The effects of forestry practices on ectomycorrhizal fungal communities and seedling establishment

Integrated studies on biodiversity, podzol profile, clear-cut logging impacts and seedling inoculation

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Front cover picture: Hyytiälä forest site (top left), photo J. Heinonsalo. Clear-cut logging at Hyytiälä (top right), photo H. Ilvesniemi. Podzol profile in Hyytiälä (bottom left), photo M. Färdig. St. Quirin field site (bottom right), photo J. Heinonsalo. *Suillus*-morphotype (in the center), photo J. Heinonsalo.

Abstract

Ectomycorrhizal fungi form symbioses with all the major tree species in the boreal forest zone and they are of key importance in nutrient acquisition, plant protection against root pathogens and drought stress. Their diversity and the impacts of forestry practises on these highly important organisms is therefore of great interest.

The aim of the study was to describe ectomycorrhizal biodiversity in boreal forest soils, and to determine biodiversity responses to different forestry practises. A specific focus was on the carbon allocation, bacterial carbon source utilization and vertical ectomycorrhizal species distribution in the podzol profile. Additionally, inoculation performance of certain, selected ectomycorrhizal fungal isolates obtained from the microcosm and field experiments was tested, and the performance and community-level influence of inoculated ectomycorrhizal fungal and mycorrhizal helper bacterial strains, a combination which is already in commercial use in France, was also investigated under field conditions.

Three microcosm experiments, one combined tube and pot experiment and two field experiments were accomplished. All experiments were performed in a Scots pine forest in southern Finland, or using soil collected from that forest, except the second field experiment, which was carried out in the Vosges region in France. Radiolabelled $^{14}CO_2$ was used in the carbon allocation study; Biolog® microtitre plates were used in the bacterial carbon source utilization experiments; and gross morphotyping following PCR-RFLP fingerprinting and sequencing were used in all the studies dealing with ectomycorrhizal identification.

Photosynthetically fixed carbon was shown to be equally distributed in roots and mycorrhizas in humus, E- and B-horizons suggesting that roots and ectomycorrhizal fungi significantly support biological activity in podzol horizons. The data also support theories of the important roles of ectomycorrhizal fungi in podzolization and weathering processes. Congruently, bacterial communities associated with roots and ectomycorrhizas utilised a wide range of carbon souces, measured using CLPP (community level physiological profiling) analysis, suggesting their active role in E- and B-mineral horizons.

Root-associated ectomycorrhizal fungal communities of the seedlings growing in the field and microcosms were diverse: in total, 53 taxa were determined. Shifts in community structure occured after clear-cut logging and podzol horizon-specific species were identified. Four taxa (representing in most cases a single fungal species) were dominant in the field, namely an unknown *Piloderma* sp., *Suillus variegatus*, *Phialocephala fortinii*-aggregate and *Cenococcum geophilum*. Based on the field experiment, it is concluded that the shift in ectomycorrhizal community structure observed in the seedling roots after clear-cut logging is not due to the lack of inoculum in the clear-cut soil but due to changes in the soil environment.

It was shown both in tube and pot experiments using Finnish forest isolates, and in the French field experiment using well-studied fungal and bacterial strains, that seedling inoculation with ectomycorrhizal fungi can have a positive impact on seedling growth even though no detectable differences in indigenous bacterial and fungal communities can be found.

Gross morphotyping following PCR-RFLP fingerprinting and sequencing was shown to be a suitable method for forest-scale ectomycorrhizal biodiversity studies. Ectomycorrhizal fungal diversity detected in microcosm experiments was shown to mimic relatively well the diversity observed in the field experiment, although most of the rare species identified in the field experiment were not found in the laboratory conditions.

Tiivistelmä

Ektomykorritsasienet muodostavat symbioosin pohjoisen havumetsävyöhykkeen metsäpuiden kanssa. Sienijuuren muodostava symbioosi on kasveille elintärkeä, sillä se auttaa puita ravinteidenotossa, suojaa kasvitauteja vastaan sekä parantaa kuivuudensietoa. Väitöskirjatyön tarkoituksena oli selvittää ektomykorritsasienilajistoa suomalaisessa mäntymetsässä molekyylibiologisin menetelmin, sekä tutkia miten erilaiset metsähoidolliset toimenpiteet vaikuttavat lajiston monimuotoisuuteen. Erityisen kiinnostuksen kohteena oli kasvin yhteyttämän hiilen jakautuminen juuriin ja mykorritsoihin, bakteeriyhteisöjen hiilenkäyttö sekä mykorritsalajiston jakautuminen podsolimaannoksen eri kerroksissa. Yhtä Vogeesien alueella Ranskassa tehtyä kenttäkoetta lukuunottamatta kaikki kokeet tehtiin keskisuomalaisessa mäntymetsässä tai laboratoriossa käyttäen em. koealalta kerättyä maata.

Kasvin yhteyttämän hiilen todettiin jakautuvan suhteessa tasaisesti juuriin ja mykorritsoihin podsolimaannoksen eri kerroksissa. Kun myös juurten ja mykorritsojen pinnoilta uutettujen bakteeriyhteisöjen havaittiin käyttävän aktiivisesti erilaisia hiiliyhdisteitä energianlähteinään, näyttää siltä, että kasvi ja ektomykorritsasienet ylläpitävät biologista aktiivisuutta podsolimaannoksessa, myös sen alemmissa mineraalikerroksissa. Nämä havainnot tukevat aiempia tutkimustuloksia, jotka viittaavat ektomykorritsasienten vaikuttavan merkittävästi podsolimaannoksen muodostumiseen ja mineraaliaineksen rapautumiseen.

Ektomykorritsasienten lajisto oli tutkituissa olosuhteissa monimuotoista: yhteensä löytyi 53 taksonia, joista useimmat edustavat yhtä sienilajia. Avohakkuu aiheutti taimien juurissa lajistomuutoksia vaikka lajien määrä ja monimuotoisuus sinänsä eivät kontrollimetsän ja avohakkuukäsittelyn välillä muuttuneetkaan. Avohakkuun reuna-alue, joka on pystyynjääneiden puiden juurten vaikutuspiirissä, oli lajistoltaan monimuotoisin. Neljä taksonia dominoi kenttäkokeessa: *Piloderma* sp., *Suillus variegatus*, *Phialocephala fortinii*-ryhmä ja *Cenococcum geophilum*. Podsolimaannoksen kerrokset erosivat osittain lajistoltaan ja näyttikin, että mineraalimaassa esiintyy enemmän runsaasti ulkorihmastoa muodostavia lajeja.

Sekä laboratoriossa tehdyt siirrostuskokeet suomalaisilla sienikannoilla että Ranskassa tehty kenttäkoe osoittivat, että taimien siirrostaminen tunnetuilla mykorritsasienikannoilla voi parantaa taimien kasvua. Siirrostuskokeiden ei havaittu muuttavan metsämaahan siirrettyjen taimien juuristojen mykorritsasienilajistoa eikä bakteeriyhteisöjen rakennetta.

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List of original publications

This thesis is based on the following articles, which in the text are referred by their Roman numerals.

I Heinonsalo, J., Hurme, K.-R. and Sen, R. (2004) Recent ^{14}C-labelled assimilate allocation to Scots pine seedling root and mycorrhizosphere compartments developed on reconstructed podzol humus, E- and B-mineral horizons. Plant and Soil 259 (1): pp 111-121.

II Heinonsalo, J., Jørgensen, K.S. and Sen, R. (2001) Microcosm-based analyses of Scots pine seedling growth, ectomycorrhizal fungal community structure and bacterial carbon utilization profiles in boreal forest humus and underlying illuvial mineral horizons. FEMS Microbiology Ecology 36: 73-84.

III Heinonsalo, J and Sen, R. Scots pine ectomycorrhizal fungal inoculation potential in podzol specific humus, eluvial and illuvial horizons sampled one and four growth seasons after forest clear-cut logging. Submitted manuscript.

IV Heinonsalo, J., Koskiahde, I. and Sen, R. Scots pine bait seedling performance and root colonizing ectomycorrhizal fungal community dynamics before and over four years after forest clear-cut logging. Manuscript.

V Heinonsalo, J., Frey-Klett, P., Pierrat, J.-C., Churin, J.-L., Vairelles, D. and Garbaye, J. (2004) Fate, tree growth effect and potential impact on soil microbial communities of mycorrhizal and bacterial inoculation in a forest plantation. Soil Biology and Biochemistry. 36: 211-216.

Authors contribution:

J. Heinonsalo has designed the experiment of Paper I; in Papers II, III and IV the planning of the experimentation has been carried out together with R. Sen. In Paper V, the field trial was originally started and planned by P. Frey-Klett and J. Garbaye and the experimentation carried out in 2000 was planned by J. Heinonsalo together with P. Frey-Klett and J. Garbaye. J. Heinonsalo carried out most of the laboratory work presented in papers I-V. He is corresponding author in all the papers and has interpreted the results and written the papers together with supervisors, R. Sen in Papers I-IV and P. Frey-Klett and J. Garbaye for Paper V.

Abbreviations and definitions

Abundance The number of organisms in a population, combining ‘intensity’ (density within inhabited areas) and ‘prevalence’ (number and size of inhabited areas) (Begon et al. 1996).

Advanced regeneration
Seedlings present in a forest understorey that, following clear-cut logging, are subsequently used for forest regeneration (Jones et al. 2003).

Community The species that occur together in space and time (Begon et al. 1996).

Community structure
The list of species and their relative abundances in a community (Begon et al. 1996).

Evenness Also known as equitability, describes how equally abundant the species are in a community (Magurran et al. 1996).

Morphotype
In this study: ectomycorrhizas that are grouped on the basis of similar morphological characteristics (colour, ramification, shape, hyphal structures).

PCR Polymerase Chain Reaction method, developed by Mullis and Faloona (1987), which enables exponential amplification of target DNA sequences.

Population A group of individuals of one species in an area (Begon et al. 1996).

rDNA Region of chromosomal DNA that codes for ribosomal RNA. Although variation occurs among different species and between prokaryotes and eukaryotes, rRNA genes usually have multiple copies (up to several hundreds) in a chromosome (Levin, 1997).

RFLP Restriction Fragment Length Polymorphism. Method, in which PCR-amplified DNA is digested using restriction enzymes to smaller fragments.

Species A collection of closely related strains sufficiently different from all other strains to be recognized as a distinct unit (Brock et al. 1994).

Species richness
The number of species present in a community (Begon et al. 1996).

Taxa In this study: fungal PCR-RFLP patterns that fall within a defined cluster of a constructed dendrogram. A taxon generally consists of a single species, but can in some cases, may also include several, closely related species.

1. Introduction

1.1. Background

Biological diversity or biodiversity refers, in its general sense, to all aspects of variety in the living world (Begon et al. 1996). Therefore, the term includes the genetic diversity within species, species diversity, and diversity of different communities and ecosystems. The ecological importance of biodiversity is complicated to determine, but it is commonly suggested that for an ecosystem functioning under changing environmental conditions, it is preferable to try to maintain as high diversity as possible. From an ethical perspective, the protection of living organisms, i.e. conservation of their habitats, is also important, and the idea of saving natural life for future generations has gained general acceptance.

In recent decades, the importance of conserving and studying the biological diversity on earth has been recognized, and 183 nations signed an international agreement, the Convention on Biological Diversity (CBD), at the Earth Summit in Rio de Janeiro in 1992. The objectives of the convention were described as follows: '*The objectives of this Convention, to be pursued in accordance with its relevant provisions, are the conservation of biological diversity, the sustainable use of its components and the fair and equitable sharing of the benefits arising out of the utilization of genetic resources, including by appropriate access to genetic resources and by appropriate transfer of relevant technologies, taking into account all rights over those resources and to technologies, and by appropriate funding*.' (http://www.biodiv.org/convention/articles.asp). In relation to the convention, the nations committed themselves to preserve and improve the biodiversity in their countries. This includes identification and monitoring the species and taking the biodiversity into account in agriculture, forestry and fisheries.

Based on this commitment, the Academy of Finland, in collaboration with ministries, industry, foundations and organisations, initiated a research programme in 1997 called the Finnish Biodiversity Research Programme (FIBRE), (Markkanen et al. 2002). The aim was the interdisciplinary study of biodiversity in Finland thus implementing the international Convention on Biological Diversity, signed by the government of Finland. One aspect of this research programme was to improve knowledge of the groups of organisms that are poorly known and of how the impact of human actions, such as forestry, affect these organisms.

Work presented in this thesis forms part of a FIBRE research project that focussed on characterisation of the diversity of below-ground root symbiotic ectomycorrhizal fungi and assessment of the effects of clear-cut logging on this diversity. The project was entitled '*Tree root associated microbial diversity in undisturbed and clear-cut Scots pine forests: interactions and impacts on nutrient cycling and seedling growth*'.

1.2. Boreal forest environment

1.2.1. Forest soil as a physical and chemical environment

Soils are heterogenic environments and provide a wide variety of niches for living organisms due to differences in physical, chemical and biological parameters. Soil consists of mineral material, organic matter in various states of decay, water, a gaseous

atmosphere, the roots of plants, and microbial and animal biomass (Killham, 1994). The parent material of mineral soil particles is present either at the site as bedrock below the soil horizons or it may have originated elsewhere and been transported to the site e.g. by glacial activities. The parent material originally defines the chemical structure of the soil matrix and particle distribution that is the physical texture of the soil (FitzPatrick, 1986; Fischer and Binkley, 2000). However, the vegetation, microbes and animals alter the soil through a wide range of biological activities. Photosynthetically bound organic compounds, mainly generated by vascular plants, not only alter the chemistry of the soil directly when entering the soil via root exudation (Grayston et al. 1997) or litterfall (Finer et al. 2003) and humus layer formation, but also provide energy to the soil ecosystem, allowing a wide diversity of degradative, competitive and metabolic processes to occur (Fisher and Binkley, 2000). The vegetation may differentially affect the soil which results in changes in soil properties (Priha 1999). It has been estimated, based on tree girdling (Högberg et al. 2001), carbon budgeting (Ågren et al. 1980) and ^{14}C-experiments (Rygiewicz and Anderson, 1994) that a major part (30-60%) of the plants' photosynthates enter the soil and support soil respiration that results from metabolic activities of plant roots, microbes and animals.

Water and temperature are the most important factors in soil formation (FitzPatrick 1986). These factors together affect the type of biological activity in soil, the direction of water flow (upwards as evapo-transpiration or downwards as drainage) influences the soil horizon formation, and the amount of water present affects the soil solute concentration resulting in differences in retention or leachate potentials of nutrients. Temperature has a strong influence over biological activity and water chemistry, having an overwhelming importance in the soil environment (FitzPatrick, 1986; Fisher and Binkley, 2000). The extremes, frozen tundra soil and dry, arid soil in the desert, clearly highlight the effects of water and temperature on soil ecosystems.

In the boreal forest zone, podzols (see FAO-Unesco, 1997 and Soil Survey Staff, 1998 for details) represent the predominant soil type. The formation of podzol soil profile is typical in acid mineral soils, which are poor in base cations (Petersen, 1976). An important factor in the formation of this profile is that precipitation exceeds evaporation, which results in humid conditions and water flow through the soil matrix (Petersen, 1976).

The structure of a typical Finnish ferric podzol soil profile, classified as a Haplic Arenosol by FAO-Unesco and a Typic Haplocryod by Soil Survey Staff (Ilvesniemi et al. 2000), is presented in Figure 1. The definition of podzol soil type and the theory behind the podzolisation process had been earlier reviewed by Petersen (1976) and more recently by Lundström et al. (2000a). Briefly, the upper litter horizon is formed from input of litter that originates from the standing vegetation together with dead animal and fungal remains. In the humus (O) horizon, the litter is already transformed to unidentifiable humic substances mainly by litter-decomposing activities of soil fungi and animals. Bacteria are also involved in the process, but are not decomposers of the primary lignin and cellulose structures that make up the bulk of plant derived litter.

Under these organic horizons, an eluvial (E) horizon is being formed. From this layer, great part of the base cations and Fe and Al are leached, being translocated into the deeper illuvial (B) horizon. The processes and mechanisms responsible for soil profile formation are not entirely known, but both chemical and biological parameters are thought to influence the process (Lundström et al. 2000a). The accumulation of base cations and Fe and Al in the B-horizon is thought to be caused by the complexiation of these compounds with organic acids, which are probably formed by ectomycorrhizal fungi

(Lundström et al. 2000b). These horizons (O, E and B) form directly on the bedrock or are supported by the C-horizon which consists of unaltered parent material.

Figure 1. Podzol soil profile. O= humus layer, E= eluvial and B= illuvial horizon. Photo: M. Färdig.

Podzol soils are typical of relatively poor sites where concentrations of available soil nutrients are highly limited. However, some trees such as Scots pine (*Pinus sylvestris* L.), are adapted to these conditions and have developed mechanisms to be able to survive and successfully compete in these environments. The foundation of this success is that such plants allocate photosynthetically-fixed carbon compounds to mycorrhizal, root-symbiotic fungi, which help the plants to mobilize nutrients in these recalcitrant soils (Smith and Read 1997).

1.2.2. Biological components of the forest soil ecosystem

Plant roots and animals

Plant roots influence the soil environment in four main ways. The penetration of roots into soil matrix forms channels, especially when the roots die and decompose. These channels improve water and gas movement (Moore et al. 1986). Water uptake by plants via roots affects the water content of soil, considerably altering e.g. soil aeration, particle structure and chemistry. Root systems also stabilise the soil, particularly in areas with high topographical differences and thereby reduce soil erosion and landslides (Fisher and Binkley, 2000). Soil is dramatically influenced by both root litter production, which increases the organic carbon pool in the soil, and the root-associated biological activity (Killham, 1994).

The primary function of soil animals in the soil ecosystem is the ‘processing’ and mixing of soil detritus (Killham, 1994) thus improving the decomposition process by physical, chemical and microbiological means (in the animal gut the soil organic material is exposed to chemicals and decomposing microbial flora). A dominant source of energy for soil animals is the microbial biomass of which the external mycelium of ectomycorrhizal fungi represent a major fraction (Siira-Pietikäinen, 2002). The microfauna (less than 100 μm in dimension), also called protozoa, consists of flagellates, ciliates, amoebae and sporozoa (Paul and Clark, 1989; Killham, 1994). Protozoa live mainly by grazing soil microbes, bacteria and fungi. The most important members of larger soil fauna, meso- (from 100μm to 1-2 mm) and macrofauna (greater than 1-2 mm) are the earthworms,

enchytraeid worms, nematodes, arthropods (e.g. mites and Collembola), molluscs and mammals.

Microorganisms

Although fungi generally exceed bacteria in terms of biomass in boreal forest soil (van Elsas et al. 1997), bacteria colonize specific soil microenvironments in great numbers, e.g. particles (Brock et al. 1994), root surfaces, rhizosphere and mycorrhizospheres (Timonen et al. 1998, Heinonsalo et al. 2000). In addition to interactions with plant roots and mycorrhizal fungi, soil bacteria interact with other soil fungi and animals as well. Bacteria are responsible for several key processes in soil such as nitrification, denitrification and nitrogen fixation (Paul and Clark, 1989). The exponential growth pattern of bacteria leads to the formation of dense colonies of single or multiple species when nutrients are available. Bacteria also typically form biofilms, where microbial colonies are attached to a surface, encased in an adhesive, usually polysaccharide material (Brock et al. 1994). In biofilms, bacteria modify their environment to form specific niches for different types of bacteria (Tolker-Nielsen and Molin, 2000) and create an organism-like structure based on multiple interactions of coexisting bacterial communities (Coghlan, 1996).

A part of the soil biota belongs to the phyla *Archaea*. Although it was earlier thought that Archaea only survive under very extreme conditions (in high salinity, temperature or pressure, or in anaerobic conditions) (Brock et al. 1994), it has been recently shown that Archaea exist also in boreal forest soils and mycorrhizosphere (Jurgens et al. 1997; Bomberg et al. 2003). The exact function of the members of the kingdom *Crenarchaeota* found in the soil ecosystem still remains to be reported (Jurgens and Saano, 1999).

Fungi are eukaryotic organisms that form the major part of microbial biomass in boreal forest soils. Fungi are divided into five major groups: Deuteromycetes (Fungi imperfecti), Oomycetes, Zygomycetes, Ascomycetes and Basidiomycetes. The function, reproduction and ecological function of the species belonging to different taxonomic groups are, outside a limited number of ‘model’ species, poorly understood. In 1995, approx. 72 000 fungal species were known and it has been estimated that there may be about 1.6 million fungal species (Carlile et al. 2001). However, functional groups such as saprotrophs (wood- and litter-decomposing fungi), parasitic, pathogenic and symbiotic (e.g. mycorrhizal) fungi are all known to exist in the soil environment (Carlile et al. 2001).

Wood and litter-decomposing fungi are saprotrophic and thus obtain their energy from dead organisms. Wood-decomposing fungi are specialized in using lignin or cellulose compounds as their energy source. Wood-decomposers are generally divided to white-rot (degrade lignin), brown rot (degrade cellulose) and soft rot fungi (degrade cellulose causing wood to lose its mechanical strength) (Dix and Webster, 1995). These organisms are mainly responsible for initiating the degradation process of woody material, which eventually ends in its complete mineralization.

Litter-decomposing fungi have similar abilities as wood-rotting fungi, but they live in an ecologically more heterogenic environment, the litter layer (Dix and Webster, 1995; Steffen, 2003). As such, these fungi as a group are capable of degrading a wider range of compounds and they need to be more competitive in the soil environment that is also populated by many other organisms.

In contrast to saprotrophic fungi, mycorrhizal fungi generally tend to obtain a major part of their energy resources directly from the host plant (Harley and Smith, 1983). Mycorrhizal fungi form a symbiotic relationship with many plant species. The earliest evidences of mycorrhizal associations are from the early Devonian era, approx. 400 million

years ago, which is when the first land plants were developing. It has been estimated that this ancient form of symbiosis can be found in approx. 80% of land plants (Brundrett 2002).

Several different types of mycorrhizal associations exist (Harley and Smith, 1983). The main division is based on the type of cellular contact: in endomycorrhizas the fungal structures are formed within plant cells, while in ectomycorrhizas, the fungi are restricted to the inter-cellular space in cortical and epidermal layers of fine roots and do not puncture the cell memrane. There are several different kinds of endomycorrhizas: VA (vesicular-arbuscular, often called only arbuscular), ectendo-, arbutoid, monotropoid, ericoid and orchid mycorrhizas (Harley and Smith, 1983). The specificity for their host plants varies, but in the boreal forest zone tree species generally form ectomycorrhizal (sometimes ectendomycorrhizal) symbioses while shrubs are commonly ericoid mycorrhizal, and herbs and grasses are arbuscular mycorrhizal (Harley and Smith, 1983; Smith and Read, 1997). Certainly, in boreal forest soils, fungi forming several types of mycorrhizas can coexist (Öpik et al. 2003).

Symbiosis in where both partners benefit from the association is called mutualism (Begon et al. 1996). Generally in mycorrhizal symbiosis, the fungal symbiont (mycobiont) gains energy from the host plant in the form of photosynthetically fixed carbon compounds while the plant receives nutritional benefits (e.g., improved phosphorus and nitrogen uptake) (Harley and Smith, 1983), protection against competitive plant pathogens (Marx, 1969; Smith and Read, 1997; Sen, 2001) and drought stress (Garbaye, 2000). It is noteworthy that, in all cases of mycorrhizal symbioses, the benefits to either of the partners are not clear, and in some cases the relation can be neutral or even parasitic. Additionally, the specific consequences of the mycorrhizal associations to the host are difficult to estimate under natural conditions in the long run, where several other parameters vary considerably e.g., competition between other organisms, time of the symbiosis, age of symbionts, and climatic conditions.

1.3. Ectomycorrhizas[1]

1.3.1. Roles of ectomycorrhizal fungi

Ectomycorrhizal fungi can influence the host plant in numerous ways. Decades ago, it was demonstrated that ectomycorrhizal fungi improve plant nutrition by enhancing nitrogen, phosphorus and potassium uptake, amongst others (Harley and Smith, 1983). Although the possibility of ectomycorrhizal involvement in nitrogen uptake was proposed by Frank (1894), until the last 20 years further investigations focused on the capture of phosphorus (Smith and Read, 1997). Low availability of nitrogen is a characteristic feature of many ecosystems dominated by ectomycorrhizal symbioses, such as boreal forests (Tamm, 1991). Inorganic nitrogen can be taken up directly by plants, but ectomycorrhizas have been shown to improve N uptake (Rygiewicz et al. 1984; Martin et al. 2001). Since saprotrophic fungi and bacteria readily utilise inorganic nitrogen, these forms are competed for in soil. Ectomycorrhizal fungi have a competitive advantage in harvesting these nutrients compared to saprotrophic organisms, because they have a direct access to plant photosynthates and do not therefore need to compete as much for energy.

[1] Ectomycorrhiza is the composite organ formed by the host plant root and ectomycorrhizal fungi (Harley and Smith, 1983)

An ecologically very interesting and important hypothesis is that ectomycorrhizal fungi possess an ability to access to organic nitrogen forms. Many plant species have been shown to be able to use amino acids and proteins with the help of their mycorrhizal symbiont, but not in non-mycorrhizal conditions (Smith and Read, 1997). Bending and Read (1995) demonstrated in microcosms, using fresh organic matter from the FH horizon of a pine forest, that colonization of the introduced organic substrate by ectomycorrhizal fungi significantly reduced its nitrogen concentration. This finding is very important since most of the nitrogen in forest humus is in organic forms while 'free' inorganic (NO_3^-, NH_4^+) nitrogen is present, under normal undisturbed conditions, in very low concentrations (Tamm, 1991).

Nutrients, particularly N and P, are taken up by ectomycorrhizal mycelium through active absorption and specific cell membrane transporters following degradation by exoenzymes (e.g. phosphatases) (Smith and Read, 1997). Nutrient exchange between fungus and plant occurs using cell-to-cell contact in ectomycorrhizas, especially in the *Hartig net*[2] where fungal mycelium occupies the intercellular space between cortical cells of the colonised fine root (Harley and Smith, 1983).

Fluctuations in the availability of water commonly occur in natural environments and drought periods can cause considerable stress symptoms for trees (Garbaye, 2000). Non-mycorrhizal root tips, which are covered with 'slimy' mucilage, offer only limited buffering against drought. In contrast, mycorrhizal root tips, which are typically ensheathed by a hyphal structure called the *mantle*[3] and which often are connected to extensive network of *extraradical or extramatrical mycelium*[4] with or without *rhizomorphs*[5], are much less sensitive to dry soil conditions. Duddridge et al. (1980) showed for the first time that fungal mycelia could take up and transport water from as far as 8 cm from the root. Such distances, developing from each individual ectomycorrhizal root tip, increase the plant's access to soil water resources significantly. There are species-specific differences in mycorrhizal fungal influence on a plant's tolerance to water stress and therefore maintaining fungal biodiversity indirectly guarantees better water efficiency at the ecosystem level (Garbaye, 2000).

Ectomycorrhizas also protect host plant roots against root pathogens and root herbivorous soil microfauna. Mycorrhizal fungi reduce the likelihood of pathogens invading the root through competition, physical barrier formation, support of associated antagonistic microorganisms unfavourable for pathogens or secretion of toxic compounds (Harley and Smith, 1983). Improved general fitness of mycorrhizal plants also decreases the risk of pathogenic attack.

Increased shoot biomass of mycorrhizal vs. non-mycorrhizal seedlings has been demonstrated over the last 100 years, starting at the time when the symbiosis and its major functions were discovered (Harley and Smith, 1983). Specific ectomycorrhizal species and strains can give competitive advantages (Le Tacon et al. 1992) and in certain cases improved shoot growth can positively influence seedling survival and later competitive success. This phenomenon is the basis of inoculating forest tree seedlings to be used in forest regeneration (Landis et al. 1989a), since it is often critical that the

[2] Hartig net is formed by the fungal growth between plant cortical cells and it provides a large surface of contact between the two symbionts (Harley and Smith, 1983)

[3] Mantle or fungal sheath defines the layer of fungal tissue that develops on the surface of the mycorrhizal root. It varies greatly between fungal species both in forms and thickness (Harley and Smith, 1983)

[4] Extraradical or extramatrical mycelium extends from the mantle into the surrounding soil forming a connection between the mantle and soil (Smith and Read, 1997).

[5] Rhizomorphs are linear aggregates of hyphae which specialize in the uptake and transport of water and nutrients (Smith and Read, 1997)

seedlings are not suppressed in the first years by the ground vegetation in the reforestation site.

1.3.2. Fungal mycelium and the mycorrhizosphere

The growth pattern of fungal hyphae is characteristically apical with subapical branching (Carlile et al. 2001). The branching habit of individual hyphae in the mycelium results locally in efficient harvesting of nutrients but mycelial strands or rhizomorphs are needed if new sources of nutrients are to be reached. In unfavourable conditions, *sclerotia*[6] can be produced by some species (Carlile et al. 2001).

Since the hyphal network colonises large volumes of soil, and since plant photosynthates flow into this hyphal network, it is obvious that the presence of mycorrhizal extramatrical mycelium in soil alters its environmental conditions compared to non-colonised, 'bulk soil'. The term mycorrhizosphere (Linderman, 1988) has been used to describe the soil volume influenced by the mycorrhizal mycelium. Thus, tree root-associated mycorrhizal fungi increase greatly the volumes of soil that can be effectively exploited by the host tree (Smith and Read, 1997). This critical feature forms the basis for the observed positive influences gained through mycorrhizal symbiotic association between fungi and plants. Access to remote and recalcitrant nutrient sources and water with help of fungi is essential for the host trees.

Fluctuations in soil fungal biomass occur seasonally (Berg et al. 1998). The mycelial growth pattern in soil was proposed (Shearer, 1995) and later shown (Lindahl, 2001) to be dynamic, involving competition between different types of fungi and different fungal species. Environmental and biological factors (e.g. host plant diversity and competition) influence fungal community dynamics, but reciprocal fungal competitive patterns can also affect above-ground host plant diversity and productivity (van der Heijden et al. 1998).

Many soil animals graze mycorrhizal mycelium, which can have either positive (Setälä, 1995) or neutral/negative impact on tree growth performance (Ek et al. 1994). Mycelium also supports bacteria and location-specific bacteria have been identified in mycorrhizospheres (Nurmiaho-Lassila et al. 1997; Timonen et al. 1998; Bertaux et al. 2003). Further, differences in bacterial communities extracted from bulk soil, rhizosphere and mycorrhizosphere soil compartments have been detected in different podzolic forest and oil-contaminated soils (Timonen et al. 1998; Heinonsalo et al. 2000; Paper II). Bacteria have been shown to specifically support mycorrhiza formation (Garbaye and Bowen, 1987; Meyer and Linderman, 1986) and a theory for the mode of action of mycorrhiza helper bacteria (MHB) has been presented by Garbaye (1994). Nitrogen fixing genes have recently been detected in endosymbiont bacterial strain of mycorrhizal fungus (Minerdli et al. 2001) suggesting mycorrhizosphere-associated nitrogen fixation in soil. In conclusion, it is evident that fungal mycorrhizosphere is a particular, extensive habitat supporting a variety of bacterial types and having specific functions of mostly unknown ecological importance.

1.3.3. Ectomycorrhizal fungal dispersal

Ectomycorrhizal fungi can disperse and form new mycorrhizas either via living hyphal connections, special resting structures like sclerotia, or spores (Brundrett, 1991). Living hyphae, which form part of the plant-supported hyphal network, is probably the most

[6] Sclerotia are resting bodies, which can persist for years, formed by the fungal mycelium (Carlile et al. 2001)

common source of inoculum in forest conditions, where mycorrhizal roots are already available. Dahlberg and Stenlid (1995) also suggested, that in the presence of an existing mycorrhizal community in an established forest, mycelial colonization probably dominate over spore colonization. Under conditions where the living hyphae are not available, e.g. after clear-cut logging, the major sources of inoculum for new tree seedlings may be sclerotia and spores. If the regeneration happens soon after logging (e.g. within a few years), the remaining stumps and dying roots have been shown to support living hyphae of ectomycorrhizal fungi (Hagerman et al. 1999a) and new seedlings can achieve mycorrhizas that way.

Spores and sclerotia can survive in soil for a very long time and can be activated by suitable conditions e.g. increased soil moisture, heat shock after forest fire or stimulatory compounds in root exudates. Spore activation is followed by spore germination (Carlile et al. 2001). The emanating primary monokaryotic mycelium of a basidiospore is already capable of forming mycorrhizae (Kope and Fortin, 1990), but normally mycorrhiza formation is generated by secondary heterokaryotic mycelium that arise from the mating of two sexually compatible primary mycelium. Little is known about forms of mycelium responsible of mycorrhiza formation by Ascomycetes, which are numerous in ectomycorrhizal communities (e.g. Tedersoo et al. 2003). Some fungal species are known to spread successfully via spores while others form mycorrhizas preferentially via living connections or sclerotia (Brundrett, 1991, Dahlberg, 1997).

The succession of ectomycorrhizal fungi is mainly connected to the dispersal ability of different fungi. The hypothesis of early- and late-stage fungi (Mason et al. 1983), originating from a study of Mason et al. (1982) carried out in former agricultural soil, suggested that some species colonise the host tree roots earlier, while some others can form the symbiosis only in later growth stage of the trees. This hypothesis is no longer widely supported in the scientific community, but the principle of certain successive traits of ectomycorrhizal fungi is reasonable. Nara et al. (2003a,b) studied primary succession of ectomycorrhizal fungi in pristine soil developed by volcanic activities having no earlier mycorrhizal history. They found a clear trend of succession that was detected both in mycorrhizas in roots and in the sporocarp production. The conditions in a secondary successional site, where ectomycorrhizal inoculum from previous forest exists, are very different from these primary sites and thus the mechanisms of succession remain poorly defined. However, understanding the functional differences between different ‘stage’ fungi and the reasons for changes in ectomycorrhizal community structure due to e.g. man-made disturbances is an important and a demanding future task.

1.3.4. Ectomycorrhizal biodiversity

More than 1000 fungi are estimated to form ectomycorrhizas in the Scandinavian forests (Knudsen and Hansen, 1991), a number that greatly exceeds the number of ectomycorrhizal hosts in the region. Since many ectomycorrhizal fungal species do not form epigeous (above-ground) sporocarps but inconspicuous or hypogeous (below-ground) ones, it is probable that the actual species richness is much higher. Thus, the possible species combinations and intra- or inter-specific interactions in these highly diverse communities are enormous and have been a subject of increasing interest in the last 20 years (Dahlberg, 2001). The community studies of ectomycorrhizas normally aim either at simply describing the species present under specific conditions or at observing the effects of natural or anthropogenic disturbances on the community structure.

Many ectomycorrhizal community studies have focussed on mycorrhizas in the humus horizon. Although the fine roots of some tree species, e.g. Norway spruce, are more superficially distributed than others (see e.g. Mikola et al. 1966), the importance of

mycorrhizal fungal communities in the mineral horizons should not to be neglected (Taylor, 2002). Only in recent years have some community-level studies in boreal forests using modern molecular methodology highlighted the importance of these deeper soil horizons (Paper II; Dickie et al. 2002; Landeweert et al. 2003; Rosling et al. 2003).

Ectomycorrhizal fungal identification has traditionally been based on observations on the morphological characters of mycorrhizal root tip, morphotyping (e.g. Agerer, 1987-1998). If morphotyping is carried out with care, a good species identification is obtained (e.g. Sakakibara et al. 2002). However, detailed morphotyping involving the use of microscopy requires long experience and training and in many cases in published literature, only gross morphotyping using easily detectable characters has been used. In those cases, morphotyping does not separate similar looking species and even the detailed analysis cannot uncover strain-level differencies. Due to those deficiencies, the use of molecular methods has been increasing. Analysing the ITS-region of the ribosomal RNA gene, which has multiple copies in chromosomal DNA, using PCR reactions and specific primers (see for methodological details in recent reviews by Dahlberg (2001) and Horton and Bruns (2001)), it has been possible to identify fungal species and strains from even single ectomycorrhizal root tips. The range of molecular biological methods available is wide and new methods are being actively developed.

Due to improvements in species identification methodology, community-level studies have produced new, critical knowledge of ecosystem functioning and the roles of mycorrhizas in e.g. plant community diversity (van der Heijden et al. 1998), nutrient uptake (e.g. Wallander et al. 1997; Näsholm et al. 1998), tolerance against disturbances (Peter et al. 2001) or microbial interactions in the rhizosphere (Whipps, 2001). Despite the increasing number of studies published on ectomycorrhizal fungal communities, there are still numerous questions to be answered. Do certain members of the community carry out specific functions in the soil ecosystem or does the high species richness ensure the functioning of the system even if some members are lost? Can the community structure of undisturbed forest be maintained e.g. by modifying forestry practises, and is it worth of it? Should the species having only low abundance be conserved as a ‘reserve for disturbances’? How do different groups of plants and fungi compete in the soil? Is the plant community structure formed as a result of below-ground competition or the other way round? Many such important questions are still to be answered.

1.3.5. Research on ectomycorrhizal fungi in Finnish forests

Finland belongs to the boreal forest zone and our main tree species, Scots pine (*Pinus sylvestris* L.), Norway spruce (*Picea abies* L. Karst) and silver or downy birch (*Betula pendula* L. or *B. pubescens*), covering 64.7, 24.0, and 9.0% of forest soil, respectively (Peltola, 2001), are all ectomycorrhiza-formers.

Mycorrhizal research has a long tradition in Finland and the pioneer of the subject in Finland was Peitsa Mikola, who described the form and introduced the term ‘ectendomycorrhiza’ (Harley and Smith, 1986; Smith and Read, 1997). Mikola and Olavi Laiho extensively studied mycorrhizas from a forestry perspective since 1960’s (e.g. Mikola and Laiho, 1962; Laiho, 1970; Mikola 1970; Laiho et al. 1987; Mikola, 1988). There has been a long tradition of mycological inventories in Finland and distributions of ectomycorrhizal sporocarp-formers are fairly well known (Ohenoja, 1978; Salo, 1979; Ohenoja, 1984; Ohenoja and Koistinen, 1984; Hintikka, 1988; Ohenoja, 1988; Väre et al. 1996). Finnish scientific journals like *Karstenia* and *Annales Botanici Fennici* frequently publish results from such Finnish mycological studies. However, long-term and large-scale below-ground inventories on ectomycorrhizal communities are almost lacking in Finland and no field-scale studies using modern, DNA-based identification methods have been

published. Thus, most of the knowledge gained from field experiments involving such assessments of below-ground ectomycorrhizal communities of Scots pine, Norway spruce and silver or downy birch is from elsewhere, mainly from Sweden (e.g. Kårén et al. 1997, Mahmood et al. 1999; Jonsson et al. 1999, Kranabetter, 1999; Kõljalg et al. 2000; Fransson et al. 2000, Peter et al. 2001).

1.4. Practical perspective

1.4.1. Forestry practices

Stem-wood harvesting

In Finland, three major kinds of regeneration felling are performed: clear-cutting, seed tree and shelterwood logging. Approximately 73.5% of the regeneration areas are clear-cut, while 24.5% are left in the seed tree or shelterwood position in 2000 (Peltola, 2001).

In the clear-cut logging operation, the stems of the dominant tree species are completely removed but the undergrowth and sometimes the naturally regenerated seedlings under the previous canopy are left to grow on. The slash is also normally left on the site and nowadays also some grown-up broadleaved trees (like aspen and mountain ash) or dead trees are left standing, to preserve biological diversity (Fig. 2 A).

In the seed tree position, approximately 100 stems/ha of the trees of the previous generation (normally Scots pine) are left standing to the site to improve the seed fall and therefore natural regeneration (Fig. 2 B). To improve Norway spruce seedling development, which need shelter to develop successfully, approx. 100-300 stems/ha are retained as shelterwood (Fig. 2 C). These trees are logged after a few years where there is successful regeneration (Valkonen et al. 2001).

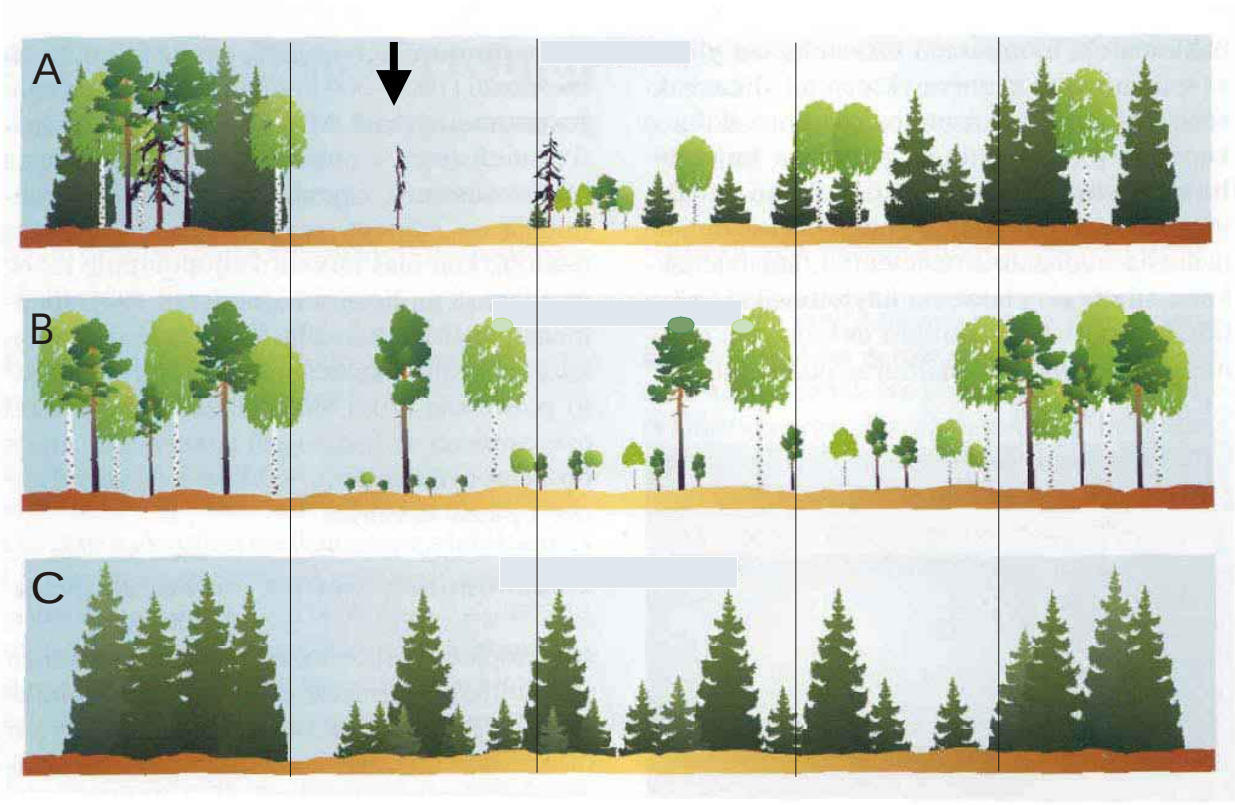


Figure 2. Clear-cut (A), seed tree (B) and shelterwood (C) logging. In the figure, site development progresses from left to right, i.e. from uncut forest through different felling (arrow) to second generation mature forest. Reproduced with modifications from Valkonen et al. (2001), with permission.

Site preparation

After the logging, soil at the sites is usually prepared artificially to improve the growth conditions for regenerating seedlings. Site preparation generally results in increased soil temperature that enables a significant increase in the length of the growth period, decreased competition by other plants, and direct access to more stable water conditions in the exposed mineral soil. Sometimes site preparation involves drainage of the site, because removal of the standing forest causes increased soil moisture to levels that are detrimental to optimal seedling growth (Fisher and Binkley, 2000; Valkonen et al. 2001). The most common methods of site preparation used in Finland are harrowing or disc trenching, ploughing, mounding, and prescribed burning. The areas treated in 2000 with the above-mentioned methods were approx. 93 000 ha, 2000 ha, 25 000 ha and 500 ha, respectively (Peltola, 2001). Method selection depends mainly on the thickness of the humus and litter layer, and the humidity of the site as well as the tree species to be regenerated. Generally, the less intensive methods (e.g. harrowing, see Fig. 3) can be used on sites with thinner humus layers and drier soils. A schematic presentation of the most common methods (harrowing and patchy and drain mounding) is given in Figure 3.

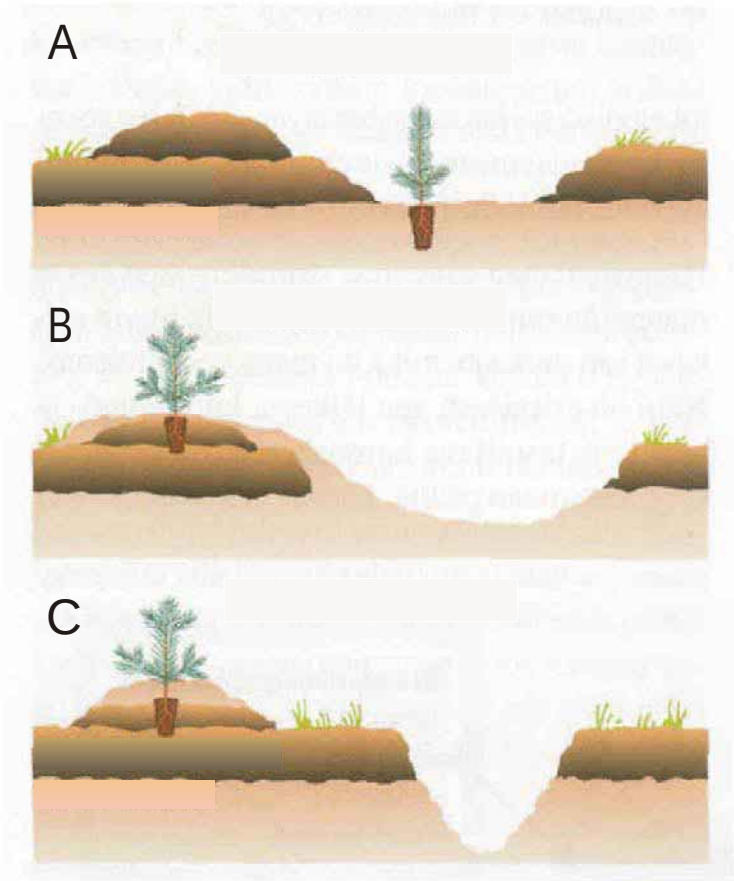


Figure 3. Harrowing (A), patchy (B) and drain mounding (C). Dark brown layer in the picture illustrates humus and beige colour mineral soil horizons. Reproduced with modifications from Valkonen et al. (2001), with permission.

Forest regeneration

As mentioned earlier, in approx. 25% of the forest area felled in 2000 in Finland (c. 50 000 ha), seed trees or shelterwood are preserved. This proportion is also termed natural regeneration since no other action (seeding or planting) is taken for seedling establishment. In 2000, roughly 34 000 ha was regenerated by seeding (mainly Scots

pine) and planting was performed in 83 000 ha (32% for pine, 57% spruce, 11% the rest) (Peltola, 2001). Seeding is often performed using selected seeds at the same time as the soil preparation is done, whereas seedlings are planted later in the same summer or even a few years later.

1.4.2. Effects of clear-cut logging

Soil chemistry and physics

The removal of standing forest obviously has a dramatic influence on soil chemistry. The cessation of the flow of photosynthetically fixed carbon to soil via roots and mycorrhizas, the rapid initiation of the decomposition of roots in soil and litter, and the deposition of slash on the soil surface greatly affect the chemical environment. The consequences are, however, dependent on the compound in question. The concentration of key nutrients, nitrogen, phosphorus and potassium, can increase, decrease or remain unaffected (Smethurst and Nambiar, 1990; Bradley et al. 2001; Simard et al. 2001; Bock and Van Rees, 2002; Piirainen et al. 2002a,b) in soil solution. Soil pH has been shown to increase (Pietikäinen and Fritze, 1995; Smolander et al. 1998; Bradley et al. 2001), while Simard et al. (2001) reported non-significant decrease in pH at three sites after clear-cutting. Traditionally, leaching of key nutrients out of the soil profile is thought to be a major risk connected to clear-cut felling from a chemical perspective. However, in an extensive study carried out over a whole catchment area, the effects of Norway spruce clear-cut felling on leachate loss of nutrients was only minor, with most of the nutrients being tightly held in the soil profile, particularly in the B horizon (Piirainen 2002 and references therein). Similar conclusions of vertical re-translocation of nutrients in the podzol profile (nutrients leached from humus are retained in deeper mineral layers) were also proposed in an extensive study of Johnson et al. (1997).

The physical conditions of the site change after clear-cutting, since the ground water levels are commonly shown to increase (Fisher and Binkley, 2000) and the soil temperature conditions are altered mainly due to increased irradiation and altered evapo-transpiration (Ballard, 2000), which are in turn connected to the changes in the moisture levels of soil. Soil can also be compressed either due to the activities of the heavy logging machinery (Fisher and Binkley, 2000) or changes in soil biota that are largely responsible for controlling the granular structure of soil particles (Killham, 1994). Changes in the preferential water flow through macropores, due to the soil compaction, have also been reported (Moore et al. 1986). Soil compaction may cause alterations in soil atmosphere since the aeration is hindered and if this is accompanied by water logging, different chemical processes (e.g. anaerobic) can take place and drastically alter soil chemistry and nutrient status.

Soil microbiology

The effects of clear-cut forest harvesting on soil microbiology, including soil animals, have been widely studied. After clear-cutting, bacterial-driven nitrification was shown to be enhanced (Smolander et al. 1998; Paavolainen and Smolander, 1998) or unchanged, due to the low pH (Simard et al. 2001). Ammonification (Frazer et al. 1990) and denitrification increased after clear-cutting (Paavolainen and Smolander, 1998). Total microbial biomass decreased in mixed Norway spruce/ Scots pine, western hemlock/ amabilis fir and Norway spruce forest, respectively (Pietikäinen and Fritze, 1995; Bradley et al. 2001, Siira-Pietikäinen et al. 2001) while Smolander et al. (1998) detected a rapid and short-lasting increase in microbial C and N concentrations due to clear-cutting in a Norway spruce stand.

Ergosterol concentrations, which have been used to estimate fungal biomass, have been shown to decrease after clear-cutting (Pietikäinen and Fritze, 1995) as did the fungal-specific phospholipid and fatty acid (PLFA) concentrations (Bååth et al. 1995). Siira-Pietikäinen et al. (2001) showed that the fungal-to-bacterial ratio, based on PLFA analysis, decreased after clear-felling and was caused by the reduction in the amount of fungal component. Wood-decomposing and litter-decomposing fungi are often supposed to benefit from clear-cutting although studies focusing on their species diversity and biomass are almost lacking (Marshall, 2000). The abundance and diversity of mycorrhizal fungi are generally negatively affected by clear-cutting (Hagerman et al. 1999a; Durall et al. 1999; Byrd et al. 2000) since their dominant source of carbon, current photoassimilate, is interrupted following drastically changed environmental conditions. However, the mycelial inoculum can remain viable in the decaying roots several years after clear-cut logging (Hagerman et al. 1999a). As was mentioned earlier, mycorrhizal fungal inoculum can also be maintained in soils in the form of sclerotia and spores.

Many soil animals commonly graze on bacteria and fungi and therefore animals are generally negatively affected by clear-cut felling (Marshall, 2000). Siira-Pietikäinen et al. (2001) concluded that it is mainly the decrease in ectomycorrhizal fungal mycelium that causes the decrease of certain important groups of soil animals. However, they also observed that microbes and enchytraeids are capable of maintaining their populations in exposed mineral soils, i.e. after site preparation following stem-wood logging.

In conclusion, the effects of clear-cutting on the soil biota are highly variable, depending on group of organisms in question. The recovery of microbial communities clearly occurs after the clear-cut. Sundman et al. (1978) estimated a recovery period of 8-13 years after stem-wood harvest.

1.4.3. Plant production and regeneration success

Seedlings are nowadays produced in large commercial tree nurseries mainly as container seedlings. The proportion of bare-rooted seedlings has decreased over the last 20 years. Today, container seedlings represent 98% of Scots pine, 86% of Norway spruce and 89% of silver birch production in Finland (Peltola, 2001). The production takes place in tree nurseries where large quantities of seedlings are produced in controlled conditions using artificial growth substrate, irrigation, fertilization, pest control and sometimes day-night shift treatments to promote seedling growth (Landis et al. 1989a, b; Rikala, 2002). Approximately 50 million pine seedlings were produced in Finland in 2000 (Peltola, 2001). Seedlings spend one to three years in the nursery.

Plant diseases may be the biggest problem in nurseries (Landis et al. 1989b) and their prevention in the large monoculture seedling beds has traditionally been achieved using bacteriocides and fungicides. Maintenance of nursery hygiene is also of major importance. However, the quality of the seedlings is not always satisfactory and the mortality of pine seedlings 5-15 years after planting in the forest site can be as high as 60% (Rikala, 1993), in problem cases even higher. A term ‘transplantation shock’ has been used to describe a suite of symptoms in seedlings including yellow, short needles and reduced growth, arising from relocation from nursery to forest soil (Rikala and Huurinainen, 1990).

Under nursery conditions, ectomycorrhizal fungi live in an artificial environment and are affected by pest control, watering and fertilization (Landis et al. 1989a, b). Generally, all these factors are unfavourable to most ectomycorrhizal fungi, but certain types are commonly found in the nurseries, e.g. *Thelephora* spp. and ectendomycorrhizal species

(Laiho, 1965; Landis et al. 1989b). Unfortunately, most of the so-called nursery fungi are not represented as dominant members in forest mycorrhizal communities, which suggest a poor competitive capacity after transplanting.

1.4.4. Forest tree inoculation

Controlled mycorrhization (Le Tacon et al. 1987) has been used in some countries with the aims of introducing growth-promoting ectomycorrhizal fungi into nurseries, and modifying seedling production procedures respectively, in order to achieve improved growth responses and rapid post-transplantation adaptation in the reforestation site. In North America, the research and practical applications have been established for decades (Landis et al. 1989b), whilst in Europe the procedure has been in commercial use at least in France since early 1990's (Le Tacon et al. 1997).

The potential advantages of controlled mycorrhization in the nursery are not only the positive growth response of the seedlings, but also reduced need for fertilization, pest control and shorter time needed to grow ready-to-sell seedlings. However, optimised protocols and skills in nursery practises are needed to obtain successful results.

Seedling performance in the field after ectomycorrhizal inoculation has been variable in European field studies. In the best cases, six years after outplanting an increase in tree volume of approx. 90% has been observed, while in other conditions inoculated seedlings performed worse in the field compared to control ones (Le Tacon et al. 1992). Variable environmental growth conditions and differences in local mycoflora obviously explain part of the variation. More research is required to understand the reasons for the variability, and to improve the inoculation success in the field.

It is important that the planting of seedlings that have been inoculated with specific mycorrhizal strains, typically of exotic origin, into nature should not cause any negative impacts on the local, indigenous microflora. Ecological knowledge on the interactions of existing community and introduced strains should therefore be improved to prevent the risk of adverse effects. Understanding the survival and competitive abilities of selected strains under field conditions is of high priority in the risk management of controlled mycorrhization. The potential problems are in many ways analogous to those in studies on field release of genetically modified microorganisms.

2. Aims of the study and structure of the experimentation

The main aim of the study was to describe ectomycorrhizal biodiversity in boreal forest soils, and to determine biodiversity responses to different forestry practises. The more specific aims were

1. To describe the ectomycorrhizal species present in a Scots pine dominated forest, using both microcosms in the laboratory and field experiments.

2. To specifically focus on the allocation of carbon to, and the distribution of ectomycorrhizal fungal species throughout the podzol profile.

3. To study changes related to clear-cut logging in the ectomycorrhizal community structure over time.

4. To test inoculation performance of certain, selected ectomycorrhizal fungal isolates obtained from the microcosm and field experiments.

5. To evaluate the performance and microbial community-level influence of inoculated ectomycorrhizal fungal and mycorrhizal helper bacterial strains under field conditions in France.

The structure of the experimentation is schematically presented in Figure 4 where the numbers refer to the original publications listed on page 6. UR indicates unpublished results. Detailed experimental aims are presented in corresponding original articles.

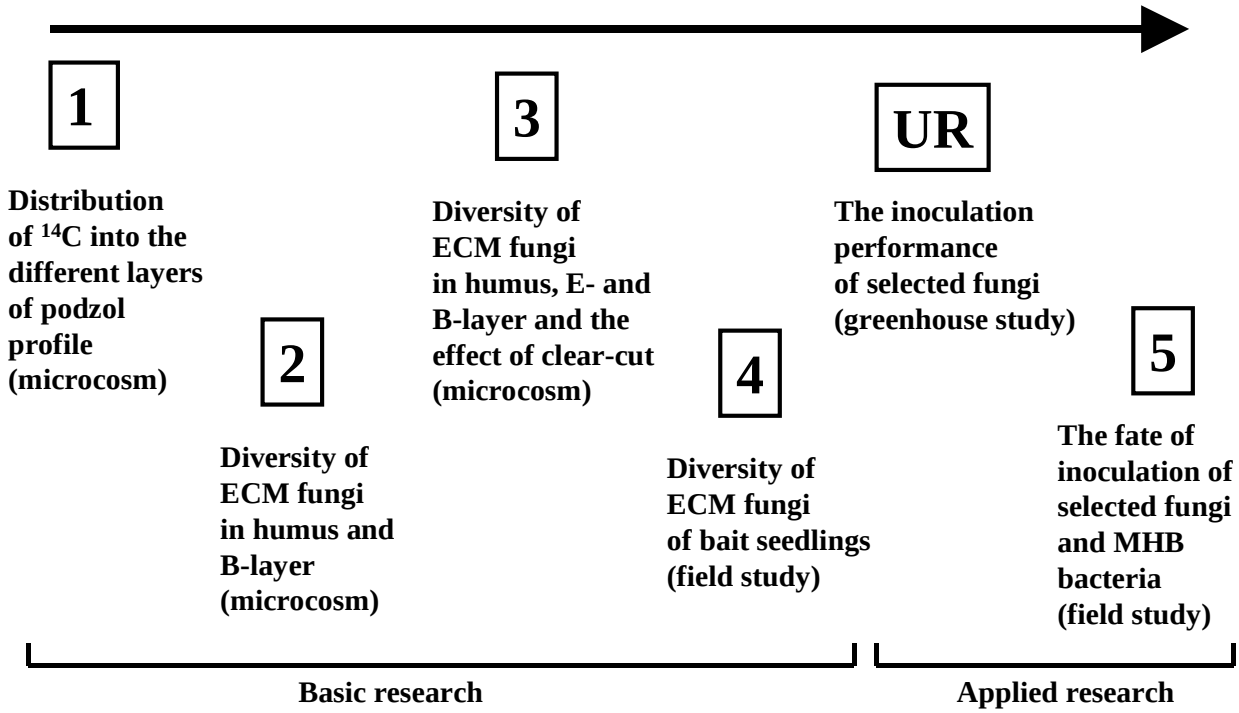


Figure 4. The schematic presentation of research aims and experimentation. The direction of the arrow indicates the logic behind the project where the basic knowledge generated on ectomycorrhizal diversity, obtained from microcosm and field experiments, was later used in applied studies. ECM= ectomycorrhizal fungi.

3. Materials and Methods

The methods used in different studies are listed in Table 1. The materials and methods of an unpublished experiment (UR), the results of which are later discussed, is described separately.

The studies were performed in two different forest sites, one at Hyytiälä in central Finland (Paper IV), the other in the Vosges region in France (Paper V). The microcosm studies were performed in the laboratory (Papers I-III), using soil collected from the Hyytiälä site.

The Hyytiälä ('Mämmilampi') site (see Figure 5) is located in the vicinity of the Hyytiälä Forestry Field Station of the University of Helsinki, in southern Finland (61°84′N, 24°26′E). At the closed canopy stand (approx. 4 ha), the dominating tree species was approx. 100-year-old Scots pine (*Pinus sylvestris* L.), interspersed with younger Norway spruce (*Picea abies* (L.) Karst.) and occasional silver birch (*Betula pendula* Roth.). The ground vegetation was dominated by *Vaccinium myrtillus* and *V. vitis-idaea*, the most common mosses being *Hylocomium splendens* and *Pleurozium schreberi*. The soil was a podzol (Typic Haplocryod/ Haplic Arenosol) comprised of an upper 5-cm mor/humus layer (O) and an approx. 5-cm eluvial (E) horizon supported above an approx. 30-cm illuvial (B) horizon. Detailed descriptions of the site can be found elsewhere (Ilvesniemi et al. 2000). Part of the site (2 ha) was clear-cut harvested in February 1998.

The St.Quirin field site (see Figure 6) is located in the Vosges region in northeastern France. The beech (*Fagus sylvatica*) forest was clear-cut in the spring 1995 and the harvesting residues were removed from the experimental area. Re-growth of understorey after clear-cut mainly consisted of *Juncus* sp., *Senecio* sp., *Rubus* sp., *Luzula* sp. and grass (mostly *Deschampsia cespitosa*). The site was experimentally replanted with Douglas fir (*Pseudotsuga menziesii*).

Figure 5. Hyytiälä field site (control forest) in June 1998.

Figure 6. The field site of St.Quirin, in Vosges, France in April 2000.

Table 1. The presentation of the methods used in the studies. Detailed descriptions of the methods mentioned are given in corresponding Papers.

Experimental setup	Paper I	Paper II	Paper III	Paper IV	UR	Paper V
Microcosm/laboratory experiment[7]	x	x	x		x	
Field experiment				x		x
Analytical methods						
Weather monitoring				x		
Plant biomass	x	x	x	x	x	
Plant height and diameter at ground level						x
Mortality assessment				x		x
^{14}C-labelling	x					
Bacterial inoculation						x
Bacterial total counts		x				x
Community level physiological profiling (CLPP using Biolog plates)		x				x
Selective plating and enrichment cultures for *P. fluorescens* BBc6R8						x
Ectomycorrhizal fungal inoculation					x	x
Ergosterol analysis						x
Exploration types of external mycelium	x		x	x	x	
Mycorrhizal morphotype analysis[8]		x	x	x	x	x
Ectomycorrhizal PCR-RFLP identification		x	x	x	x	x
Ectomycorrhizal ITS sequencing			x	x	x	
Statistical methods						
ANOVA followed by Tukey HSD or Bonferroni	x	x	x	x	x	x
t-test		x				
Non-parametric tests		x	x	x		
Principal component analysis (PCA)		x	x	x		
Correspondence analysis (CA)		x				
Discriminant analysis (DA)						x
Single linkage ordination (dendrogram formation)		x	x	x	x	

Materials and methods of inoculation study (UR)

Based on the ectomycorrhizal species diversity data obtained from Papers II, III and IV, four potentially suitable pure culture strains (morphotypes M, E, K and AC, see Paper IV) were selected and identified using PCR-RFLP fingerprinting. The strains were subcultured to numerous Hagem's agar (Modess, 1941) plates to obtain sufficient amount of inoculum. After the cultures were grown for five weeks on agar, sterile four-week-old Scots pine seedlings growing in the Leca®-BW agar tubes (see Paper II) were inoculated with one of the four selected strains. Control seedlings were left uninoculated but were otherwise treated similarly. Twenty five replicate tubes of each treatment were

[7] The photographs of growth chambers used in Papers I-III are presented in Appendix 1
[8] The photographs of the most common morphotypes found in Paper IV are given in Appendix 2.

constructed. The tubes were moistened twice with ½ MMN liquid media (Marx, 1969) during the growth period.

After two months in the tubes, the mycorrhizal status of the seedlings was visually determined. Pure culture strains, which clearly formed mycorrhizas, (morphotypes M and E) were chosen for the second phase: six randomly selected tubes of control seedlings and seedlings inoculated with cultures M and E were taken, and put aside. The rest of the tubes were harvested and the biomass of the seedlings measured. The strains selected for the second phase of the experiment showed similar growth patterns as the uninoculated control seedlings while the two rejected strains showed a clear trend of reduced shoot biomass.

In the second phase, the seedlings were gently removed from the tubes and carefully planted to 5 x 5 x 7 cm pots containing a reconstructed podzol profile comprising of 150 g fw B-horizon soil in the bottom, 50 g fw of E-horizon soil above the B layer and finally 10 g fw of humus above the mineral soil layers. Soil was collected from two locations in the Hyytiälä clear-cut site, stored in +4°C for about a year and homogenized before use in the podzol reconstruction. The potted seedlings were randomly put in three cooled growth boxes (Paper II), two pots of each treatment/box. During the 4.5 months growth period, the pots were circulated regularly between different boxes to assure standard growth conditions. The pots were watered three times a week with distilled water, so that the surface soil was saturated with water for a short period.

After the growth period, the seedlings were carefully removed from the pots and the roots were washed. Then, the ectomycorrhizal status of the seedlings was recorded with emphasis on detection of introduced morphotypes. Five samples were taken from each morphotype, and the shoot and root biomass measured. The original strains used were sequenced for an exact identification (see summary table 2): morphotype M- strain was confirmed as *S. variegatus* (accession number AJ630031) and type E as *P. fortinii* (number AJ630032).

4. Results and Discussion

4.1. Biodiversity

4.1.1. Carbon allocation in podzol profile (Paper I)

In Paper I, quantification of photosynthetically fixed carbon in the different rhizosphere, mycorrhizosphere and soil compartments indicated an equivalent carbon allocation to roots and mycorrhizas, in organic and mineral podzol soil horizons. Autoradiographic visualization of this phenomenon is presented in Figure 7. Therefore, it seems possible that conclusions concerning carbon allocation below-ground can be made based on the root and mycorrhiza distribution data. Earlier work, which is mostly carried out in artificial growth substrates (Finlay and Read, 1986; Wu et al. 2001; Leake et al. 2002), is here extended into the natural podzol soil conditions.

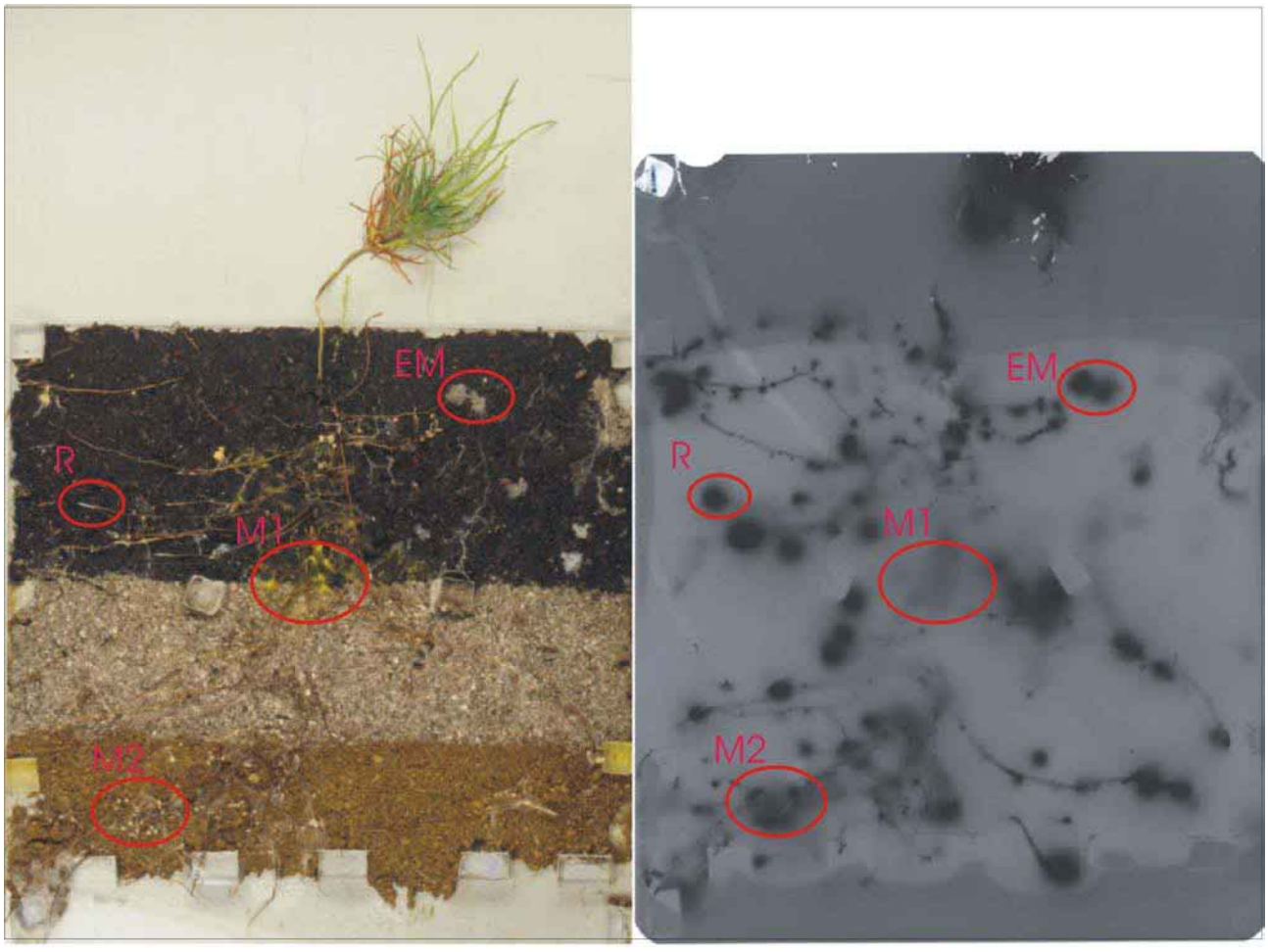


Figure 7. A reconstructed podzol profile microcosm (left) and a corresponding, representative autoradiography image (right) visualizing the allocation of labeled ^{14}C into the plant-root-fungal-soil system. Note the allocation of ^{14}C into marked locations: M1; local grouping of mycorrhizas in humus colonized by a yellow morphotype (*Piloderma croceum*-type) which do not show significant label incorporation, M2; grouping of mycorrhizas displaying a *Suillus*-morphotype showing strong label incorporation in the B-horizon. EM; Strong ^{14}C- label incorporation into external fungal mycelium in humus horizon. R; highly labeled non-mycorrhizal lateral root tip.

Root distribution

Scots pine root distribution in podzol profiles has been well studied under the field conditions. Persson (1983) reported higher fine root and total root biomass in mineral soil compared to F/H layer both in young and mature stand. Makkonen and Helmisaari (1999, 2001) studied fine root biomass and production in 15-, 35-, 38- and 100-year-old Scots pine stands and concluded that the majority of roots were located in mineral soil layers. The data of Ilvesniemi and Liu (2001), in a study also performed at the Hyytiälä forest station, support earlier findings: the surface area of roots, as a percentage of the total, was approx. 36% in humus and 64% in mineral horizons. Ågren et al. (1980) showed by measuring the annual carbon budget of Scots pine that, from the yearly net photosynthetic production used for growth, 63% was allocated to roots and only 37% to above-ground parts. As Persson (1983) concluded that greatest death and replacement of fine roots was found in mineral soil and Helmisaari (1995) reported that 78% of the total tree biomass was above-ground and 22% below-ground, it can be clearly seen that the below-ground carbon cycle, both in organic and mineral soil, is both rapid and active and a major part of the allocated carbon supports the biological activity of soil. This conclusion was also confirmed in a recent forest girdling experiment by Högberg et al. (2001).

Mycorrhiza distribution and microbial activity

Mikola et al. (1966) studied the distribution of mycorrhizas in podzol soil profiles to a depth of 5 cm in mineral soil. They found that 58.3 % of mycorrhizas were in the mineral horizon and suggested that there were considerable numbers at even greater depths in the soil profile. Egli (1981) studied the distribution of oak mycorrhizas up to the depth of 200 cm and found mycorrhizas at all depths and concluded that some types were specific to a particular depth. Similar vertical specificity and distribution has been shown in Paper II and by Dickie et al. (2002), Landeweert et al. (2003) and Rosling et al. (2003) in corresponding field studies in the US and Sweden.

Many authors have measured microbial biomass and activity in podzol horizons: Söderström (1979) reported a similar amount of metabolically active fungal mycelium in the organic and mineral horizons, when presented on an area basis (per m^2). In the laboratory, the presence of the plant was found to activate substrate-induced and basal respiration in mineral soil while in organic soil there was no significant differences or the response was the reverse (Priha et al. 1999). In a similar field study, microbial activity in mineral soils was shown to be relatively more active in less fertile than in richer site under Scots pine (Priha et al. 2001). Fritze et al. (2000) studied distribution of microbial biomass and phospholipid fatty acids in podzol profiles in Hyytiälä forest station, in Finland. Although they concluded that microbial biomass decreased with increasing depth, the sum of microbial biomass in mineral soil samples greatly exceeded the microbial biomass estimates measured in humus in all but one site. Therefore, although humus seems to be a microbially more active horizon on a volume basis, the large volume and depth of mineral soil horizons makes these compartments particularly important.

^{14}C-experiments

Using ^{14}C-labelling methodology, carbon allocation to roots, mycorrhizas, mycorrhizal mycelium and soil has been shown by several authors (Miller et al. 1989; Durall et al. 1994; Rygiewicz and Andersen, 1994; Warembourg and Estelrich, 2000). The rapidity of carbon allocation to the below-ground mycorrhizal system (Johnson et al. 2002), role of mycorrhizal mycelium as an important carbon sink (Wu et al. 2002), differential allocation within the mycelial system (Leake et al. 2001) and transport of labelled photosynthates between mycorrhizal plants connected by mycelial networks have also

been demonstrated (Finlay and Read, 1986; Simard et al. 1997; Wu et al. 2001). Differences in carbon sink strength of different ectomycorrhizal fungi were shown by Bidartondo et al. (2001) and mycorrhizal morphotype community changes after lowered carbon supply by Saikkonen et al. (1999). As Dosskey et al. (1990) reported, different ectomycorrhizal fungal species can change the rate of photosynthesis of the host plant. Therefore, it seems important to establish future integrated studies, where temporal carbon allocation to different compartments is analysed together with relevant ectomycorrhizal fungal species under natural growth conditions. To our knowledge, our study (Paper I) is the first to visualize and quantify podzol horizon-related ^{14}C-carbon allocation in rhizosphere and mycorrhizosphere compartments.

Ecological function of carbon allocation

Although we and others now show significant carbon allocation and microbial activity in soil and podzol horizons, what is the ecological function and importance of this activity? The following two hypotheses will be discussed: the possible roles of biological activity in *podzolization* and *weathering* processes.

Different theories have been proposed to explain the podzolization process (reviewed recently by Lundström et al. 2000a). Two major mechanisms were earlier thought to explain the downward movement of Al and Fe in the soil profile: the formation of complexes with organic acids and transport as inorganic colloidal sols (Lundström et al. 2000a). This movement results in the formation of E- and B-horizons in the podzol profile. Based on a literature review and the results of a multidisciplinary research program on podzolization, Lundström et al. (2000b) concluded that it is now generally accepted that Al and Fe migrate downwards in the form of organic complexes. These complexes are mainly formed by humic, fulvic or low molecular weight organic acids (LMW). The role of mycorrhiza in the production of LMW (e.g. citric, acetic, oxalic acids) was hypothesized to be highly significant, as was the ability of these fungi to transport Al and Fe to the O horizon (Lundström et al. 2000b).

The degradation of organic complexes is presumably caused by microbial decomposition resulting in the horizon formation (Lundström et al. 2000b). van Hees et al. (2002) reported that organic acid complexes can be degraded in B-horizons, which would support the theory proposed by Lundström et al. (2000b). In Paper II, increased bacterial numbers in rhizosphere and mycorrhizosphere compartments in the B-horizon were shown, as well as specific bacterial communities in those compartments. The conclusion of microbial degradation is further supported by the studies on microbial activity in mineral soil that were discussed in an earlier chapter.

Ectomycorrhizal mycelia secrete organic acids (Lapeyrie et al. 1987; Ahonen-Jonnarth et al. 2000) and it has been suggested that the ectomycorrhizal effect on soil weathering is due to exploratory abilities of hyphae (Leyval and Berthelin, 1991). Jongmans et al. (1997) started an on-going scientific debate on the active role of ectomycorrhizal fungi in the weathering process by showing that in mineral particles tunnels were of equivalent diameter to fungal hyphae; these were claimed to be proof of direct access of mycorrhizal fungi to mineral nutrients. Landeweert et al. (2001) and van Breemen et al. (2000a, b) supported this hypothesis by showing strong evidence pointing to major ectomycorrhizal influence in weathering. Hoffland et al. (2002) presented evidence for a fungal role in weathering in a chronosequence study in North Sweden and Lundström et al. (2000b) also proposed that the exudation of LMW by ectomycorrhizal fungi was the main weathering process including pore formation on the mineral surfaces in E-horizon. Our data identifying *S. variegatus* mycorrhizas and extramatrical mycelium in the upper eluvial E mineral (Paper III) and in the B layer (Papers II and III) adds further

support for the role of these species and associated bacterial communities in the mineral weathering process, organic complex degradation and phosphorus uptake that were suggested but not confirmed in the above mentioned studies. However, the presence of the ectomycorrhizal hyphae in the tunnels of the mineral particles has not been proven, and for example Wallander and Wickman (1999) could not confirm Jongmans' hypothesis on ectomycorrhizal activity in pores, in spite of their finding that ectomycorrhizal seedlings were more efficient in weathering of biotite. Rosling et al. (2004) showed clear species-specificity in substrate acidification by different ectomycorrhizal fungi.

Species-specific differences in ectomycorrhizal carbon uptake were detected in Paper I and therefore it seems possible that carbon exudation and associated bacterial communities are also different between the mycorrhizospheres of different ectomycorrhizal species, a conclusion presented already by Timonen et al. (1998). Since rhizosphere bacteria are also known to produce organic acids (Leyval and Berthelin, 1991) and as the origin of the low molecular weight organic acids and their reactions in soil are not fully understood (Jones DL et al. 2003), the role of rhizosphere and mycorrhizosphere bacterial communities in soil processes should be further investigated. In conclusion, the equal carbon allocation to roots and mycorrhizas in podzol humus, E- and B-horizon shown in Paper I adds further evidence for an important role of ectomycorrhizal fungi in the fundamental processes of soil formation and plant nutrition.

4.1.2. Bacterial communities in podzol soil horizons (Paper II and unpublished results)

Significantly increased bacterial numbers in different Scots pine rhizosphere and mycorrhizosphere compartments on humus, E- and B-horizon, compared to bulk soil, were identified. Compartment-specific community level physiological profiles, obtained with the Biolog® method and analysed using principal component analysis (PCA), were also detected (Paper II and unpublished results).

Bacteria, among other microorganisms, are known to enrich in the rhizosphere of plants, compared to surrounding uncolonised 'bulk' soil (e.g. Atlas and Bartha, 1986; Grayston et al. 1997). As most of the boreal forest trees form ectomycorrhiza, the rhizosphere is influenced strongly by mycorrhizal fungi. Therefore, Linderman (1988) proposed in his review that the mycorrhizosphere effect, that is enhanced microbial activity in the soil around mycorrhizae and mycelium, is of central importance in soil microbiology. He also stated that clearly distinct bacterial communities have been found in rhizosphere and mycorrhizosphere soils.

Bacterial associations with mycorrhizal fungi can be endosymbiotic or bacteria can densely colonize or form biofilms on the hyphae (Perotto and Bonfante, 1997; Nurmiaho-Lassila et al. 1997; Mogge et al. 2000). Bacteria are even thought to utilise mycorrhizal mycelium for their distribution in soil matrix (Perotto and Bonfante, 1997). Garbaye (1994) presented data indicating that certain bacterial species or strains promote mycorrhiza formation and called them mycorrhiza helper bacteria (MHB). Since Timonen et al. (1998) showed that the bacterial diversity and communities in the mycorrhizosphere are location- and species-dependant and as Bomberg (2003) showed recently, for the first time, that Archaea were present in the Scots pine mycorrhizosphere but not in the rhizosphere in Finnish forest soil microcosms, it seems evident that the mycorrhizosphere is clearly a particularly important niche in forest soil ecosystem. Olsson et al. (1996a,b) and Olsson and Wallander (1998) studied VA- and ectomycorrhizal effects on bacterial activity in different sandy soils and concluded that, in the case of VA-mycorrhiza, the presence of

mycelium did not affect bacterial activity, whereas with ectomycorrhizas, negative, neutral and positive effects on bacterial activity, and occasional changes in biomass and community structure, were recorded. Although bacterial parameters in mineral horizons of podzol soil profile have been widely studied (see previous chapter), to our knowledge no detailed comparisons amongst bacterial communities in bulk soil, rhizosphere or mycorrhizosphere compartments in the different podzol horizons have been presented, prior to Paper II and our unpublished results.

Bacterial community level physiological profiling (CLPP), performed using Biolog® GN microtitre plate assay, was introduced by Garland and Mills (1991). Since then, the method has been used widely in soil microbiology (see e.g. Heinonsalo et al. 2000 and Preston-Mafham et al. 2002 for references). Although the CLPP patterns have to be produced with special care (see critique and recommendations in Preston-Mafham et al. 2002 and Paper II) and interpreted carefully, the method is still regarded as being suitable for describing and comparing differences in bacterial communities.

The CLPP results presented in Paper II showed three clusters: cluster 3 contained humus rhizosphere samples, cluster 2 bulk and rhizosphere samples from B-horizon mineral soil and cluster 1 contained the rest of the samples, i.e. bulk humus and all mycorrhizosphere samples. This clustering, indicates firstly that distinct bacterial communities occur in different compartments. Secondly, it points out that mycorrhizosphere communities were similar, regardless of the substrate on which mycorrhiza and mycelium was growing. In contrast, rhizosphere communities differed markedly in relation to where the roots were growing. In unpublished results (Figure 8) using the same material as in Paper III (for methods, consult Paper II), rhizosphere CLPPs differed clearly between humus and mineral horizon samples whereas bulk and mycorrhizosphere compartments are not separated. A general trend of separation between humus and mineral soil samples is obvious. Interestingly, the CLPPs obtained from unplanted control microcosms fell within the bulk and mycorrhizosphere compartment samples, indicating significant saprotrophic microbial activity in soil.

In conclusion, our findings that the presence of roots and mycorrhizal fungi enrich active bacterial communities in different podzol horizons and that these communities are partly location dependant, indicate that plant/fungus-associated bacteria are abundant in soil and potentially have specific and significant ecological functions which still need to be identified. The contribution of these bacterial communities to podzolization and weathering processes that were discussed in previous chapter represent one of their potential functional roles that needs further investigation.

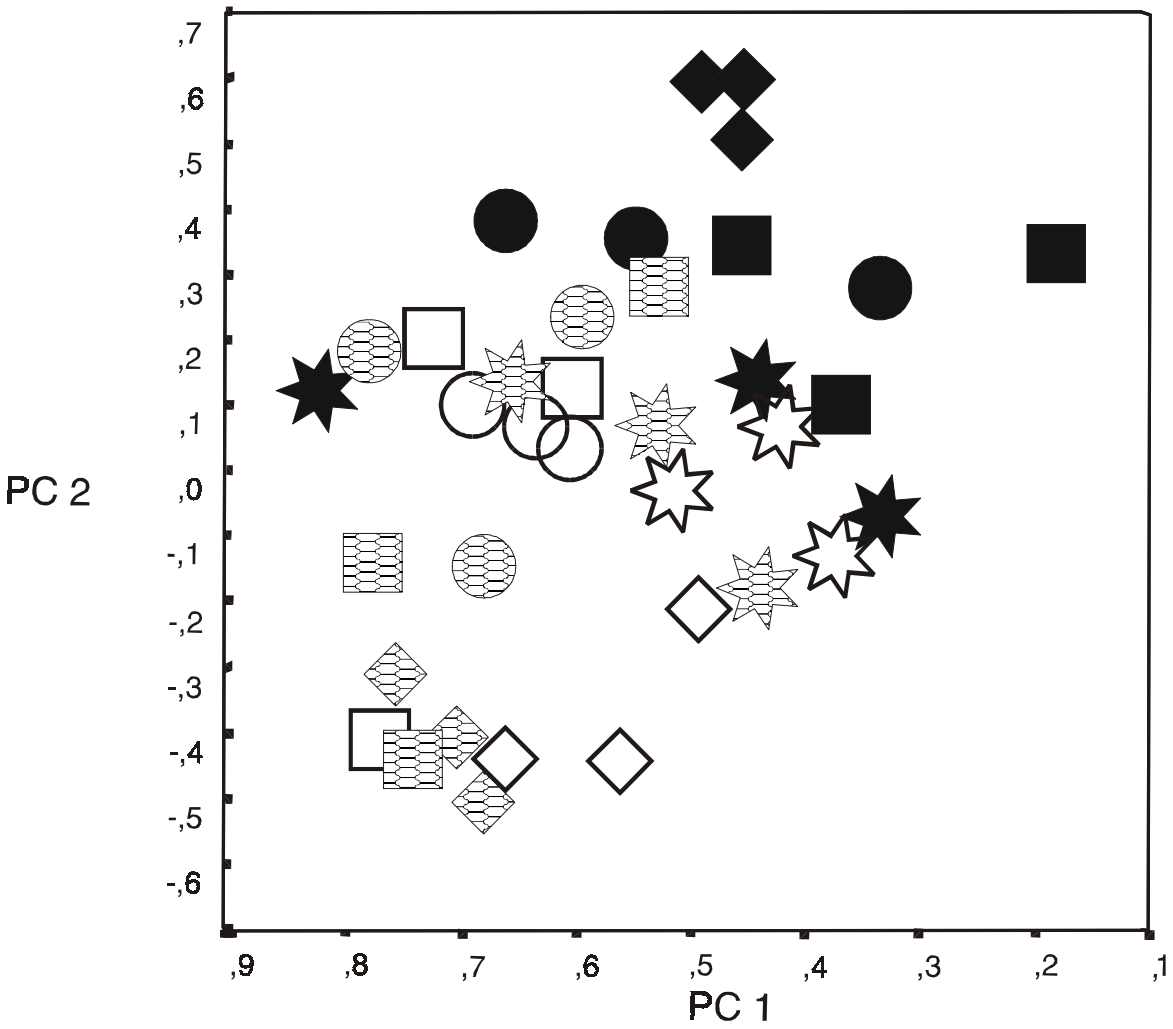


Figure 8. The projection of the first two axes obtained from principal component analysis (PCA) identifying bacterial community relationships in Scots pine rhizosphere, mycorrhizosphere and bulk soil compartments. Variance explained by axis PC 1 is 34.4% and by PC 2 9.0%. The codes indicate the following: star= non-planted control microcosms, circle= bulk soil from planted microcosm, diamond= rhizosphere and square= mycorrhizosphere samples from planted microcosms. Full symbols indicate samples from humus (O) layer, tiled from eluvial (E) horizon and open symbols from illuvial (B) horizons from respective microcosms.

4.1.3. Ectomycorrhizal community structure: field vs. microcosm assessment (Papers II, III and IV)

Knowledge of communities is important in understanding ecosystem functioning

Fungal communities in soil are extremely diverse since the great majority of over 80 000 fungal species so far identified are supposed to occur in soil at some stage of their life-cycle (Bridge and Spooner, 2001). Additionally, over 5 000 species of ectomycorrhiza forming fungi have been described (Molina et al. 1992). Since Trappe (1977) estimated that Douglas-fir (*Pseudotzuga menziesii*), a tree with a wide geographic distribution, is associated with over 2 000 species of ectomycorrhizal fungi, it is clear that when studying the diversity of ectomycorrhizal fungi, one is dealing with an enormous number of interacting species. Therefore, there have been attempts to re-direct the biodiversity research from a purely species-centered view towards approaches aiming at describing the functional diversity of an ecosystem, raising new questions such as how much/little

redundancy there is in the ecosystem and what is the relative importance of each functional group (Walker, 1992)?

The importance of ectomycorrhizal diversity to ecosystem functioning was discussed by Leake (2001) who noted that only a handful of papers had focused on this topic. Jonsson et al. (2001) concluded that the effects of ectomycorrhizal diversity on plant productivity are context dependent and can be positive, negative or neutral. However, they stressed that an individual ectomycorrhizal fungal species can have an influence on ecosystem properties. Baxter and Dighton (2001) concluded that greater ectomycorrhizal diversity *per se* increased carbon allocation to roots and phosphorus uptake of grey birch. However, fungal competition in soil has hardly been studied (Shearer, 1995) and although proposed by numerous authors (e.g. Bruns, 1995; Dahlberg and Stenlid, 1995; Buscot et al. 2000; Leake, 2001; Horton and Bruns, 2001), there is clearly a shortage of studies aimed at resolving questions concerning mycorrhizal functional groups and the effects of mycorrhizal fungi on ecosystem functioning. To be able to test various hypotheses on ecosystem function, Dahlberg and Stenlid (1995) and Taylor (2002) pointed out the great need to better understand the spatial and temporal patterns of ectomycorrhizas and mycelium in soil. Agerer (2001) also stressed the central importance of mycorrhizal mycelium and presented a proposal for functional classification of ectomycorrhizal mycelial systems based on fungal exploration types.

To be able to achieve the demanding goal of understanding the role of ectomycorrhizas in ecosystem functioning, ectomycorrhizal communities need to be sampled and analysed properly and reliably under field conditions to obtain more knowledge about the species we are actually dealing with. Dahlberg (2001) reviewed the current literature on community ecology of ectomycorrhizal fungi and highlighted the dramatic increase in ectomycorrhizal community studies since the 1990's and reported that only a third of the 50 studies published involved the use of molecular identification methods. Even if the number of studies is rising, it is clear that to be able to resolve extensive functional groups and to test their performance, there still remains far too little knowledge e.g. on the species richness and evenness in different sites, on what species there are and on how they respond to different disturbances. However, there is no doubt that the recent development of molecular methods coupled with the continued reduction in running costs, will allow researchers to produce large data sets which can be used in the future to better understand soil ecosystem functioning.

Ectomycorrhizal diversity detected in this thesis

In this thesis, the aim was to produce new information on the ectomycorrhizal fungal species in a Scots pine forest with a special emphasis on vertical podzol horizon-specific distribution patterns and to investigate the effect of forest clear-cut logging on this species diversity. The ectomycorrhizal fungal species, identified either by PCR-RFLP fingerprinting alone or together with sequencing, were studied both in the laboratory (Papers II and III) and under field conditions over a five-year period (Paper IV). The vertical distribution of ectomycorrhizal types was investigated in the microcosm experiments. The results from these experiments were obtained using Scots pine as so-called bait seedlings to characterize the ectomycorrhizal inoculum potential of the studied soils. Jonsson et al. (1999b) showed that species colonizing seedlings and trees are similar, so our field results can be used to reach tentative conclusions about the ectomycorrhizal fungal community in the mature control forest.

When ITS-RFLP identification data from different experiments was taken together, 53 different RFLP-taxa were observed (see the summary in Figure 9 and Table 2) to form ectomycorrhizas with Scots pine seedlings. This figure is well in accordance with other

studies of similar intensity using PCR-RFLP and sequencing methodology (Jonsson et al. 1999a,b; Dahlberg et al. 2001; Peter et al. 2001; Tedersoo et al. 2003). In the five-year field experiment, 47 taxa were identified (Paper IV) on a total of 152 seedlings; in first microcosm experiment (Paper II) 10 taxa were identified on 41 seedlings and in second (Paper III) 12 taxa on 27 seedlings. Representative samples of taxa, with a pre-defined abundance cut-off (≥ 5%) in any one treatment in any one year in the field experiment, together with representative samples from all the taxa in Paper III were sequenced. As can be seen from the summary table, the most common taxa in the field were also found in the microcosms; however, five additional RFLP-taxa were specific to microcosms. A major part of the rare field taxa were not detected in microcosm experiments at all. The observed diversity has to be regarded as very high since roots of only relatively small seedlings were analysed. This supports the report of Allen et al. (1995) where they estimated that ectomycorrhizal species richness was 10-100 times higher than host plant diversity in coniferous boreal forest.

Few key species dominate

The general trend in the experiments was that a few key species dominate whereas the majority of the taxa identified were more or less rare. This phenomenon, very common in the ecological studies, has been reported in earlier ectomycorrhizal community studies (Jones et al. 1997; Gehring et al. 1998; Horton and Bruns, 1998; Erland et al. 1999; Hagerman et al. 1999a,b; Jonsson et al. 1999 a,b; Jonsson T. et al. 1999; Mahmood et al. 1999; Dahlberg, 2001; Dahlberg et al., 2001). The four most commonly found species in the field experiment were taxa 23 (unknown *Piloderma*), 35 (*Suillus variegatus*), 5 (*Phialocephala fortinii* cluster) and 32 (*Cenococcum geophilum*). However, as can be seen in the rank-abundance analysis (Figure 10), considerable variation in the occurrence of the key species was apparent. *C. geophilum* together with Corticeaceous, Russulaceous and Thelephoraceous fungi have been found to be the most common in boreal forests (Dahlberg, 2001). In Swedish studies on Scots pine, Jonsson et al. (1999b) found that *C. geophilum*, *Piloderma croceum*, *S. variegatus* and an unidentified *Piloderma* were present in the roots of all seedlings and trees they studied. Jonsson et al. (1999a) similarly reported, in a study focusing on the effects of wildfire on the ectomycorrhizal communities, that RFLP-taxon X1 (an unidentified *Piloderma*?), *C. geophilum*, *S. variegatus, Cortinarius* spp. and *Phialocephala fortinii* were the most frequently found mycorrhizal types. Kårén (1997) showed also that *C. geophilum*, *P. croceum* and *Tylospora fibrillosa* (taxa 3 in Paper IV) were among the most common mycorrhiza forming fungi. Strikingly, our results further confirm that the same few species are the most common mycorrhiza forming fungi throughout the Fennoscandian Scots pine boreal forests.

An important question is whether there are threatened mycorrhizal species in the Scots pine forests and how does the clear-cut felling affect these species. In our field approach (Paper IV), representative mycorrhizas of most common taxa were sequenced in order to assess the effects of treatment, but the rare types were not intentionally

Table 2. (Next two pages) The summary of the field experiment and two microcosm experiments, based on the combined dendrogram presented in Fig. 9. O= humus, E= eluvial and B= illuvial horizon. Con= control forest, IF= interface zone and CC= clear-cut area. The filled taxas were the most common (frequency above 5%) in the field experiment. Dark colour with a cross descibed the zone/horizon where the taxa was found. Inoc.= unpublished inoculation experiment (UR). RFLP match refers to the RFLP database species (Kårén et al., 1997) which clustered closely together with the sample (Fig. 9). Blast match refers to the EMBL sequence database accession with highest homology to the sequence in question. ECM= ectomycorrhizal fungi.

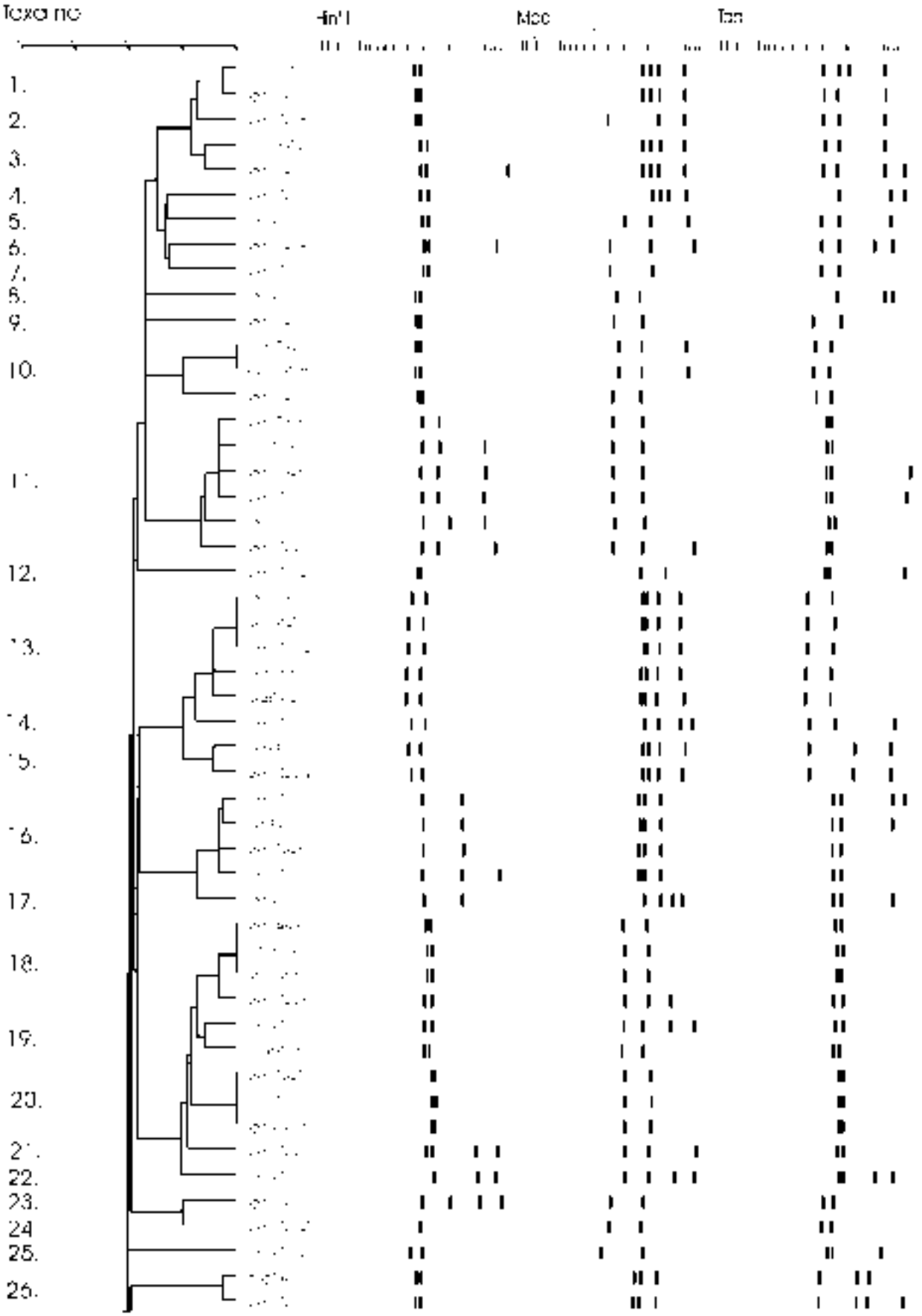
Fig.9	Paper IV				Paper II			Paper III							Inoc.
Taxa	Taxa	Con	IF	CC	Taxa	Con		Taxa	Con			IF	CC		
						O	B		O	E	B	O	O	E	B	
1	10			+												
2	9		+	+												
3	**11**			+												
4	**3**	+	+	+												
5	2	+	+													
6	4	+														
7	**1**	+	+	+												
8	17		+													
9	18			+												
10	19			+												
11	31			+	8	+	-	3		+		+	+	+		
12	26			+												
13	**12**	+	+		7	+	-									
14	**13**	+	+													
15	**14**	+		+												
16	**15**	+	+	+	6	+	-	4	+	+						
17	16	+		+												
18	**5**	+	+	+	4	+	+									Strain E
19	6	+	+													
20	**5**	+	+	+	5	-	+									
21								6	+	+	+	+	+			
22	7	+														
23								5	+							
24	25		+													
25	20	+														
26	30	+	+													
27	**8**	+	+	+												
28								12		+						
29	24			+												
30	39		+													
31	38	+	+													
32	**21**		+													
33	**22**	+														
34	**34**		+													
35	28	+		+												
36	27		+	+												
37								10				+	+			
38	29	+	+													
39	41	+	+													
40					10	-	+	9			+					
41	43			+												
42	**23**	+	+	+	9	+	-	11					+	+		
43	**44**		+	+												
44	45			+												
45	**33**		+													
46	**35**	+	+	+	1	+	+	1	+	+	+	+	+	+	+	Strain M
47	**36**		+	+	2	+	+	2		+						
48	37		+													
49	40		+	+	2	-	+	8							+	
50	42	+														
51	**32**	+	+	+	3	+	-	7	+			+	+			
52	46		+													
53	47		+													

[1]Ama.mus/por/reg= *Amanita muscaria*, *A. porphyria*, *A. regalis*; Cen.geo= *Cenonoccum geophilum*; Cor.fer/leu/sem= *Cortinarius fervidus*, *C. leucophanes*, *C. semisanguineus*; Cor. sp.= *Cortinarius* sp.; Der.dem= *Dermocybe semisanguinea*; Geo.car= *Geopyxis carbonaria*; Heb.lon= *Hebeloma longicaudum*; Hym.eri= *Hymenoscyphus ericae* -aggregate; Lac.mus/rus/vie= *Lactarius musteus*, *L. rufus*, *L. vietus*; Lcc.lac= *Laccaria laccata*; Pax.inv= *Paxillus involutus*; Phi.fin= *Phialophora finlandia*; Phi.for= *Phialocephala*

RFLP match[1]	Blast (Paper IV)[1]	Blast (Paper III)[1]	Blast (inoc.)[1]	Accession number
Cor.fer				
Cor.sem	Der. sem			AJ633111
	Tyl.fib			AJ633107
	Cor. sp.			AJ633106
Ama.mus/reg				
The.ter		The.ter./Tom.sub		AJ630021/ AJ630030
Rus.vin/bad/art	Rus.vin			AJ633112
	Rus.vin			AJ633113
Rus.dec	Rus.dec			AJ633114
Pil.cro	Pil.cro	Pil.cro		AJ633115/ AJ630022
Phi.for	Uncultured ECM		Phi.for	AJ633108/ AJ630032
Geo.car				
Pic.bic	Phi.fin/ Hym.eri			AJ633109
		Phi.for		AJ630024
		Cor. sp.		AJ630023
Tri.sap				
Ama.por	Ama.por			AJ633110
Lcc.lac		Lcc.lac		AJ630020
Cor.leu				
Lac.mus/vie	Lactarius sp.			AJ633116
Lac.ruf	Lactarius sp.			AJ633117
	Pse.hum			AJ633121
Heb.lon				
		Toms. sp.		AJ630028
		Tyl. sp.		AJ630027
Pax.inv				
	uncultured (Pil. sp.)	uncultured (Pil. sp.)		AJ633118/ AJ630019
	unknown ECM			AJ633124
	Tyl.ast			AJ633120
Sui.var	Sui.var	Sui.var	Sui.var	AJ633122/ AJ630018/ AJ630031
Sui.bov	Sui.bov	Sui.bov		AJ633123/ AJ630029
Rhi.vul?				
		Rhi. sp.		AJ630026
Cen.geo	Cen.geo	Cen.geo		AJ633119/ AJ630025

fortinii; Pic.bic= *Piceirhiza bicolorata*; Pil.cro= *Piloderma croceum*; Pil. sp.= *Piloderma* sp.; Pse.hum= *Pseudotomentella humicola*; Rhi.vul= *Rhizopogon vulgaris*; Rhi. sp.=*Rhizopogon* sp.;Rus.art/bad/dec/vin= *Russula artesiana*, *R. badia*; *R. decolorans*, *R. vinosa*; Sui.bov/var= *Suillus bovinus*, *S. variegatus*; The.ter= *Thelephora terrestris*; Tom.sub= *Tomentella sublilacina*; Toms sp.= *Tomentellopsis* sp.; Tri.sap= *Tricholoma saponaceum*; Tyl.ast= *Tylospora asterophora*; Tyl.fib= *Tylospora fibrillosa*; Tyl. sp.=*Tylospora* s

Figure 9.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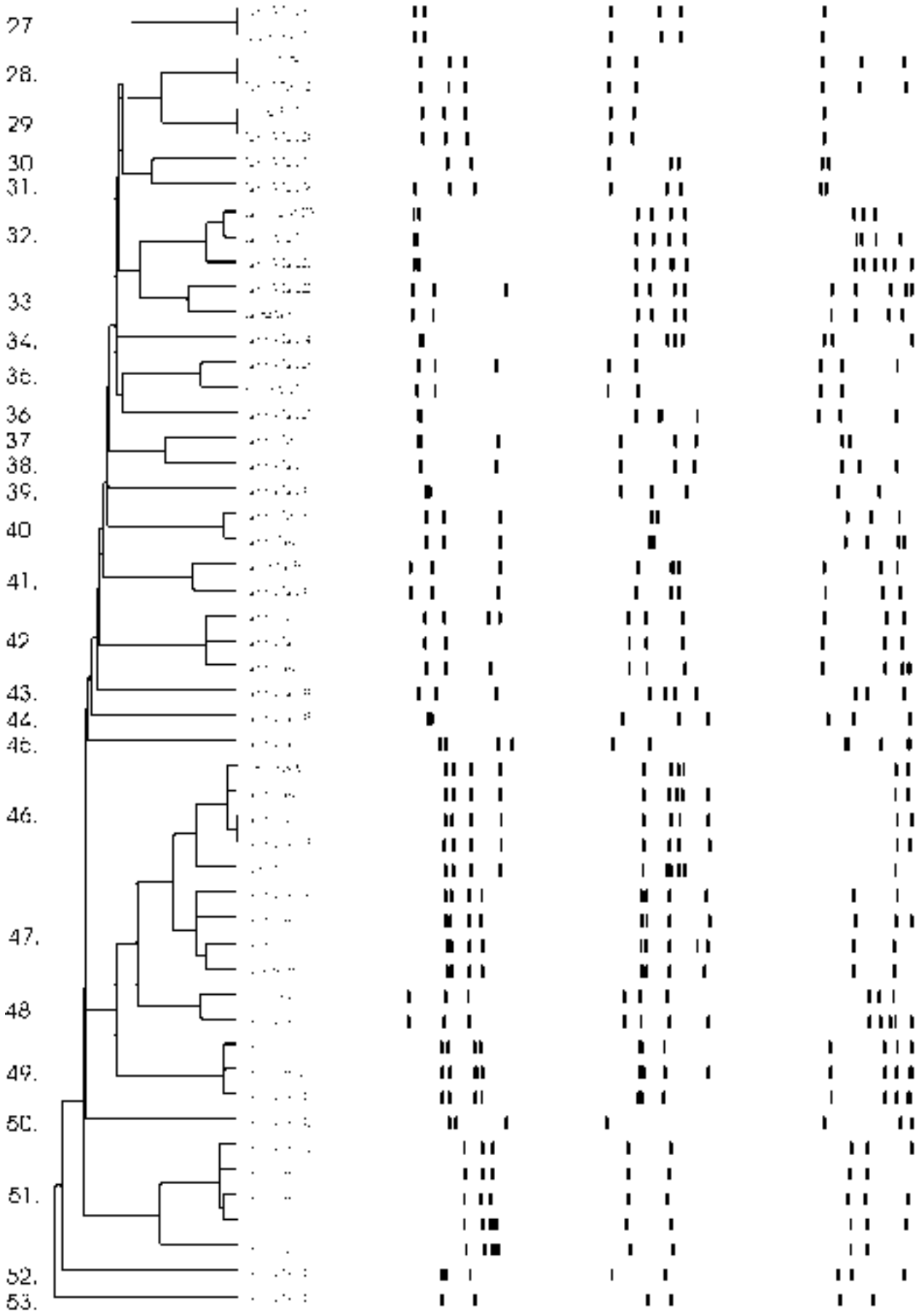
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Figure 9. (Two previous pages) The dendrogram combining representative samples from all the taxa determined in Papers II, III and IV, and the selected strains used in UR. The taxa numbering corresponds with those in Table 2 where the abbreviations used for RFLP database species names are also explained. *Hinf* I, *Mbo* I and *Taq* I are restriction enzymes used in the RFLP analysis.

identified. However, from the 34 taxa, which were not among the most common in the field and were therefore not sequenced, 15 provided a match either with the RFLP-database or sequences obtained for the Paper III (see Table 2). It seems, based on our results, that fungi that are on the endangered species list in Finland ('Red list'; Rassi et al. 2001) are not commonly found in the pine dominated forest that had been subjected to standard Finnish forestry practises. Among the non-identified types found, both in the control forest or interface and clear-cut zones, it is possible that some species are endangered. Research focusing on the presence of rare fungi as ectomycorrhizal symbionts should also be intensified since little is known about abundance of many fungi in soils.

Vertical distribution of species

In the podzol profile studies (Papers II and III), it seems that the types forming strong rhizomorphs (particularly *Suillus variegatus* and *S. bovinus*), dominate in the deeper mineral horizons while in humus the abundance of species representing the different exploration types (Agerer, 2001) is more even. In the field (Paper IV), the non-rhizomorph forming ('hairy') types have higher relative abundaces in the clear-cut treatment, whereas in the control forest, the rhizomorphic types are abundant. This is an interesting field observation, since it is contradictory to the microcosm results, which indicated that rhizomorph-formers were relatively more abundant in mineral soil. In the clear-cut site, seedlings were clearly larger and their roots certainly penetrated the mineral horizons; therefore they were expected to support increased proportions of rhizomorphic types. It seems that mycorrhizal communities change in different treatments in a functional way, which can be seen in the shift in the exploration types. However, the putative functional differences of different exploration types are not clearly understood.

The hypothesis for succession based on sequential colonization by early- and then late-stage fungi (Last et al. 1984), has been criticized since these groupings are not clearly defined. Recently, Nara et al. (2003a,b) studied primary succession (Fisher and Binkley, 2000) of ectomycorrhizal fungi in Mt. Fuji soil that had not been previously colonised by other fungi or living organisms. In such circumstances, the theory of Last et al. (1984) was supported. However, in most cases in nature, forest regeneration occurs in soils where there is plenty of ectomycorrhizal inoculum because the site has been previously occupied by ectomycorrhiza-formers (secondary succession). In these cases, the 'early- vs. late-stage-succession' is not evident. On the contrary, it would be important to study in more detail whether the different exploration types reflect the secondary succession in ectomycorrhizal communities and whether different species belonging to the same exploration type behave in a functionally similar manner.

Horizon-specific ectomycorrhizal fungi were detected in the microcosm experiments. In two experiments, 15 RFLP-taxa were found in total, of which four were humus, and four mineral soil specific. Humus-specific species were *Russula vinosa*, *Cortinarius* sp., *Tomentellopsis* sp. and *C. geophilum*. In mineral soil, E- horizon-specific species were *Laccaria laccata* and B- horizon specific *Rhizopogon* sp., unknown ECM root tip (in Rosling et al. 2003) and uncultured *Tylospora* (in Tedersoo et al. 2003), respectively. Three species, which were humus-specific in Paper II, where the E-horizon was not studied, were found either in humus or in E-, but never in the B-horizon in Paper III. Those species were: *Thelephora terrestris* (=*Tomentella radiosa*, see Kõljalg et al. 2000), *Piloderma croceum* and an uncultured *Piloderma*. Rosling et al. (2003) also made

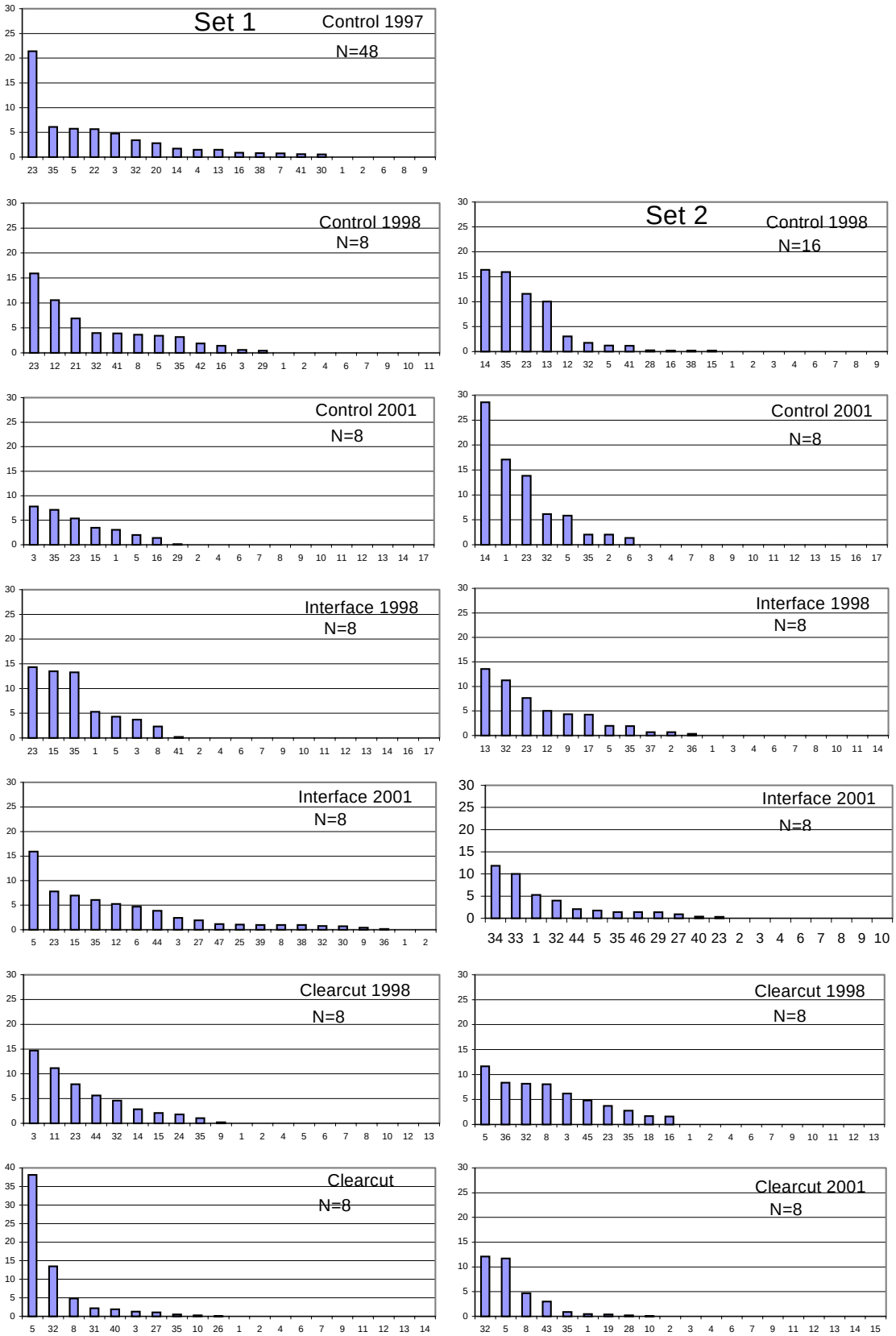

Figure 10. A rank-abundance histogram of identified Scots pine seedling root colonizing fungal taxa from Set 1(left) and Set 2 (right) per treatment and per year in Paper IV. X-axis values indicate the number of taxa, as described for Paper IV in Figure 9. Y-axis values indicate the abundance (%). The taxas omitted from the histograms have zero abundance. N= number of replicate seedlings analysed.

the distinction between species present in humus and E-horizon and the species present deeper in podzol profile. Species, which were detected in all the horizons were *S. variegatus*, *S. bovinus* and *P. fortinii*, the former and the latter were also found to be among the most common ones in the Hyytiälä field experiment. Ahonen-Jonnarth et al. (2000) studied organic acid production of certain fungi in elevated aluminium and heavy metal concentrations. Interestingly, they found that *S. variegatus* (present in all horizons in Paper III) and *R. roseolus* (close homology with taxa 8 detected in B-horizon in Paper III) were associated with highest levels of oxalic acid. In the B-horizon, the Al-ligands are known to be enriched (Lundström et al. 2000a,b) and this could mean that oxalic acid production would be increased in that horizon. The correlation of these results are interesting considering the ectomycorrhizal fungal participation in the weathering process.

Our findings on vertical distribution of mycorrhizas are well in accordance with all the other studies that have focused on the same question (Dickie et al. 2002; Rosling et al. 2003 and Landeweert et al. 2003). Tedersoo et al. (2003) suggested that species such as *Tomentellopsis submollis*, *Piloderma fallax* (=*croceum*) and *Tomentella crinalis* are able to grow saprotrophically on sterile rotted wood, indicating saprotrophic capabilities. Interestingly, some of these species were detected in the humus layer in our studies. It seems evident that some niche-differentiation occurs and thereupon different species have functional differences, which presumable are important for the functioning of the ecosystem.

Interesting species

Cenococcum geophilum is found to be one of the most common ectomycorrhizal species world-wide (Horton and Bruns, 2001) and it has been shown to have high genetic diversity on both local and global scales (LoBuglio et al. 1991; Jany et al. 2002). These conclusions were fully supported in our studies, where *C. geophilum* was commonly found in different treatments, notably always in the humus layer, and the genetic heterogeneity within that particular RFLP-cluster was always high. However, sequences from different sub-groupings within the broad *Cenococcum*-cluster in our studies were always identified as *C. geophilum*. It has been suggested that *C. geophilum* was drought tolerant and could provide drought protection to host root tips (e.g. Coleman et al. 1989).

Suillus variegatus seemed to be the ‘all-round mycorrhiza’ in our studies. It was one of the most common fungi in the field and could be found in almost every microcosm in every experiment regardless of the source of soil. However, while *S. variegatus* has been suggested to be dominant over *S. bovinus* in older forests based on sporocarp surveys, the situation is the reverse in younger forests (Dahlberg and Stenlid, 1990; Dahlberg, 1997). In our case, it is logical then that *S. variegatus* is common in control forest but, based on the mentioned reports, *S. bovinus* should be more abundant in the clear-cut site. However, since it was shown that the abundance of rhizomorph-forming fungi generally diminishes in the clear-cut, it could be that *S. variegatus*, whose inoculum was probably left alive in the root tips for some years (Hagerman et al. 1999a), is still maintained in the clear-cut, but that the proportion declines over time. Possibly, when the regenerated stand is some years older, the succession suggested by Dahlberg (1997) will take place. An alternative possibility is that this successional pattern does not operate below-ground.

Phialocephala fortinii, *Piceirhiza bicolorata* and *Geopyxis carbonaria* formed a common RFLP-cluster in Papers II, III and IV. However, the cluster formation was dependent on the samples analysed, reflecting the unstable clustering with regard to these Ascomycete species. For instance, in the summary presented in Figure 9, the field taxon 5 is located in two separate taxa although in the original data analysis, involving all the

samples of the field experiment, a clear single cluster as e.g. in Paper II was obtained. Obviously, these species are difficult to separate by PCR-RFLP and detailed analyses should be made using sequencing technology for a definitive identification.

P. fortinii has been shown to be genetically highly variable and polyphyletic (Grünig et al. 2002) and it belongs to a group of fungi referred to as DSE (dark septate endophyte) fungi. They have an unclear association with plants (Jumpponen and Trappe, 1998a). *P. fortinii* has been suggested to be pseudomycorrhizal or pathogenic (Wang and Wilcox 1985; Wilcox and Wang, 1987) or having potentially mutualistic, mycorrhiza-like associations with partial Hartig net and a thin mantle (Jumpponen and Trappe, 1998a; Jumpponen et al., 1998; Jumpponen, 2001). Since *P. fortinii* is one of the most common root endophytes (Stoyke et al., 1992) with a global distribution (Jumpponen et al., 1998a), its ecological role is potentially significant. Further studies are needed to confirm the nature of the association formed with different host plants. The fungus forming *P. bicolorata*-mycorrhiza has also been shown to cluster together with *Phialophora finlandia* in the *Hymenoscyphus ericae*-aggregate (Vrålstad et al., 2000; Vrålstad et al., 2002a). Since *H. ericae* forms ericoid mycorrhiza with ericoid plants and has a wide range of important metabolic activities, its close relationship with fungi forming *P. bicolorata* -ectomycorrhiza is very interesting. However, although it was hypothesized that *H. ericae*-aggregate may connect ericoid and ectomycorrhizal plants, no single strain was shown to form both ecto- and ericoid mycorrhizas (Vrålstad et al. 2002a). *P. fortinii* was also found in some *P. bicolorata* –mycorrhizas, from which mycobiont sequences normally aggregate together with *Phialophora finlandia* and *H. ericae* (Vrålstad et al. 2002b). *G. carbonaria* also forms similar looking structures in *Pinus contorta* as *P. fortinii* (Jumpponen et al. 1998a) and has been shown to form, depending on the conditions, moderately pathogenic or mutualistic associations with host plants (Egger and Paden, 1986). Since Vrålstad et al. (1998) were able to confirm that *G. carbonaria* can form biotrophic association with Norway spruce, they proposed that this wide-spread post-fire fungus could form ectomycorrhiza with spruce and ectendomycorrhiza with Scots pine. In conclusion, the complex group of DSE fungi, which form a wide range of associations with their host plants, should be specifically studied since their wide distribution world-wide suggest an important role in terrestrial ecosystems.

4.1.4. Dispersal strategies of ectomycorrhizal fungi and importance of inoculum type in the clear-cut (Papers II, III and IV)

Jones et al. (2003) reviewed the current literature on inoculum sources in forest soil and discussed the effects of forest clear-cut logging on ectomycorrhizas in young seedlings. They comprehensively discuss the factors affecting different sources of inoculum in the clear-cut sites, living hyphae, sclerotia and dying mycorrhizas, and spores. In clear-cut soil, secondary succession of ectomycorrhizal fungal communities takes place. The succession of ectomycorrhizal fungi has been thought to depend on the dispersal strategies (Newton, 1992). Spores and sclerotia are known to be available after forest harvesting, but depending on the period of time between logging and seedling regeneration, mycorrhizal mycelium may also remain active in the roots of the cut trees (Hagerman et al. 1999a). At the forest edge, the roots of living trees may indeed extend up to 15 meters into the opening (Jones et al. 2003), allowing the nearest seedling to have access to living hyphae of a similar group of ectomycorrhizal fungal species as in the mature forest (Jonsson et al. 1999b). The same thing is true near so-called refuge trees (Kranabetter, 1999; Hagerman et al. 2001) left standing in the site. In spite of the presence

of a range of inoculum, there can still be a shift in the inoculation potential at the site. Dahlberg and Stenlid (1995) concluded that establishment success depends largely on whether the ectomycorrhizal community is open or closed. Closed communities of mature forests, where large genets dominate, can be highly resistant to invasion by spores, whereas in open communities present in clear-cut areas, spores and sclerotia may be an important source of inoculum.

In the microcosm experiments, it was possible to study inoculation potential of disturbed soil, because sieved and stored soil from the field site has obviously no ectomycorrhizal hyphal connections to standing trees and has therefore characteristics more similar to the prepared soil in the clear-cut site. These results should optimally be interpreted only together with corresponding field results. Based on the species diversity data obtained in Papers II and III, where the ectomycorrhizal inoculation potential of soils from different podzol horizons from control, interface or clear-cut treatments was compared, it can be concluded that the origin of the soil (treatment) did not significantly influence the ectomycorrhizal community structure but the horizon did. Similarity in inoculum potential between control and clear-cut soil was reported also by Dahlberg and Stenström (1991). These observations support findings concluding that inoculum does not limit the ectomycorrhiza formation, at least within a few years after clear-cut logging (Jones et al. 2003).

In the field experiment, a part of the seedlings were already planted before the clear-cut logging. Since the seedlings were originally planted into the closed canopy forest, it was only following clear-cutting that some of them could be labelled as interface and clear-cut seedlings. We can therefore presume that all of the seedlings had similar exposure to indigenous inocula and could potentially maintain the same ectomycorrhizal community in their root system. A similar hypothesis was tested by Kranabetter and Friesen (2002) who transplanted seedlings from mature forest to openings and Hagerman et al. (2001) who studied advanced regeneration seedlings of Douglas fir. In their studies, the communities changed and the same thing occurred in our experiment. Four years after clear-cutting, the community structure had clearly changed in the different treatments: in the control forest, rhizomorph-forming fungi became significantly more common on seedling roots, whilst the reverse occurred in the clear-cut forest. Interestingly, seedling roots in the interface zone showed an intermediate response. Mostly, different RFLP-types dominated the clear-cut seedlings compared to the counterparts growing in the interface or control forest. Therefore, the observation that originally similar diversity was not maintained in the seedlings suggests that other than inoculum-derived factors define the shift in the community structure.

Jones et al. (2003) concluded that it has not been proven that the lack of inoculum in the clear-cut soil would cause the frequently observed change in the community structure. Instead, they pointed out that the changes in the environmental factors in the clear-cite site and soil may be as important for the community changes as the alterations in inoculum. Our conclusion based on combined results from microcosm and field experiments fully support these conclusions. Our suggestion is that the governing factor is carbon demand of the fungi. Therefore, the mycelial structures formed by the fungi using host-derived carbon may play a successional and functional role. The potential importance of refuge plants, also those generally regarded as ericoid mycorrhizal, should be further studied since reasons for the high abundance of *P. fortinii* –type fungi in the clear-cut are as yet not clearly understood.

4.2. Effects of forestry practices

4.2.1. Effects of clear-cut logging on seedling biomass (Paper I, II, III, IV)

Scots pine is commonly known to be a shade-intolerant pioneer tree species and it does not readily regenerate under a closed canopy (Valkonen et al. 2001). The reasons why Scots pine grows better in openings than in control forest will not be discussed here. Instead, seedling growth differences in the interface zone and adjacent clear-cut areas caused by different treatments are of interest and possible reasons for those differences are considered. In addition, the potential of soil from different field origins to support seedling growth, as studied in the laboratory, is also discussed.

In the field experiment (Paper IV), Set 1 consisted of seedlings planted into the forest before clear-cutting, thus the inoculum available in the first growing season was the equivalent for all Set 1 seedlings. Nevertheless, over subsequent years the mycorrhizal community clearly changed in the roots of the seedlings both in the interface and in the clear-cut area as compared to the control forest. This was also reflected in the biomass of the seedlings. Although in the control forest in Set 1 the seedling biomass was clearly very low, it is more interesting that the interface seedlings did not grow as well as the clear-cut counterparts. In Set 2, planted after the clear-cut, no significant differences were observed between the interface and clear-cut seedlings. These data showed that the advanced regeneration decreased the seedling growth in the interface, most probably due to changes in the ectomycorrhizal community. In contrast, seedlings from Set 1 in the clear-cut area grew relatively better than the corresponding ones from Set 2. Interestingly, the abundance of rhizomorphic types in the interface seedlings in Set 1 was similar to that of control seedlings whereas in Set 2, no statistically significant difference was found in the abundance of rhizomorphic types between interface and clear-cut seedlings.

In all of the microcosm experiments (Papers I, II and III), it could be seen that the origin of the soil did not have any significant influence on the seedling biomass. An interesting result was the elevated uptake of CO_2 by the plants growing in the microcosms containing clear-cut soil (Paper I). In this treatment, part of the carbon was clearly incorporated into plant biomass, which was slightly (not significantly) higher than in corresponding control seedlings, but increased root respiration and microbial activity could also be an alternative explanation.

Varying levels, both increased or decreased, in available nutrients can be detected in the soil after clear-cutting (Mann et al. 1988; Bock and Van Rees, 2002). In the study of Piirainen (2002), the leaching of different nutrients from different podzol profile horizons was studied in one catchment area in north-eastern Finland before and after *Picea abies* clear-cut logging. Practically no increase was detected in the leaching of nutrients out of podzol profile. However, in the organic layer increased nutrient levels (e.g nitrogen and phosphorus) were detected, which were then leached but subsequently bound in deeper mineral horizons. In our experiments, it has to be kept in mind that the microcosm-based findings might have a methodological bias of uncertain importance, in relation to soil nutrient concentrations. Soil sampling, storage at +4°C and processing (sieving and mixing) may have altered the amount and form of nutrients, probably mainly as a result of microbiological activity. Based on our results, we hypothesize that since no growth differences were observed in the laboratory, the increased seedling growth in the opening was caused by changes in field environmental conditions.

4.2.2. Ectomycorrhizal community structure after the clear-cut felling (Papers III and IV)

In the literature, most reports indicate that the ectomycorrhiza formation may decrease and community structure clearly change after clear-cut logging (Perry et al. 1987; Ingleby et al. 1998; Durall et al. 1999; Hagerman et al., 1999a; Byrd et al., 2000; Jones et al. 2003). Our diversity data support these earlier findings: from the 53 taxa identified in the different experiments performed, 32 were found either in control forest in the field experiment, or in the microcosms containing soil from control forest. In the interface zone and in the clear-cut area, 34 and 28 taxa were found (Table 2), respectively. From all species identified, eight, nine and eight were specific to control, interface and clear-cut zones, respectively. Clear treatment specificity was found, therefore, which strongly supports the generally observed finding of a shift in community composition after various disturbances (Mahmood et al. 1999; Jonsson et al. 1999a; Jonsson T. et al. 1999; Dahlberg et al. 2001; Peter et al. 2001). The shift in community structure was seen most clearly in the field experiment (Paper IV), where the dominance of taxa 23 (unknown *Piloderma*) and 35 (*Suillus variegatus*) in the control and interface treatments seems to be shifted to dominance of taxa 5 (*Phialocephala fortinii* cluster) and 32 (*Cenococcum geophilum*) in the clear-cut area (see Figure 10). Increased abundance of MRA and E-strain fungi (many of them belong to DSE e.g. *P. fortinii*) and *C. geophilum* in the clear-cut site was also reported by Hagerman et al. (1999b). It is noteworthy, that for seedling Set 1, which was planted before forest clear-cutting (Paper IV), it took a longer time to shift to the 'clear-cut species community' while the seedlings of Set 2 had, by the end of the 1998 growth season, a similar community to that in 2001. In addition, an increased proportion of rhizomorph-forming fungi was detected in control forest seedlings whereas in the clear-cut area, rhizomorph formers almost disappeared. The changes observed in the clear-cut community is also supported by findings of Kranabetter and Friesen (2002) who showed that seedlings grown earlier in mature forest and later transplanted into openings, could not maintain the 'forest community' but the structure changed to consist of more rapidly growing species. It is possible that those species demand less carbon and this sort of shift may be inevitable after clear-cut logging. It would be logical that rhizomorph-forming ectomycorrhizal fungal individuals, that have large mycelial biomass to support, are likely to obtain relatively greater proportion of their carbon requirements from the overstorey trees in closed canopy forest. Following clear-cut, seedlings associated with such fungi may be unable to provide sufficient carbon to maintain their energy demand. After disappearance of rhizomorph-forming fungi, other non-rhizomorphic, clear-cut fungal communities may colonize the fine roots. However, carbon demand amongst rhizomorph-forming fungi can differ, which was also shown to be the case between *Piloderma* sp. and *Suillus* sp. in Paper I.

The total numbers of different morphotypes and RFLP-taxa were highest in the interface zone and Simpson's diversity indices from both morphotype and RFLP-data were also highest in the interface treatment towards the end of the experimental period, which supports the results of Hagerman et al. (1999b) and Durall et al. (1999). It can also be concluded that species richness and diversity did not change between the control and clear-cut treatments while the interface zone was the most diverse habitat. It is noteworthy that if the species richness and diversity data had been expressed on a per gram root fresh weight or root length basis, seedlings in the control forest would have the highest values since the control forest seedlings remained very small for the duration of the field experiment, (see Table 2 in Paper IV).

In the laboratory microcosm experiment (Paper III) where the inoculum potential of control, interface and clear-cut zone soils were compared one and four growth seasons

after the clear-cut logging, no clear treatment-related changes in the inoculum potential were found. This indicates that the quantity or quality of soil inoculum was not changed as a result of clear-cut logging, at least within four years after logging. The shift in species dominance detected in the field experiment could not be confirmed in microcosm simulations.

Based on the literature (Jones et al. 2003) and our results, it seems that the clear-cut treatment does not decrease the general biodiversity in terms of diversity indices or species richness but a shift in the composition of the ectomycorrhizal communities does occur over time. Some types of ectomycorrhizas are treatment specific and the abundance of these taxa differ amongst the control forest, interface or clear-cut zones. Reasons for the community shift are not entirely clear but it seems that differences in environmental conditions, inoculum availability and seedling physiology, including the ability to allocate carbon to mycorrhizal symbionts, are the main factors influencing the community change. For seedlings growing at the forest edge, benefits from both the nearby standing trees (cf. the studies on continuity: Jonsson et al. 1999b; Dahlberg et al. 2001) and favourable environmental conditions in the open clear-cut site are factors that may have promoted high ectomycorrhizal species diversity in their root systems. However, it is very important to note that the shift in communities was to be seen only after two to three years after logging (see also Hagerman et al. 1999a,b). Therefore, sufficiently long experimental periods are needed to detect community shifts.

4.2.3. Site preparation and seedling establishment (Paper III)

In Paper III, the first set of microcosms (Set 1) grew for nine months, and the second set (Set 2) for six months. No major increase in the biomass of the seedlings growing in E- and B-horizons can be seen between these two experiments, thus potentially indicating that the growth in the three months period between six and nine months was slow. On the contrary, the shoot fresh weight of the nine-month-old seedlings of Set 1 growing on humus is almost five-fold that of the corresponding six months old seedlings of Set 2. However, these laboratory results are not directly comparable since the observation is made from two separate microcosms experiment, performed four years apart. However, it is hypothesized that the establishment period for the seedlings, whether they are planted in organic or mineral soil, is around six months. After that period, it is hypothesized that organic humus layer provides significantly better growth conditions for seedling development.

Örlander et al. (1998), who studied the effect of different soil preparation methods on seedling survival and establishment in the field, suggested that the ability to take up water is critical for early seedling establishment and growth while later, the nutrient uptake becomes an increasingly important factor. In our microcosms, water availability was maintained through regular watering, which is likely to have improved seedling survival in the humus layer. In general, the humus layer is not the best location for new, developing pine seedlings (e.g. Örlander et al. 1998; Valkonen et al. 2001). In the same field site in Hyytiälä that we have used, de Chantal et al. (2003) compared the effect of three different site preparation methods on Scots pine emergence from seeds together with subsequent seedling establishment and mortality rates. They concluded that the Ae/B horizon was the most suitable for regeneration by seeding. In the ectomycorrhizal community study presented in Paper III, the E-horizon community was similar to the humus community, a trend that was also found by Rosling et al. (2003). It would be logical that when planted into the exposed E-horizon, seedlings would develop a similar mycorrhizal flora in their roots as in humus and this would give those seedlings an advantage, as soon as they

reach organic soil. By contrast, Dahlberg (1990) found no evidence that the presence of an organic layer can affect the establishment and development of ectomycorrhizas on outplanted seedlings. However, this study lasted only one summer, and the biomass data of the seedlings was not provided.

Based on our results, it can be hypothesized that the presence of humus is a prerequisite for good seedling growth, after the establishing period of approximately one growing season. Therefore, a site preparation method (e.g. harrowing) where seedlings are planted into the upper mineral layer, and which would allow young seedlings to access the humus layer within one growing season, would provide optimal regeneration conditions. This hypothesis should, however, be tested under relevant field conditions. It would also be interesting to determine the role of ectomycorrhizal fungi in frost heaving of seedlings, which is reported to be an important cause of winter mortality of young seedlings (de Chantal et al. 2003).

4.2.4. Inoculation experiment (Paper V and UR)

Selection of suitable ectomycorrhizal fungal strains for inoculation (Results of UR)

In the first phase of the experiment, Scots pine seedlings were inoculated with four ectomycorrhizal fungal strains in sterile tubes. After two months of growth, only strains M and E formed mycorrhizas (determined visually but not quantified) and since the growth performance of seedlings colonized by these strains was not affected compared to non-inoculated seedlings (Figure 11), these two types were chosen for the second phase of the experiment.

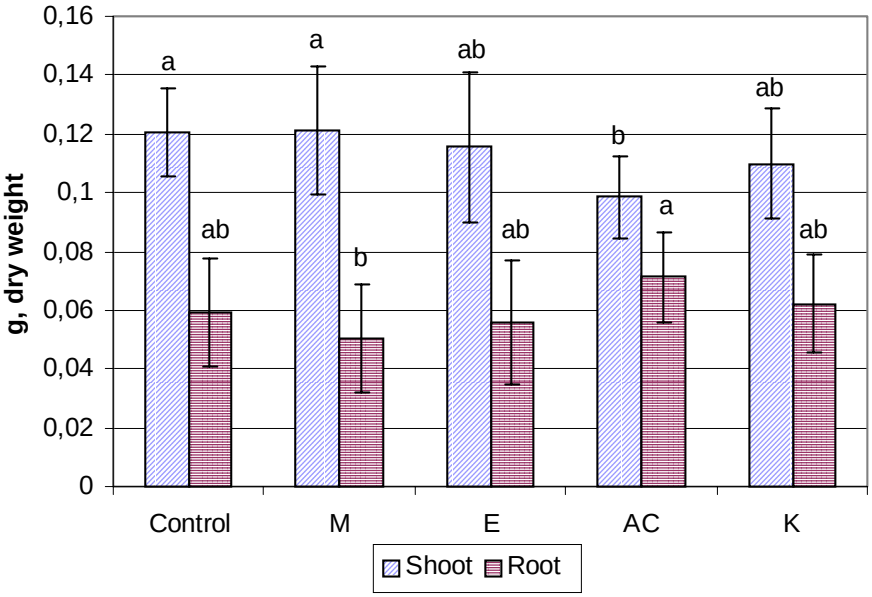

Figure 11. Biomass (g DW) of inoculated seedlings after the sterile tube experiment. M, E, AC and K are codes for pure cultures of different ectomycorrhizal fungal morphotypes inoculated in the tubes. Different letters above the standard deviation bars indicate statistically significant differences within shoot or root fraction according to Oneway ANOVA (shoot, P= 0.001; root: P= 0.008) followed by Tukey's *post hoc* test.

In the second phase, randomly selected non-mycorrhizal seedlings and seedlings mycorrhizal with strains M and E were planted into pots filled with unsterile clearcut podzolic soil. After 4.5 months of growth, the root and total biomass of the seedlings inoculated with strain E was significantly lower compared to control seedlings and seelings inoculated with strain M (Figure 12). However, no significant differences were

detected in the relative abundance of different morphotypes indicating that the abundance of M, E and other morphotypes were unaffected by the inoculation (Figure 13).

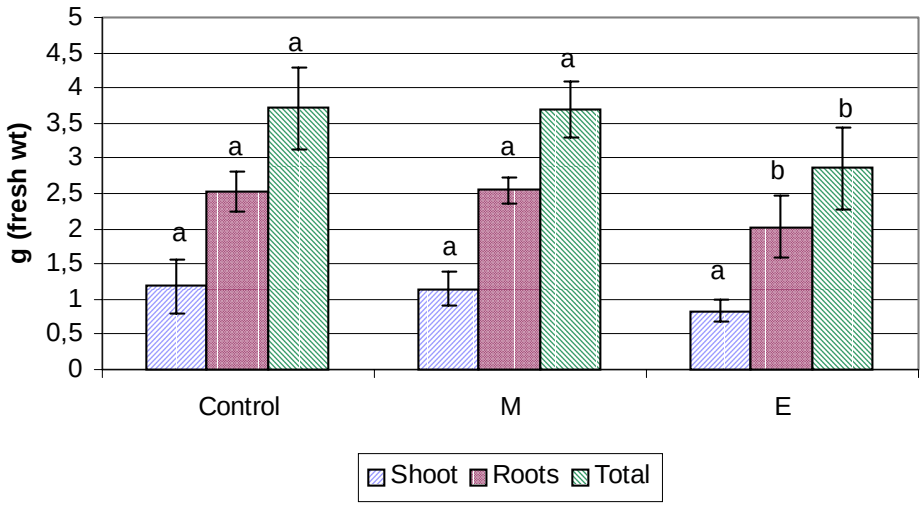

Figure 12. Biomass (g FW) of the seedlings grown for 4.5 months in pots with unsterile clear-cut soil. Control= originally uninoculated seedlings, M= seedlings inoculated with morphotype M (*S. variegatus*) and E= with morphotype E (*P. fortinii*). Different letters above the standard deviation bars indicate statistically significant differences. Oneway ANOVA (shoot: P= 0.094; root: P= 0.020; total: P= 0.021) followed by Tukey's *post hoc* test was performed separately for shoot and root fraction and for total biomass.

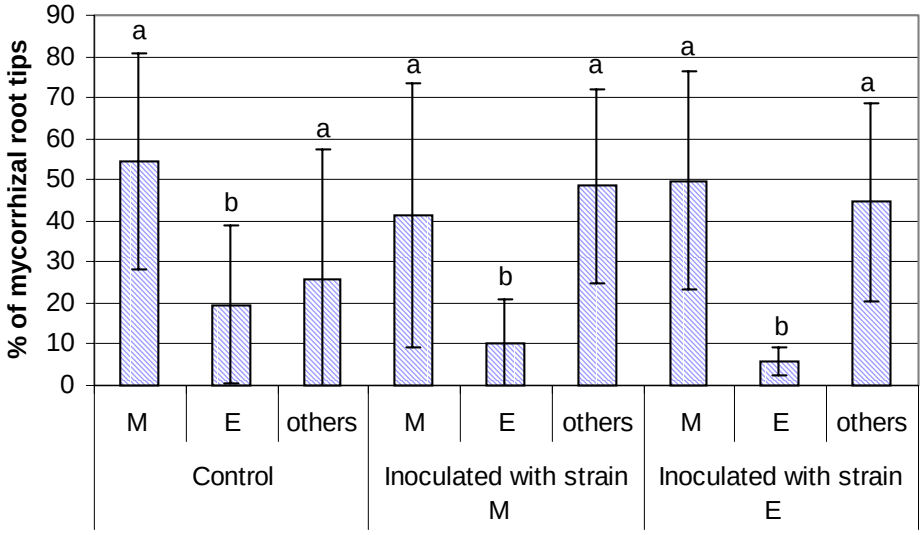

Figure 13. The abundance of introduced morphotypes (M and E) and other indigenous mycorrhizal types in the roots of Scots pine seedlings in clear-cut soil containing pots after 4.5 months growth (UR). Different letters above the standard deviation bars indicate statistically significant differences according to two-way ANOVA. Treatments did not differ statistically (P= 0.930) but the morphotype groups did (P= 0.000) without interaction (P= 0.162). Control= non-inoculated seedlings.

The results of this unpublished experiment show how a large, ecological data set (Papers II, III and IV) can be used in screening for suitable ectomycorrhizal strains useful for inoculation purposes. As Trappe (1977) concluded, 'the more completely we learn the autecology of ectomycorrhizal fungi, the more intelligently we can select the species for inoculation of nurseries'. However, the screening of different species should be more extensive and tests on inoculum production, inoculation methods, optimal growth conditions, most efficient overall protocol and, last but not least, field tests should be done both in the nurseries and in the forest site. Nevertheless, the preliminary experiment

described above, proposes one possible assay to initiate inoculation screening on a sound ecological basis and highlights the need for further applied studies.

Effects of inoculation on plant growth

In the laboratory experiment (UR), where new strains suitable for Scots pine seedling inoculation were screened, the seedling growth response following inoculation of the two tested strains was different, depending on whether the strains were growing in mycorrhizal association with seedling in axenic or in unsterile conditions. In the tubes, growth of mycorrhizal seedlings did not differ from non-mycorrhizal control seedlings. However, when transplanted into the unsterile clear-cut soil, seedlings inoculated with *P. fortinii* (strain E) had a reduced shoot and total biomass compared to control seedlings and seedlings inoculated with *S. variegatus* (strain M). *S. variegatus* was shown to perform similarly to control seedlings three years after outplanting in the field, in an experiment where some other mycorrhizal strains improved seedling growth significantly (Stenström and Ek, 1990). Jumpponen and Trappe (1998b) inoculated *Pinus contorta* seedlings with *P. fortinii* both in aseptic and non-sterile open pot cultures. In aseptic conditions, a gradient of sucrose was tested and in pot cultures, the seedlings grew in peat-vermiculite mixture with inoculum. They concluded that the inoculum-related growth response was dependent on the experimental conditions. In aseptic conditions, they found strong host tree growth promotion while in the unsterile pot experiment, no increase in growth could be seen. In our case, where no carbon gradient was used in the aseptic experiment and where natural soil was used, the trend was similar: less growth enhancement in unsterile conditions.

The main aims of controlled inoculation are to improve seedling growth and increase the survival of the inoculated seedlings in the field (Trappe, 1977). Interestingly, no increase in mortality due to *P. fortinii* was observed in experimental conditions by Jumpponen and Trappe (1998b) or by us, although it is not clear whether *P. fortinii* is parasitic, pathogenic mutualistic or pseudomycorrhizal fungi (Jumpponen and Trappe 1998a).

A four-year-long field experiment where seedlings had been inoculated with *Laccaria bicolor* S238N strain and/or *Pseudomonads fluorescens* BBc6R8 mycorrhizal helper bacterial strain was performed in France (Paper V). The strains used had earlier been shown to improve seedling growth in greenhouse and nursery conditions (Duponnois et al. 1991; Frey-Klett et al. 1999) and in the field (Villeneuve et al. 1991). In our experiment, the fungal inoculation again produced a significant growth response compared to control seedlings, even after four years in the field site. Clearly, using this particular fungal strain and tree species in these conditions, tree growth was stimulated by the inoculation.

Although many positive seedling growth responses have been observed in inoculation studies (Landis et al. 1989a), overall, the growth response of trees in the field to inoculation in Europe and North America has been variable (Molina and Chamard, 1983; Landis et al. 1989a; Le Tacon et al. 1992). Le Tacon and colleagues presented eight experiments from France, Sweden, Spain and UK, in which inoculation showed positive growth responses in four experiments (height or shoot volume), in two growth was more or less unchanged and in two there was a negative impact on growth. The authors concluded that, after transplantation, the vigour of the natural ectomycorrhizal inoculum is the most important factor determining the success or failure of the inoculation and that the introduced fungi have to be adapted to the ecological conditions of the reforestation site. This conclusion would support our method for searching for suitable strains and test them under unsterile conditions using natural soil. A thorough knowledge of the ecology of the

selected strains could improve the probability of their survival and symbiotic success in the reforestation sites after transplantation.

Survival of the inoculated strains and effects on indigenous communities

The survival of introduced natural or genetically modified microorganisms in uncontrolled field or nursery conditions is not apparent and depends on multiple environmental and biological factors. Inconsistent results have been obtained since in some cases, the introduced strains (ectomycorrhizal fungal) have been found years after inoculation (Stenström et al. 1990; Selosse et al. 1998) while in others, the strain (bacterial) has disappeared within a few weeks (Frey-Klett et al. 1997). Also, transient changes in microbial community diversity have been detected after introducing genetically engineered bacteria in soil (Bej et al. 1991; Vahjen et al. 1995).

In the pot experiment (UR), the inoculated morphotypes were detected but no differences in their abundance between different treatments was found, suggesting that the indigenous morphotypes took over and that the inoculation did not affect dramatically the mycoflora of the seedlings. However, more specific community analysis would be needed to verify this preliminary conclusion. In the field experiment (Paper V), the inoculated strains were not detectable in the reforestation site, four years after outplanting. The methods used, selective plating and enrichment culturing for the bacterial strain and morphotyping with PCR-RFLP verification for the ectomycorrhizal inoculant strain, are sensitive in detecting introduced organisms in the environment and the sampling procedures followed were extensive (in the field, 17 different sampling occasions from each treatment for rhizosphere and soil samples were performed). However, it cannot be excluded that in these two experiments the strains were present in the soil and could be found using for example highly sensitive DNA-based markers.

In the field, the impact of the introduced bacterial and fungal strains on the local microflora was measured using a range of methods i.e. Biolog community level physiological profiling, total bacterial counts, ectomycorrhizal morphotyping and ergosterol concentration measurements. Based on these analyses, the inoculation did not cause changes in the microflora either in quantity or in quality. This result is in accordance with the observation of the disappearance of the introduced strains.

Conclusions

Based on both tube and pot experiments using ectomycorrhizal fungal isolates from a Finnish forest, and a French field experiment using well-studied fungal and bacterial strains, it seems that inoculation can have a positive or negative impact on seedling growth even when no detectable differences in bacterial and fungal communities can be found. In Finland, nursery seedlings are commonly colonised by ectomycorrhizal fungi adapted to conditions found in the nursery. Their contributions to seedling performance after outplanting are of crucial importance. The pot experiment highlights the importance of controlled mycorrhization: if uncontrolled mycorrhizal fungi are associated with roots, strains that can reduce the seedling performance may dominate. By contrast, some other strains could have positive impact on seedling growth and survival.

4.3. Methodological considerations

4.3.1. Comparison of morphotyping, PCR-RFLP and sequencing.

The application of molecular methods in ectomycorrhizal community studies have been proposed by several authors (Egger, 1995; Bridge and Spooner, 2001) and their use is common in many laboratories around the world today. In their review, Horton and Bruns (2001) proposed analysing ectomycorrhizal fungal communities using crude morphotyping, ITS-RFLP fingerprinting and sequencing. These methods were chosen in this thesis and were performed in the same manner throughout in all the experiments. Therefore, the results of morphotyping and PCR-RFLP fingerprinting can be compared and the reliability of this approach can be evaluated. For Papers III and IV, the PCR-RFLP clustering was verified by direct sequencing of one to four representative samples (the very same DNA extracts) from each major taxon. Finally, a dendrogram was constructed including the representative samples from each taxa presented in Papers II, III and IV and the strains used in the unpublished inoculation experiment UR (Figure 9 and Table 2). Using this large, combined data set, general methodological considerations can be discussed.

As already discussed in Papers II, III and IV, gross morphotyping is a good method to go through large numbers of root tips. Morphotyping was also suggested to give reliable diversity estimates of the individual seedlings or microcosms. Individual seedlings or microcosms, which are fairly uniform and homogeneous sampling units, contain limited variation and diversity of ectomycorrhizal symbionts, which makes it easier to obtain reliable morphological identification. All the experiments confirmed, however, that if morphotyping is used in fast screening and only general characteristics (colour, shape, hyphal and mantle structure) are recorded, the identification is not exact enough to use the results alone for species comparisons between individual seedlings or microcosms analysed separately. Therefore, it is extremely important to standardize each sampled morphotype group by using PCR-RFLP or sequencing, particularly in the case of core sampling where the variation and diversity of different ectomycorrhizal types is high. Morphotyping, even based on a rapid superficial characterization, will provide important additional information (see e.g. Agerer, 2001) on the type of fungi in question, if compared to random root tip sampling. Sakakibara et al. (2002) confirmed that detailed morphological classification can give very accurate species identification and that for the efficient use of research funds, the use of molecular methods can be restricted to the most problematical morphological groups.

PCR-RFLP fingerprinting was shown (e.g. taxa 5 and 6 in Paper IV) of not being accurate enough for identification of all ectomycorrhizal species groups. There is no doubt that, since the costs of direct sequencing have decreased dramatically in the last few years, it is preferable to use sequencing as an identification method. However, the advances in new methodology does not mean that the older typing methods are rendered obsolete. This was clearly highlighted in the comparable analysis of our RFLP-taxa and sequences. In total, the 26 most common taxa were confirmed by sequencing the same samples that had produced the specific RFLP pattern. In all cases except one (see Table 2), the sequencing confirmed the RFLP-match or, if no RFLP-match had been obtained, found a reasonable species identification in the sequence database. This comparative analysis suggests that the RFLP-method is useful but pointed out also the importance of constructing large databases including species from both basidio- and ascomycotinaceae (Tedersoo et al. 2003). Nevertheless, as was mentioned above, the recent advances in sequencing technology and lowered costs may reduce the interests in further development of the RFLP-databases.

In conclusion, the use of morphotyping of defined sample units as a stratifying sampling method for PCR-RFLP fingerprinting followed by sequencing of representative samples from obtained RFLP-taxa, was found to be a reliable and relatively cost-efficient method for ectomycorrhizal species identification in large scale experiments.

4.3.2. Microcosm vs. field experimentation

The aim of using standardized laboratory growth conditions, in order to determine the effects of the selected treatments does include, in certain cases, unnatural variables that might result in biased observation of studied phenomenon. However, it is often too laborious or costly to carry out such experiments or impossible to study detailed mechanisms in the field. It is also often preferable to test new hypotheses under small scale conditions in the laboratory before investing large amount of resources in comprehensive field studies. Bearing these thoughts in mind, it is important to compare laboratory and field experiments to know how close to the natural situation we can get using standardized laboratory conditions. In this thesis, by using comparable methodology, material and treatments, it was possible to estimate the validity of microcosm methodology for biodiversity studies. Microcosms have been used successfully to study specific mechanisms, but although combined approaches have been suggested (Buscot et al. 2000), this is one of the few cases when the method was used parallel with field scale biodiversity experimentation (see also Teuben and Verhoef, 1992; Jones et al. 1997).

The biomass of the seedlings was clearly higher in the microcosm experiments than in the field, when the seedlings of similar age were compared. For example, the shoot fresh weight of the field control forest seedlings never reached the average weight of microcosm seedlings growing in the laboratory conditions for six months. In contrast, the interface and clear-cut site grown Scots pine seedlings reached the level of their microcosm counterparts in the second or third year in the field. This observation points out the differences in growth conditions, despite the efforts made in simulating the light intensity and growth temperature in the growth chambers. In the paper by Tammi et al. (2001), where similar microcosms but no growth chamber cooling were used, the average dry weight of Scots pine seedlings after a five month growth period was 5.5 g which is clearly a higher value than fresh weight biomass after six months in Paper III. Therefore, it seems that the temperature in laboratory conditions could be a major factor influencing plant growth and carbon allocation. In microcosms, the understorey vegetation was almost absent which might have also influenced in the differences between laboratory and field seedling growth. In conclusion, the optimal and constant environmental conditions greatly favour, at least in the beginning, the growth of laboratory seedlings. It seems also, that the laboratory conditions simulate more closely the interface and clear-cut site conditions since the seedling growth in these zones were closer to those seen in the laboratory.

The ectomycorrhizal inoculum potential, i.e. the diversity of ectomycorrhizal fungi in the root system of the Scots pine was the main aim of the biodiversity part of the project. As can be clearly seen in the combined analysis presented in Figure 9, the microcosms simulated fairly well the situation found in the field. The most common species detected in the field (see Table 2), were also found colonizing seedlings in the microcosms. However, the treatment-related shift observed in the field experiment could not be verified in the microcosm experiments. This clearly indicates that the most difficult task is to simulate control forest conditions in the laboratory. This is logical since in the forest, the effect of the mature trees on the soil microbiology is considerable and for example the disturbance of the living hyphal connections crucially alters the interactions in the soil.

Keeping the pitfalls of the microcosm methodology in mind, it is possible to use them successfully in ecological studies, including determining ectomycorrhizal inoculum

potential for young seedlings. Since it was reported by Jonsson et al. (1999b) that the bait seedlings reflect the ectomycorrhizal community structure in the roots of the large adjacent trees well, we could extrapolate our findings and suggest that microcosms can be used in estimating ectomycorrhizal community structure in the root systems of both bait seedlings and larger trees in conditions where soil is disturbed (see also Jones et al. 1997). The best way to work on the community structure and mechanisms of interactions is, however, to integrate studies using both micro- or mesocosms and field experiments.

4.3.3. Sampling in ectomycorrhizal community studies

Field experimentation is an extremely challenging task for microbial ecologists, mainly due to the significant scale differences between the organisms studied and their geographical distribution. The sampling effort has been thoroughly discussed by Taylor (2002). It was pointed out that it is important to bear in mind variation in time and space in addition to the focus of the experiment. If the aim is to look at the keystone species, the sampling intensity is lower than if the focus is in the richness and diversity of the mycorrhizas, including the rare species. Morris et al. (2002) reviewed the literature of the last 25 years dealing with studies on microbial biodiversity. They concluded that sound sampling strategy (and its clear description in the paper), statistical analyses and multiple hypothesis testing would greatly improve the quality of biodiversity studies. This statement is supported by Taylor (2002), who additionally discussed the inherent difficulties in defining the identity of ‘species’, ‘individual’ and ‘population’ or ‘community’ in the case of ectomycorrhizal fungi. Definitely, the importance of sampling strategies, being basically the most important phase in environmental experimentation, needs to be stressed in the case of ectomycorrhizal community studies. This thesis showed that, through the designation of defined sampling units (bait seedlings or microcosms) and combined laboratory and field assays, it is possible to obtain good estimates of the ectomycorrhizal communities of value in forest biodiversity and silvicultural management without intolerable effort and cost.

5. Conclusions

-Photosynthetically fixed carbon was shown to be equally allocated to the roots and mycorrhizas in humus, E- and B-horizons suggesting that roots and ectomycorrhizal fungi significantly support biological activity in podzol horizons. The data also provide evidence that ectomycorrhizal fungi are abundant and active enough in E- and B-horizons to be involved in podzolization and weathering processes.

-Bacterial communities associated with roots and ectomycorrhizas utilised a wide range of carbon sources, measured using CLPP analysis, suggesting that they are potentially also active in E- and B-mineral horizons.

-Root associated ectomycorrhizal fungal communities in seedlings growing in the field and microcosms were diverse, with a total of 53 taxa determined. Podzol horizon-specific species were identified.

-Based on the field experiment, it is concluded that any shift in ectomycorrhizal fungal community structure observed in the seedling roots after clear-cut logging is not due to the lack of inoculum in the clear-cut soil but due to the changes in environmental growth conditions.

-Based on both tube and pot experiments using Finnish forest isolates, and a French field experiment using well-studied fungal and bacterial strains, it seems that inoculation can have a positive impact on seedling growth even though no detectable differences in indigenous bacterial and fungal communities can be found.

-Gross morphotyping following PCR-RFLP fingerprinting and sequencing was shown to be a suitable method for forest-scale ectomycorrhizal biodiversity studies. Ectomycorrhizal fungal diversity detected in microcosm experiments was shown to mimic relatively well the diversity observed in the field experiment, although most of the rare species identified in the field experiment were not found in the laboratory conditions.

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8. Appendix 1.

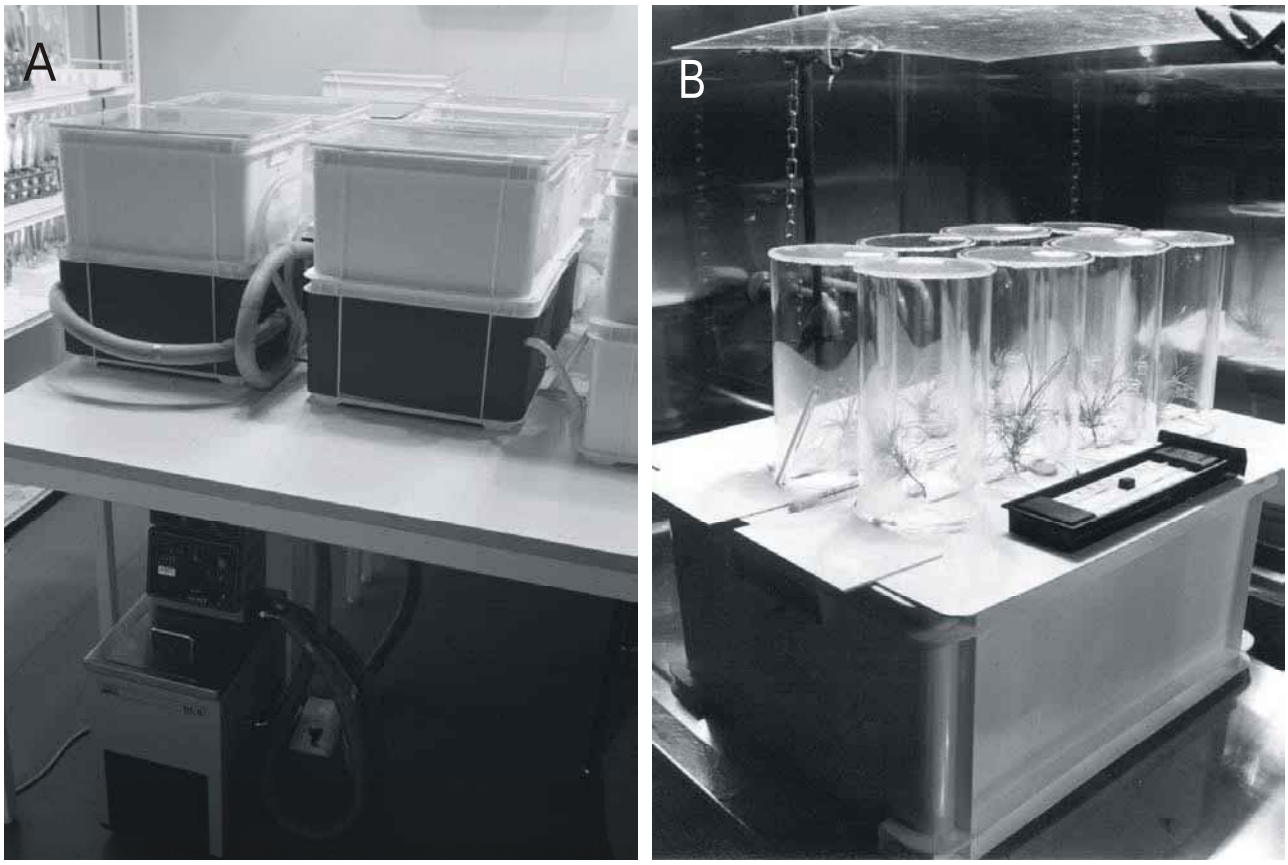

Figure 14. (A) Growth chambers used in microcosm experiments I-III. The cooling unit (under the table) circulates cold water through the tubes in the isolated bottom parts of the boxes. (B) Shoot isolating chambers used for ^{14}C-labelling in Paper I. The $^{14}CO_2$ was released in the one-liter sealed perspex chamber around the shoot preventing direct contact of label with below-ground parts of the microcosms.

9. Appendix 2.

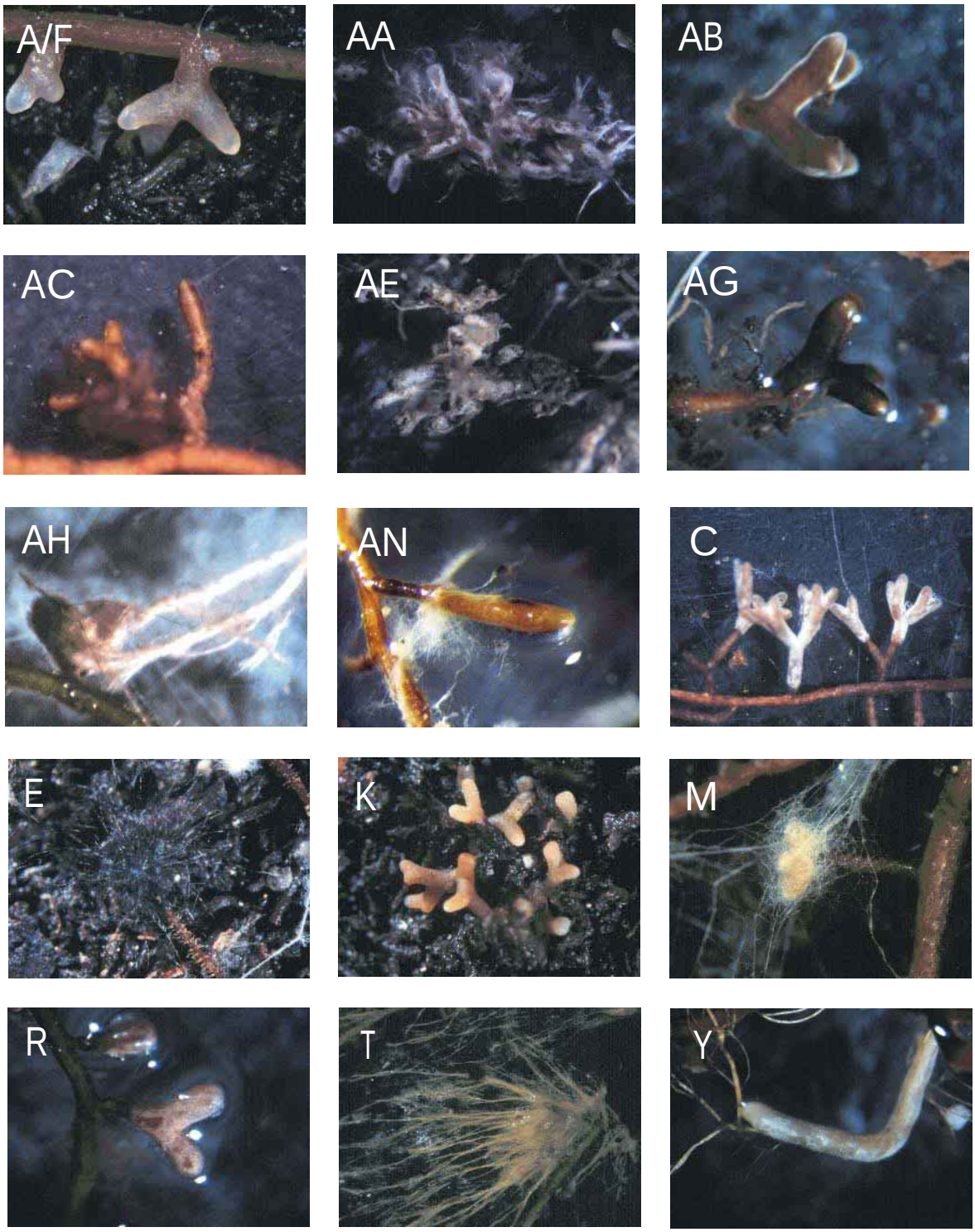

Figure 15. The photographs of the most common morphotypes (abundance in any one treatment in any one year ≥5%, see Paper IV for details) observed in the Hyytiälä field experiment. No photograph of morphotype O was available. The coding corresponds to the ones used in Papers II-IV and UR.