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Nutrient Cycling in Boreal Forests - a Mycological Perspective

Studies on phosphorus translocation within and between mycelia of saprotrophic - and mycorrhizal fungi

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Abstract

In order to understand the mechanisms controlling ecosystem diversity, production and responses to disturbance, improved knowledge about the movement and transformation of nutrients is essential. Most currently used models of nutrient cycling in boreal forests have been developed with agricultural ecosystems in mind. Boreal forest ecosystems are characterised by a high abundance and diversity of basidiomycetous fungi. These fungi occur rarely in agricultural soils but play a pivotal role in boreal forests as decomposers of organic matter and symbiotic associates of plants. The ecophysiology of basidiomycetous fungi has to be considered, when constructing nutrient cycling models for boreal ecosystems. Decomposer fungi and symbiotic mycorrhizal fungi have traditionally been placed in distinct functional categories and treated separately. This separation has no phylogenetic justification however, and fungi from the two groups share a similar mycelial morphology as well as the same microsites on the forest floor. This thesis describes experiments in which radioactive phosphorus was used in combination with non-destructive electronic autoradiography to study nutrient translocation in soil microcosms containing saprotrophic- and ectomycorrhizal fungi. Bidirectional phosphorus translocation in fungal rhizomorphs was observed, showing that nutrients may circulate throughout basidiomycetous mycelia, enabling net translocation from sources to sinks. Studies of mycelial interactions between ectomycorrhizal fungi and saprotrophic fungi suggested that ectomycorrhizal fungi can interact antagonistically with other soil fungi. Interactions were associated with transfer of significant amounts of phosphorus between the interacting mycelia. Ectomycorrhizal fungi were able to mobilise radioactive phosphorus from labelled saprotrophic mycelium and to transfer the acquired phosphorus to their host plants. Wood decomposing fungi were similarly able to mobilise phosphorus from mycorrhizal mycelium and to translocate the acquired phosphorus to colonised wood blocks. The net direction and rate of phosphorus transfer between interacting mycelia was shown to depend on the availability of resources to the interacting fungi. To explain the observed phosphorus transfer it is hypothesised that interacting basidiomycetous fungi may obtain nutrients by killing and degrading each other's mycelia. This highly competitive foraging behaviour, in combination with the ability to translocate resources over considerable distances, makes basidiomycetous fungi well adapted to the spatial heterogeneity and low nutrient availability of the boreal forest floor. A new model of nutrient cycling in boreal forests is proposed that allows for nutrient retention in soil fungi and intense competition for nutrients between soil organisms. Symbiotic association with ectomycorrhizal fungi that can effectively compete with other soil organisms for organic nutrient sources, enables plants to acquire nutrients without the need for large scale nutrient mineralisation.

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Key words: Basidiomycete, ectomycorrhiza, wood rotting fungi, translocation, mycelial interactions, microcosm, autoradiography, phosphorus, nutrient cycling, *Hypholoma fasciculare, Suillus variegatus, Paxillus involutus, Pinus sylvestris*

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Contents

Throughout the thesis summary, bold arabic numbers in brackets refer to sections in the summary.

Appendix

Papers I-IV

The thesis is based on the following papers, which throughout the summary will be refered to with bold roman numerals:

I Lindahl, B., Finlay, R. & Olsson, S. 2001. Simultaneous, bidirectional translocation of ^{32}P and ^{33}P between wood blocks connected by mycelial cords of *Hypholoma fasciculare. New Phytologist 150.* 189-194.

II Lindahl, B., Stenlid, J., Olsson, S. & Finlay, R. 1999. Translocation of ^{32}P between interacting mycelia of a wood decomposing fungus and ectomycorrhizal fungi in microcosm systems. *New Phytologist 144,* 183-193.

III Lindahl, B., Stenlid, J. & Finlay, R. 2001. Effects of resource availability on mycelial interactions and ^{32}P-transfer between a saprotrophic and an ectomycorrhizal fungus in soil microcosms. *FEMS Microbiology Ecology,* in press.

IV Lindahl, B., Taylor, A.F:S. & Finlay, R.D. 2002. Defining nutritional constraints on carbon cycling in boreal forests – towards a less "phytocentric" perspective. *Plant and Soil,* in press.

1. Aims of the thesis

This thesis describes basidiomycetous fungi as key organisms responsible for many central processes in boreal forests. The discussion is centered around two major functional groups of basidiomycetes; wood and litter decomposers on the forest floor, and ectomycorrhizal fungi, stressing similarities between these groups more than the differences. The experiments described in the thesis used different kinds of soil microcosms in combination with radioactive tracer isotopes to investigate problems in two areas; 1. translocation of resources in rhizomorphic mycelia and 2. interspecific mycelial interactions between soil basidiomycetes. The aims of the experiments were:

- to show that phosphorus may be translocated bidirectionally in basidiomycetous rhizomorphs, enabling phosphorus circulation throughout mycelia (**I**).
- to study the morphological responses of ectomycorrhizal and saprotrophic soil fungi to interspecific mycelial interactions (**II, III & IV**).
- to investigate whether interactions between saprotrophic and ectomycorrhizal fungi are associated with transfer of phosphorus between the interacting mycelia (**II & III**).
- to study the effect of differences in resource availability on mycelial interactions between soil fungi (**III**).

In addtion to the articles describing original experiments, the thesis also includes a review article, in which existing nutrient cycling theories are discussed in the light of current mycological knowledge (**IV**).

2. Some terms and definitions

Basidiomycete - Properly used, the term includes all fungi in the division *Basidiomycota*. In the general, ecological characterisations of basidiomycetes used in this thesis, the sub-divisions *Teliomycotina* and *Ustilagomycotina* are however not considered, due to their highly specialised, parasitic lifecycles. In these ecological discussions, the term basidiomycetes refers to members of the sub-division *Basidiomycotina* only.

Rhizomorph - The term rhizomorph is used for all mycelial structures that involve parallel alignment of anastomosing hyphae, found outside fruitbodies, including structures with an integrated apical growing point (rhizomorphs *sensu stricto*) as well as structures that develop behind a diffuse mycelium (elsewhere often referred to as cords or strands).

Nutrients - Following the practice adopted in many ecological publications, the term nutrients is used for substances other than carbohydrates that organisms require to fulfil their life cycle.

Carbohydrates - The term refers to substances that only consist of carbon, oxygen and hydrogen.

Resources - The terms encompasses all substances needed to fulfil the life cycle including nutrients, carbohydrates and water.

3. Introduction to fungi in general and basidiomycetes in particular

3.1. What is a basidiomycete?

Fungi are eukaryotic, usually multicellular organisms that are characterised by chitin-containing cell walls and a mycelial growth form. The fungal mycelium is built up from tubular cells joined end to end to form filaments called hyphae. Hyphae or thin bundles of hyphae form the branched networks called mycelia (Deacon, 1997, pp 1-4). Only in special cases do hyphae align together to form proper tissues (*e.g.* in the fruitbodies we know as mushrooms). The fact that fungal cells are not protected within a skin or a cuticle, but are directly exposed to the surrounding environment, separates fungi from most plants and animals and relates them to unicellular organisms sucha as bacteria or protozoa. Like bacteria, fungi produce degradative enzymes that are exuded to the surrounding environment, in contrast to plants, which rarely produce external, degradative enzymes, and to animals (and protozoa) which release enzymes to internal cavities (stomachs, intestines or vacuoles). Together with bacteria and protozoa, fungi are popularly termed microorganisms or microbes. Nevertheless it is very important to remember that fungal mycelia can be large; mycelia growing on the forest floor often extend over distances on a metre scale (Thompson & Rayner, 1983; Dowson et al., 1989a; Zhou *et al.,* 2001). In some cases, mycelia can grow extremely large; An individual of *Armillaria bulbosa* (Honey fungus) has been found to be 635 m across and at least 1500 years old (Smith *et al.*, 1992).

The fungi are situated next to animals in the evolutionary tree (Van de Peer *et al.,* 2000). The fungal kingdom is subdivided into four groups: basidiomycetes, ascomycetes, zygomycetes and chytrids. This thesis will mainly consider basidiomycetous fungi. Basidiomycetes are characterised by their often conspicuous fruitbodies. Most mushrooms that are commonly found in forests, such as boletes, chanterelles, agarics and bracket fungi are basidiomycetes. Some examples of ascomycetes are morels, lichens and many moulds while zygomycetes and chytrids rarely form macroscopic structures. Early

basidiomycete ancestors are thought to have inhabited wooden substrates (Hibbett *et al.*, 2000) and, with the exception of a few ascomycetes, basidiomycetes are the only fungi that can cause large-scale degradation of wood (Rayner & Boddy, 1988). This is due to their capacity to produce enzymes that can degrade lignin, a highly recalcitrant constituent of wood. Basidiomycetes may form large mycelia that can span throughout the length of a decaying tree trunk or extend over several square metres of the forest floor. In basidiomycetous mycelia growing in soil, hyphae are sometimes organised into linear aggregates called rhizomorphs (Boddy, 1993). These can be simple, with just a few hyphae growing together, or thicker (up to 5 mm) and differentiated into complex structures. Uptake of carbohydrates and nutrients seems to be restricted to the non-rhizomorphic parts of the mycelium, where individual hyphae excrete enzymes and take up resources that are made available by the activities of the enzymes (Wessels, 1993; Unestam, 1995). The more resistant rhizomorphs maintain the physical integrity of the mycelium, facilitating transport of various substances between its different parts (**I**; Boddy, 1999).

3.2. Carbohydrate sources utilised by basidiomycetes

In order to decompose wood and other plant tissues, many basidiomycetes produce enzymes - cellulases, that degrade cellulose (a major constituent of plant cell walls) to glucose, which can be assimilated to provide energy and structural carbon. Those fungi that lack the ability to produce cellulases (*e.g.* many ascomycetous or zygomycetous moulds and yeasts) have to rely on simple sugars or other less recalcitrant compounds. There is a fierce competition for these high quality carbohydrates (a sandwich left in a moist plastic bag is rapidly colonised by a variety of different mould fungi). Fungi that can only use simple sugars have to allocate a large fraction of their acquired resources to spore production and rapid dispersal, in order to colonise and utilise attractive substrates before they are used up by other fungi or bacteria. Fungi allocating a large fraction of their resources to dispersal and less to degradation of recalcitrant substrates can be termed R–strategists. Most basidiomycetes, in contrast, allocate a smaller fraction of their resources to spore dispersal. Most of the biomass is vegetative mycelium and only a small fraction is found in fruitbodies. Basidiomycetous individuals are often long lived (Smith *et al.*, 1992; Dahlberg & Stenlid, 1995) and spore production is usually limited to short annual events. Generally, basidiomycetes instead invest their resources in production of long lived mycelia that can withstand competition from other fungi. With their high enzymatic capacity, they degrade cellulose and other complex macromolecules, enabling them to remain active in the substrate long after all simple sugars have been used up. Fungi with these characteristic properties can be termed C-strategist fungi. (Cook & Rayner, 1984, pp. 92-108)

Some basidiomycetes have evolved an intricate way to acquire carbohydrates without decomposing recalcitrant plant polymers, but at the same time without having to compete with R-strategist fungi for simple sugars. The key to the problem is to cooperate with plants. Coniferous trees and some deciduous trees form ectomycorrhizal symbioses with a large number of basidiomycetes and a few ascomycetes. The term ectomycorrhizal; "mucor" meaning fungus and "rhizon" meaning root, relates to plant roots, where fungal hyphae weave more or less dense mantles around the root tips, growing between the root cells but never inside them. The prefix "ecto" separates this type of symbiosis from endomycorrhiza, where the fungal hyphae penetrate the cell walls of the host plant and proliferate within the cells (Smith & Read, 1997).

Ectomycorrhizal fungi receive simple carbohydrates directly from their host trees, and in return the fungi supply the trees with nutrients and water (Smith & Read, 1997). More than 95% of the root tips of boreal forest trees are ectomycorrhizal (*e.g.* Fransson *et al.*, 2000). In the mycorrhizal root tips, the nutrient absorbing regions of the root are completely covered by fungal mantles and are thus isolated from the soil solution. This means that the trees are almost completely dependent on their associated fungi for nutrient uptake. The importance of the mycorrhizal fungi for the performance of the trees is also highlighted by the fact that around 10–20% of the carbon assimilated by the tree has been estimated to be allocated to the fungal symbionts (Smith & Read, 1997, p. 253). The dependence of mycorrhizal fungi on the current photosynthesis of their host trees for carbohydrates is clearly illustrated by a field experiment in which the trees were girdled to interrupt the flow of photosynthetic products below ground. In forest areas with girdled trees, the production of ectomycorrhizal fruitbodies was negligible compared with adjacent control areas (Högberg *et al.*, 2001). Ectomycorrhizal fungi thus have access to a source of carbohydrates that is unavailable to other fungi. Instead of allocating resources to the production of wood degrading enzymes, ectomycorrhizal fungi have to allocate resources to the colonisation of plant roots. They also transfer a more or less substantial fraction of their acquired nutrients to the plant roots in order to support their host. Recent phylogenetical studies have shown that the ability to form ectomycorrhizal symbiosis has emerged on several independent occasions during the evolution of the present basidiomycetous species (Hibbett *et al.*, 2000). Distantly related genera such as *Boletus, Russula* and *Cantharellus* are mycorrhizal, although their common ancestors are thought to have been saprotrophic (decomposer) fungi. Many fungi, that today are saprotrophic (*e.g. Agaricus*) are thought to have had ectomycorrhizal ancestors, indicating that the ability to form mycorrhiza has not only been gained, but has also been lost on several independent occasions. This finding emphasizes that ectomycorrhizal fungi are not a phylogenetically distinct group, but an assembly of very different fungi that have independently developed a symbiotic lifestyle. As ectomycorrhizal symbiosis has traditionally been studied mainly from the perspective of plant physiologists, these fungi have almost been treated as

alterations of the plant roots with focus on the behaviour of the plant with and without fungal symbionts present. One of the aims of this thesis is to examine ectomycorrhizal fungi within a holistic view of basidiomycetes, stressing the similarities between saprotrophic and symbiotic fungi and the need to study these organisms in their own right, not simply as appendages of plant root systems.

3.3. Nutrient sources utilised by basidiomycetes

Basidiomycetes generally acquire carbohydrates through degradation of cellulose or from symbiotic relationships with tree roots, but fungi also require other resources. Nutrients, such as nitrogen and phosphorus, can be taken up in inorganic forms from the soil solution but, as in the case of high quality carbon sources, there is strong competition for inorganic nutrients. This is particularly true in boreal ecosystems, where easily available nutrients are scarce. Plant litter, in which saprotrophic basidiomycetes degrade cellulose to obtain assimilable carbohydrates, also contains complex, nutrient containing polymers such as proteins, nucleic acids, phospholipids etc. Fungi use extracellular enzymes to break down these macromolecules to assimilable, low molecular weight compounds. Often, however, the limited amounts of nutrients available in the decomposing plant tissues are not enough to allow efficient colonisation and degradation of the substrate (especially in woody substrates that are very nutrient poor). Other substrates in the surrounding environment; dead microorganisms, microfauna or even highly recalcitrant humus compounds, may be degraded and the obtained nutrients transported throughout the mycelium to meet the demands of the fungus. The utilisation of fungal mycelium as a nutrient source by basidiomycetous fungi is one of the main topics of this thesis (**II, III** & **IV**).

Due to their symbiotic relationship with their tree hosts, ectomycorrhizal fungi are not dependent on cellulose degradation to obtain carbohydrates. As enzyme production involves a considerable cost for the fungi, it is understandable that fungi that develop a symbiotic lifestyle lose their redundant capacity for rapid cellulose degradation (Colpaert & van Tichelen, 1996). The demand for nutrients may, however, be even larger for mycorrhizal fungi than for saprotrophic fungi, since the former usually support their host plants as well as themselves. There is thus no reason to assume that the abilitiy to degrade complex nutrient containing substrates and to assimilate nutrients in organic form should also be lost, as fungi evolve from a saprotrophic to a symbiotic lifestyle. This is in accordance with what has been found in laboratory studies; a wide range of ectomycorrhizal fungi produce extracellular proteases, can assimilate amino acids and can thus grow on proteins as a single source of nitrogen (Abuzinadah *et al.* 1986; Abuzinadah & Read, 1986a, 1986b and 1989; Finlay *et al.,* 1992, Näsholm *et al.,* 1998). Besides proteins, ectomycorrhizal representatives have been found to be able to degrade a range of other nutrient containing macromolecules such as chitin, nucleic acids and polyphenols (reviewed by Leake & Read, 1997). Utilisation of organic nitrogen by ectomycorrhizal fungi was suggested at the end of the 19th century

by Frank (1894) and demonstrated in laboratory experiments 50 years ago by the Uppsala professor Elias Melin (Melin & Nilsson, 1953). Nevertheless, this fact is still poorly acknowledged by most scientists outside the research area of mycology. This is problematic, since the association with fungi that have access to organic nutrients could make ectomycorrhizal plants independent of inorganic nutrients. Almost all established models of nutrient cycling assume that only inorganic nutrients are available to plants and that plant nutrient uptake is dependent on saprotrophic fungi or bacteria to release inorganic nutrients to the soil solution. The quantitative effect of mycorrhizal symbiosis on plant nutrient uptake, due to the much larger nutrient absorbing surface area of the fungal mycelium compared with the naked root system, is now widely acknowledged. However, the association with fungi also has important qualitative effects, in that organic nutrient sources, that would be unavailable to the tree alone, can be utilised with the help of the fungi (Read, 1991).

3.4. Basidiomycetes and the boreal forest

The boreal forest is the largest terrestrial biome of the earth, covering 17% of the total land surface. In boreal ecosystems basidiomycetous fungi are characteristically dominant in the soil compared to temperate forests, grasslands or agricultural soils, where bacteria are more abundant and fungi less prominent (Swift *et al.*, 1979, p. 23; Elliott *et al.*, 1993; Frostegård & Bååth, 1996). As basidiomycetes are the main organisms responsible for wood decomposition, wood inhabiting basidiomycetes are abundant in all forest ecosystems throughout the world (Rayner & Boddy, 1988). However, during evolution, a multitude of species has evolved that live not on wood but on the ground. These are usually termed soil fungi, litter fungi or terricolous fungi; terms that can be misleading, as there is a gradual change in substrate preference from boles through twigs and leaves to physically disintegrated matter. Fungi that produce fruitbodies on the forest floor can decompose large pieces of wood that are buried in the soil, and other fungi with their fruitbodies on wood can extend their mycelium out into the soil, colonising litter. Nevertheless, a key feature of the boreal forest is the high abundance and diversity of soil dwelling, basidiomycetous fungi (of which some have evolved further into ectomycorrhizal fungi).

Due to the cold climate, litter turnover rates are low, causing boreal soils to become depleted of easily available nutrients (Van Cleve & Yarie, 1986). In environments where available nutrients are scarce, plants are favoured that maximise their nutrient utilisation efficiency (often at the expense of photosynthetic- and carbohydrate utilisation efficiency). To use acquired nutrients more efficiently, leaves must have a low nutrient content and nutrient losses due to leaf abscission must be minimised (Aerts, 1995). Boreal ecosystems are therefore characterised by evergreen plants. Evergreen plants have tough leaves that are unpalatable to herbivores. The leaves are rich in structural lignin and contain large amounts of secondary substances like terpenes and tannins

(Aerts, 1995). Polyphenolic compounds, such as lignin and tannins, form recalcitrant complexes with proteins and amino acids (Handley, 1961; Bending & Read, 1996). The large amounts of nutrients immobilised in polyphenolic complexes cause nitrogen availability in the soil to decrease further. There thus seems to be a positive feedback loop involving decreased nutrient availability, slow turnover of foliage and increased litter polyphenolic content (Aerts, 1995, Northup *et al.* 1995). As a consequence, the forest floor below boreal plants (mainly coniferous trees and ericaceous dwarf shrubs) is covered by nitrogen poor, acidic litter that is rich in polyphenols. The low litter quality and the cold climate lead to accumulation of organic matter on the forest floor in the form of humus (Swift *et al.* 1979, pp. 15-24).

With their developed enzymatic capacity to degrade lignin and other polyphenolic complexes and a preference for acidic conditions (Swift *et al.* 1979, pp. 244-247), basidiomycetous fungi are perfectly adapted to live on the boreal forest floor. The low pH means that burrowing earthworms; the primary mixing agents of the mull soils of temperate forests and grasslands, are rare (Huhta, 1998). Mixing of the soil is likely to put organisms that are built up from filamentous hyphae at a disadvantage, and in soils with earthworms, bacteria are favoured at the expense of fungi. The low degree of mixing means that boreal mor soils are layered, with fresh litter on the top and more decomposed matter further down in the soil profile. The spatial heterogeneity of the soil should favour basidiomycetous fungi with rhizomorphs through which resources can be translocated between different parts of the mycelium (**I**), allowing foraging for different resources in different types of substrate. Last, but not least, the low nutrient availability favours plants living in symbiotic association with ectomycorrhizal fungi. The ability of many ectomycorrhizal fungi to degrade organic, nutrient containing macromolecules, makes the nutrients therein available to both the fungi and their associated plants. Many ectomycorrhizal species also have enzymes that can degrade polyphenolic complexes (Haselwandter *et al.,* 1990; Bending & Read, 1996; Chen *et al.* 2001), releasing bound nitrogen. Importantly, the capacity to degrade lignin and other polyphenolic compounds is not unique to basidiomycetes. Some ascomycetes also have this ability, including the fungi forming endomycorrhizal associations with ericoid plants such as heather and lingonberries (Leake & Read, 1989;. Bending & Read, 1996). The ericoid mycorrhizal symbiosis is essential for this group of plants to be codominant with coniferous trees in boreal ecosystems.

4. Morphology of basidiomycetous mycelia and translocation within mycelia

4.1. Mycelial morphology

Fungal hyphae grow only at the hyphal tips, where enzyme exudation and nutrient uptake also usually take place (Wessels, 1993; Unestam, 1995). Thus, both resource acquisition and resource consumption (for growth, respiration etc.) take place at hyphal tips. When a mycelium extends through a heterogeneous substrate, it reacts to the surrounding environment by altering its growth morphology. In substrates with low resource availability, mycelial growth is sparse and extension rates are high, leaving a minimum of mycelial biomass and hyphal tips in the poor substrate. In contrast, when the mycelium grows through a rich substrate, branching is frequent and a dense mycelium extends slowly through the substrate, leaving many hyphal tips behind that can further explore the rich substrate (Thompson & Rayner, 1982 & 1983). The boreal forest floor is a heterogeneous environment, not only due to the vertical stratification of the soil, but also due to the fact that resources enter the forest floor as discrete packages, such as needles, twigs and dead roots. Mycorrhizal fungi also obtain resources from discrete sources - living root tips. As a fungus finds one of these supply packages (here termed resource units), it colonises it with dense mycelium, rich in hyphal tips. As the resource unit is fully colonised, the fungus continues to grow with a sparse mycelium, until a new resource unit is encountered. When the mycelium grows sparsely, between resource units, rhizomorphs are often formed behind the growing mycelial front, connecting the front with the rest of the mycelium. The diffuse mycelium that does not take part in rhizomorph formation dies; a process called autolysis (Thompson & Rayner, 1983). Behind the diffuse growing front, the mycelium will thus consist of patches of dense, highly branched mycelium in the attractive substrates connected by a network of rhizomorphs. Mycelial growth in heterogeneous substrates is exemplified below.

In an experiment by Dowson *et al.* (1989b), where a mycelium of the soil dwelling, wood degrading basidiomycete *Phanerochaete velutina* grew out from a wood block over a soil surface, the diffuse growing edge of the mycelium advanced radially outwards from the wood block (the inoculum), leaving rhizomorphs behind. When a fresh wood block (a bait) was presented to the mycelium, the fungus colonised it with dense, highly branched mycelium. After colonisation of the bait, not only the redundant, non-rhizomorphic mycelium behind the front autolysed, but all mycelium gradually disappeared leaving only a single rhizomorph to connect the two wood blocks. New mycelial fronts extended from the bait, to continue the exploration of the soil for new resource units (Figure 1).

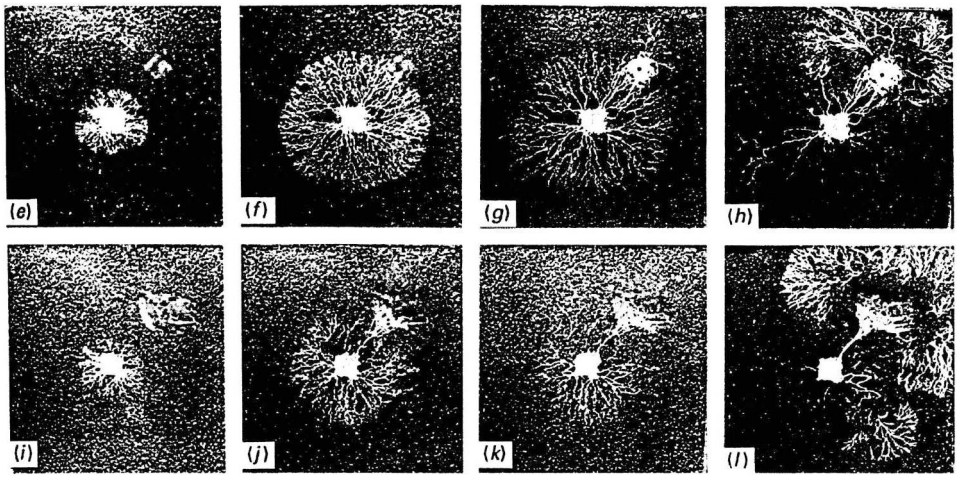

Figure 1. Time series of photos taken 11, 25, 44 an 71 days after the introduction of wood blocks inoculated with *Hypholoma fasciculare* into 20x20cm soil microcosms. The photos show how the fungus grows out from the inoculum with rhizomorphic mycelium and colonises a wooden bait (e-h) or beech leaves (i-l). The two resource units remain connected by coarse rhizomorphs, while non-connecting mycelium regresses (Reproduced from Dowson *et al.,* 1989b with the publisher's permission).

Bending & Read (1995a) conducted an experiment using the, now classical, microcosm design first developed by Duddridge *et al.* (1980). Flat Perspex plates were covered with a thin layer of peat, in which ectomycorrhizal fungi grew in association with pine seedlings. Mycorrhizal mycelia developed from the colonised root tips and extended over the peat surface. This microcosm design is ideal for studies of mycelial morphology of ectomycorrhizal fungi. Organic forest floor material was introduced as discrete patches in the peat. When the mycelium of the mycorrhizal fungus *Suillus bovinus* encountered the patches, it formed mats of dense mycelium that proliferated through the forest floor material. This experiment showed that not only saprotrophic fungi "forage" in the forest floor for high quality organic substrates, but also ectomycorrhizal fungi. Extraction of enzymes from patches of forest floor material showed that the activities of proteases, polyphenol oxidases and phosphomonoesterases were higher (170-300%) in patches colonised by the ectomycorrhizal fungus *Paxillus involutus* compared to non-colonised control patches (Bending and Read, 1995b). These enzymes are active in mobilisation of nitrogen and phosphorus from complex organic sources. Formation of dense mycelial patches by ectomycorrhizal fungi in response to high quality organic substrates has also been descibed by Unestam (1991), Read (1992), Leake *et al.* (2001) and (**II** & **III**). A striking example of morphological shifts in basidiomycetes is the formation of ectomycorrhizal root tips. Just as in the earlier example with a wood degrading fungus, many ectomycorrhizal fungi advance through the soil with diffuse mycelial fronts, leaving rhizomorphs behind that connect the growing fronts to their carbon sources - the mycorrhizal root tips. When a non–colonised root tip is encountered, the mycelium increases the branching frequency to form

a dense mantle around the root. After colonisation of the root tip, the mycelium continues to extend through the substrate, leaving colonised root tips integrated in a network of rhizomorphs (Figure 2).

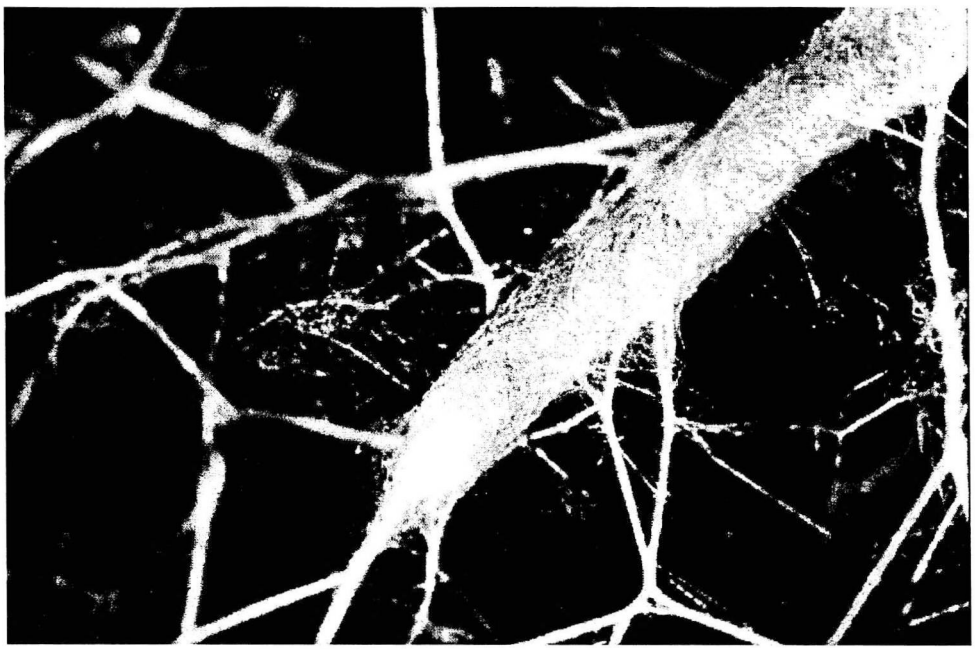

Figure 2. The photo shows a part of a mycelium of the ectomycorrhizal fungus *Dermocybe cinnamomea* including a *Picea abies* root tip, the surface of which is covered by a mantle of densely proliferating hyphae. Rhizomorphs, connected to the mantle, translocate photosynthetically derived carbohydrates to the rest of the mycelium as well as soil derived nutrients to the host plant (photo: Andy Taylor).

4.2. Resource translocation

The mycelial growth form enables fungi to colonise large volumes of substrate with a minimum of biomass while maintaining a physically integrated unit. To consider a fungal mycelium as one integrated entity is, however, pointless if there is no communication or transport of resources between different parts of the mycelium. Without intercellular transport or some other form of communication between cells, the fungal mycelium could be treated as a colony of independent cells. Olsson (1995) designed an experiment to test the ability of 60 different fungi to translocate resources over a distance of a few cm. Fungal mycelia were cultivated in elongated trays filled with agar, in which there was a gradient in glucose concentration from one end of the trays to the other. Similarily, there was a gradient in nutrient concentration in the opposite direction. Some of the tested fungi (43%) could not translocate resources across their mycelium and grew only in the middle of the trays, where both glucose and nutrients were present. Many of the tested fungi however grew just as well on the gradient agar as on homogeneous control agar, indicating that they were able to translocate resources

throughout their mycelium. Of the eight tested basidiomycetes, seven were able to grow well on the glucose free side of the trays and six were able to grow on the nutrient free side. Translocation of water, carbohydrates and nutrients between distant parts of the mycelium thus enables fungi to acquire resources in one place but utilise them in another. This ability is likely to be highly beneficial to organisms living in a spatially heterogeneous environment like the boreal forest floor. The importance of translocation to mycorrhizal fungi is obvious, as they have to translocate plant-derived carbohydrates to the hyphal tips in order to grow outside the roots. They also have to translocate nutrients to the roots in order to be able to support their host plants.

Translocation is believed to be most efficient in rhizomorphs. A classic example is a mycelium of the species *Armillaria mellea* (Honey fungus) that extended a rhizomorph for at least 10 m along a water tunnel without access to any carbohydrate sources along the way (Findlay, 1951). Using radioactive (^{14}C, ^{32}P, ^{42}K) or stable (^{15}N) tracer isotopes, rhizomorph translocation of carbohydrates and nutrients has been studied in several basidiomycetous species and has been reviewed by Jennings (1987), Cairney (1992), Boddy (1993, 1999) and Boddy & Watkinson (1995). Rhizomorphs can be built up in many different ways, but a typical design is a core of large diameter hyphae, so called vessel hyphae, surrounded by a sheath of thinner hyphae (Figure 3). Often the rhizomorphs are coated in hydrophobic substances that isolate them from the surrounding soil solution (Unestam, 1995).

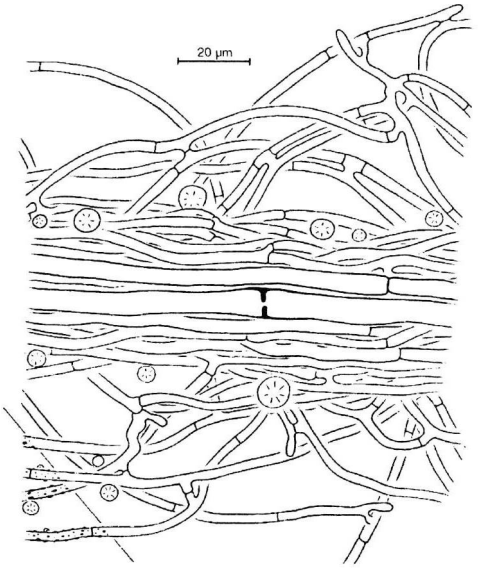

Figure 3. Drawing of a transection of a *Suillus variegatus* rhizomorph showing the large diameter core hyphae surrounded by thinner hyphae. (Reproduced from Raidl, 1997 with the publisher's permission).

4.3. Directionality of translocation

4.3.1. Inherent directionality of rhizomorphs - an obsolete concept?

In this part of the thesis, a hypothesis is advanced that the direction of net translocation in rhizomorphs is not determined by inherent structural polarities but by differences in resource availability and consumption in different parts of the mycelium. This idea has been proposed by Cairney (1992) and Olsson (1999).

The directionality of translocation in rhizomorphs; whether translocation of specific compounds occurs acropetally, meaning towards the tips, or basipetally, meaning from the tips and backwards, has received much attention. The end of a rhizomorph that is attached to a resouce unit or "food base" is considered to be the basipetal end, and the end fanning out into the growing mycelial front is termed the acropetal end. A foraging mycelial front can however rapidly colonise a new resource unit, turning the acropetal end of the rhizomorph into a basipetal end. The mycelium can encounter substrates with a variety of different qualities, ranging from highly nutritious to inert or even toxic, making it very difficult to clearly differentiate between growing mycelial fronts and mycelium colonising a resource unit. Rhizomorphs connect areas of the mycelium that are rich in hyphal tips with each other, and hyphal tips are sites of both resource acquisition and resource consumption (**4.1**), making the directionality of a rhizomorph an obsolete concept. The manner in which rhizomorphs are formed also suggests their lack of inherent polarity. With the exception of *Armillaria* and some other fungi (*e.g. Marasmius*) with rhizomorphs that extend like plant roots with highly integrated growing tips, rhizomorphs are formed behind a extending front of more or less diffuse mycelium (Thompson & Rayner, 1983). Rhizomorphs form through the differentiation of a handful of adjacent hyphae, from which hyphal branches grow out parallel with the "founder hyphae". These secondary branches can be aligned antiparallel as well as parallel with the founder hyphae (Figure 4). Aligned hyphae connect to each other through hyphal bridges, anastomoses, to form a rhizomorph (Raidl, 1997). Most rhizomorphs do not thus grow at the tip, but are formed through the thickening of already existing hyphal bundles. This often antiparallel alignment of hyphae in rhizomorphs implies that, although individual hyphae are polar, there is usually not a clear, inherent directionality in rhizomorphs.

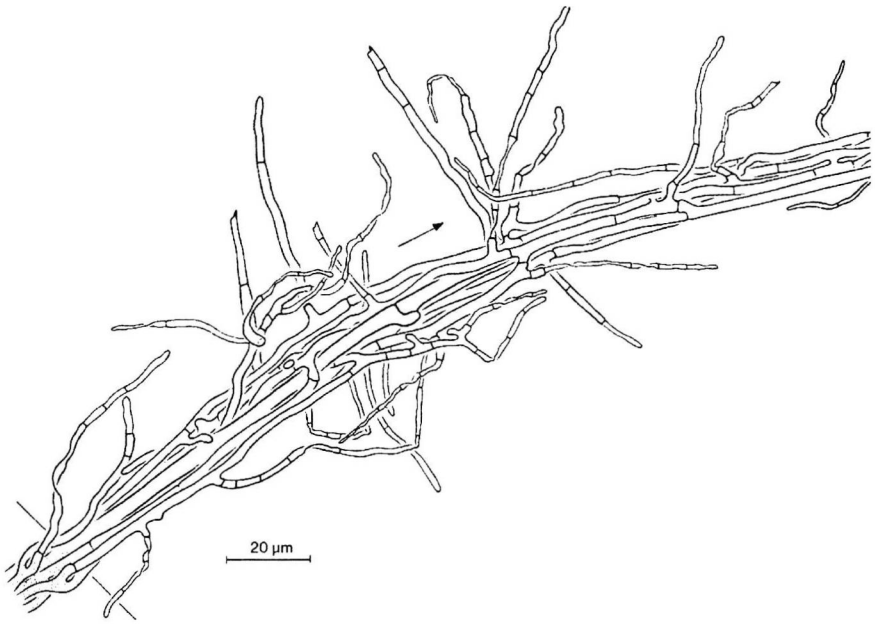

Figure 4. Drawing of a rhizomorph of *Amanita muscaria* (Fly agaric). The arrow show the growth direction of the founder hyphae. (Reproduced from Raidl, 1997 with the publisher's permission).

If there is no clear directionality in rhizomorphs, transport of a specific resource should be possible in any direction. Bidirectional transport was, surprisingly enough, first found in rhizomorphs of *Armillaria*; one of the few fungi with rhizomorphs that do grow at the tip. Granlund *et al.* (1985) found that ^{14}C, supplied as glucose to the base of an *Armillaria* rhizomorph, was translocated towards the tip at the same time as ^{3}H, also supplied as glucose but to the tip of the same rhizomorph, was translocated towards the base. Fluxes of the two isotopes were similar. A long series of experiments, studying translocation of ^{32}P between wood blocks connected by rhizomorphs (mostly in *Phanerochaete velutina)*, was conducted by Wells *et al.* (reviewed by Boddy, 1999). From these experiments it is evident that phosphorus can be transported from a wood block towards growing mycelial fronts as well as from mycelial fronts towards a wood block. In most of these experiments, however, the tracer isotope was introduced into the experimental systems together with large amounts of non-radioactive phosphorus, causing polarities in the mycelia induced by the phosphorus additions. In one experiment however, the addition of radioactive phosphorus at one site was compensated for by additions of corresponding amounts of non-radioactive phosphorus to the rest of the system (Wells *et al.* 1998a). In this experiment, even when the addition of the radioisotope did not affect the patterns of phosphorus availability in the system, the radioisotope seemed to spread throughout the mycelium away from the site of

addition, irrespective of where the ^{32}P was applied. Similarily, Olsson & Gray (1998) found that ^{32}P, added to fungal mycelia cultivated on agar, was translocated in different directions dependent on the site of isotope addition.

4.3.2. Paper I: simultaneous, bidirectional translocation of phosphorus

To further explore the bidirectional character of ^{32}P tranlocation patterns in rhizomorphs, the experiment presented in (**I**) was designed. In this experiment, wood blocks, inoculated with the rhizomorph forming, wood rotting fungus *Hypholoma fasciculare* were introduced into trays (1x2x20cm) filled with sieved forest floor material. The fungus extended from the wood blocks (the inocula) through the organic matter until it encountered wooden baits, situated 12 cm from the inocula. The fungus colonised the baits, leaving rhizomorphs to connect them to the inocula. Phosphorus has two radioactive isotopes with half-lives suitable for laboratory work, that were used in combination to test the bidirectionality of phosphorus translocation in rhizomorphs of *Hypholoma*. The mycelium at the inoculum was supplied with ^{33}P-orthophosphate in a small droplet of water. At the same time, a droplet containing ^{32}P-orthophosphate was added to the mycelium covering the bait. Both tracer isotopes were added carrier free (or almost), meaning that very little phosphorus was added to the system (picomoles). Translocation of the radioactive isotopes was followed over 30 days, using an electronic autoradiography method, developed for this experiment, that enables separation of the radiation from the two isotopes based on differences in their energy spectra.

A time series of electronic autoradiography scans showed that ^{32}P was translocated from the bait to the inoculum at the same time as ^{33}P was translocated from the inoculum to the bait (**I**, fig. 3 & 4). The main conclusion from (**I**) is thus that phosphorus circulates in the mycelium of *Hypholoma fasciculare*. The total phosphorus transport is larger than the net translocation between different parts of the fungus. Bidirectional phosphorus transport has, as mentioned, been found in rhizomorphs of *Phanerochaete velutina* (Wells *et al. 1998a)* and also in the ectomycorrhizal fungi *Suillus variegatus* and *Paxillus involutus* (**II** & **III**), although in these other studies, translocation in different directions was measured in separate, but identical, systems. Together these findings suggest that circulation of phosphorus is a general feature of rhizomorphic mycelia.

Why do fungi transport phosphorus back and forth throughout their mycelia? Circulation of resources may be an efficient way to regulate transport between different parts of an organism. Without circulation, a hyphal tip with high demand for a particular resource must send some kind of signal through the mycelium to increase the provision of this resource. Resources must then be routed from areas of uptake to the area with high demand. If instead all available resources are circulated throughout the whole mycelium, there is no need for signals. The hyphae take up what they need and leave the remaining resources to circulate freely. In this aspect the networks of rhizomorphs found in many basidiomycetes could be similar to the vascular transport system of animals.

Another finding of the experiments described in (**I**) is that most of the phosphorus taken up remained at the site of addition. This is in accordance with the findings of Clipson *et al.* (1987) and Olsson & Gray (1998) who observed that ^{32}P was immobilised after uptake, when added to rhizomorphs in the field or to agar cultures respective. In the experiments described in (**I**), as well as in the experiments described by Olsson & Gray (1998), phosphorus was slowly released from the site of immediate immobilisation. Once released, the phosphorus was rapidly translocated to other parts of the mycelium. Experiments using NMR (nuclear magnetic resonance) to identify chemical forms of phosphorus in fungal hyphae suggest that polyphosphates are the main translocated forms (Ashford *et al.*, 1994). The rapid immobilisation directly after uptake could be due to the fact that mobilised orthophosphate has to be converted to polyphosphate before it can be translocated. Alternatively, mobilised orthophosphate is rapidly incorporated into immobile macromolecules such as phospholipids and nucleic acids. There is a slow, continuous turnover of these compounds, resulting in conversion of the radioisotope back to simple forms which may be translocated away from the addition site.

4.3.3. A translocation model

Rhizomorphic mycelia can be subdivided into mycelial subunits, connected to each other by rhizomorphs. In the model of mycelial translocation proposed here, all resources circulate throughout the mycelium and the fluxes of resources are dependent on the rate of circulation as well as the concentration of resources in the circulation stream. The flux of a resource out of a mycelial subunit should depend on the concentration of that particular resource in the mobile pool of the subunit, and the flux of a resource into a mycelial subunit should depend on the concentration of the resource in the mobile pool of the rest of the mycelium. If the concentration in the mobile pool of a subunit is lower than in the rest of the mycelium, the flux into the subunit will be larger than the flux out from the subunit, and there will be a net flux into the subunit, which can be termed a sink for this particular resource. If, on the other hand, the concentration in the mobile pool is higher in the subunit than in the rest of the mycelium, there will be a net flux out of the subunit, which can be termed a source. As an effect of circulation in the mycelium, there will thus be net fluxes of resources from sources to sinks. Different resources could be circulated using the same transport mechanism, but the net fluxes will be specific to each particular resource. A mycelial subunit could thus be a source for one resource and a sink for another, without the need for independent transport mechanisms.

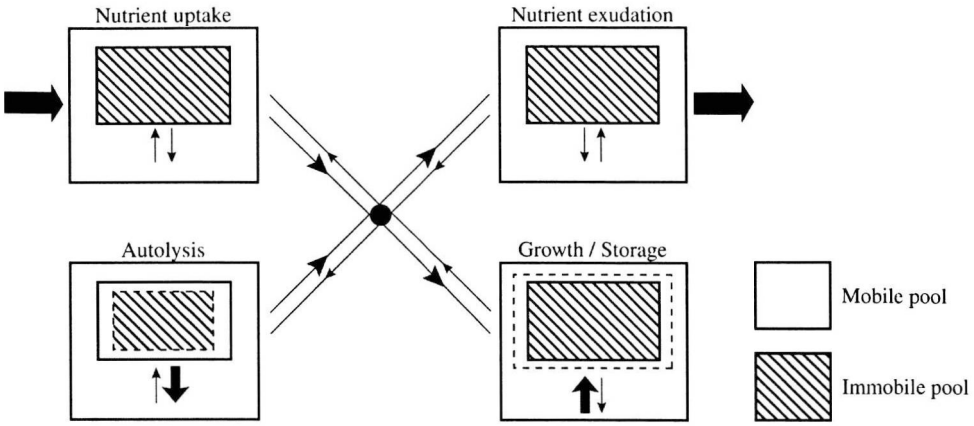

Figure 5. A schematic representation of resource translocation in a mycelium with four subunits connected by rhizomorphs. Arrows represent resource fluxes between mycelial subunits or between a mobile pool and immobile compounds within subunits. Resources are circulated throughout the mycelium as outlined in (**4.3.3**). Differences in resource concentrations in the mobile pool cause net translocation from sources to sinks.

What factors affect the concentrations of resources in the mobile pool of a mycelial subunit? A high rate of resource mobilisation from outside the mycelium increases the concentration in the mobile pool whereas excretion to the outside lowers the concentration. Incorporation of resources into immobile macromolecules decreases the concentration in the mobile pool. During mycelial growth, rapid incorporation of resources into immobile tissues takes place, and the concentration in the mobile pool decreases. Correspondingly, rapid conversion of immobile compounds to mobile forms will increase the concentration in the mobile pool; a situation which could occur in association with degenerative processes during mycelial senescence. Resources will thus be redistributed from areas of uptake or mycelial degeneration to areas of intensive growth or exudation (Figure 5).

4.4. Mechanisms of translocation

Several different mechanisms of translocation in individual hyphae, as well as in rhizomorphs, have been proposed. The dry rot fungus (*Serpula lacrymans*), dreaded for its detrimental effect on wooden houses, can colonise dry wood by wetting the wood with water droplets formed at the hyphal tips (Clarke *et al., 1980*)(the latin *lacrymans* means "weeping"). The ability to transport water from moist places to dry wood implies bulk flow of water through the hyphae. Translocation of ^{3}H-labelled water through rhizomorphs of the ectomycorrhizal fungus *Suillus bovinus* was demonstrated by Duddridge *et al.* (1980), and Brownlee *et al.* (1983) showed that an ectomycorrhizal mycelium could support a pine seedling, growing in a dry substrate, with enough water to keep it vital, as

long as mycelial contact with a moist substrate was maintained. Water transport in rhizomorphs has been suggested to occur mainly through the coarse "vessel hyphae" at the centre of the rhizomorphs (Figure 3; Duddridge *et al.,* 1980). These hyphae are usually devoid of cytoplasm, and often only remnants of the crosswall septa remain. Water transport in rhizomorphs thus probably occurs through apoplastic pathways with relatively low hydraulic resistance. If bulk flow of water though rhizomorphs commonly occurs, dissolved carbohydrates and nutrients could be translocated along with the water stream. Brownlee & Jennings (1982) investigated the mechanisms behind resource translocation in rhizomorphic mycelium of *Serpula*, extending from a wood block over an inert surface. They showed that when the osmotic potential in the solution surrounding the wood block was increased, formation of water droplets at the hyphal tips as well as translocation of ^{14}C (added as glucose), ^{32}P (added as phosphate) and ^{42}K was interrupted. This finding confirms that the solutes moved through the rhizomorphs in a pressure driven bulk flow of water. Jennings (1987) proposed the following mechanism: At sites of cellulose degradation, uptake of glucose increases the osmotic potential in the mycelium, causing water to enter the hyphae. At the growing mycelial front, the osmotic potential is lower, due to incorporation of low molecular weight compounds into macromolecules during hyphal growth. The low osmotic potential at the hyphal tips causes water to leave the hyphae (droplet formation). The resulting pressure gradient throughout the rhizomorph causes bulk flow of water, carrying solutes from carbohydrate sources to sinks. The same mechanism was proposed for mycorrhizal fungi by Unestam & Sun (1995), who observed exudation of water droplets, similar to those described in *Serpula*, at the hyphal tips of different ectomycorrhizal species growing in association with pine seedlings in microcosms. Exudation was induced by exposure of the plant shoots to light, and the exuded droplets were withdrawn when the shoots were shaded. In analogy with the theory of Jennings (1987), transfer of photosynthetic products from the plant to the fungus could increase the osmotic potential in the mycorrhizal mantle causing water to enter the hyphae and a pressure gradient to form away from the mycorrhizal root tips (Sun *et al.,* 1999).

An alternative transport mechanism, taking place within the cytoplasm of living cells (along symplastic pathways), was first described by Shepherd *et al.* (1993a), who found a system of tubular vacuoles in hyphal tips of the mycorrhizal fungus *Pisolithus tinctorius*. The vacuoles are motile and depend on microtubuli to squeeze their content through hyphae with peristaltic movements. Different tubular elements within single hyphae transport their content in different directions, facilitating simultaneous, bidirectional translocation. Motile, tubular vacuoles have now been described in a wide range of fungi from all divisions (Rees *et al.* 1994). The vacuolar system is present in rhizomorphs (Allaway & Ashford, 2001), and has been observed to penetrate the dolipore septa that separate cells, enabling intercellular transport (Shepherd *et al.,* 1993b).

Rhizomorph translocation of carbohydrates, phosphorus and potassium in *Serpula* (Brownlee & Jennings, 1982), and of carbohydrates in *Suillus* (Finlay & Read, 1986a) and *Phanerochaete* (Wells *et al.,* 1995), has been observed at rates between 20 and 300 cm/h. The transport rates estimated in these experiments appear to be one or two orders of magnitude higher than the rate of peristaltic movements observed in motile vacuoles (Hyde *et al.* 1997). There was evidence that translocation in *Serpula* can be mediated by bulk flow of water (as described above). In experiments with *Phanerochaete velutina* extending into soil from wood blocks, ^{14}C translocation rates and fluxes increased when the wood blocks were thoroughly wetted before addition of the tracer isotope (Wells *et al.,* 1995). This could, as the authors suggest, be due to limited ^{14}C-glucose uptake by mycelium colonising dry wood blocks, caused by surface tension and air bubbles in the mycelium. It could also, however, be an effect of increased water potential in the wood causing increased water uptake into hyphae colonising the wood and a steeper pressure gradient throughout the rhizomorphs, suggesting that carbohydrate translocation by bulk flow also occurs in *Phanerochaete*. Finlay & Read (1986a) proposed, based on the high transport rates, that carbohydrates in *Suillus* rhizomorphs were translocated by bulk flow through vessel hyphae. Accumulation of radioactivity in the growing fronts of the extraradical mycelium, when the plant shoot was supplied with $^{14}CO_2$, implies that translocation of the radioisotope mainly occurred away from the plant roots. In the discussion of a similar experiment, Brownlee *et al.* (1983) argued that since the ^{14}C–translocation occurred in the opposite direction to the supposed direction of the water flow, that is towards the transpiring plant, the carbohydrates must be transported through symplastic pathways. In mycorrhizal fungi, reversals of the water flow direction could however occur on a diurnal basis (Unestam, personal communication). According to this hypothesis, transpiration from the plant leaves during daytime causes a low water potential in the roots. Due to the low water potential in the root cells, water leaves the fungal hyphae in the ectomycorrhizal root tips, causing a pressure gradient to be formed towards the roots. Water would thus be transported from the substrate to the plant via mycorrhizal rhizomorphs (Duddridge *et al.*, 1980; Brownlee *et al.,* 1983). At night however, transpiration decreases and a pressure gradient is built up away from the root tips, maintained by a high osmotic potential and subsequent transport of water into the hyphae in the mycorrhizal root tips (Sun et al. 1999). Water could be provided by the plant roots, as water is taken up by deep taproots and translocated to surface roots (the so called "hydraulic lift"). The night time bulk flow of water from tap roots to surface roots and further out into the mycorrhizal mycelium has been demonstrated by Querejeta *et al.* (2001). In the experiments conducted by Brownlee *et al.* (1983) as well as Finlay & Read (1986a) the plant shoots were enclosed in tight plastic boxes during labelling. Plant transpiration should thus have been low, similar to the night time situation described above, enabling bulk flow of water and therein dissolved carbohydrates towards the extraradical mycelial fronts.

In an experiment by Granlund *et al.* (1985), using rhizomorphs of *Armillaria,* grown in tubes filled with culture solution, translocation rates of ^{14}C and ^{32}P were lower than in the experiments discussed above. The measured rates of 1 - 3.5 cm/h are of the same order of magnitude as the peristaltic movements of tubular vacuoles. In this experiment carbohydrates were transported in two directions simultaneously in a manner similar to the bidirectional phosphorus translocation found in *Hypholoma* (**I**). While diurnal shifts in flow direction could hypothetically cause bidirectional bulk flow in mycorrhizal fungi, the simultaneous bidirectional translocation found in saprotrophic fungi by Granlund *et al.* (1985) and (**I**) is not easily fitted into the bulk flow model of Jennings (1987). Simultaneous, bidirectional translocation through bulk flow of water would have to involve opposite pressure gradients in separate elements of a rhizomorph, maintained by different osmotic potentials in adjacent hyphae. Taking into account the high frequency of anastomoses between hyphae within a rhizomorph, this scenario is highly unlikely. Timonen *et al.* (1996) estimated translocation rates of ^{32}P in mycelia of the ectomycorrhizal fungus *Paxillus involutus* to 7.5 mm/h. Autoradiographic experiments with ectomycorrhizal fungi (*Suillus* and *Paxillus*) in microcosms have shown that ^{32}P can be translocated from mycelium in the mycorrhizal root tips to extraradical mycelium as well as from the substrate towards the plant roots (Finlay & Read, 1986b; **II** & **III**). The bidirectional character of ^{32}P translocation together with the slower translocation rates suggest that phosphorus is translocated using a transport mechanism other than apoplastic bulk flow. The system of motile vacuoles is a strong candidate, especially since the vacuoles have been shown to contain polyphosphate (Ashford *et al.*, 1994).

In conclusion, current knowledge of translocation in rhizomorphic basidiomycetes supports the model suggested by Cairney (1992). Bulk flow of water seems to occur frequently in rhizomorphs of basidiomycetes. The flow is driven by pressure gradients maintained by osmotic differences attributed to uptake of glucose or, in the case of mycorrhizal fungi, by plant transpiration. Carbohydrates seem to be rapidly translocated along with the water fluxes, which are directed from carbohydrate sources to sinks. Phosphorus, on the other hand, seems to circulate throughout mycelia, presumably as polyphosphate through motile vacuoles. Phosphorus generally moves through rhizomorphs at lower rates (0.75-3 cm/h) than carbohydrates (20–300 cm/h). Exceptions to this pattern are the study of *Serpula* by Brownlee & Jennings (1982), in which phosphorus appeared to follow the bulk flow of water together with carbohydrates, and the study of *Armillaria* by Granlund *et al.* (1985), in which carbohydrates were translocated bidirectionally. Due to the absence of radioactive isotopes with a suitable half life, nitrogen translocation is less studied than translocation of carbon and phosphorus. Olsson & Gray (1998) demonstrated bidirectional translocation of an ^{14}C-labelled amino acid analogue (aminoisobutyric acid) in mycelia cultivated on agar. Jentschke *et al.* (2001) found that translocation of nitrogen, potassium and magnesium increased when phosphorus was added to the

extraradical mycelium of *Paxillus involutus*, suggesting that these substances were transported by the same mechanism as phosphorus. Nitrogen, as well as magnesium and potassium, has also been found associated with polyphosphates in fungal vacuoles (Ashford *et al.* 1994; Kottke *et al.* 1995). In field experiments, where mesh bags with ^{15}N-labelled plant leaves or needles were incubated on the forest floor, ^{15}N was translocated out of the mesh bags at the same time as the total nitrogen content in the bags increased (Berg, 1988; Gebauer *et al.,* 2000). The simultaneous transport of nitrogen in and out of the mesh bags is another indication that nitrogen is also circulated throughout fungal mycelia (**4.3.3**)

5. Mycelial interactions and nutrient transfer between interacting fungi

5.1. Interactions between fungi and other organisms

Morphological flexibility and translocation of resources make basidiomycetes well adapted to the spatially heterogeneous environment of the boreal forest floor (**4**). Higher levels of complexity are added to our understanding of the environment in which fungal mycelia are active, if we consider the presence of other organisms and their interactions with the fungi. Interactions between organisms change resource availability and alter the physico-chemical environment. When an interaction is beneficial to both organisms, it is termed mutualistic. When beneficial to one organism but detrimental to the other, the interaction is termed parasitic or predatory. If the interaction is detrimental to both parts, it is of a competitive nature (Campbell *et al.*, 1999, p. 1111).

Interactions between basidiomycetous fungi and plants are relatively well documented. These interactions can be of a mutualistic nature, as in the case of most ectomycorrhizal associations. Many fungi live as parasites on living plants, and in some cases plants can be parasitic on fungi (certain achlorophyllous plant species obtain their demand for carbohydrates and nutrients from fungi; Leake, 1994). The occurrence of competition between plants and fungi for resources has to a large extent been overlooked but is now receiving increasing attention and is discussed in (**IV**). Many insects, mites and worms feed on fungal hyphae (Anderson, 1975), but fungi can also use animals as nutritional resources; several studies have described how some basidiomycetous fungi can trap nematodes (Dackman *et al.,* 1992) and digest them to obtain nutrients. Symbiotic relationships between fungi and animals have been observed, where mycotrophic (fungal feeding) insects (*e.g.* Talbot, 1977) or mammals (Maser *et al., 1978*) act as dispersing agents, spreading fungal mycelium to new resource units. A classic example of interactions between fungi and bacteria is the strong antagonism displayed by *Penicillium* species and many other ascomycetes. Most people are familiar with the use of the bactericidal secondary metabolites, produced by these

fungi, for pharmaceutical purposes. Bacterial metabolites may also be detrimental to basidiomycetes (*e.g.* Thrane *et al.,* 1999) and antagonistic as well as mutualistic interactions between soil bacteria and ectomycorrhizal fungi have been observed (Garbaye, 1994).

5.2. Morphological responses to mycelial interactions

5.2.1. Interactions under axenic conditions

As the mycelial density in boreal forests is high and fungi play central roles in many ecosystem processes, interactions within the fungal kingdom are likely to be of high relevance to our understanding of the functioning of boreal ecosystems. Interactions between basidiomycetous fungi have mainly been studied in wood rotting species (reviewed by Boddy, 2000), partly due to the potential of these fungi for use as biocontrol agents against forest pathogens. When mycelia of two different wood decomposing fungi meet on agar plates, the fungi generally respond with antagonistic reactions. At sites of mycelial contact, there is usually a shift in mycelial morphology with increased branching frequency resulting in the formation of a barrier of dense mycelium. Mycelial interactions are also often associated with pigmentation of the mycelium. In some cases, extension of the interacting mycelia stops at the sites of contact and a deadlock situation occurs. In other cases, one of the fungi is successful in overcoming the antagonistic reactions of the opponent and overgrows the other fungus with mycelial fans or rhizomorphs. Overgrowth of one fungus by another results in lysis of the overgrown mycelium. Only during transient periods, do mycelia of two different individuals coexist within the same limited substrate volume. Wood dwelling fungi thus display a kind of territorial behaviour. Agar plate interactions between different ectomycorrhizal fungi or between ectomycorrhizal fungi and saprotrophic fungi have been studied by Shaw *et al.* (1995) and Baar & Stanton (2000). Interactions resulted in mutual inhibition or intermingling of the mycelia and hyphal lysis in one or both of the fungi. In interactions involving ectomycorrhizal and saprotrophic fungi, mycelial damage, if any, only occurred in mycorrhizal mycelia. Sometimes, however, ectomycorrhizal fungi inhibited the growth of saprotrophs. Shaw *et al.* (1995) also studied interactions between ectomycorrhizal fungi, growing in association with plants under axenic conditions. They found that mycorrhizal root formation by *Lactarius rufus* was completely inhibited by interaction with other mycorrhizal fungi. Similarly, the presence of *Laccaria laccata* significantly reduced mycelial extension rates of *Suillus bovinus* and root colonisation rates of both *Suillus bovinus* and *Paxillus involutus*. Mycorrhiza formation by *Paxillus* was also drastically reduced when agar plugs with mycelium of the saprotrophic fungus *Collybia maculata* were introduced into the system.

5.2.2. Interactions in natural substrates

The observations from wood decomposers interacting on agar have been complemented with experiments using woody substrates, in which interactions between two fungi results in deadlock or the exclusion of one fungus by the other (Boddy, 2000). In contrast to interactions in wood, interactions between basidiomycetous fungi in soil or litter have been studied in only a handful of cases. Dowson *et al.* (1988) studied interactions between rhizomorph forming, wood degrading fungi growing out into soil from two opposing wood blocks. Interaction responses were similar to those observed on agar, but not always as obvious. When physical contact between mycelia occurred in the soil, discoloration and lytic responses of one or both of the interacting mycelia usually occurred at the contact site. Lysis of rhizomorph segments, as a consequence of antagonistic interactions, was followed by death of mycelium that lost its translocative connection to the wood block. In some species combinations, a deadlock situation developed where neither of the mycelia was able to advance further after contact. In other combinations, one or both of the interacting mycelia advanced towards the opposing wood block, where they were sometimes able to replace the original occupant, resulting in its eventual exclusion from the entire microcosm.

In laboratory and field experiments, Frankland *et al.* (1995) studied interactions between the litter fungus *Mycena galopus* and other litter colonising basidiomycetes. In this study, mycelial barriers and pigmentation of mycelium were also observed in the interaction zones. Interactions resulted in deadlock, or in some cases, overgrowth. An estimation of the vertical distribution of *Mycena* mycelium in the field was obtained by tracing fruitbody stems to their origin. In areas where *Marasmius androsaceus* coexisted with the *Mycena*, fruitbodies of the latter were rooted significantly deeper in the litter than when *Marasmius* was not present, suggesting exclusion of *Mycena* from the upper horizons by *Marasmius*. In laboratory experiments in which the two species colonised sterile spruce litter, the proportion of needles colonised by *Mycena* increased, due to the presence of a collembola (*Onychiurus latus*) that grazed preferentially on *Marasmius* ycelium.

Wu *et al.* (1999) studied interactions between the ectomycorrhizal fungi *Pisolithus tinctorius, Suillus luteus* and a third, unidentified ectomycorrhizal fungus in soil microcosms containing pine seedlings. As the mycelium of the unidentified fungus advanced across the microcosms, it overgrew mycelium of *Pisolithus,* resulting in subsequent discoloration and disappearance of the *Pisolithus* mycelium and rhizomorphs. The unidentified fungus also colonised root tips already colonised by *Pisolithus,* and eventually completely replaced *Pisolithus* on the roots.

In a series of experiments (**II, III & IV; 5.3.2)** interactions were studied in soil microcosms containing an ectomycorrhizal fungus; *Suillus variegatus* or *Paxillus*

involutus, colonising seedlings of *Pinus sylvestris,* and the soil dwelling, wood decaying fungus *Hypholoma fasciculare*. When challenged with ectomycorrhizal mycelium, the wood degrading fungus often produced dense mycelial barriers in the soil, in particular when the saprotroph had access to large amounts of resources in the form of colonised wood and stored nutrients (**III**, fig. 1). The barriers were similar to those commonly formed by *Hypholoma* in response to interactions with various other fungi on agar (Griffith *et al.,* 1994). In many cases, when the resources available to the *Hypholoma* were restricted, the mycorrhizal fungi overgrew the saprotrophic soil mycelium and formed patches of dense mycelium in the area where the two mycelia overlapped (**II**, fig. 1 & **III**, fig. 2). These mycorrhizal mycelial patches were similar to those observed by Unestam (1991), Read (1992), Bending & Read (1995a) and Leake *et al.,* (2001) in response to enrichment of the substrate with organic nutrients of high quality (**4.1**). There was large variation between different experiments in the morphological outcome of the interactions between mycorrhizal and saprotrophic fungi, even when apparently identical experimental systems were used. In some of the experiments, extension of the soil mycelium of the wood decomposer was clearly inhibited due to interactions with ectomycorrhizal mycelium (**IV**, fig. 3) Rapid senescence of saprotrophic mycelium could also be observed in the interaction zones (**IV**, fig. 5). Autolysis (apoptosis) of mycelia occurs commonly however, even in non-interacting mycelia, in association with rhizomorph formation (**4.1**), and additional experiments must be performed to confirm the occurrence of mycelial interference by ectomycorrhizal fungi against saprotrophic fungi.

Experiments involving interactions between an ectomycorrhizal- and a wood decomposing fungus have also been conducted by Leake *et al.* (2001). In peat microcosms, mycelia of *Suillus bovinus,* growing in association with pine seedlings, were confronted with mycelia of *Phanerochaete velutina*, extending across the peat from wood blocks. Extension rates and vigour of the mycorrhizal mycelium were drastically reduced as an effect of interactions with the saprotroph. The antagonistic effect of the saprotroph on the mycorrhizal fungus was confirmed by feeding the plant shoot with a pulse of ^{14}C-labelled carbon dioxide. The amounts of ^{14}C incorporated into the extraradical mycelium were reduced by two thirds in interacting mycorrhizal systems compared with non-interacting control systems.

Competitive interactions can be categorised into exploitation competition, in which uptake of resources by one organism decreases the amount of resources available to the other (Lockwood, 1992), and interference competition, where one organism prevents the other from acquiring resources from the common resource pool (Wicklow, 1992). Interactions between wood decomposing fungi are clear examples of interference competition, since fungi prevent other fungi from decomposing and taking up resources from a substrate by excluding them from the substrate. Wood fungi thus compete directly for space and only indirectly for

carbohydrates and nutrients. The studies by Frankland (1995), Dowson *et al.* (1988) and Leake *et al.* (2001) show that saprotrophic fungi can display interference competition, not only in wood, but also in litter or soil. Furthermore, Wu *et al.* (1999) and (**IV**) suggest that ectomycorrhizal fungi also can interact antagonistically with other soil fungi, monopolising root tips in a volume of soil by excluding other mycorrhizal fungi, or inhibiting mycelial growth of saprotrophic fungi in the soil. As mycorrhizal- and saprotrophic fungi to a large extent use different carbon sources, competition between fungi from these two groups should occur mainly for soil nutrients.

5.3. Nutritional interactions between mycelia

5.3.1. Competition between soil fungi for nutrients

Most studies of competition between fungi mainly consider competition for energy sources. Saprotrophic fungi compete with each other for substrates rich in cellulose. Similarly, ectomycorrhizal fungi compete with each other for living root tips. Fungi may however compete, not only for sources of carbohydrates, but also for nutrients. In ectomycorrhizal fungi, it is the extraradical mycelium that forages and competes for nutrients in the soil (Read, 1992). In (**IV**) it is proposed that saprotrophic fungi also take part in the competition for soil nutrients. As saprotrophic fungi degrade wood or plant litter, they obtain nutrients as well as carbohydrates. However, the amounts of nutrients in the poor boreal plant litter are often not enough to allow efficient degradation of the substrate, and at early stages of decomposition, litter with higher nutrient content tends to decompose more rapidly than nutrient poor litter (Berg 1986; Cotrufo *et al.* 1995). This is even more pronounced in wooden substrates (Merrill & Cowling, 1966). In (**4.3.3**) it is proposed that basidiomycetes, by circulating nutrients in their mycelium, cause net translocation of phosphorus and nitrogen from sites of high availability to sites of high demand. In several experiments (reviewed by Boddy, 1999), the ability of different rhizomorph forming fungi to take up ^{32}P from soil and transport it into wood blocks has been demonstrated. Field experiments have shown that the amounts of nitrogen and phosphorus in wood and nutrient poor litter tend to increase during early stages of decomposition (**IV**, fig. 2; Staaf & Berg, 1982; Fahey, 1983; Yavitt & Fahey, 1986; Laiho & Prescott, 1999). Together these findings suggest that saprotrophic fungi translocate nutrients from the surrounding soil into newly colonised, nutrient poor substrates, to increase the rate of colonisation and cellulose degradation. Saprotrophic fungi could thus compete with each other, with mycorrhizal fungi and with other soil organisms for soil nutrients. Occurrence of competition for nutrients between ectomycorrhizal and saprotrophic fungi was proposed by Gadgil & Gadgil (1971) to explain observed increases in decomposition rates, when all roots that entered a soil volume were cut. Reduction in mycorrhizal activity, due to the interrupted flow of carbohydrates to the root tips, was hypothesised to increase nutrient availability to saprotrophic fungi and thereby increase decomposition rates.

A wide range of different potential nutrient sources can be distinguished in boreal forest soils. Inorganic ions in the soil solution as well as organic forms of nutrients in dead plant tissues or in humic material may be assimilated by soil fungi. An additional pool of soil nutrients is incorporated in living and dead fungal mycelium; in the F/H horizon of a boreal forest, 15-20% of the nitrogen and 18% of the organic phosphorus have been estimated to be incorporated into fungal hyphae (Bååth & Söderström, 1979). Fungi may thus compete for nutrients in the soil at the same time as they themselves constitute a significant pool of nutrients for other organisms. Fungal mycelia, growing in substrates in which nutrients are easily available, deplete the substrate as they take up nutrients and translocate them away or incorporate them into the mycelium. Fungi, growing in substrates where easily available nutrients are scarce, could, on the other hand, enrich the substrate as they translocate nutrients into the substrate or mobilise recalcitrant, or physically protected, nutrients incorporating them into mycelium. In an environment poor in easily available nutrients, this mycelium in itself constitutes a high quality nutrient source to other organisms.

5.3.2. Papers II and III: transfer of phosphorus between interacting saprotrophic and ectomycorrhizal mycelia

In (**II** & **III**), a series of experiments is described, in which ectomycorrhizal fungi and a saprotrophic fungus interact in soil microcosms. The mycorrhizal fungi *Suillus variegatus* and *Paxillus involutus* grew in association with the roots of *Pinus sylvestris* seedlings and the saprotrophic fungus *Hypholoma fasciculare* extended from blocks of birch wood (**5.2.2**). The interactions took place in narrow plastic trays (20x2x1cm) filled with sieved, non-sterile humus material from the floor of a mixed coniferous forest. Humus is poor in easily available nutrients, and the microcosms were watered with de-ionised water. The main hypothesis underlying the experiments was that the fungi, in an environment with limited access to nutrients, would utilise the mycelium of the interacting fungus as a nutrient source. This hypothesis was tested by labelling either of the interacting mycelia with ^{32}P and testing whether the radiotracer was transferred to the other, initially non-labelled mycelium. The tracer isotope was added to saprotrophic mycelium covering the wood block or to mycorrhizal mycelium surrounding a root tip (in small plastic cups beneath the roots). Electronic autoradiography was then used to follow translocation of the radioactive phosphorus in the microcosms over time periods of up to 30 days.

In the first experiments (**II**), the mycorrhizal fungi rapidly overgrew the saprotrophic mycelium and formed dense mycelial patches in the interaction zones (**II**, fig. 1). The radioactive isotope was added to the microcosm at the time of first physical contact between the interacting mycelia. Regardless of which fungus the ^{32}P was added to, radioactivity was rapidly translocated to the interacting mycelial fronts. When ^{32}P was added to the saprotrophic mycelium on the wood block, radioactivity could be detected in mycorrhizal roots 10 days after addition of the tracer isotope, indicating transfer of phosphorus from the

saprotrophic mycelium to the mycorrhizal fungus. Radioactivity could be detected in the shoot of the pine seedling 20 days after tracer isotope addition (**II**, fig. 2). A mathematical regression model was developed to relate the amounts of ^{32}P transferred between the fungi to the amounts of ^{32}P available in the interaction zone. Simple regression could not be used, as the activity in the interaction zone in many cases increased during the experiments due to continuous translocation of ^{32}P to the interacting mycelial fronts. Data analysis showed that the average transfer rate of ^{32}P from labelled saprotrophic mycelium to the mycorrhizal root tips was 0.8% of the activity in the interaction zone per day. After 28 days, 12% of the activity outside the wood block was found in plants colonised by *Paxillus*. The corresponding figure for microcosms with *Suillus* was 14%. When mycorrhizal mycelium was labelled with ^{32}P, radioactivity above background level could be measured in the wood blocks 15 days after isotope addition, indicating transfer of phosphorus from the labelled mycorrhizal mycelium to the saprotrophic fungus. The transfer rates were however very low; around 20 and 100 times lower for systems with *Suillus* and *Paxillus* respectively than the transfer rates observed in the opposite direction.

Holmer & Stenlid (1993) found that the outcome of interactions between wood rotting fungi was dependent on the relative sizes of the volumes of wood that the fungi colonised. With the aim of studying a broader range of interactions between saprotrophic and ectomycorrhizal mycelia, experiments were set up in which the saprotroph (*Hypholoma fasciculare*) colonised wood blocks of two different sizes; 1.6 and 0.44 cm^3. The outcome of interactions in microcosms with small wood blocks was similar to that observed in the first experiment (**II**), although variations in morphology between replicates were large. In many cases the mycorrhizal fungus (*Suillus variegatus*) formed dense mycelial patches in the interaction zones (**III**, fig. 2). In microcosms with large wood blocks, the saprotroph however overgrew the mycorrhizal mycelium producing dense mycelial fronts (**III**, fig. 1). *Hypholoma* mycelium growing from large wood blocks thus seemed to have a larger inoculum potential (more energy available at the site of interaction) than mycelium growing from smaller wood blocks. Transfer of ^{32}P between the fungi was studied using electronic autoradiography as described above. The average transfer rate of ^{32}P from labelled saprotrophic mycelium to the mycorrhizal roots decreased from 0.30% to 0.13% of the activity in the interaction zone per day, when large wood blocks were used instead of small ones. In contrast, the average rate of ^{32}P transfer from labelled mycorrhizal mycelium to the wood blocks increased from 0.15% to 0.37% of the activity in the interaction zone per day, when large wood blocks were used.

In conclusion, there was significant exchange of phosphorus between interacting mycelia of saprotrophic and ectomycorrhizal fungi. Although transfer of ^{32}P in the first experiments (**II**) was strongly polarised towards the mycorrhizal fungi, some transfer always occurred in both directions, and the transfer rates were affected by the inoculum potential of the fungi involved. Mycelium with

high inoculum potential seemed to lose less phosphorus to its antagonist and capture more phosphorus from its antagonist. Thus, the net flux of phosphorus to an interacting fungus seems to increase, as the inoculum potential of the fungus is increased.

5.3.3. Mechanisms of nutrient exchange between interacting mycelia

The experiments described above show that fungal mycelium is a potentially important source of phosphorus for soil fungi. Even when ^{32}P was added directly to a living saprotrophic fungus, ectomycorrhizal fungi were able to compete successfully for the radiolabelled phosphorus. Considering the large amounts of fungal mycelium found in boreal forest soils, the rapid transfer of mycelial phosphorus to mycorrhizal root tips highlights the importance of fungal mycelium as a nutrient source for ectomycorrhizal plants. There are several possible mechanisms behind the observed phosphorus transfer:

1. The transferred, radioactive phosphorus may originate from dead hyphae of the labelled fungus. Cytoplasmic content could be released in association with hyphal lysis and phosphorus-containing molecules could be taken up in organic form directly by the non-labelled fungus. Phosphorus containing macromolecules such as phospholipids, nucleic acids and polyphosphates could be mobilised after enzymatic degradation.
2. Living hyphae of the labelled fungus could exude phosphate to the soil solution that could be taken up by hyphae of the non-labelled fungus growing in close vicinity.
3. Organic phosphorus in dead, labelled hyphae could initially be taken up by bacteria and microfungi. When their demand for phosphorus is saturated, these organisms could exude labelled orthophosphate that could subsequently be taken up by the non-labelled basidiomycete.

All fungi in the interaction microcosms rapidly took up the added ^{32}P label. If phosphorus was exuded by the hyphae, exudation and uptake must therefore have occurred simultaneously. Simultaneous exudation and uptake of carbohydrates was demonstrated by Sun *et al.* (1999) in mycelia of *Suillus bovinus* growing on agar. The same authors also found that droplets, exuded at hyphal tips, contained phosphorus. Exudation (hypothesis 2) can thus not be ruled out as the mechanism behind the phosphorus transfer and exudation.

The observed high transfer rates, however, make phosphorus mobilisation from dead, labelled mycelium a more appealing hypothesis. As the soil in the microcosms was not sterilised, it is impossible to tell whether the tracer isotope was taken up directly by the non-labelled fungi in organic form (hypothesis 1) or whether it was first converted to orthophosphate by other microorganisms (hypothesis 3). An interesting observation is that the uptake of ^{32}P from labelled mycelium by mycorrhizal seedlings was about 15 times faster that the uptake of ^{32}P measured by Timonen *et al.,* (1996), when the radioisotope was added in

tracer amounts to peat colonised by *Paxillus involutus* growing in association with pine seedlings. The low availability of ^{32}P in inorganic form suggests that the tracer isotope was transferred between the interacting mycelia in organic form (hypothesis 1), but the interaction experiments must be repeated under axenic conditions to test this hypothesis. The capacity of saprotrophic basidiomycetes to degrade complex organic substrates is undisputed. Evidence has accumulated to suggest that many mycorrhizal fungi can also degrade and utilise complex organic nutrient sources (**3.3.** & **6.3**). Direct degradation and utilisation of fungal mycelium as a nutrient source for ectomycorrhizal fungi was proposed by Abuzinadah *et al.* (1986). The ericoid mycorrhizal symbiont *Hymenoscyphus ericae* is known to produce enzymes that degrade chitin; a nitrogen containing polymer that constitutes a large fraction of fungal cell walls (Leake & Read, 1990; Kerley & Read, 1995). *Hymenoscyphus* is able to utilise fungal mycelium as a sole nitrogen source to support itself as well as its host plant (Kerley & Read, 1997 and 1998). Experiments by Dighton *et al.* (1987), Leake & Read (1990) and Hodge *et al.* (1995) suggest that ectomycorrhizal fungi can also mobilise nitrogen from chitin.

If the transfer of ^{32}P between the interacting mycelia was due to utilisation of phosphorus in dead hyphae, either directly or mediated by other microorganisms, the question arises as to whether the availability of dead hyphae increased due to the interaction. Autolysis of redundant mycelium commonly occurs in rhizomorph forming fungi (**4.1**). Old hyphae that do not take part in the formation of rhizomorphs usually lyse, even in the absence of other fungi. Basidiomycetous fungi may be able to withdraw phosphorus and other nutrients from hyphae before they autolyse (Cowling & Merrill, 1966). Cells close to the hyphal tips generally have a higher cytoplasmic content than cells further back from the tips, which are to a large extent vacuolised (Schnürer & Paustian, 1986) indicating reallocation of resources towards the growing hyphal front. Mycelial interference during interactions may result in lysis and death of freshly formed mycelium that is rich in hyphal tips. The amounts of nutrients made available by successful interference may thus be considerably larger than the amounts associated with autolysis of scenescent mycelium. In the interaction experiments discussed here, most interactions resulted in some kind of dense mycelial growth by one of the fungi, making it difficult to evaluate possible mycelial damage to the other fungus. In some of the experiments, mycelial extension of *Hypholoma* more or less stopped after overgrowth by mycorrhizal mycelium (**IV**) and, in the experiments with larger wood blocks, growth of mycorrhizal mycelium was drastically reduced (**III**). Additional experiments need to be performed to confirm that interactions between saprotrophic and mycorrhizal fungi result in increased turnover of soil mycelium.

Although more experiments are needed to firmly establish that basidiomycetous fungi can kill and degrade the mycelia of each other to obtain nutrients, a brief discussion of terminological problems could be of interest.

These types of interactions are clearly not special cases of interference competition, since competitive interactions should be detrimental to both organisms. When fungi use each other as nutrient sources, one fungus may benefit from the presence of the other. Interactions which are beneficial to one organism but detrimental to the other are generally termed parasitic or predatory. Parasitism usually refers to interactions in which both organisms remain alive throughout the interaction, often with one living fully or partly inside the other. Predation refers to interactions where one organism is killed and ingested by the other. Griffin (1972) used the term "exploitation", defined as "the utilisation of the cells of one organism by another, the exploited cells being either living or dead, but if the latter, killed by the exploiter". This is a general term, which nicely encompasses parasitism and predation, as well as the fungal antagonism discussed here. The same term is however used for competition through rapid exploitation of resources (Lockwood, 1992) and the risk of misunderstanding is obvious. Instead I suggest that the term predation should be expanded, not only to include situations were one organism is ingested by another, but to encompass all situations where an organism or parts of an organism are killed by another and thereafter consumed by the killer, the mechanism of consumption being irrelevant. If this terminology is accepted, fungal predation differs from other predatory interactions in that the prey, after being killed, is first digested and then ingested instead of the other way around (this is the case also for some animals such as spiders). Predation of fungi by other fungi also differs in that the predator - prey relationship can shift depending on circumstances so that the predator becomes the prey, or perhaps more commonly, the interacting fungi are both predators and prey at the same time (**5.3.2**).

6. Nutrient cycling in boreal forests

6.1. Traditional model of nutrient cycling

Basidiomycetous fungi constitute a major fraction of the microbial biomass in boreal forest soils (**3.4**) and their ecophysiology must be taken into consideration when discussing microbial soil processes in boreal ecosystems. Traditional models of nutrient cycling were developed with agricultural ecosystems in mind. Agricultural soils are less acidic, more homogeneous and characterised by a higher bacteria to fungi ratio compared to boreal forest soil (Frostegård & Bååth, 1996). In (**IV**) it is concluded that recent findings in basidiomycete ecophysiology, some of which have been presented in the preceding sections, open the way for new models of nutrient circulation in boreal ecosystems, that explain observed experimental results better than traditional models. Nutrient circulation in terrestrial ecosystems involves movement of nutrients between many different pools. Generally there is a slow transfer of nutrients from large abiotic pools such as bedrock minerals (phosphorus, potassium, magnesium etc.),

or from the atmosphere (nitrogen), into biotic pools. Nutrients then circulate more or less rapidly through the biological pools, until they 1. end up in recalcitrant organic compounds with very long turnover rates, 2. are immobilised in clay minerals, 3. leave the system dissolved in water or 4. return to the atmosphere. Plant biomass and dead plant tissues constitute two large biological nutrient pools and the way in which nutrients move from plant litter to living plants is a central theme in nutrient circulation. The ideas behind traditional nutrient cycling models (as described in general text books in soil ecology such as Kilham, 1994 and Myrold, 1998) are briefly outlined below.

Dead plant litter is degraded to assimilable, organic compounds by the enzymatic activities of microorganisms. After uptake, nutrients are either incorporated into microbial biomass (immobilised), or released as inorganic ions to the soil solution (mineralised). According to traditional models, nutrients become available for uptake by plant roots, only when they have been mineralised by microorganisms and occur in inorganic forms in the soil solution. The partitioning of nutrients between immobilisation and mineralisation is thought to depend on the C/N-ratio of the decomposed substrate. When degrading substrates with a high C/N-ratio, microorganisms incorporate all assimilated nutrients into their biomass. The C/N ratio of the substrate, including the microorganisms therein, decreases, as carbon is respired during decomposition. When the C/N ratio of the substrate has decreased below a threshold level (around 20), carbon skeletons of nutrient containing organic molecules are used to maintain respiration, and nutrients excess to requirements are exuded in inorganic form, to avoid accumulation in the microbial cells.

When applied to boreal ecosystems, this simple model conflicts with current mycological knowledge in two major respects: First, the theory that the relation between mineralisation and immobilisation depends on the C/N-ratio of the decomposed substrate is not applicable to large, translocating mycelia that can mobilise carbohydrates and nutrients from different, spatially separated sources (**4**). Secondly, the traditional definition of plant available nutrients as inorganic nutrients is not valid for plants associated with mycorrhizal fungi that can utilise various organic nutrient sources (**3.3** & **5**).

6.2. Reallocation of resources during litter decomposition

When the C/N ratio of decomposing litter is used as a parameter in traditional nutrient cycling models, the degraded plant litter is analysed together with the microorganisms in the litter. Such analyses provide a false picture of the substrate quality, since already mobilised nutrients are not available for uptake. Using analysis of chitin content, Frankland *et al.* (1978) measured the production of fungal biomass by the basidiomycete *Mycena galopus* in decomposing litter of *Betula* and *Fraxinus*. The carbon use efficiency of the fungus (the fraction of assimilated carbon that is incorporated into biomass) was estimated to be 26% in

Betula litter and 34% in *Fraxinus* litter. These figures indicate that fungal biomass production during decomposition is not negligible. Assuming that the nitrogen content of the mycelium in the litter is similar to the nitrogen content of sporocarps of litter degrading fungi, which is around 6% (Gebauer & Taylor, 1999), the nitrogen concentrations in the non–decomposed litter need to be 1.6% and 2% for *Betula* and *Fraxinus* respectively, in order to cover the nitrogen demand of the fungal mycelium. Observed nitrogen concentrations in *Betula* and *Fraxinus* litter are around 1% and 1.5% respectively (Aber & Melillo, 1980). This simplified calculation suggests that the decrease in C/N ratio observed during decomposition does not reflect a change in substrate quality, but a movement of nitrogen from the decomposing litter tissue with high C/N ratio to fungal tissue with lower C/N ratio.

Studies of decomposition of ^{15}N labelled litter in litterbags (made from nylon mesh), placed on the forest floor, support the picture obtained from the simple carbon-nitrogen budget above. In litter bags with pine needles (Berg, 1988) or beech leaves (Gebauer *et al.,* 2000), the amounts of ^{15}N in the bags declined at relative rates similar to the rate of litter mass loss. At the same time as the amounts of ^{15}N in the litter bags decreased, the total amounts of nitrogen increased. A likely explanation of this is that the original nitrogen in the litter, as it is taken up by the decomposer fungus, enters a mobile nitrogen pool that is circulated within the mycelium (**I** & **4.3.3**). As a major part of the mycelium is found outside the litter bag, a major part of the ^{15}N is translocated out of the bag. The parallel decline in ^{15}N and litter mass again suggests that the C/N ratio of the substrate itself remains the same during decomposition.

The increase in total nitrogen in the litter bags suggests a net import of nitrogen into the decomposing litter through fungal mycelium, as commonly found during the early phases of decomposition (**IV**, fig. 2; Staaf & Berg, 1982; Fahey, 1983; Yavitt & Fahey, 1986; Laiho & Prescott, 1999). If we compare the figures in the calculations above, we find that the nitrogen demand of the fungus is larger than the amounts made available from decomposition of the leaf litter. The translocation model presented in (**4.3.3**) predicts that when the rate of incorporation of nitrogen into immobile compounds is higher than the rate of assimilation to the mobile pool, the nitrogen concentration in the mobile pool will decrease, causing net translocation to this part of the mycelium. To summarise, all nitrogen (and probably phosphorus) that becomes available during litter decomposition is likely to be taken up and incorporated into the mycelium of the decomposer fungi. The nutrients available in the litter are usually not enough to enable efficient utilisation of the cellulose in the litter and additional nutrients are therefore often translocated to the mycelium in the decaying litter.

The initial nutrient import into decomposing litter eventually slows down and changes into a loss of nutrients (**IV**, fig. 2; Staaf & Berg, 1982). In what forms do the nutrients leave the litter and what are the mechanisms behind the nutrient

losses? A fraction of the nutrients acquired from the litter by the decomposer fungus may be translocated out of the decayed litter by the primary decomposer, as suggested by the ^{15}N studies described above. Reduced growth rates and vacuolisation of senescing hyphae may increase the concentration of mobile nutrients in the shrinking cytoplasm, causing net translocation from mycelium in well decayed litter towards more actively growing mycelium (**4.3.3**). Decomposer fungi may thus translocate nutrients vertically from well decayed litter deep in the organic soil horizons to more recently colonised litter at the surface. Redistribution of nutrients within mycelia of rhizomorphic wood decaying fungi from well decayed wood to newly colonised wood was suggested by Boddy & Watkinson (1995) and demonstrated for ^{32}P in *Phanerochaete velutina* by Wells *et al.* (1998b). Vertical translocation of ^{15}N was demonstrated in a field experiments by Hart & Firestone (1991). All nutrients in senescing mycelium cannot however be reallocated. During decomposition, nutrients are incorporated into highly recalcitrant polyphenolic complexes - humic compounds (Swift *et al.,* 1979, pp. 207-215), which accumulate at the bottom of the organic horizon or are leached out of the soil. Significant amounts of nutrients also remain in the litter incorporated into fungal cell walls and membranes (Bååth & Söderström, 1979).

The mycelium remaining in the litter is a resource used by other organisms. Organisms that utilise fungal mycelium can be divided into two groups; 1. organisms that use mycelium as their major source of carbohydrates as well as nutrients and 2. organisms that have access to other sources of carbohydrates and use the mycelium primarily as a nutrient source. The first group encompasses mycophagous soil animals, bacteria and non-translocating fungi. When fungal mycelium is utilised by these non-translocating organisms, traditional models of nutrient cycling (**6.1**) are valid and the organisms that live on mycelium may accumulate a surplus of nutrients, leading to mineralisation. For example, ammonium production is usually increased due to the presence of soil animals, enchytraeids in particular (Huhta *et al.*, 1989). The second group consists of translocating, basidiomycetous fungi, which use the dead fungal mycelium primarily as a source of nutrients (**II, III & 5.3**). These fungi have access to other carbohydrate sources; living plant roots in the case of mycorrhizal fungi and woody debris or fresh litter in the case of saprotrophic fungi. The C/N ratio of the degraded mycelium is thus of little relevance to the nutritional balance of the whole fungus. When dead mycelium is degraded by translocating basidiomycetes, nutrients are not likely to be released in inorganic forms. Surplus nutrients may instead be translocated to sites where they are used either to maintain growth and decomposition of nutrient poor substrate (**III**), or to support a mycorrhizal plant host (**II**).

Nutrients that are transferred from dead plant tissues to fungal tissues during decomposition are thus removed from the decaying litter by four major mechanisms: 1. nutrients are leached out of the litter, usually in recalcitrant

forms (humic compounds), 2. nutrients are translocated out of the litter by the primary decomposer, 3. nutrients are translocated out of the litter by a fungus that uses the mycelium of the primary decomposer as a nutrient source, 4. nutrients are mineralised and released by bacteria, non-translocating fungi or soil fauna that utilise the mycelium of the primary decomposer.

Fungivorous soil animals are likely to feed preferentially on living hyphae and probably contribute continuously to the production of inorganic nutrients. Bacteria and non-translocating fungi probably gain access to nutrients in fungal mycelium only when the mycelium is dead (with the exception of some mycoparasitic fungi). Antagonistic basidiomycetous fungi that "predate" other fungi should have a competitive advantage compared with non-translocating microorganisms, since they can attack their "prey" when it is still alive (**II, III** & **5**). In my opinion, nutrients in boreal forest soils are mineralised mainly by soil fauna, grazing on mycelia, or in association with disturbances that kill large amounts of fungal mycelium, such as rapid freezing-thawing or drying-wetting cycles, which eliminate fungal competition and provide bacteria with large amounts of substrate. During more stable conditions, where competition between basidiomycetous fungi for nutrients is high, most nutrient losses from litter are probably due to translocation through fungal mycelia rather than mineralisation (**IV**).

6.3. What are "plant available nutrients"?

Traditional nutrient circulation models, centered around mineralisation, have never been thoroughly questioned, as only inorganic forms of nutrients have been considered to be available to plant roots. Myrold (1998) reasoned that, as plants thrive in most soils, substantial net mineralisation of nutrients has to occur. The possibility that ectomycorrhizal fungi may provide plants with nutrients assimilated in organic form, however, allows for new models in which mineralisation and plant uptake of inorganic nutrients play a less important role. In accordance with the ideas in the preceding section, Heal & Harrison (1990) proposed that in ecosystems with nutrient poor litter, microbial retention and translocation cause reduced nutrient turnover external to the microflora leading to reduced availability of inorganic nutrients to plant roots. Competition for nutrients between microorganisms and plants has also been suggested by Kaye & Hart (1997). Heal & Harrison (1990) further suggested mycorrhizal symbiosis and uptake of organic fractions as possible compensatory mechanisms of plants in response to reduced availability of inorganic nutrients. The term "plant available nutrients" thus needs to be critically reviewed (**IV**).

In traditional models, nutrient uptake by plant roots from the soil solution plays a central role. Ectomycorrhizal roots in boreal forests are however more or less isolated from the soil solution by the mycorrhizal mantles that cover the nutrient absorbing root tips (**3.2**). Nutrients are delivered to the plant mainly through its

mycorrhizal associates. As mycorrhizal fungi must be considered a part of the microbial community, the transfer of nutrients from the microbial pool to the plants actually occurs between cells in physical contact with each other inside the mycorrhizal root tips. The term "plant available nutrients" is therefore problematic, as it can refer to either nutrients available to the plant root cells at the mycorrhizal interface or to nutrients available to the mycorrhizal plant-fungus association considered as a whole. The forms in which nutrients are delivered to the plant cells within the mycorrhizal root tips are not fully clarified (Chalot & Brun, 1998). Laboratory experiments suggest that the partitioning of nutrients between the two partners in mycorrhizal associations is variable and dependent on the species involved (Colpaert *et al.,* 1996).

If we choose the less strict definition of "plant available" and consider what nutrients are available to the mycorrhizal plant-fungus association, current knowledge is still very poor. Laboratory studies suggest that the enzymatic capacity of many of the investigated mycorrhizal fungi is considerable and that a wide range of organic substrates may be utilised (**3.3**), but still only a handful out of several thousand species has been examined. Näsholm *et al.* (1998) have shown that the amino acid glycine is taken up in intact form by mycorrhizal boreal plants in the field, using a double labelled ($^{15}N/^{13}C$) substrate. Even though there is little other evidence that organic nutrient sources are used to a large extent in the field, there is no evidence at all that they are not used. In a laboratory experiment by Durall *et al.* (1994), various ^{14}C labelled substrates were incubated under axenic conditions together with four different ectomycorrhizal fungi growing in association with Douglas fir *(Pseudotsuga menziesii*). All tested fungi were able to degrade hemicellulose and cellulose and three of the fungi caused significant losses of ^{14}C from labelled fir needles, suggesting that direct decomposition of plant litter by ectomycorrhizal fungi can occur. In a similar experiment by Colpaert & Van Tichelen (1996), rates of weight loss of fresh beech leaves were significanly higher when the leaves were colonised by the ectomycorrhizal fungus *Suillus bovinus* in association with *Pinus sylvestris* compared with controls without basidiomycetous fungi. Decomposition was however much higher in the presence of the saprotrophic fungus *Lepista nuda*, indicating that saprotrophic fungi have a much larger capacity for degradation of fresh plant litter. Mycorrhizal fungi may therefore be restricted to mobilising nutrients from litter at advanced stages of decomposition, when most nutrients have already been incorporated into saprotrophic fungal mycelium (**6.2**). Here, the mycorrhizal fungi may take advantage of their access to photosynthetically derived carbohydrates to successfully engage in competetive, possibly "predatory" interactions with saprotrophic fungi (**II** & **5.3**). Nutrients incorporated into humus compounds may also be available to mycorrhizal plants (**3.4**). This is particularly true for ericoid mycorrhizal associations.

New models of nutrient circulation in boreal ecosystems should, as a complement to the traditional nutrient mineralisation mechanisms, consider

alternative mechanisms that allow for intense competition for nutrients between fungi, plants and other soil organisms. In these models, nutrient circulation could be described in terms of a food web, in which fungi interact antagonistically with each other in succession. During each interaction, nutrients contained in overgrown mycelium are taken over by the replacing fungus. The plants take part in this food web through their mycorrhizal associates. When mycorrhizal fungi are successful in interactions with other fungi and thereby acquire some nutrients, a fraction will be transferred to the host plants (**IV**, fig. 4).

7. Conclusions

In short, the thesis can be summarised by the following conclusions:

- Phosphorus (and probably other nutrients) may be translocated bidirectionally in rhizomorphs, enabling circulation throughout mycelia and net translocation from sources to sinks (**I**).
- Some ectomycorrhizal fungi may respond to interactions with saprotrophic soil fungi by overgrowing the saprotrophic mycelia with dense mycelial patches (**II** & **III**). The reductions in mycelial extension rates that are sometimes observed in saprotrophic fungi, as a response to interaction with ectomycorrhizal fungi (**IV**), suggest that ectomycorrhizal fungi may be antagonistic and display interference competition against other soil fungi. However, saprotrophic soil fungi may also interfere with ectomycorrhizal fungi through overgrowth of mycorrhizal mycelium with dense saprotrophic mycelium, resulting in reduced mycorrhizal mycelial growth (**III**).
- Interactions between ectomycorrhizal and saprotrophic fungi may be associated with transfer of phosphorus between the interacting mycelia. Transfer occurs in both directions simultaneously but the transfer rates may be different, causing net translocation of phosphorus between the interacting mycelia (**II** & **III**). Due to this transfer, significant phosphorus translocation may occur from litter, via saprotrophic mycelium to ectomycorrhizal fungi and further to their host plants (**II**). Phosphorus, already mobilised by mycorrhizal mycelium, may also be translocated to saprotrophic mycelium in woody litter (**III**). A mechanism behind the phosphorus transfer is suggested whereby basidiomycetous fungi kill and degrade the mycelium of other fungi, acting as fungal "predators".

- The outcome of mycelial interactions between saprotrophic and ectomycorrhizal fungi is affected by the inoculum potential of the saprotroph, which is in turn dependent on the resource availability to the saprotrophic fungus (**III**).
- New models of nutrient cycling in boreal forest have to allow for the occurrence of saprotrophic fungi that compete with plants and other soil organisms for nutrients. Boreal plants may be dependent on the ability of their associated mycorrhizal fungi to interfere with saprotrophic fungi and to mobilise nutrients from complex organic substrates such as fungal mycelium, in order to successfully compete with saprotrophic fungi for nutrients (**IV**).

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