-508.

Falkengren-Grerup, U., Linnermark, N., & Tyler, G. 1987. Changes in acidity and cation pools of south Swedish soils between 1949 and 1985. *Chemosphere*. **16**: 10-12.

FAO-UNESCO. 1988. *Soil Map of the World*. Revised legend. FAO, Rome.
Finlay, R.D., Ek, H., Odham, G. and Söderström, B. 1988. Mycelial uptake, translocation and assimilation of 15N-labelled ammonium by ectomycorrhizal *Pinus sylvestris* plants infected with four different ectomycorrhizal fungi. *New Phytol*. **110**: 59-66.
Finlay, R.D., Ek, H., Odham, G. and Söderström, B. 1989. Uptake, translocation and assimilation of nitrogen from 15N-labelled ammonium and nitrate sources by intact ectomycorrhizal systems of Fagus sylvatica infected with *Paxillus involutus*. *New Phytol*. **113**: 47-55.
Fleming, L.V. 1983. Effects of soil trenching and coring on the formation of ectomycorrhizas on birch seedlings around mature trees. *New Phytol.* **98**: 143-153.
Fleming, L.V. 1985. Experimental study of sequences of ectomycorrhizal fungi on birch (Betula sp.) seedling root systems. *Soil Biol. Biochem*. **17**: 591-600.
Fox, F.M. 1986. Groupings of ectomycorrhizal fungi of birch and pine, based on establishment of mycorrhizas on seedlings from spores in unsterile soils. *Trans. Brit. Mycol. Soc*. **87**: 371-380.
Frank, A.B. 1885. Über die auf Wurzelsymbiose beruhende Ernährung gewisser Baume durch unterirdische Pilze. *Berichte der Detsche Botanische Gesellschaft*. **3**: 128-45.
France, R.C. and Reid, C.P.P. 1983. Interactions of nitrogen and carbon in the physiology of ectomycorrhizae. *Can. J. Bot*. **61**: 964-984.
Garbaye, J. 1994. Helper bacteria: a new dimension to the mycorrhizal symbiosis. *New Phytol.* **128**: 197-210.
Gardes M, Bruns T. 1993. ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. *Molecular Ecology* **2**: 113-118.
Gardes M, Bruns T. 1996. Community structure of ectomycorrhizal fungi in a *Pinus muricata* forest: above- and below-ground views. *Can. J. Bot.* **74**: 1572-1583.
Gardes M., White, T.J, Fortin, J.A., Bruns, T.D, Taylor, J.W. 1991. Identification of indigenous and introduced symbiotic fungi in ectomycorrhizae by amplification of nuclear and mitochondrial ribosomal DNA. *Can. J. Bot.* **69**: 180-190.
Gibson, F. and Deacon, J.W. 1988. Experimental study of establishment of ectomycorrhizas in different regions of birch root systems. *Trans. Brit. Mycol. Soc*. **91**: 239-251
Giovannetti, G. Roth-Bejerano, N., Zanini, E., Kagan-Z. 1994. Truffles and their cultivation. *Horticultural Rev*. **16**: 71-107.
Gleason, H. A. 1939. The Individualistic Concept of the Plant Accossiation. *Bulletin of the Torrey Botanical* Club. **53**: 7-26.
Grime, J.P. 1974. Vegetation classification by reference to strategies. *Nature*. **250**: 26-30.
Gronbach E. 1988. Charakterisierung und Identifizierung von Ektomykorrhizen in einem Fichtenbestand mit Untersuchungen zur Merkmalsvariabilität in sauer beregneten Flächen. *Bibliotheca Mycologica* **125**. 1-217.
Grosse-Brauckmann H and Grosse-Brauckmann, G. 1978. Zur Pilzflora der Umgebung von Darmstadt vor 50 Jahren und heute (Ein Vergleich der floristischen Befunde Franz Kallenbachs aus der Zeit von 1918 bis 1942 mit dem gegenwärtigen Vorkommen de Arten. *Z. Mykol*. **44**: 257- 269.
Gryta H., Debaud, J.C., Effosse, A., Gay, G. & Marmeisse, R. 1997. Fine scale structure of populations of the ectomycorrhizal fungus *Hebeloma cylindrosporum* in coastal sand dune forest ecosystems. *Molecular Ecology*. **6** : 353-364.

Gulden, G. Hoiland, K., Bendiksen, K., Brandrud, T.E., Foss, B.S., & Jenssen, H.B. 1992. Macromycetes and air pollution: mycocoenological studies in oligotrophic spruce forests in Europe. Bibl. Mycol. Bd. 144. Stuttgart: J. Cramer.
Haas, H. 1932-33. Die bodenbewohenden Grosspilze in den Waldformation einiger Gebiete von Württemburg. *Beih. Bot. Centralblatt.* **50**: 35-134.
Hallbäcken, L. and Tamm, C.O. 1986. Changes in soil acidity from 1927 to 1982-1984 in a forest area of South-West Sweden. *Scand. J. For. Res*. **1**: 219-232.
Hallingbäck, T. 1994. *The macrofungi of Sweden*. Uppsala: Swedish Threatened Species Unit, Swedish University of Agricultural Sciences.
Hannertz, M., and Hånell, B. 1997. Effects on the flora in Norway spruce forests following clearcutting and shelterwood cutting. *For. Ecol. Manage.* **90**: 29-49.
Hansen L, Knudsen H, 1992. Nordic Macromycetes vol. 2. *Polyporales, Boletales, Agaricales, Russulales*. Copenhagen: Nordsvamp.
Hartig, T. 1840. Voll standige Naturgeschichten der forstlichen Culturpflanzen Deutschlands. A. Forstnersche Verlagbuchhandlung, Berlin.
Helm, D.J, Allen,E.B. and Trappe, J.M. 1996. Mycorrhizal chronosequence near Exit Glacier, Alaska. *Can. J. Bot*. **74**:1496-1506.
Hintikka, V. 1988. On the macromycete flora in oligotrophic pine forests of different ages in South Finland. *Acta Bot. Fennica.* **136**: 89-94.
Hjortstam, K., Larsson, K.H., and Ryvarden L. 1988. The Corticiaceae of North Europe. Vol 8. *Phlebiella, Thanatephorus-Ypsilonidium*. Oslo: Fungiflora.
Holmen, H., Nilsson, Å., Popovic, B. and Wiklander, G. 1976. The optimum nutrition experiment in Norrliden. A brief description of an experiment in a young stand f Scots pine. (Pinus silvestris L.). Dept. For. Ecol. and For. Soils. Research notes 26.
Hora, F.B. 1959. Quantitative experiments on toadstool production in woods. *Trans. Brit. Mycol. Soc*. **42**: 1-14.
Ingleby K, Mason PA, Last FT, Fleming LV. 1990. Identification of ectomycorrhizas. ITE research publication no. 5. Institute of Terrestrial Ecology, Edinburgh, Scotland.
Johnson, B.N., and McGill, W.B. 1990. Comparison of ergosterol and chitin as quantitative estimates of mycorrhizal infection and *Pinus contorta* seedling response to inoculation. *Can. J. Bot*. **20**: 1125-1131.
Keizer, P.J; Arnolds, E. 1994. Succession of ectomycorrhizal fungi in roadside verges planted with common oak (Quercus robur L.) in Drenthe, The Netherlands. *Mycorrhiza*. **4**: 147-159.
Keller, G. 1996. Utilization of inorganic and organic nitrogen sources by high-subalpine ectomycorrhizal fungi of *Pinus cembra* in pure culture. *Mycol. Res*. **100**: 989-998.
Kelley, K.R. & Stevenson, F.J. 1996. Organic forms of N in soil. Humic substances in terrestrial ecosystems. Ed: A. Piccolo. Elsevier Science. pp. 407-427.
Laiho, O. 1970. *Paxillus involutus* as a mycorrhizal symbiont on forest trees. *Acta Forestalia Fennica*. **106**: 1-73.
Lange, M. 1978. Fungus flora in August. Ten year observation in a Danish beech wood districts. *Bot Tidsskr*. **73**, 21-54.
Last, F.T., Mason, P.A., Ingleby, K. & Fleming, L.V. 1984. Succession of fruitbodies of sheathing mycorrhizal fungi associated with *Betula pendula*. *Forest Ecology and Management*. **9**: 229-234.
Lehto, T. 1984. Effect of liming on the mycorrhizas on pine. Folia For, no 609, 20 pp.
Lindeberg, G. 1948. On the occurrence of polyphenol oxidases in soil inhabiting Basidiomycetes. *Physiologia Plantarum*. **1**: 196-205.

Linderman, R.G. & Call, C.A. 1977. Enhanced rooting of woody plant cuttings by mycorrhizal fungi. *J. Am. Soc. Horticultural Sci*. 1977, **102**: 629-632
Linderman, R. G. 1988. Mycorrhizal interactions with the rhizosphere microflora: The mycorrhizosphere effect. *Phytopathology*. **78**: 366-371.
Lorillou, S., Botton, B. & Martin, F. 1996. Nitrogen source regulates the biosynthesis of NADP-glutamate dehydrogenase in the ectomycorrhizal basidiomycete *Laccaria bicolor*. *New Phytologist*. **132**: 289-296.
Mac Arthur, R. & Wilson, E.O. 1967. *The Theory of Island Biogeography*. Princeton, NJ, USA. Princeton University Press.
MacMahon, J.A., Schimpf, D.J., Andersen, D.C., Smith, K.G., Bayn, R.L. Jr. 1981. An organism centered approach to some community and ecosystem concepts. *J. Theor. Biol.* **88**: 287-307.
Magurran, A.E. 1988. Ecological diversity and its measurement. London : Croom Helm, 1988. Princeton University Press.179 p.
Majdi, H., and Bergholm, J. 1995. Effects of enhanced supplies of nitrogen and sulphur on rhizosphere and soil chemistry in a Norway spruce stand in SW Sweden. *Water Air Soil Pollut.* **85**: 1777-1782.
Majdi, H., and Kangas, P. 1997. Demography of fine roots in response to nutrient applications in a Norway spruce stand in SW Sweden. Ecoscience, **4**: 000-000 (April issue).
Majdi, H., and Persson H., 1995. A study on fine-root dynamics in response to nutrient applications in a Norway spruce stand using the minirhizotron technique. Z. Pflanzenernaehr. Bodenk. **158**: 429-433.
Markkola, A.M., Ohtonen, R., Tarvainen, O., and Ahonen-Jonnarth, U. 1995. Estimates of fungal biomass in Scots pine stands on an urban pollution gradient. *New Phytol*. **131**: 139-147.
Martin, F., Delaruelle, C. and Hilbert, J.L. 1990. An improved assay to estimate fungal biomass in ectomycorrhizas. *New Phytol*. **94**: 1059-1064.
Martin, F., and Botton, B. 1997. Nitrogen metabolism of ectomycorrhizal fungi and ectomycorrhiza. In: Mycorrhiza: A Synthesis. *Adv. Plant Pathol*. **8**.
Maser, C., Trappe, J.M. & Nussbaum, R.A. 1978. Fungal-small mammal interrelationships with emphasis on Oregon coniferous forests. *Ecology*. **59**: 799-809.
Mason, P.A., Last, F.T., Pelham, J. and Ingleby, K. 1982. Ecology of some fungi associated with an ageing stand of birches (Betula pendula and B. pubescens) *For. Ecol. Manag*. **4**: 19-39.
Massicotte, H.B., Peterson, R.L., Ackerley, C.A., and Melville, L. H. 1993. Structure and ontogeny of *Betula alleghanensis-Pisolithus tinctoris (*Pisolithacae) and *Piloderma bicolor* (Corticiaceae). *Mycorrhiza* **3**. 1-11.
Mehus, H. 1986. Fruitbody production of macrofungi in some Norwegian forest types. *Nord. J. Bot*. **6**: 679-701.
Melin, E. 1925. *Untersuchungen über die Bedeutung der Baummykorrhiza*. Jena, Germany: Gustav Fischer.
Menge, J.A. and Grand, L. F. 1978. Effect of fertilization on production of epigeous basidiocarps by mycorrhizal fungi in loblolly pine plantations. Can. J. Bot. 56, 2357-2362.
Miller, O.K., Jr. 1983. Ectomycorrhizae in the *Agaricales* and *Gasteromycetes*. *Can. J. Bot.* **61**: 909-916.

Molina, R., Massicotte, H., Trappe, J.M. 1992. Specificity phenomena in mycorrhizal symbiosis: Community-ecological consequenses and practical implications. In: Allen MF, ed. *Mycorrhizal Functioning - an integrated plant-fungal process.* New York: Chapman and Hall, 357-423.

Mullis, K.B. and Faloona, F.A. 1987. Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. *Methods Enzymology* **155**: 335-50.

Newell, S.Y., Arsuffi, T.L. and Fallon, R.D. 1988. Fundamental procedure for determining ergosterol content of decaying plant material by liquid chromatography. *Appl. Environ. Microbiol*. **54**: 1876-1879.

Nilsson, L.O. and Wiklund, K. 1994. Nitrogen uptake in a Norway spruce stand following ammonium sulphate application, fertigation, irrigation, drought and nitrogen-free fertilization. *Plant Soil*, **164**, 221-229.

Norkrans, B. 1950. Studies in growth and cellulolytic enzymes of Tricholoma**.** *Symbolae Botanicae Uppsaliensis***.** **11**. pp. 126.

Nylund, J.E. and Wallander, H. 1992. Ergosterol analysis as a means of quantifying mycorrhizal biomass. In *Methods in Microbiology* vol 24. (ed. by J.R. Norris, D.J. Read & A.K. Varma). London: Academic Press.

Nylund, J.,E., Dahlberg, A., Högberg, N., Kårén, O., Grip, K., and Jonsson, L. 1995. Methods for studying species composition of mycorrhizal fungal communities in ecological studies and environmental monitoring. *In*: Stocchi V, Bonfante P, Nuti M, eds. *Biotechnology of Ectomycorrhizae*. New York: Plenum Press. 229-240.

Paolocci, F., Cristofari, E., Angelini, P., Granetti, B., and Arcioni, S. 1995. The polymorphism of the rDNA region in typing ascocarps and ectomycorrhizae of truffle species. *In*: Stocchi V, Bonfante P, Nuti M, eds. *Biotechnology of Ectomycorrhizae.* New York: Plenum Press. 171-184.

Pielou, E.C. 1966. Species diversity and pattern diversity in the study of ecological succession. *Journal of Theoretical Biology*. **10**: 370-383.

Plassard, C., Mousain, D. and Salsac, L. 1983. Estimation of chitin on ectomycorrhizas of maritime pine (*Pinus pinaster*) associated with *Pisolithus tinctorius*: evaluation of the mycelial mass and mycorrhiza formation. *Can. J. Bot*. **61**: 692-699.

Ramstedt, M. & Söderhäll, K. 1983. Proteinase, phenoloxidase and pectinase activities in mycorrhizal fungi. *Trans. Br. Mycol. Soc*. **81**: 157-161.

Read, D.J. 1987. In support of Frank's organic nitrogen theory. *Angewandt Botanik*. **61**: 25-37.

Read, D.J. 1992. The mycorrhizal mycelium. *In*: M.F. Allen (Ed.): *Mycorrhizal functioning. An integrated plant-fungal process*. London: Chapman & Hall. p. 102-134.

Ritter, G., and Tölle, H. 1978. Stickstoffdüngung in Kiefernbeständen und ihre Wirkung auf Mykorrhizabildung und Fruktifikation der Symbiosepilze. Beitr. Forstwirtschaft. **4**: 162-166.

Robertson, N.F. 1954. Studies on the mycorrhiza of *Pinus sylvestris*. *New Phytologist*. **53**: 253-283.

Romell, L.G. 1938. A trenching experiment in spruce forest and its bearing on problems of mycotrophy. *Sv. Bot. Tidskr*. **32**: 89-99.

Rousseau, J.V.D., Sylvia, D.M., and Fox, A.J. 1994. Contribution of ectomycorrhiza to the potential nutrient-absorbing surface of pine. *New Phytol*. **128**: 639-644.

Rühling, Å., and Tyler, G. 1991. Effects of simulated nitrogen deposition to the forest floor on the macrofungal flora of a beech forest. *Ambio*, **20**: 261-263.

Salmanowicz, B. and Nylund, J.E. 1988. High performance liquid chromatography determination of ectomycorrhizal infection in Scots pine. *Eur. J. For. Patol*. **18**: 291-298.
Seitz, L.M.H.E, Mohr, R. Burroughs and Sauer, D.B. 1977. Ergosterol as an indicator of fungal invasions in grains. *Cereal Chem*. **54**: 1207-1217.
Smith, S.E., and Read, D.J. 1997. *Mycorrhizal symbiosis*. London: Academic press.
Sneath, P.H.A, Sokal, R.R. 1973. *Numerical Taxonomy.* San Francisco: W.H. Freeman.
Sousa, W.P. 1979. Experimental investigation of disturbance and ecological succession in a rock intertidal algal community. *Ecological Monographs*. **49**: 227-254.
Såstad, S.M. and Jenssen, H.B. 1993. Interpretation of regional differences in the fungal biota as effects of atmospheric pollution. *Mycol. Res*. **97**: 1451-1458.
Tamm, C.O. 1991. Nitrogen in terrestrial ecosystems. Questions of productivity, vegetational changes and ecosystem stability. *Ecological Studies* **81**. Berlin: Springer-Verlag.
Termorshuizen, A.J. 1993. The influence of nitrogen fertilisers on ectomycorrhizas and their fungal carpophores in young stands of *Pinus sylvestris*. *For. Ecol. Manage*. **57**: 179-189.
Termorshuizen, A.J., and Schaffers, A.P. 1987. Occurrence of carpophores of ectomycorrhizal fungi in selected stands of *Pinus sylvestris* in The Netherlands in relation to stand vitality and air pollution. *Plant and Soil*. **104**: 209-217.
Termorshuizen, A.J., and Schaffers, A.P. 1990. Succession of mycorrhizal fungi in stands of Pinus sylvestris in the Netherlands. *Agr. Ecosyst. Environ*. **28**: 503-507
Tilman, D. 1982. *Resource Competition and Community Structure*. Princeton, NJ: Princeton University Press.
Trojanowski, J., Haider, K. and Hüttermann, A. 1984. Decomposition of 14C-labelled lignin, holocellulose and lignocellulose by mycorrhizal fungi. *Archives Microbiology*. **139**: 202-206.
Tyler, G. 1985. Macrofungal flora of Swedish beech forest related to soil organic matter and acidity characteristics. *For. Ecol. Manage*. **10**: 13-29.
Wallander, H. 1995. A new hypothesis to explain allocation of dry matter between mycorrhizal fungi and pine seedlings in relation to nutrient supply. *Plant Soil*, **168-169**, 243-248
Wallander, H. and Nylund, J.E. 1992. Effects of excess nitrogen and phosphorous starvation on the extramatrical mycelium of ectomycorrhizas of *Pinus sylvestris* L. *New Phytol*. **120**, 495-503.
Wang, C.J.K. and Wilcox, H.E. 1985. New species of ectomycorrhizal and pseudomycorrhizal fungi: *Phialophora finlandia, Chloridium paucisporum* and *Phialosephala fortinii*. *Mycologia* **77**, 951-958.
Watling, R. 1997. The business of fructification. *Nature*. 385: 299-300.
West, A.W., Grant, W.D, Sparling, G.P. 1987. Use of ergosterol, diaminopimelic acid and glucosamine contents of soils to monitor changes in microbial populations. *Soil Biol. Biochem*. **19**: 607-612.
White, T.J, Bruns, T., Lee, S., and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ. eds. *PCR Protocols: a Guide to Methods and Applications.* New York: Academic Press, 315-322
Whittaker, R.H. 1965. Dominance and diversity in land plant communities. *Science*. **147**: 250-260.
Whittaker, R.H. 1970. Communities and Ecosystems. New York: MacMillan.

Wiklund, K., Nilsson, L.O., and Jacobsson, S. 1995. Effect of irrigation, fertilization, and artificial drought on sporocarp production in a Norway spruce stand. *Can. J. Bot*. **73**: 200-208.

Wilkins, W.H. 1948. Survey of literature on fungus sociology and ecology. *Ecology*. **29**: 376-383.

Visser, S. 1995. Ectomycorrhizal fungal succession in jack pine stands following wildfire. *New Phytol*. **129**: 389-401.

Vogt, K.A., Grier, C.C., Meier, C.E., and Edmonds, R.L. 1982. Mycorrhizal role in net primary production and nutrient cycling in *Abies amabilis* ecosystems in western Washington. *Ecology*, **63**: 370-380.

Vogt, K.A., Bloomfield, J., Ammirati, J.F. and Ammirati, S.R. 1992. Sporocarp production by basidiomycetes with emphasis on forest ecosystems. In: Carroll CG, Wicklow DT. eds. The fungal community, its organization and role in the ecosystem. New York: Marcel Dekker, 563-581.

Väre, H. Ohenoja, E., and Ohtonen, R. 1996. Macrofungi of oligotrophic Scots pine forests in northern Finland. *Karstenia*. **36**: 1-18.

Weete, J.D., and Weber, D.J. 1980. Lipid biochemistry of fungi and other organisms. New York, Plenum Publishing Corp.

Wikars, L.O. 1997. Effects of forest fire and the ecology of fire-adapted insect. PhD-thesis. Uppsala University.

Wilcox, H.E. 1968. Morphological studies of the roots of red pine, Pinus resinosa. II. Fungal colonisation of roots and development of mycorrhizae. *Am. J. Bot*. **55**: 686-700.

Wästerlund, I. 1982. Försvinner tallens mykorrhizasvampar vid gödsling? *Sv. Bot. Tidsskr*. **76**: 411-417.

Yamada, A. and Katsuya, K. 1996. Morphological classification of ectomycorrhizas of *Pinus densiflora. Mycoscience* **37**: 145-155.

Zak, B. 1973. Classification of ectomycorrhizae. In: Ectomycorrhizae (Eds. G.C. Marks, T.T Kozlowski). New York, USA: Academic Press. pp. 43-78.

Zak, B. 1976. Pure culture synthesis of Pacific madrone ectendomycorrhizae. *Mycologia*. **68**: 362-369.

Acknowledgements

I am sincerely grateful to my supervisor, Jan-Erik Nylund, who introduced me to the fascinating world of mycorrhizal fungi. Thank you for your support, enthusiasm and guidance through an endless sea of brownish, grey-brownish and brown-greyish mycorrhizas on the way to completing this thesis!

I am indebted to Anders Dahlberg, Lena Jonsson, Ulla Ahonen-Jonnarth, Håkan Wallander, Roger Finlay, Nils Högberg and all other colleagues at the department for your help with the field and lab work as well as with the preparation of the papers and this thesis summary, for inspiring discussions and – most importantly –for creating a wonderful atmosphere. I also want to sincerely thank Karin Grip and Maria Jonsson, who made this thesis possible by their hard work in the molecular lab. Ulf Olsson helped me with statistics. Dan Broström and Kjell Olofsson carried out the sporocarp inventories at Siljansfors and Norrliden. Ulf Johansson helped me with field work at Skogaby. Christer Karlsson helped me to find suitable stands in Siljansfors. Hugues Massicotte helped me to classify the mycorrhizas dealt with in paper II.Elisabet Wennberg assisted with the HPLC analyses. Peter Roberntz made all the drawings in this thesis. David Tilles edited my English in papers II and IV and in the thesis. Jim Trappe and friends helped to make my stay in Corvallis memorable. Finally, I am grateful to the following herbaria and persons for providing herbarium material: Botanical Garden & Museum at University of Oslo (Gro Gulden), Botanical Museum and Library at University of Copenhagen (Henning Knudsen), Department of Systematic Botany at University of Gothenburg (Stig Jacobsson), Fytoteket at the University of Uppsala (Svengunnar Ryman). Dept. of Biology at University of Oulu (Esteri Ohenoja), Jan Vesterholt and Stig Svensson.

To my parents: None of this work would have been possible without your love and support.

Finally, my love to Anna, for your support and patience.