Litter Decomposing Fungi
in Boreal Forests

Their Function in Carbon and Nitrogen Circulation

Johanna Boberg

Faculty of Natural Resources and Agricultural Sciences
Department of Forest Mycology and Pathology
Uppsala

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Cover: Fruit bodies of *Mycena epipterygia* (Photo: Cajsa Nygren), respiration measurements of decomposing pine needles and pine litter on the forest floor (Photo: Johanna Boberg)

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Abstract

Decomposition of soil organic matter connects the global carbon (C) cycle with the turnover of nitrogen (N), but exactly how the availability of N affects the soil C turnover is unclear. In boreal forest soils, fungi are essential to litter decomposition. Most litter decomposing fungi form large mycelia and have a well developed capacity to translocate resources, such as carbohydrates and nutrients, within their mycelia. Translocation by litter fungi may enable efficient utilization of spatially separated substrates differing in respect to their quality and thus have a major influence on decomposition and nutrient dynamics.

In order to isolate the different mechanisms controlling C and N turnover by litter decomposing fungi, controlled laboratory studies were conducted with the known, common litter fungi *Marasmius androsaceus* and *Mycena epipterygia*. Furthermore, some litter fungi with unknown functional capability were tested for their ability to decompose pine needles and potential effects of interspecific interactions for C and N dynamics were also assessed.

The low initial N content of recently abscised pine needles limited the catabolism as well as growth and needle decomposition by both *M. androsaceus* and *M. epipterygia*. Accordingly, when colonizing new needle litter both fungi translocated assimilated N into the needles leading to a net gain in the total N pool. This way, both fungi were able to increase their growth in the new substrate. In addition, both fungi translocated C to low C:N ratio substrates, thereby overcoming local C deficiency and subsequently restricted mineralization of N. When able to connect substrates of different quality, both fungi increased their efficiency by allocating a larger proportion of assimilated C into biomass production, thereby reducing the release of CO_2. Interspecific antagonistic interactions prevented mycelial linking of substrates colonized by different species, and thereby hampered translocation. This thesis demonstrates that the capacity of litter decomposing fungi to translocate resources within their mycelia may have a decisive influence on the C and N cycle in boreal forests.

Keywords: C cycling, C-use efficiency, fungal translocation, litter decomposing fungi, *Marasmius androsaceus*, *Mycena epipterygia*, N cycling, N-mineralization, *Pinus sylvestris*

Author's address: Johanna Boberg, SLU, Department of Forest Mycology and Pathology, P.O. Box 7026, 750 07 Uppsala, Sweden
E-mail: Johanna.Boberg@mykopat.slu.se

Dedication

Till Miriam och Lovisa

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I   Boberg, J., Finlay, R.D., Stenlid, J., Näsholm, T., Lindahl, B.D. (2008). Glucose and ammonium additions affect needle decomposition and carbon allocation by the litter degrading fungus *Mycena epipterygia*. *Soil Biology & Biochemistry* 40, 995-999.

II Boberg, J.B., Finlay, R.D., Stenlid, J., Lindahl, B.D. (2009). Fungal C translocation restricts N-mineralization in heterogeneous environments. *Functional Ecology*, doi 10.1111/j.1365-2435.2009.01616.x

III Boberg, J.B., Ihrmark, K., Lindahl, B.D. Decomposing capacity of some ascomycetes common in *Pinus sylvestris* needle litter. (manuscript).

IV Boberg, J.B., Finlay, R.D., Stenlid, J., Ekblad, A., Lindahl, B.D. Fungal reallocation of nitrogen during litter decomposition in stratified systems. (manuscript).

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1 Introduction

The global carbon (C) cycling has received increasing attention mainly due to elevated CO_2 levels in the atmosphere and associated global warming. Soils constitute a major pool of terrestrial organic C (Post *et al.*, 1982) of which about 30% is found in ecosystems in high northern latitudes (Jobbagy & Jackson, 2000). The main pathway by which C is transferred from soils to the atmosphere is through soil respiration (Schlesinger & Andrews, 2000). The turnover of soil organic C is connected to the nitrogen (N) cycle in a complex relationship, but exactly how the availability of N effects soil C storage and release is unclear (Neff *et al.*, 2002). Studies to elucidate the mechanisms are further complicated by increased deposition of atmospheric N and the elevated atmospheric CO_2 levels caused by anthropogenic activities (Hyvönen *et al.*, 2007; Reich *et al.*, 2006). Heterotrophic respiration, by organisms utilizing organic C as a substrate for metabolism, constitute approximately half of the total CO_2 flux from soils to the atmosphere in boreal forests (Högberg *et al.*, 2001). It is therefore important to identify factors influencing decomposition of organic matter in soil. At an ecosystem level, decomposition of organic matter interlink the global C cycle with the nutrient cycle in soils (Berg & McClaugherty, 2003). Factors affecting decomposition are therefore also important since the availability of nutrients may limit primary production and thereby affects forest productivity.

Fungi are the major decomposers of forest litter (Rayner & Boddy, 1988), but the identity and ecology of most litter fungi are still poorly understood. In spite of their importance for decomposition, the specific physiology of litter fungi is often disregarded in process-oriented research on nutrient cycling, and all microorganisms are instead treated as a single functional group.

The effect of N availability on C turnover in soils, driving mechanisms and influencing factors have been studied extensively (*e.g.* Knorr *et al.*, 2005). Most of these studies investigate effects on entire complex microbial communities either directly in the field or in laboratory studies using mixed and sieved soil material. The results are often contradictory and difficult to interpret. Disturbances and additions of readily assimilable substrates may result in drastic alterations of the microbial community by favouring opportunistic taxa. Observations made under such disturbed conditions could be due to the activities of otherwise rare and dormant microorganisms. Field experiments are also complicated by the fact that microbial communities in forest soils are not only comprised of various groups of saprotrophic microorganisms, but also of mycorrhizal fungi, which obtain C from their symbiotically associated plant hosts (Smith & Read, 1997). Furthermore, microbial growth and C allocation patterns are difficult to assess in experiments where microbial biomass turns over rapidly in soil food-webs.

This thesis attempts to isolate the different factors and mechanisms influencing and controlling C and N turnover by litter decomposing fungi in boreal forest. All the studies were conducted as detailed laboratory studies under controlled conditions and with identified, artificially inoculated decomposer organisms.

2 Background

2.1 The boreal forest

Boreal forests cover a major part of the terrestrial northern hemisphere (Bonan & Shugart, 1989). The dominant vegetation consists of coniferous trees, producing highly lignified litter with high content of polyphenolic substances and a low N concentration (Aerts, 1995). The acidic podzols commonly found in boreal forests are usually poor in mineral nutrients, particularly N and primary production is generally limited by this element (Tamm, 1991). The high contents of recalcitrant substances in the litter together with low N concentrations have a negative effect on the rate of litter decomposition. This together with climatic factors leads to an accumulation of organic matter in the soil (Swift *et al.*, 1979). Limited mixing of the soil profile due to the absence of burrowing earthworms, results in stratification of soil organic material at different stages of degradation. Litter decomposition in boreal forests is, therefore, characterized by a high degree of spatial heterogeneity.

In boreal forest ecosystems, microorganisms, *e.g.* bacteria and fungi, carry out more than 95% of litter decomposition (Persson *et al.*, 1980). Fungi generally dominate the microbial biomass and respiration in boreal forest soil, due to the acid, nutrient poor soils and associated recalcitrant litter (Joergensen & Wichern, 2008; Högberg *et al.*, 2007; Ekblad & Nordgren, 2002; Frostegård & Bååth, 1996). Basidiomycetous litter fungi are considered especially important due to their production of ligninolytic enzymes essential for degradation of plant material (Osono & Takeda, 2002). Ascomycetous fungi constitute a large part of the fungal community in forest litter (Lindahl *et al.*, 2007; O'Brien *et al.*, 2005), although the lignin

degrading capacity of most ascomycetes appears to be limited (Osono & Takeda, 2002).

The community of saprotrophic litter decomposers has been observed to be restricted to the upper litter layer, whereas the fungal community in the deeper layers is typically dominated by mycorrhizal fungi (Lindahl *et al.*, 2007; O'Brien *et al.*, 2005). Mycorrhizal fungi form symbiotic associations with higher plants from which they obtain photoassimilated C and in return the fungus supplies the plant with nutrients (Smith & Read, 1997).

2.2 Litter decomposition

Decomposition is broadly defined as the physical, chemical and biological mechanisms that transform organic material into increasingly stable forms (Berg & McClaugherty, 2003). In this thesis, however, the term decomposition will be used to signify the degradation of macromolecules into assimilable compounds through microbial activity. In soil, saprotrophic microorganisms depolymerize organic matter into assimilable compounds using extracellular enzymes in order to obtain the nutrients and C they need for energy and growth (Jennings, 1995). Thus, the plant litter C assimilated during decomposition may be either mineralized to CO_2 or converted to microbial biomass. Of the mineral nutrients, N is needed in the highest amounts by litter decomposing fungi followed by phosphorus (P) (Dowding, 1981).

A major part of the total litter input to forest soils are non-woody plant residues such as leaves, fruit and reproductive structures (Swift, 1982). The total litter fall in a Scots pine forest was estimated to 1-1.5 tonnes ha^{-1} year^{-1}, of which the needles accounted for 69-87% of the total amount (Vestgarden *et al.*, 2004). Complete structural disintegration of coniferous needle litter takes about 7 years compared to most deciduous litter which takes between 9 months, and 3 years to be fully disintegrated (Boddy & Watkinson, 1995).

2.2.1 Litter components and degradative enzymes

Plant litter consists of several groups of compounds and the relative amounts of the different constituents vary depending on the plant part (leaves, stem *etc*) and species. Broadly, the dominant C rich components are soluble organic compounds, hemicellulose, cellulose, and lignin (Berg & McClaugherty, 2003). Soluble organic compounds consist of sugars, low molecular weight phenolics (tannins), hydrocarbons and glycerides. These are generally easily degraded or lost from the litter through dissolution and leaching during the first year of decomposition (Berg *et al.*, 1982).

Cellulose is the most abundant natural polymer on earth and makes up about 40% of plant wall material (Eriksson *et al.*, 1990). Cellulose consists of straight chains of *D*-glucose residues and is degraded into monomers or oligomers by hydrolytic enzymes before it can be taken up and metabolized by the fungi (Baldrian & Valaskova, 2008). Hemicelluloses, constituting approximately 15% of Scots pine needles, are linear or branched polymers containing several different sugar units (Berg & McClaugherty, 2003). Hemicellulose requires a more complex set of different hydrolytic enzymes than cellulose for its decomposition (Eriksson *et al.*, 1990), but is degraded at higher or similar rates to the latter (Berg & McClaugherty, 2003).

Lignin is a complex aromatic polymer built up from phenyl propanoid units but is not a well defined compound because of the lack of systematically repeated units (Eriksson *et al.*, 1990). The term 'lignin' is commonly used to signify the fraction obtained by proximate analysis, *e.g.* sulphuric acid hydrolysis used to assay 'Klason lignin' (*e.g.* Berg *et al.*, 1982). This fraction contains not only 'true' lignin but also tannins and cutin (Preston *et al.*, 1997) and may also include humic substances and fungal melanins (Osono, 2007). Lignin is oxidized in a very complex process including several enzymatic and nonenzymatic reactions (Baldrian & Valaskova, 2008). A major part of the cellulose in plant material is protected by lignin. Lignin degradation, thus, make the polysaccharides within available for degradation and utilization (Jennings & Lysek, 1996). There are large costs associated with the synthesis and activation of ligninolytic enzymes and no organism has been found to use lignin as a sole C source (Hatakka, 2001; Kirk & Farrell, 1987). Since synthesis of extracellular enzymes requires large amounts of both C and N their production is thought to be tightly regulated in order to minimize unnecessary losses (Sinsabaugh, 2005).

N in coniferous forest soil is mainly found in organic forms such as protein and amino sugars (chitin and murein) (Leake & Read, 1997). A large pool of organic N is also sequestered in complex organic forms in the humus layer (Tamm, 1991). Extracellular enzymes such as proteases (Nygren *et al.*, 2007) and chitinases (Lindahl & Finlay, 2006) are commonly studied in association with availability and uptake of organic N.

2.2.2   Succession of fungi in decomposing litter

Studies of fungal succession have until recently been performed by incubating litter or organic matter and isolating the fungal hyphae growing out into pure culture (Osono & Takeda, 1999; Frankland, 1998). The main

drawback of this method is the discrimination against slow growing and non-culturable fungal species (Lindahl & Boberg, 2008). Recently, molecular methods have been developed enabling DNA based identification of the whole fungal community in forest litter (Lindahl *et al.*, 2007; O'Brien *et al.*, 2005). Studies using the 'isolation method', together with recent molecular studies and observations of enzymatic activity in the soil profile, have revealed a typical pattern of litter decomposition.

When the newly shed litter reaches the forest floor, it is already colonised by endophytic fungi. In pine needle litter, for example, the well known endophytic fungus *Lophodermium pinastri* is frequently found. The ecological role of endophytes is not clear, but many stay in the dead litter and some have saprotrophic capabilities (Osono, 2006). In this early stage of decomposition, mainly soluble sugars and other low molecular weight compounds are lost from the litter (Berg *et al.*, 1982). Enzymes linked to soluble saccharides are also prominent but then rapidly decline (Sinsabaugh, 2005). Some endophytic fungi in the early community also have cellulolytic capacities and have been observed to cause significant mass loss in laboratory experiments (Korkama-Rajala *et al.*, 2008). In Scots pine needles, unidentified ascomycetous species belonging to either the Leotiomycetes, primarily within Helotiales, and Dothideomycetes dominate the fungal community at early stages (Lindahl *et al.*, 2007). Knowledge of the functional capacities of these fungi is, however, very limited. The first fungal community in the recently shed litter is soon accompanied by early basidiomycetous fungi. Species within the genera *Athelia* and *Sistotrema* are frequently found (Lindahl *et al.*, 2007; O'Brien *et al.*, 2005) and another early, very common and well spread litter fungus is *Marasmius androsaceus* (Korkama-Rajala *et al.*, 2008; Lindahl *et al.*, 2007; Holmer & Stenlid, 1991).

During the second phase of litter decomposition, cellulolytic enzymes are active and the main degradation of the polymer occurs. Laccase activity can also be observed relatively early in decomposition of litter with high contents of phenolic compounds (Sinsabaugh, 2005). Typical litter basidiomycetes such as species of the genera *Mycena*, *Clitocybe* and *Collybia* are prominent during this stage of decomposition (Osono, 2007).

Short description of the main litter decomposing fungi studied in this thesis

Marasmius androsaceus (L.: Fr.) Fr., occasionally called *Setulipes androsaceus* (Koukol *et al.*, 2008), has been reported from both temperate and boreal regions in widely distributed areas, throughout North America and Europe but also in remote areas such as Hawaii (Desjardin *et al.*, 1992). It is found on both deciduous and coniferous litter but mainly on the latter in most areas. *M. androsaceus* is characterized by the formation of numerous black rhizomorphs (see section 2.3.1) often seen connecting twigs and needles on the forest floor. The conspicuous rhizomorphs have also given the fungus its colloquial name 'Horse hair fungus' ('Tagelbroskskivling' in Swedish) and have been found to be used as nest lining by birds in north eastern United States (McFarland & Rimmer, 1996). The fungus colonize freshly fallen litter from established mycelial networks that can exceed 1 m in size (Holmer & Stenlid, 1991), and has well known cellulolytic and ligninolytic capabilities (Cox *et al.*, 2001; Gordon & Petersen, 1997; Lindeberg, 1944).

Mycena epipterygia (Scop.) Gray is a species complex including around 20 varieties (indexfungorum), which are very difficult to distinguish. The species complex is found worldwide in a wide variety of habitats ranging from the arctic tundra (Miller *et al.*, 1982) to tropical montane forests (Lodge *et al.*, 2004) on both deciduous and coniferous litter but most varieties usually fruit on coniferous litter. The bright yellow stipes of the fruit bodies has given the fungus the colloquial name 'Yellowleg bonnet'. The Swedish name 'Flåhätta' is derived from the fact that the pellicle of both cap and stipe can be easily removed. *M. epipterygia* has a well developed capacity to degrade both cellulose and lignin (Steffen *et al.*, 2000; Lindeberg, 1961). Photo by Cajsa Nygren.

In later stages of decomposition the typically litter decomposing fungi appear to be absent and instead mycorrhizal fungi have been found to dominate the fungal community in the humus-layer of both deciduous and coniferous forests (Lindahl *et al.*, 2007; O'Brien *et al.*, 2005). Due to problems with culturability of most mycorrhizal fungi (Nygren, 2008) this functional group escapes detection by traditional, culture based methods. Opportunistic species within the genera *Trichoderma* and *Penicillium* have been isolated frequently in well decomposed litter using different incubation methods (Santo *et al.*, 2002; Frankland, 1998), but were absent from a molecular based study of fungal communities in decomposing Scots pine litter (Lindahl *et al.*, 2007).

2.2.3 Interspecific interaction

Decomposing fungi rarely occur as single species populations on substrates is forest soils. Instead interspecific interactions constantly occur as different fungi compete for space and resources. Such interactions can be mediated from a distance by volatile and diffusible compounds or at hyphal or mycelial level by interference and antagonistic compounds on a local scale (Woodward & Boddy, 2008). Interspecific interaction may result in full or partial *replacement*, when one fungus gains access to the substrate occupied by the other, or *deadlock*, when neither of the fungi gains access to the substrate occupied by the other (Boddy, 2000). The outcome has been observed to vary depending on the microclimatic environment, species involved, as well as the size and state of decay of the resource occupied (Boddy, 2000). The interactions will have consequences for decomposition and nutrient mineralization, since different species affect these processes to a varying degree. Interactions may also involve a substantial C cost. In wood decomposing fungi interspecific interactions have been observed to increase respiration rates (Wells & Boddy, 2002) and increase the production of ligninolytic enzymes (Chi *et al.*, 2007; Iakovlev & Stenlid, 2000; White & Boddy, 1992). Nutrient uptake, allocation and release are also affected by interspecific interactions. Exchange of P has, for example, been observed between interacting cord forming wood decomposers, presumably as uptake of excudates or lysates (Woodward & Boddy, 2008).

Besides the obvious interactions between the different saprotrophic fungi in the litter layer, interactions between saprotrophic and mycorrhizal fungi are likely to occur, especially at the interface between the litter and humus layers. Presumably, competition between saprotrophic and mycorrhizal fungi is mainly focused on mineral nutrient acquisition since mycorrizal fungi

receive C from their host plants. They may not only compete for soil nutrients, but also capture nutrients from each other's tissues (Cairney & Meharg, 2002). Using ^{32}P and non-destructive electronic autoradiography Lindahl et al. (1999) showed that up to 25% of ^{32}P applied to a saprotrophic fungus was captured by a mycorrhizal fungus during antagonistic interaction and further translocated to the plant host. The direction of the ^{32}P transfer was, however, influenced by the size and energy supply so that a larger resource base available for the saprotrophic fungi resulted in a net P transfer from the mycorrhizal fungi to the saprotroph (Lindahl et al., 2001b).

2.3   Fungal physiology

2.3.1   Fungal translocation

The majority of fungal species grow as hyphae, forming a continuous network, the mycelium. Most filamentous fungi are able to translocate resources, such as carbohydrates, mineral nutrients and water within their mycelia (Tlalka et al., 2008; Cairney, 2005; Lindahl & Olsson, 2004; Boddy, 1999; Boddy & Watkinson, 1995; Olsson, 1995; Paustian & Schnürer, 1987).

Many species, basidiomycetes in particular, form rhizomorphs or cords that are involved in colonization of new substrates and also act as a link between substrates through which the fungus can transport water and nutrients (Boddy et al., 2009). Rhizomorphs and cords are formed as the mycelium aggregates into long linear structures. The growth pattern can differ significantly, but many species form wide diameter hyphae, vessel hyphae, which run like a channel within the rhizomorph or cord (Jennings, 1995). The outer region is often composed of closely packed hyphae, which in rhizomorphs often have thick melanized walls. True rhizomorphs, as produced by M. androsaceus, are defined as being fully autonomous and exhibiting apical growth while mycelial cords do not, but instead are formed behind the diffuse mycelial growth zone by aggregation of aligned hyphae (Rayner & Boddy, 1988). In several species of wood decomposing fungi N limitation induces formation of cords (Tlalka et al., 2008).

The mechanism and regulation of translocation are not fully understood, although there seem to be several processes operating on different scales, in different fungal species and under different conditions (Cairney, 2005). Transport in individual hyphae is thought to be a combination of diffusion, mass flow, cytoplasmic streaming and vesicular transport. In rhizomorphs or cords, mass flow has been proposed as the main mechanism of transport and

is generally much faster than in non-differentitated mycelia (Cairney, 2005). The main C rich compounds translocated are trehalose and sugar alcohols, such as mannitol and arabitol (Brownlee & Jennings, 1981), while N is presumably translocated as amino acids (Tlalka *et al.*, 2002; Finlay *et al.*, 1989; Finlay *et al.*, 1988). Bi-directional transfer of resources is enabled since different transport mechanisms may work simultaneously (Cairney, 2005). Within rhizomorphs and cords, parallel transfer within spatially separated hyphal elements has been proposed (Cairney, 1992) and simultaneous transport of tubular vacuoles may also occur in different directions within single hyphae (Allaway & Ashford, 2001). Bi-directional transfer of resources, have been observed for single compounds such as P and N (Tlalka *et al.*, 2002; Lindahl *et al.*, 2001a), as well as for carbohydrates and P (Olsson & Gray, 1998) and N (Frey *et al.*, 2003; Olsson, 1995).

Resources are typically translocated from resource-rich regions of the mycelium to resource-deficient patches, so that net translocation occurs from sources to sinks (Lindahl & Olsson, 2004). Whether it is the strength of the sink or the source that determine the amount actually being transported is not yet fully known (Schimel & Hättenschwiler, 2007).

This internal redistribution of resources allows fungi to extend out from an already colonized substrate into the surrounding environment in search of new resources, and to utilize spatially separated substrates that may differ in nutrient quality, e.g. C:N ratio (Boddy, 1999). The implication of translocation of resources throughout the mycelium is that the mycelium functions as a single entity in which the nutritional status of one part of the mycelium affects distant parts (Lindahl & Olsson, 2004). Litter decomposing fungi commonly form large mycelia (Holmer & Stenlid, 1991; Kirby *et al.*, 1990), which together with the ability to translocate resources make them well adapted to environments such as the forest floor, where substrates are heterogeneously distributed (Boddy, 1999).

2.3.2   Fungal growth dynamics

The fungal mycelium is very dynamic and fungi can redirect their growth according to substrate availability and in response to environmental factors. Litter decomposing fungi constantly grow from well decomposed litter, depleted in resources, in search of fresh resources (Frankland *et al.*, 1995). The dynamic responses of cord forming wood decomposing fungi to input of new resources have been studied comprehensively (Boddy, 1999; Wells *et al.*, 1998; Dowson *et al.*, 1989b; Dowson *et al.*, 1988). Mycelial growth is generally redistributed towards the new fresh substrate, whereas mycelium in depleted substrates ultimately regresses and is degraded through autolysis.

Not only biomass production but also nutrients such as P are redistributed (Harris & Boddy, 2005; Wells *et al.*, 1999; Wells *et al.*, 1998; Hughes & Boddy, 1996). The fairy ring fungus *Clitocybe nebularis,* colonizing deciduous litter on the forest floor, displays a similar polarized growth where resources from the senescing, proximal parts of the mycelium are re-allocated to the extending mycelial front (Dowson *et al.*, 1989a).

2.3.3 Fungal C:N ratio and C-use efficiency

The C:N ratio of an organism reflects how much N in relation to C the organism requires for biomass production. Since C is used both as a source of energy, and thereby lost as CO_2, and to produce biomass, the C:N ratio of the substrate must be higher than that of the organism in order to balance the need. The C:N ratio of saprotrophic fungi is commonly assumed to be around 10-15 (Paul, 2007). Stating such a narrow range, however, is most certainly an oversimplification, and the C:N ratio of decomposer fungi appear to depend heavily on N availability of the substrate utilized. In 1969, Levi & Cowling showed that several wood decomposing fungi were able to adapt their C:N ratio depending on the availability of the different compounds. The species *Polyporus versicolor* was, in fact, able to alter the mycelial N content from 0.1 to 8% corresponding to a C:N ratio ranging from 6 to 500 (assuming a C content of 50%). Boddy & Watkinson (1995) suggest a mycelial C:N ratio of 35 for a typical wood decomposing fungus and the C:N ratio of mycorrhizal fungi has been estimated to be around 20 (Wallander *et al.*, 2003).

The distribution of assimilated C between biomass production and energy consumption may be described as the C-use efficiency and is specifically defined as the proportion of total assimilated C that is allocated to biomass production. The C-use efficiency of decomposer organisms has implications for C-turnover since it determines the proportion of C that leaves the soil system as CO_2 in relation to the amounts that are retained in the soil organic matter pool. Using chitin analysis to estimate fungal biomass production, Frankland *et al.* (1978) estimated the C-use efficiency of the litter decomposing fungus *Mycena galopus* to range between 26 and 34% when decomposing deciduous litter. An altered C-use efficiency may be attributed to both a shift in microbial species composition (Ågren *et al.*, 2001) and to changes within single species (Boyle, 1998). Increased C-use efficiency has, for example, been observed in a wood decomposing fungus where addition of complex N sources increased fungal growth without changing wood mass loss (Boyle, 1998). A decreased C-use efficiency may be observed when respiration increases without a parallel increase in

biomass. This has been observed in yeast when C is in excess and catabolism is decoupled from anabolism, leading to overflow metabolism (Larsson *et al.*, 1995).

2.4   C and N turnover dynamics

2.4.1   The N cycle

N may enter the soil through biological N_2 fixation or deposition, mainly due to anthropogenic activities, but the major input of N is in organic form as plant residues (Tamm, 1991) (Fig 1). The organically bound N may then be mineralized into ammonium and further into nitrate if ammonium is present and soil conditions are appropriate (e.g. aerobic conditions and neutral pH). N mineralization is mediated by saprotrophic organisms and is the result of C limitation during decomposition. As the plant residues decompose, the C:N ratio of the substrate will decrease as C is released through respiration by the decomposing organisms. When the C:N ratio of the substrate reaches the point at which C becomes limiting, the microorganisms are forced to use the N rich substrate primarily as a C source. Excess N is removed using intracellular enzymes and subsequently exuded into the environment as ammonium. The release of ammonium is counteracted by the uptake of mineral N by microorganisms, i.e. immobilization. The net release of mineral N through the activity of soil decomposer microorganisms has traditionally been regarded as the major rate limiting step in the N cycle (Schimel & Bennet, 2004) since plant N uptake was thought to be restricted to mineral N.

However, N availability differs significantly in different ecosystems with considerable implications for the mechanisms controlling N turnover. In the typical boreal forest the N cycle is very tightly regulated due to the low N availability. In coniferous forest ecosystem, N mineralization has been suggested to be of minor importance for N cycling (Lindahl *et al.*, 2002) and instead depolymerization of N-containing polymers into monomers may be regarded as the major rate limiting process (Schimel & Bennet, 2004). The low levels of mineral N found in the boreal forest correspond to the capacity of plants to access organically bound N through their associated mycorrizal fungi (Read & Perez-Moreno, 2003). Direct uptake of simple amino acids by forest plants has also been observed (Näsholm *et al.*, 1998). In addition to plant uptake, N may leave the soil system either through denitrification or leached as mineral N or dissolved organic N (DON), the latter being the main source of loss from coniferous forests (Northup *et al.*, 1995).
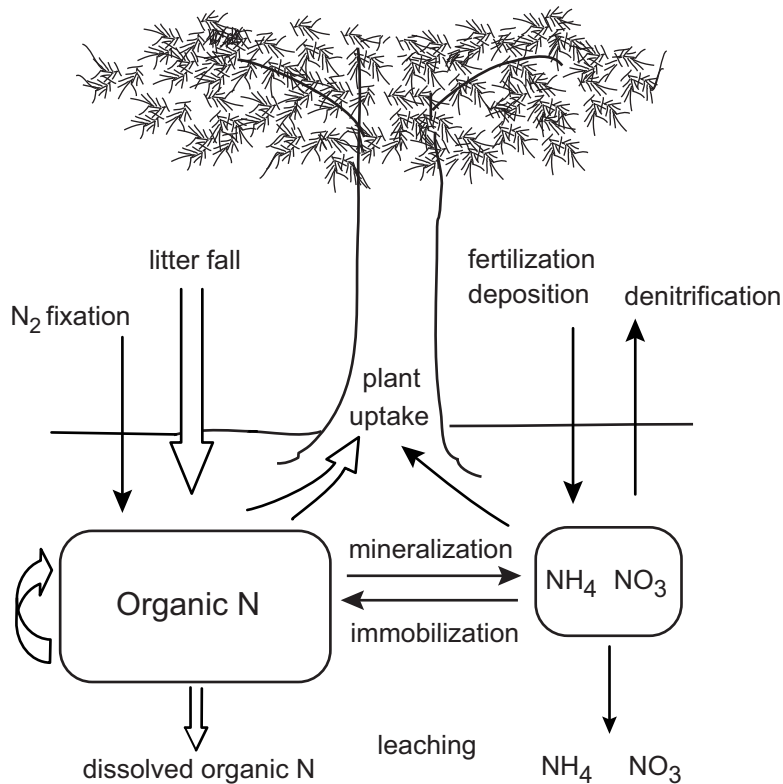
Figure 1. N–cycling in the boreal forest.

2.4.2   Effect of N on litter decomposition

Both the amount of N in the litter, endogenous N, and the amount available N in the surroundings, exogenous N, affect patterns and rates of decomposition (Berg & McClaugherty, 2003). Generally, litter with high initial N and low lignin content decomposes faster, whereas low N litter with high lignin content tend to decompose at lower rates (Zhang *et al.*, 2008b). The effect of exogenous N addition to litter decomposition has been studied extensively with inconsistent responses. The effect appears to depend on a range of factors such as initial N and lignin content of the litter (Knorr *et al.*, 2005; Berg & McClaugherty, 2003) and type of N added (mineral or organic N) (Fog, 1988), amounts applied and ambient N deposition levels (Knorr *et al.*, 2005). The inconsistent observations may also be due to the fact that many of these studies investigate responses from complex microbial communities. In field studies, responses may partly reflect

changes in the mycorrhizal community, which substantially contributes to soil respiration in forest soils (Heinemeyer et al., 2007). Similarly, laboratory incubation studies often study whole saprotrophic communities, where disturbances and addition of readily assimilable resources may generate responses from opportunistic taxa, which are otherwise rare and dormant. Generally, the decomposition rate of more recalcitrant material, with low initial N content and high lignin concentrations, has been observed to decrease, especially seen in a longer time perspective while decomposition of high quality (low-lignin) litter appears to be stimulated by N additions (Knorr et al., 2005; Fog, 1988). Similarly, the decomposition of Scots pine needles has been demonstrated to be stimulated by N additions at early stages of decomposition, when soluble and non lignified carbohydrates are decomposed (Berg et al., 1982). At later stages of decomposition, when lignin degradation is the rate limiting process, N has a negative effect (Berg & McClaugherty, 2003).

The effect of N addition on the enzymes active in decomposition commonly show as similar pattern. Hydrolysable enzymes involved in the degradation of cellulose generally increase as a response to N additions while oxidative enzymes involved in the degradation of more recalcitrant compounds decrease (DeForest et al., 2004; Waldrop et al., 2004; Carreiro et al., 2000). The latter effect is most pronounced in high lignin litter and for phenol oxidases. Whether the decreased ligninolytic enzyme production found in the field is a result of a suppressed enzyme production by the fungal community or a shift in the community structure is not known.

In common with the contradictory effects of N additions on decomposition, the effect of N on the microorganisms performing the process is complex and inconsistent. In wood decomposing fungi ligninolytic enzyme production has been observed to both decrease (Keyser et al., 1978) and increase (Kaal et al., 1995) due to N supplements. In field studies, N additions have been found to both decrease (DeForest et al., 2004; Frey et al., 2004) and slightly increase fungal biomass (Sinsabaugh et al., 2002). Although, in a recent meta-analysis, Treseder (2008), found no overall significant effect of mineral N fertilization on fungal biomass production in soil. Shifts in the microbial community structure due to N additions have been observed. Using molecular methods, a reduced richness of decomposer fungal taxa was observed in an Alaskan boreal forest due to N fertilization (Allison et al., 2007). DeForest et al. (2004) on the other hand observed no alteration of the microbial community structure using PLFA analysis.

2.4.3 Are saprotrophic fungi C or N limited?

Whether saprotrophic soil microorganisms are C or N limited has been under debate for a long time. Growth and activities of soil microorganisms have generally been assumed to be limited by the availability of carbohydrates (Smith & Paul, 1990), since the addition of readily assimilable C sources (such as glucose or acetate) results in enhanced CO_2 production (Ekblad & Nordgren, 2002; Vance & Chapin III, 2001; Aerts & De Caluwe, 1999; Flanagan & Van Cleve, 1983; Foster *et al.*, 1980). The effect of N additions are contradictory with both decreased and increased responses of decomposition rates and microbial growth observed (*e.g.* Treseder, 2008; Knorr *et al.*, 2005) (see section 2.4.2).

Schimel and Weintraub (2003) and Ågren *et al.* (2001) suggest that the contradictory responses may be due to changes in soil microbial C-use efficiency (see section 2.3.3). Schimel and Weintraub (2003) hypothesized that the lack of respiratory response or even decrease in respiration after N addition could be explained by an increased C-use efficiency, *i.e.* that the assimilated C is being used for microbial growth rather than being respired. The increase in respiration after C addition could be due to a decreased C-use efficiency, *i.e.* 'overflow metabolism' that is not associated with growth.

Potential changes in fungal C-use efficiency in response to supply and demand of C and N could possibly elucidate the contradictory responses earlier observed.

2.4.4 Fungal translocation and potential effects on C and N cycling

During the initial years of decomposition the N content of litter generally increases, not only in relative concentration, but also in absolute amounts (Moore *et al.*, 2006; Melillo *et al.*, 1989; Berg *et al.*, 1982). This is especially apparent in more recalcitrant litter types and up to an 80% increase in total N has been observed in Lodgepole pine litter (Fahey *et al.*, 1985). In Scots pine needle litter Berg *et al.* (1982) observed a more moderate increase of 30%. The net gain of N has been hypothesized to be mediated through fungal translocation (Hart & Firestone, 1990). Zeller *et al.* (1998) estimated fungal N to explain 35% of external N uptake in decaying beech litter whereas Frey *et al.* (2000) observed that ^{15}N influx into wheat straw on the soil surface was reduced by 52-86% following application of fungicides in a non-till agricultural soil. However, net increases in litter N not accounted for by fungal translocation have also been observed. Chadwick *et al.* (1998) observed net increases of N and calcium (Ca) in decomposing Scots pine needle associated with the nutrient concentrations of the underlying litter in a microcosm experiment. The authors, however, suggested that fungal

translocation was not responsible for the net import of N and Ca, since their treatment of lifting and rotating the litter every two weeks did not affect respiration or decomposition of the upper litter.

Potential fungal translocation of N into freshly fallen litter has been suggested to be a strategy to overcome N limitation in the newly shed litter, thereby increasing fungal growth and decomposition rates (Lindahl & Boberg, 2008). In the same way, growth and metabolism of the mycelium in C poor environments are likely to be supported by translocation of carbohydrates from litter with a high C:N-ratio (Persson *et al.*, 2000). Frey *et al.* (2003) found bi-directional fungal-mediated transfer of C and N between soil and wheat straw using stable isotopes and observed an 50% reduction in fungal biomass and C and N flux due to fungicide application. Bi-directional translocation of C and N has also been observed in litter fungi when grown in laboratory microcosms with opposing concentration gradients of glucose and N (Olsson, 1995). Thus, fungal mycelia may link substrates that differ with respect to C and N availability and thereby overcome resource deficiency through translocation.

N mineralization is the result of C limitation during substrate utilization and it is generally assumed that the decomposer organisms are exclusively dependent on the resources available in their immediate surrounding. Fungi, however, may potentially prevent local C limitation by translocating C from an external source. This way, even low C:N ratio substrates may be utilized without being mineralized. Instead, excess N may be translocated into substrates with high carbohydrate availability and low N content, thereby increasing fungal growth and decomposition.

Altogether, litter decomposing fungi may significantly affect nutrient and C fluxes in the litter layer by mycelial translocation and thereby have a decisive influence on decomposition rates. Fungal growth and substrate utilization may be supported in fresh litter by fungal N translocation from well decomposed litter. Similarly, fungal activity in well decomposed litter may be supported by C translocation from fresh litter, leading to restricted C limitation and reduced N losses as mineral N. By linking substrates of different quality fungi may avoid local limitation, by using external sources of carbohydrates and nutrients. Thus, resource relationships have to be interpreted with all sources and sinks of the entire mycelium taken together. This contrasts with the common view of a microorganism as being unicellular with negligible spatial extension. The specific ecophysiology of filamentous litter fungi may, thus, have a decisive and as yet unexplored influence on C and N cycling in fungal dominated ecosystems such as boreal forests.

3 Objectives

The overall aim of this work was to study the functional role of litter decomposing fungi in C and N cycling in boreal forest ecosystems. The specific objectives were:

- To study changes in C allocation patterns of litter degrading fungi in response to C and N availability and the implications for respiration, fungal growth and decomposition (Paper I and IV).

- To investigate the effects of C and N translocation in litter degrading basidiomycetes on decomposition rates and fungal metabolic efficiency (Paper II and IV).

- To study the implications of C translocation on N-mineralization in litter degrading basidiomycetes (Paper II and IV).

- To assess the effects of interspecific interactions on C and N translocation and the subsequent effects on fungal growth, decomposition and N circulation (Paper IV).

- To examine the ability of some commonly found ascomycetous litter fungi to degrade Scots pine needles (Paper III).

4   Materials and methods

A more detailed description of the methods used can be found in the different papers enclosed in the thesis and in the references cited therein.

4.1   Fungal strains and substrates

Two species of litter decomposing basidiomycetous fungi, *Marasmius androsaceus* and *Mycena epipterygia* were used in all experiments. A single strain of each species was isolated from sporocarps collected underneath Scots pine (*Pinus sylvestris*) trees in mixed forests located near Uppsala, central Sweden. Cultures were obtained by placing sterile pieces of tissues, cut from the inside of spororcarps, on Hagem agar (Stenlid, 1985).

In paper III, eight ascomycetes were tested for their decomposition abilities, five Helotiales strains; Helotiales 1, Helotiales 2, Hyaloscyphaceae 1, Hyalosphyphaceae 2 and *Mollisia cinerea*, two Dothideomycetes; *Sydowia polyspora* and Dothideomycete sp., and *Lophodermium pinastri*. One additional basidiomycete, a *Clavulina/Sistotrema* strain was also included. All strains were isolated from Scots pine needles collected from various places in central Sweden (Table 1). To confirm the identity of *M. androsaceus* and *M. epipterygia* and to identify the rest of the isolates, the ITS region of the ribosomal DNA was sequenced using the primers ITS1 and ITS4 (White *et al.*, 1990). The sequences obtained were then compared with the sequence database GenBank (Benson *et al.*, 2005). For most of the isolates, identification to species level was not possible.

Brown abscised Scots pine needles were used as a substrate in all studies. The needles were collected on sheets in a 25-year-old pine stand situated in Jädraås, central Sweden, in the autumn of 2002. The site consists of pine stands of different ages growing on a nutrient-poor sediment soil (Axelsson & Bråkenhielm, 1980). After collection the needles were air dried and then

stored frozen at –27°C until used. The pine needles contain 0.4% N and have a C:N-ratio of 135. The lignin content of Scots pine needles from Jädraås has been reported to be 25% (Johansson *et al.*, 1995). The needles were dried at 80°C (paper I, II and IV) or freeze dried (paper III) before and after the experiments to obtain changes in needle mass. To achieve sterility in the systems needles were autoclaved at 121°C for 15 min before use. Double labelled (U-[^{13}C$_2$],[^{15}N]) glycine, was used in paper II, and ^{15}N labelled NH$_4$Cl was used in paper IV, in order to trace mycelial translocation of C and N.

4.2 Experimental design

### 4.2.1 Effects of C and N addition on decomposition and C allocation (paper I)

In order to investigate whether changes in C allocation between respiration and growth (*i.e.* changed C-use efficiency) could be observed in a litter decomposing fungus as a response to C and N addition, the following experiment was set up. Axenic microcosms were prepared using Petri dishes in which pine needles were placed on C and N-free agar medium. The microcosms were inoculated with *M. epipterygia* and pre-incubated at 20°C for 56 days before receiving either ammonium, glucose or a salt solution (used as a control). The response in respiration was measured for each dish separately in sealed measuring cells for 35 days (Fig 3a). The microcosms were then harvested and needle mass loss, fungal biomass production and remaining amounts of glucose and ammonium in the medium were determined.

4.2.2 Fungal C translocation and N mineralization (paper II)

To examine whether C translocation by litter decomposing fungi would reduce N mineralization in a low C:N ratio substrate and N translocation would increase needle decomposition, axenic laboratory microcosms with spatially separated substrates were prepared (Fig 2). The substrates differed with respect to their C:N ratio; Scots pine needles (C:N ≈ 135) and glycine (C:N = 2). In order to trace translocation of carbohydrates and N, double labelled ^{13}C and ^{15}N glycine, enriched to 1 atom% excess, was used. Microcosms were prepared using split Petri dishes with agar medium; one half with added glycine and the other half with added pine needles. The microcosms were inoculated with either *M. androsaceus* or *M. epipterygia*. By growing across the barrier the fungus was able to colonize both sides of the

dish (Fig 3b). Microcosms where needles and glycine medium were connected by a common mycelium were compared with microcosms where either of the two substrates were excluded. The microcosms were incubated for approximately 3 months at 20°C in darkness until harvested. At harvest, needle mass loss, total C and N content and the ^{13}C and ^{15}N abundance of the needles as well as ammonium content of the medium were estimated.

Figure 2. Illustration of the experimental design of paper II showing the different types of microcosms prepared. Apart from basal agar-medium, the microcosms contained (a) needles and glycine in different halves, (b) only glycine in one half, (c) needles and basal medium in different halves or (d) control with neither needles or glycine (basal medium only). The grey colour represents medium with added glycine and the small circles symbolize the inoculum plugs.
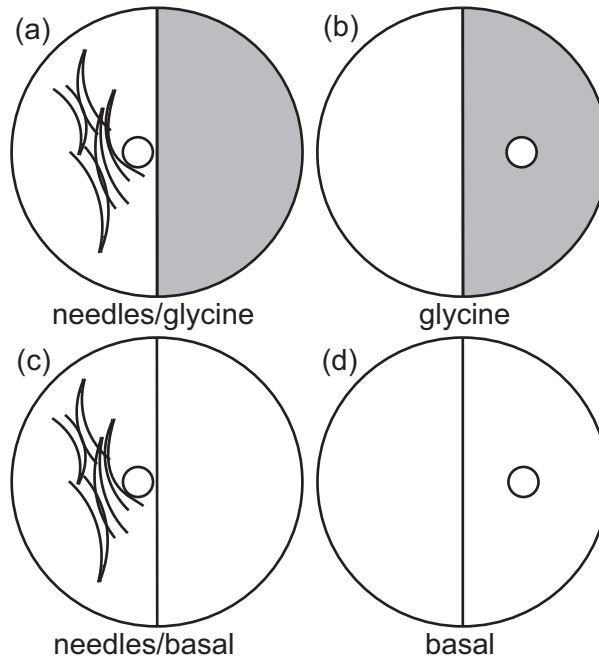
4.2.3 Decomposition test of some litter fungi (paper III)

In order to asses the potential of some commonly found ascomyceteous litter fungi to degrade Scot pine needles, Petri dishes were prepared with water agar onto which freeze dried, autoclaved pine needles were placed. The dishes were inoculated with 11 species of common litter fungi, described in section 4.1 (Fig 3d). Seven replicates were prepared for each species as well as for control dishes with uninoculated needles. The dishes were incubated at 20°C for 12 months. After harvest, needle mass loss was estimated gravimetrically. Contents of lignin, cellulose and the acid detergent fibre (ADF) soluble fraction were determined for the needles, pooled for each fungal species.
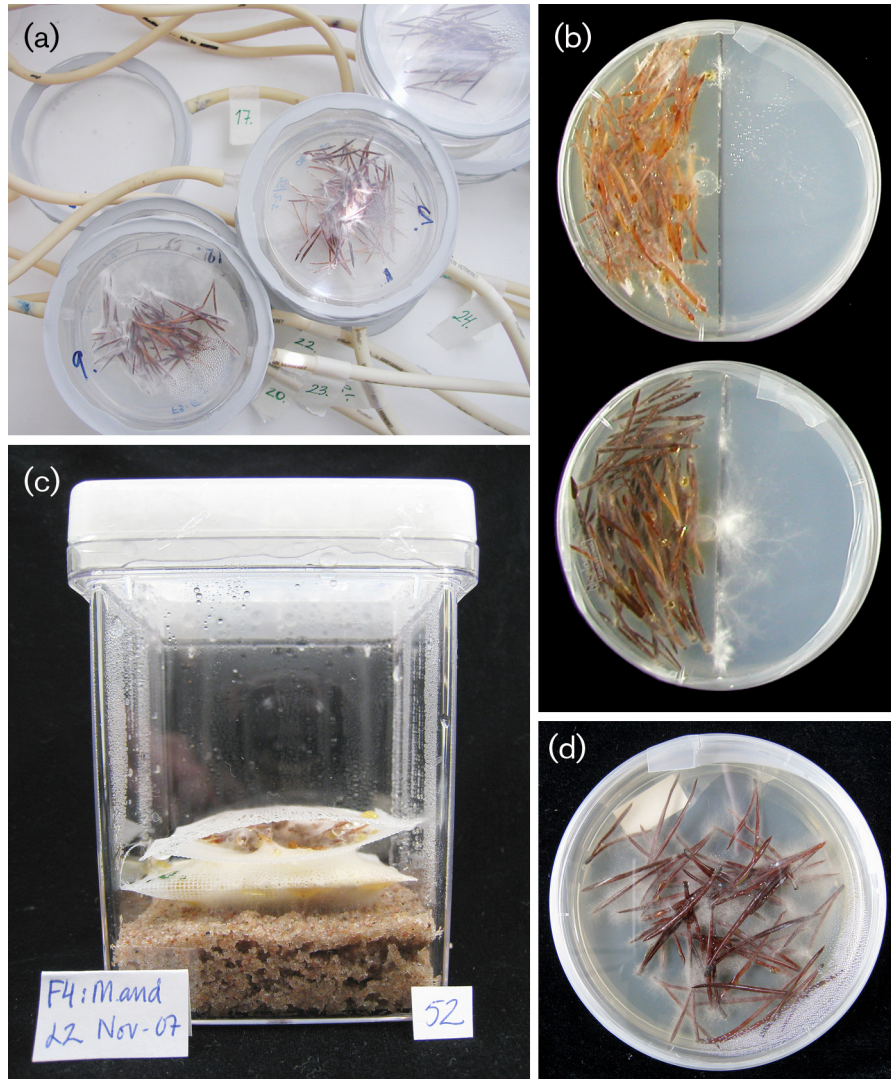
Figure 3. (a) Petri dishes with *M. epipterygia* decomposing pine needles enclosed in airtight measuring cells in order to measure respiration (paper I), (b) *M. androsaceus* (top) and *M. epipterygia* (bottom) colonizing Scots pine needles and glycine containing agar medium in the experimental microcosms of paper II, (c) Interspecific interaction between *M. androsaceus* colonizing well degraded needle litter (lower litter bag) and *M. epipterygia* colonizing less degraded litter (upper litter bag) in microcosms of paper IV and (d) Hyaloscyphaceae strain 2 colonizing pine needles at the end of the 1 year long incubation period of the decomposition test of paper III.

4.2.4 Mycelial reallocation of C and N (paper IV)

The aim of this study was to explore the potential of litter decomposing fungi to reallocate assimilated resources between substrates of different quality and the subsequent effect on fungal growth and C and N turnover. We hypothesized that the fungi would act to minimize losses of limiting N to the environment by reallocating N from mycelium within well decomposed litter to mycelium colonizing fresh resource units. We further hypothesized that C translocation from a new substrate would support fungal metabolism in a well decomposed substrate. Bi-directional translocation of C and N would thereby enable efficient utilization of the combined resources.

Axenic laboratory microcosms were prepared with pine needle litter inoculated with *M. epipterygia* or *M. androsaceus*, incubated for five months (Fig 4). In order to study the allocation of N within the microcosms, ^{15}N–labelled NH_4Cl solution (enriched to 1 atom% excess), was added to the microcosms. Control microcosms received NaCl solution instead. The litter was subsequently moved to new microcosms, a portion of newly shed litter was placed on top of the first litter and the systems were incubated for another five months.

Experimental design
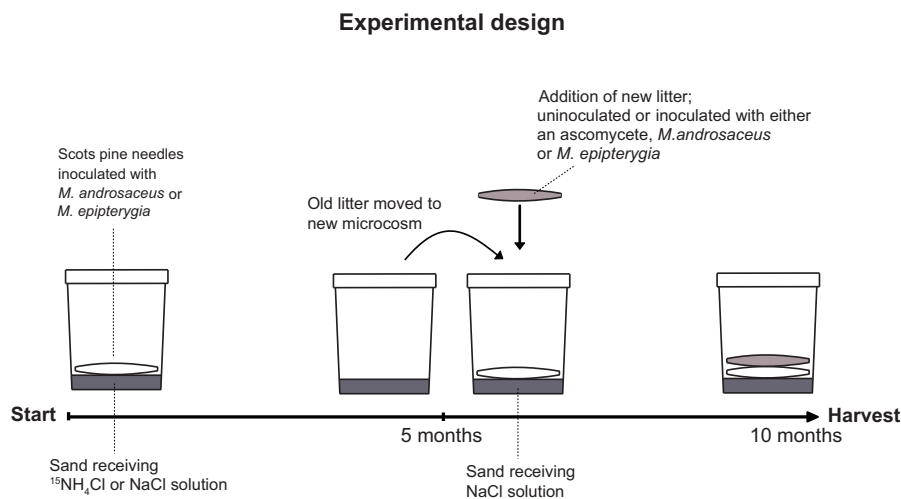
Figure 4. Illustration of the experimental design of paper IV showing the time line and a description of the sequential treatments. At start, a needle filled litter bag inoculated with either *M. androsaceus* or *M. epipterygia* was placed on sand receiving $^{15}NH_4Cl$ or NaCl. After 5 months the old litter (white) was moved to new vessels with sand receiving NaCl and a second layer of new litter (grey), uninoculated or inoculated with either an ascomycete, *M. androsaceus* or *M. epipterygia*, was placed on top of the old litter. After an additional 5 months, the experiment was terminated. Control systems in which the first litter layer was incubated over 10 months, without addition of new litter, and control systems with single litter bags incubated for each of the 5 months periods only, were also prepared.

[15]N re-allocation, total N in needles and mycelia, fungal biomass production, needle decomposition, N-mineralization as well as production of DON and DOC, were then determined and compared between microcosms with litter of different quality incubated together or separated. The effects of interspecific interactions on the determined parameters were studied in identical microcosms where the second layer of new litter was pre-inoculated with another fungal species (Fig 3c), *i.e.* an Helotialean ascomycete belonging to the family Hyaloscyphaeae (isolate BDI from paper III; Fig 3d) or another basidiomycete (*M. androsaceus* or *M. epipterygia*).

4.3 Analyses

Decomposition was estimated gravimetrically as needle mass loss with (paper IV) or without (paper I-III) correction for fungal biomass produced. The amount of fungal biomass produced in the needles was determined by chitin analysis using a method described by Ekblad & Näsholm (1996). In paper IV, chitin to fungal C and N conversion factors were obtained by measuring amounts of chitin, total C and N of the fungal mycelium growing into glass fibre bundles placed adjacent to the decomposing needles.

N mineralization in paper II and IV and remaining ammonium in the agar medium in paper I was analysed using a Flow Injection Analyser based on the method described by Svensson & Anfält (1982). Total C and N of needles and mycelia as well as total dissolved C and N (paper IV) were determined using a CN analyzer. When [13]C- and ^{15}N-abundances of needles and mycelium were determined, a CN analyzer coupled to an isotopic ratio mass spectrometer was used.

In paper I, respiration was measured in airtight measuring cells connected via a multipoint switching gas handling unit connected to an Infra Red Gas Analyser.

Remaining amounts of glucose in microcosm medium in paper I were determined using the oxidase/peroxidase method of Southgate (1976). The enzymatic reaction produces a colour change of a chromophore (o-dianisidine) detectable at 540 nm.

In paper IV, the acid detergent fiber (ADF)–sulphuric acid method (Rowland & Roberts, 1994) was used to determine amounts of lignin, cellulose and the ADF soluble fraction in the needles. Briefly, protein, lipids, pectin, starch, water soluble carbohydrates and hemi-cellulose (ADF soluble fraction) is removed using acid detergent leaving a fibre residue. Cellulose is removed by treating the fibre fraction with 72% sulphuric acid leaving the "lignin" and ash, which is removed by combustion.

5 Results and discussion

5.1 N limitation and C allocation (paper I and IV)

In paper I, addition of ammonium to needles colonized by *M. epipterygia* resulted in a rapid increase in respiration rate, which peaked after 13 days and then started to decrease. Glucose addition did not cause any immediate response but respiration started to increase after 7 days and after 17 days the respiration rate was higher than in ammonium treated systems. Cumulative over the measurement period, respiration increased by 32 and 19% for ammonium and glucose treated systems, respectively (Fig 5a). Addition of ammonium also stimulated needle mass loss by 31% and increased the needle chitin content by a factor of four (Fig 5b and c). These data suggest that the decomposition activity, catabolism, and fungal growth of *M. epipterygia* were N-limited in the unamended decomposing pine needles. In a more general context, this supports the proposal that degradation of pine litter in early stages of decay is constrained by the low N availability (Berg & McClaugherty, 2003). The data are also consistent with observations of increased growth of wood decomposer fungi (Boyle, 1998) and enhanced respiration by soil fungi (Allison *et al.*, 2009) due to N amendments.

The chitin content of fungal mycelium has been shown to increase with N content of the substrate (Nilsson & Bjurman, 1998), suggesting that the large increases in chitin after N addition may not directly correspond to increases in fungal biomass. In paper IV, however, the chitin to fungal C content did not change significantly in response to the treatments (addition of N and/or new litter and incubation length). Using the chitin to C conversion factor of 7.5 obtained for *M. epipterygia* in paper IV, the data suggest, that N additions did increase fungal C-use efficiency in paper I. Increased C-use efficiency due to enhanced N availability has been observed

not only in wood decomposer fungi (Boyle, 1998), but also for a fungal dominated community in an agroecosystem soil (Thiet *et al.*, 2006).
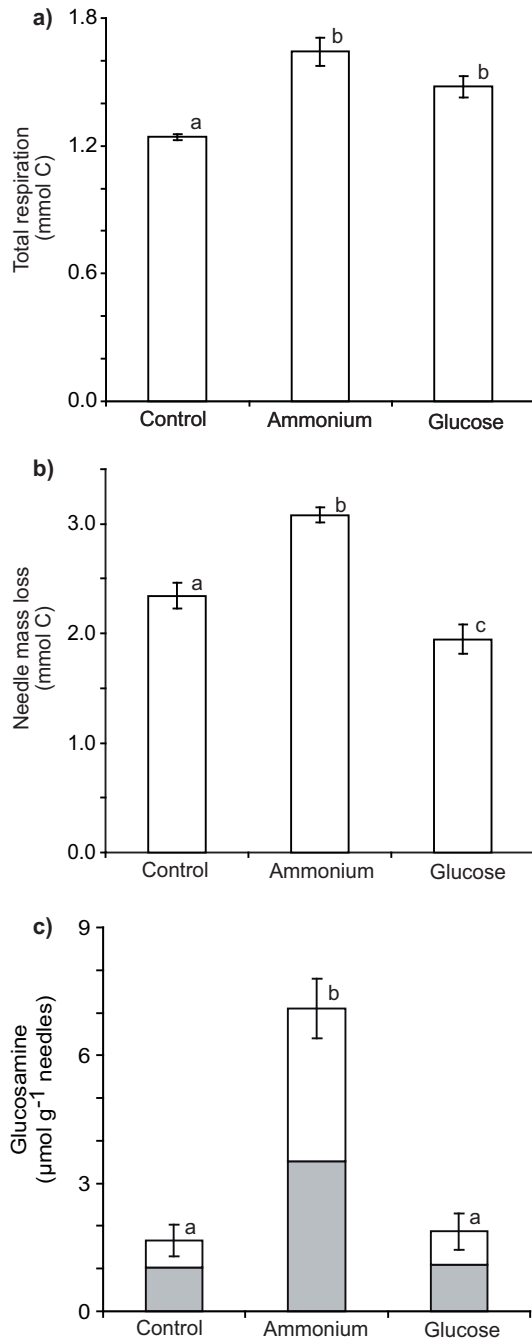
Figure 5. Cumulative respiration (a), needle mass loss (b) and glucosamine production (c) during 24 days (a) and 35 days (b,c) following the addition of 2 ml of ammonium, glucose or control solution to pine needles inoculated with *M. epipterygia*. Each bar represents the mean of 7 replicates ±SE. The distribution of glucosamine between needles (shaded area) and medium (white area) is indicated on the bars. Different letters indicate significant difference between treatments (*P*<0.05) according to Mann–Whitney test for respiration and Fishers's PLSD for mass loss and glucosamine production. Of the C respired from glucose amended systems, up to 0.5 mmol C may have originated from the added glucose.

The addition of glucose to needles colonized by *M.epipterygia* in paper I, increased respiration but needle mass loss was decreased by 17% and the chitin content indicated no alterations in fungal biomass production (Fig 5). Presumably, the added C was primarily respired since N limited fungal growth. Increased respiration paralleled by an unchanged biomass production, thus resulted in a reduction of the C-use efficiency. This observation support the hypothesis by Schimel & Weintraub (2003) that C additions to N-limited systems may result in overflow metabolism, *i.e.* respiration not associated with growth (Larsson *et al.*, 1995). Overall, *M. epipterygia* appeared not to be C limited when decomposing pine needles, supporting observations of unaltered growth of wood decomposer fungi in response to carbohydrate additions (Boyle, 1998).

These results demonstrate that changes in C-use efficiency can occur within a single fungal species and illustrates the plasticity of litter fungi to adapt their physiology to resource availability.

5.1.1 Methodological considerations

The results of paper I emphasize the importance of distinguishing respiration (*i.e.* catabolism) from growth and degradative activity since these parameters are not always proportional to each other. Allocation of added readily assimilable C to overflow metabolism may result in increased respiration after C-additions, in spite of N-limitations on growth and decomposition. Thus, using respiration as a measure of the amount of active biomass may be very misleading, and increases in respiration after additions of labile C may not necessarily indicate C-limitation.

This also has implications for methods used to estimate decomposition. Often decomposition is determined by substrate mass loss or measured as respiration, which implies that decomposition is defined to only encompass the conversion of plant C to CO_2. In this thesis, decomposition is defined to also include the conversion of plant derived C into fungal biomass. In order to assess total C utilized, substrate mass loss should be corrected for microbial biomass produced. The challenge is to obtain a reliable conversion factor from the specific components, measured to quantify biomass, to corresponding amounts of C and N. In paper IV, fungal chitin to C and fungal chitin to N conversion factors were obtained for *M. androsaceus* and *M. epipterygia*. Even though the amounts of chitin and N showed a strong correlation, the conversion factor overestimated the fungal N pool estimated in the needles and associated mycelium by up to a factor of 2. This

demonstrates the difficulty of obtaining a reliable method to correct for microbial biomass in decomposing substrates. Nevertheless, separating growth and respiration may be a first step towards elucidating the mechanisms driving fungal growth and activity, thereby increasing our understanding of ecosystem processes.

5.2 C translocation and N mineralization (paper II and IV)

In paper II, N-mineralization of glycine was significantly reduced when the mycelium of *M. epipterygia* and *M. androsaceus* was connected to pine needles (Fig. 6). Thus, in support of our hypothesis, both fungi appear able to transport C from the needles over a barrier into mycelium utilizing glycine, thereby eliminating local C limitation and subsequently reducing N mineralization. The ^{13}C excess recovered from within the needles corresponded to 17% of the added glycine. This, however, should represent a lower bound estimate of the total amount assimilated glycine, since part of the assimilated C most likely was retained in the mycelium on the glycine side and some was respired. When glycine was the only substrate supplied, only 2 and 5% were assimilated by *M. androscaeus* and *M. epipterygia*, respectively. The utilization of glycine, thus, seemed to increase when needles were present but without any significant N-mineralization.
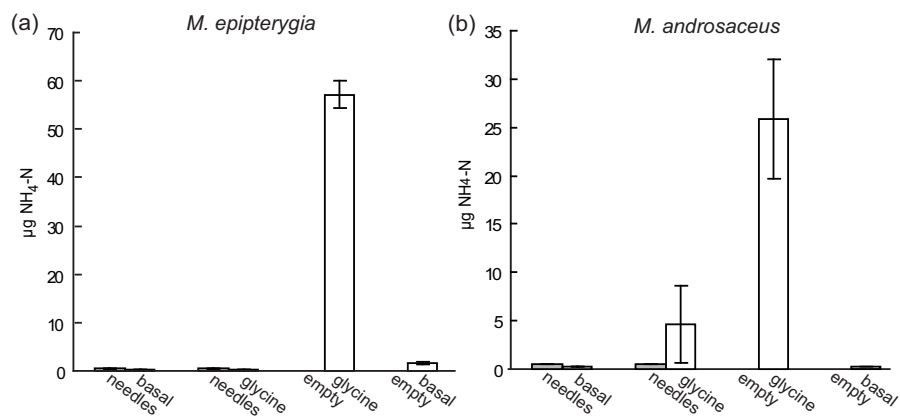
Figure 6. N-mineralization as μg NH$_4$-N produced in each half of agar microcosms containing different substrates in the following combination: needles and N-free basal medium, needles and glycine, glycine only or neither needles or glycine (basal medium only). The systems were colonized by (a) *M. epipterygia* and (b) *M. androsaceus.* Each bar represents the mean of seven replicates ± SE (three replicates for systems with basal medium only).

The ^{13}C excess obtained from the needles also indicates that C was transported bi-directionally in both fungi, which has been previously observed in rhizomorphs of the basidiomycete *Armillaria* (Granlund *et al.*, 1985). Carbohydrates, thus, appear to be readily circulated throughout the whole mycelium, something which may support the conclusion drawn from paper I that litter decomposing fungi are not generally limited by the amount of C when decomposing pine needles at early stages of decay.

In paper IV, the correlation between fungal C translocation and N-mineralization was studied in somewhat more ecologically realistic microcosms. Here, we hypothesized that systems with a single litter layer incubated for a total of 10 months would eventually experience C limitation and thereby release excess N. We assumed that addition of new litter after five months incubation would enable fungal C translocation from the new litter to the old litter in order to support fungal metabolism and subsequently reduce N-mineralization. The amounts N mineralized were overall very small, with treatment mean values ranging from 0.2-4‰ of the total initial needle N pool of the microcosms. Occasionally higher rates were found in some systems inoculated with *M. androsaceus* with a maximum of 1.2% of total needle N being mineralized in one replicate. Nevertheless, our hypothesis was supported for both *M. androsaceus* and *M. epipterygia*; N-mineralization was significantly reduced when new litter was added to the old litter in the microcosms. Taken together, these results suggest that litter decomposing fungi may restrict N-mineralization by translocating C from C-rich sources.

Coniferous litter decomposes over a long period and new litter is deposited on top of the older litter before the latter is depleted in readily available C. Only about 25% needle mass loss takes place in the first year (Berg & McClaugherty, 2003) and a continuous supply of carbohydrates should be ensured for decomposer fungi. In agreement with our findings, the concentration of mineral N in undisturbed coniferous forest soils is generally very low (Persson *et al.*, 2000). Higher N mineralization rates are often found in grasslands or agricultural soils, where litter enters the soil seasonally and is rapidly decomposed. Advanced stages of decomposition may therefore be reached before new litter is deposited, reducing the potential for nutrient translocation between litter substrates of different quality. We propose that the low mineralization rates in fungal dominated ecosystems may be attributed to resource redistribution within fungal mycelia.

Most N cycling models separate decomposing material into pools of different quality with different C:N ratios and no interaction is assumed to

occur between these substrates. Hence, traditional N cycling models are based on the assumption that decomposer organisms are exclusively dependent on a single substrate of a specific quality with a specific C:N ratio. The observed reduction in N mineralization as a result of fungal C translocation, thus, suggests that N cycling models, in which the C:N ratio of the substrate determines the fate of the N (CENTURY 2000; Zhang *et al.*, 2008a; Ågren & Bosatta, 1998), may be oversimplified. Especially, when applied to forest ecosystems in which fungi dominate the decomposer community.

An undisturbed fungal mycelium is a pre-requisite for efficient resource translocation in order to minimize losses. In the field, soil animals as well as freeze-thaw and drying-rewetting cycles may cause structural disturbance of the mycelium, thereby disconnecting resource translocation. Such disturbances have been observed to decrease both fungal biomass and C-translocation at the soil litter interface (Butenschoen *et al.*, 2007) and are often associated with increases in N mineralization (Lenoir *et al.*, 2007; Neilson *et al.*, 2001; Pulleman & Tietema, 1999; Ineson *et al.*, 1982). In paper IV, increased N mineralization due to interspecific interactions between *M. androsaceus* and *M. epipterygia* was observed. Most probably the deadlock formed between the two different species hindered supportive fungal C translocation from the new litter inducing local C-limitation in the old litter. These data support the suggestion by Woodward & Boddy (2008) that interspecific fungal interactions may be one of the main factors resulting in release of nutrients to soil together with invertebrate grazing.

5.3 Implications of N translocation for needle decomposition and fungal growth (paper II and IV)

We hypothesized in paper II that N assimilated from glycine would be transported into mycelium colonizing pine needles, in order to increase fungal growth and decomposition in the N limited substrate. However, no glycine-N was found in needles colonized by *M. androsaceus* and only small amounts were found in needles inoculated with *M. epipterygia*, as determined by the ^{15}N excess. These results suggest that limited amounts of N were translocated from the glycine, in contrast to earlier observations of N translocation in basidiomycetous fungi in laboratory studies (Tlalka *et al.*, 2002; Olsson, 1995).

Schimel & Hättenschwiler (2007) showed that the source strength controlled the amount of N being transported between two decomposing leaves in a laboratory study. This could imply that it is the ability of the

mycelium in the glycine side to provide N rather than the mycelial demand for N in needles that determines the amount of N being translocated. The supply of mobile C from the needles into the glycine side possibly created a high demand for N, associated with fungal biomass production. This proposition is actually supported by a single replicate of *M. androsaceus* which seems to be an outlier. This microcosm, receiving both needles and glycine, had an ammonium level in the medium comparable to systems with glycine only. Apparently, the fungus experienced C limitation when utilizing the glycine leading to N mineralization, although the reason for this is unknown since the ^{13}C excess data indicated efficient C translocation of C from the glycine into the needles. Surprisingly the ^{15}N excess was 10 times higher than the other replicates indicating N translocation. The fact that a surplus of N in the glycine side correlated with an observed N translocation support the hypothesis that N is translocated out of substrates only if the amount available exceeds the local demand.

In paper IV, 13% of added ^{15}N was found in single litter bags after a total 10 months incubation period. Since N may be translocated both to and from the needles simultaneously (Gebauer *et al.*, 2000), this is not an estimate of total net uptake but indicates that the added N was assimilated from the sand and imported into the needles. The extra N increased total N retained in needles and mycelium, as well as increased fungal growth and needle decomposition of both *M. androsaceus* and *M. epipterygia*. These results confirm the conclusion from paper I that both *M. androsaceus* and *M. epipterygia* are N limited when decomposing pine needles.

5.3.1   Redistribution of assimilated N

When new litter was added on top of old litter in paper IV, ^{15}N taken up in the old litter during the first incubation was redistributed to the new litter. Approximately 41 and 36% of the total ^{15}N recovered in the needles for *M. androsaceus* and *M. epipterygia*, respectively, were translocated to the added new litter and the ^{15}N excess of the old litter was subsequently reduced. This corresponded to a net N pool increase of about 6 and 1% for *M. androsaceus* and *M. epipterygia*, respectively. The redistribution of N from old to new litter significantly increased the total N pool and fungal biomass production of the new litter for both fungal species (Fig 7). Needle decomposition of the new litter appeared to follow fungal growth patterns, but was not significantly increased.
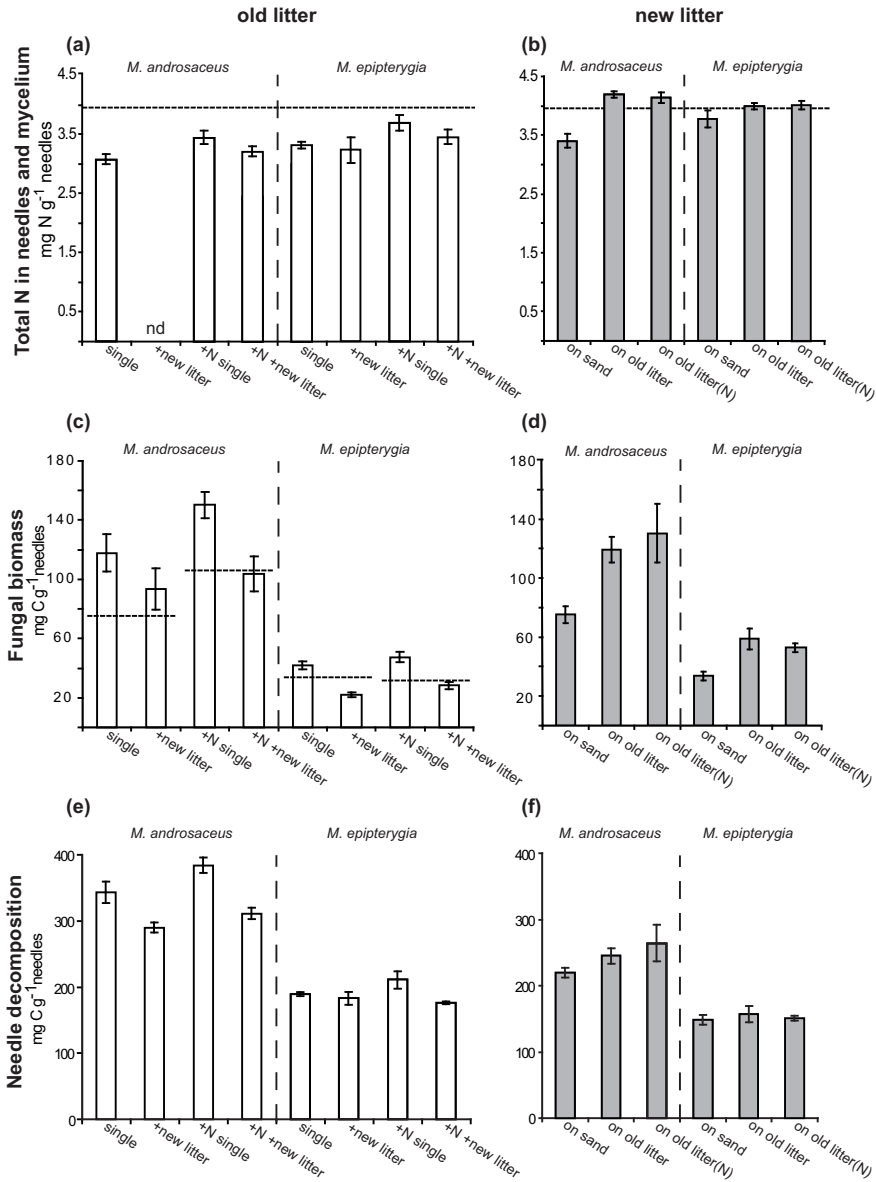
Figure 7. Total N in needles and mycelium (a and b), total fungal biomass C produced (c and d) and needle C decomposed (e and f) of old litter (open bars) and new litter (grey bars), in litter bags containing needles colonized by litter degrading fungi *M. androsaceus* or *M. epipterygia*. The N and C pools are expressed as amounts per g start needles 'Old needles' represents needles incubated for 2 x 5 months and 'new litter' represents needles which were placed on top of the old litter after 5 months. In old litter, 'single' represents single litter bags incubated for two periods (without and with N addition) and '+ new litter' represents old litter receiving new litter during the second incubation period. In new litter, 'on sand'

represents single litter bags placed on sand and incubated during the second incubation period only and 'on old litter' represents new litter placed on top of old litter (without or with N addition) during the second incubation period. Data are means $\pm$ 1 SE. In diagrams (a) and (b) the horizontal dashed line represents initial needle N content. In diagram (c) the horizontal dashed line represents fungal biomass produced during the first 5 months of incubation.

The limited amount of ^{15}N found in new litter of the interspecific interaction systems, where interaction between *M. androsaceus* and *M. epipterygia* ended in deadlock, suggest that N was transferred due to active mycelial transport rather than passive diffusion along continuous water films.

These results are in agreement with earlier findings of fungal N transfer into decomposing litter (Frey *et al.*, 2000; Zeller *et al.*, 1998), indicating that fungal translocation may substantially contribute to net N increases frequently detected in decomposing litter during early stages of decay (Moore *et al.*, 2006; Melillo *et al.*, 1989; Fahey *et al.*, 1985; Berg *et al.*, 1982). However, the net N gain observed in paper IV is lower than the net increases observed in earlier studies (Chadwick *et al.*, 1998; Berg & Staaf, 1981). The comparably low net N gain observed here could be due to lack of potential N sources, such as mosses and substrates at later stages of decomposition present in the field. In addition, in field studies and laboratory studies using inoculum comprising a microbial community, interactions between different organisms may influence both their growth and activity. By reducing accumulation of dead and living mycelium, competition, parasitism and grazing may enhance both decomposition and nutrient mobility (Woodward & Boddy, 2008), potentially increasing overall C and N turnover.

To summarize, in contrast to the lack of fungal N translocation found in paper II, the experiments in paper IV showed that both fungi were able to translocate N from well decomposed litter into new litter during colonization. It is evident that we do not fully understand the mechanism determining the net transfer of N within fungal mycelium. In paper I, the ammonium uptake corresponds to an increase of total needle-fungal N pool by a factor of more than 2, suggesting that *M. epipterygia* has a high potential capacity to take up N. The total amount of N assimilated by the fungi during the first incubation period in paper IV is, as mentioned earlier, not known. Fungal mycelium in the sand produced during both the first and the second incubation period could contribute substantially to the total N pool.

5.3.2 Resource translocation and litter fungi dynamics

In paper IV, the increased fungal growth and total N pool of the new litter was paralleled by a decrease of fungal growth and needle decomposition of the old litter. There was also a trend that the total amount of N retained in the old litter was lower (although not significantly so). After addition of new litter fungal growth ceased in old litter colonized by *M. androsaceus* and the amount fungal biomass in old litter colonized by *M. epipterygia* actually decreased. The decrease in fungal biomass was probably due to autolysis caused by chitinolytic enzymes in order to recycle N (Lindahl & Finlay, 2006). The redistribution of overall fungal activity to the new litter followed the same patterns as previously observed in cord forming wood decomposing fungi. Typically, mycelial growth as well as translocation of resources within the mycelium is directed towards new substrates, whereas the mycelium in depleted substrates regresses and is ultimately degraded through autolysis (*e.g.* Boddy, 1999).

Presumably, the high resource availability within the new substrate increased fungal growth and thereby created a strong sink for N. The high demand for N in the new litter presumably resulted in withdrawal of N from the mycelium in the old litter and thereby reduced fungal growth and decomposition there. The decreased fungal activity of the old litter was probably due to the low relative quality of the old litter which approached 60% loss of needle C in systems inoculated with *M. androsaceus*. The fungus was, however, still capable of using the well decomposed needles as a substrate, as shown by the continued mass loss and fungal growth observed in single litter bags.

The treatment of adding new litter increased fungal C-use efficiency for both fungi, but the improved efficiency did not appear to be directly related to the higher quality of the new substrate. Instead, fungal redistribution of resources between old and new litter appear to have resulted in a more efficient use of assimilated C. Yet, it appears as if fungal reallocation between litters of different quality actually decreased overall decomposition rates. In systems inoculated with *M. androsaceus* for example, addition of new litter reduced overall decomposition by 5% but increased fungal biomass by 10%. This suggests that decomposition capacity is not in itself the main aim of fungal activity. Instead, maximising growth and thereby increasing the potential to explore more substrates with a larger inoculum potential and ultimately produce fruit bodies may be the primary feature influencing ecological success.
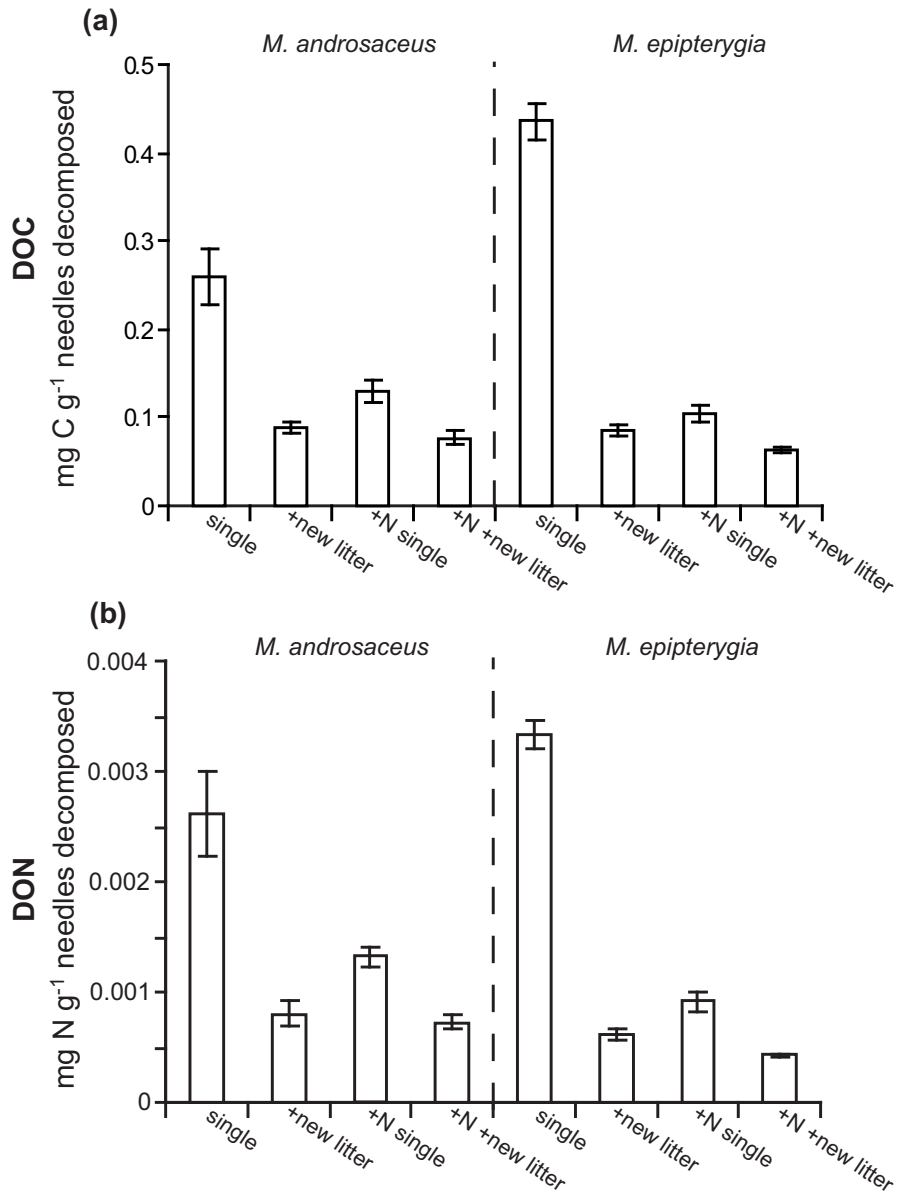
Figure 8. DOC (a) and DON (b) as amounts in the sand solution of microcosms containing needles colonized by litter degrading fungi, related to the amounts of needle C degraded during the last 5 months of a total 10 month incubation period. The systems were inoculated with either *M. androsaceus* or *M. epipterygia*. 'Single' represents systems with single litter bags incubated for 2 x 5 months (without and with N addition) and '+new litter' represents systems with old litter receiving new litter during the second incubation period. Data are means ± SE.

5.3.3 Losses of N

The losses of N from decomposing needles colonized by either *M. androsaceus* or *M. epipterygia* were overall very small (paper IV). In undisturbed systems, a major fraction was lost as DON and only small amounts as ammonium as earlier discussed (see section 5.2). These observations is in agreement with patterns of high N immobilisation rates generally observed in typically low productive ecosystems, together with a predominant loss of N in organic form (Schimel & Bennet, 2004; Northup *et al.*, 1995).

The amount of DON produced from single litter bags decreased due to N addition even though decomposition rates increased. Addition of new litter also decreased the amount of DON produced (Fig 8). In field studies, addition of N has been observed to increase the cellulolytic activity but to hamper phenol oxidase activity in high lignin litter (DeForest *et al.*, 2004; Carreiro *et al.*, 2000). Addition of new litter should have the same effect, *i.e.* reducing the production of ligninolytic enzymes by redirecting decomposer efforts to the cellulose rich new litter. Kalbitz *et al.* (2006) observed that lignin degradation during later stages of decay increase DOC production from high lignin litter such as spruce and pine needles. Thus, DON production may possibly reflect degradation of more polyphenol–rich material through oxidation, and this process may be hampered by the addition of N and/or new litter. In a study by Vestgarden (2001), the amount of DON leached from decomposing pine needles as proportion of total N tended to decrease when N availability increased.

To summarize, mineral N losses appeared to be small and a major part of the lost N occurred in organic form, either as DON but also presumably as particulate matter.

5.4 Species interactions (paper IV)

In paper IV, effects of interspecific interactions on fungal growth, needle decomposition and subsequent N circulation were investigated. According to visual inspection, the ascomycetous Helotialean strain pre-inoculated in the new litter appeared to have been completely overgrown by both *M. androsaceus* and *M. epipterygia* at the end of the experiment. The initial inoculation with the ascomycete did not affect total fungal biomass production or needle decomposition rates by the two latter species. Most likely, the ascomycete had a very low competitive strength and the ecological strategies by which Helotialean ascomycetes co-exist with basidiomycetes remain unclear.
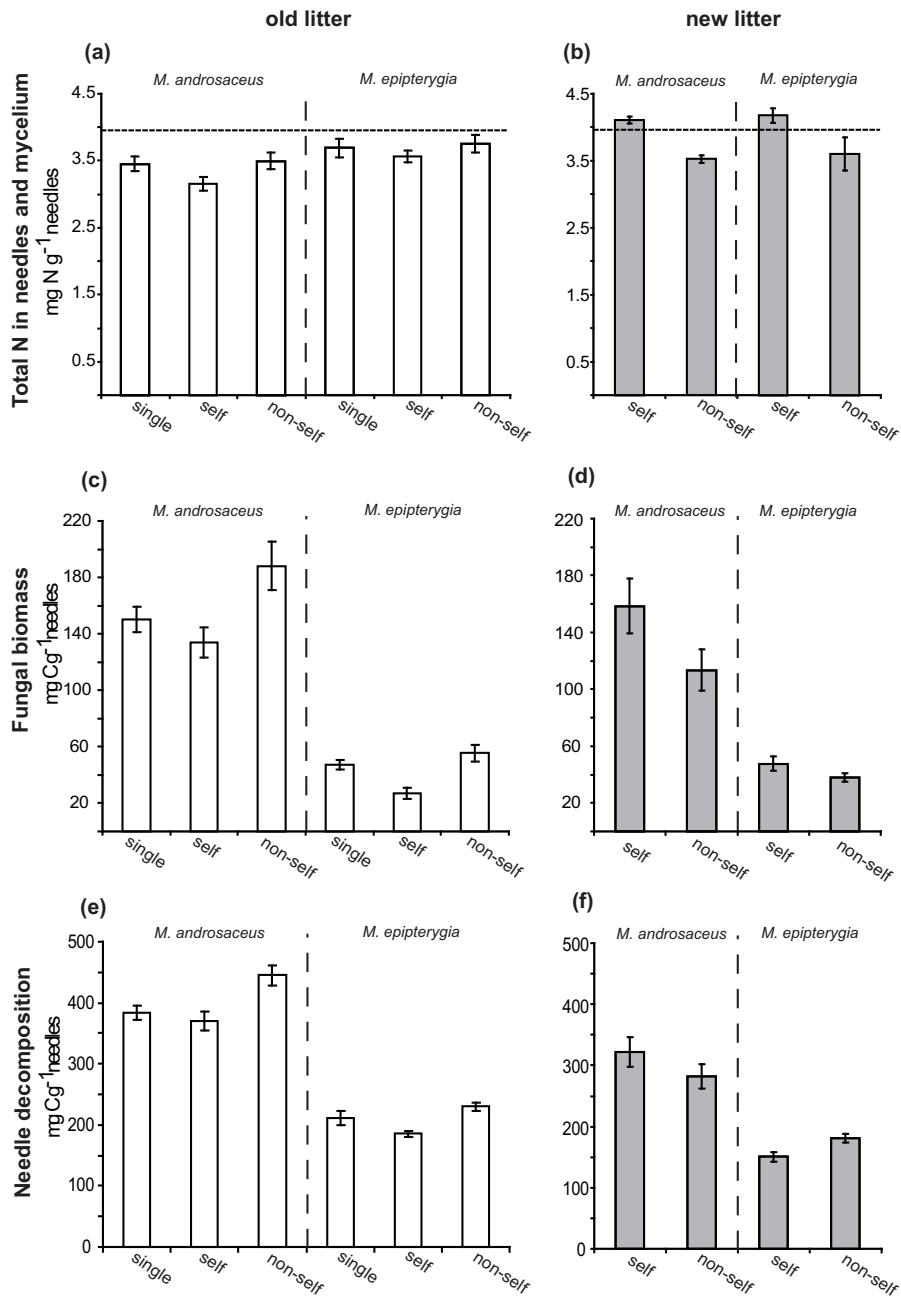
Figure 9. Total N in needles and mycelium (a and b), total fungal biomass C produced (c and d) and needle C decomposed (e and f) of old litter (open bars) and new litter (grey bars), in litter bags containing needles colonized by litter degrading fungi *M. androsaceus* or *M. epipterygia* subjected to self and non-self interaction. The N and C pools are expresses as amounts per g start needles. 'Old litter' represents needles incubated for 2 x 5 months and

'new litter' represents needles which were placed on top of the old litter after 5 months. In old litter, 'single' represents single litter bags incubated for two periods (with N addition during the first incubation period), 'self' represents old litter receiving new litter during the second incubation period inoculated with the same fungal species and 'non-self' represents old litter receiving new litter inoculated with the other fungal species (*M. epipterygia* placed on *M. androsaceus* and vice-versa). In new litter 'self' represents new litter placed on top of old litter inoculated with the same species and 'non-self' represents new litter placed on top of old litter inoculated with the other basidiomycete. Data are mean ± SE. In diagram (a) and (b) the horizontal dashed line represents initial needle N content.

Whether the ascomycete remained in the needles could be assessed using molecular methods, such as qPCR, which both detects and quantifies the number of DNA copies present.

Interaction between *M. androsaceus* and *M. epipterygia* appeared to end in deadlock, *i.e.* none of the species gained access to the substrate occupied by the other. The species in the upper new litter with no possibility of importing supportive resources, as indicated by the limited ^{15}N import and smaller N pool, appeared to be N-limited reducing fungal growth (Fig 9). This result again emphasizes the significant role of fungal translocation to increase overall fungal activity and efficiency.

In the old litter, both fungal growth and needle decomposition increased in response to the deadlock interaction. The lack of mycelial translocation between the two layers resulted in conditions in the old litter similar to single bag systems. Nevertheless, both fungal growth and needle decomposition during deadlock were higher than for the single litter bags. The higher activity must therefore have partly been induced by the antagonistic interaction itself and suggest that interaction may increase the C cost of the fungus. Increased C demand due to interspecific interaction has been observed in wood decomposing fungi as enhanced respiration (Wells & Boddy, 2002) and as increased production of ligninolytic enzymes (Iakovlev & Stenlid, 2000; White & Boddy, 1992). The deadlock interaction also increased N-mineralization rates (see section 5.2).

5.5 Decomposer ability of litter fungi (paper III)

The five Helotialean species tested here caused a pine needle mass loss of 16 to 28% during one year, which should be compared with the uninoculated control needles that lost 8% of their weight (Table 1). All except *Mollisia cinerea* caused some changes in the cellulose fraction ranging from 29 to 5% loss. The ecological strategy of these fungi is unresolved, but Hyaloscyphaceae 1 and 2 group together with a cluster of Helotialean fungi frequently encountered in the litter layer by Lindahl *et al.* (2007). These taxa

emerge in litter during early stages of decay and seem able to persist at a lower frequency in the humus layer. The Dothideomycetes, *S. polyspora* and Dothideomycete sp. caused low or, for the latter, no needle mass loss. Together with Helotialean species *M. cinerea*, these taxa had limited cellulolytic capabilities. *S. polyspora* is an endophytic or a parasitic fungus on needles but appears to stay in the needles when they are shed and mainly fruit on the ground (Sinclair & Lyon, 2005). The identity and ecology of Dothideomycete sp. is unknown and our results merely show that the strain is unable to degrade cellulose and lignin. The fact that *M. cinerea* includes a range of different varieties, earlier classified as different species, seemingly representing different ecological strategies, makes it complicated to elucidate its ecological strategy and functional traits.

Table 1. *Fungal taxa, isolate number, origin and mass loss of pine needles, cellulose, lignin and the ADF soluble fraction caused by the respective isolate after 365 days.*

Taxa	Isolate	Origin	Needle mass loss (%)[a]	Cellulose mass loss (%)[b]	Lignin mass loss (%)[b]	ADF sol. mass loss (%)[b]
Basidiomycetes						
Marasmius androsaceus	JB14	Uppsala	63.9 ± 1.8	69	76	49
Mycena epipterygia	JB13	Uppsala	51.5 ± 2.0	33	63	49
Clavulina/Sistotrema	BCE	Jädraås	32.6 ± 0.8	45	–9	35
Ascomycetes						
Lophodermium pinastri	96–78	Nacka	39.8 ± 1.8	33	25	41
Helotiales 1	BDJ	Jädraås	27.7 ± 0.6	29	–7	33
Helotiales 2	BCX	Jädraås	21.2 ± 0.7	11	–12	32
Hyaloscyphaceae 1	BDK	Jädraås	22.9 ± 0.3	19	–14	31
Hyaloscyphaceae 2	BDI	Jädraås	18.0 ± 0.2	5	–15	29
Mollisia cinerea	BCZ	Jädraås	15.5 ± 0.7	-0.3	–13	26
Sydowia polyspora	HomC	Jädraås	13.5 ± 0.3	–3	–18	26
Dothideomycetes	BBC	Uppsala	7.9 ± 0.1	–4	–14	12
Control	–	–	7.6 ± 0.7	–	–	–

[a] *% of original weight; data are mean values of 5-7 replicates ± SE*
[b] *% of control weight; data are from pooled replicates*

An unexpected finding was the capacity of *L. pinastri* to cause both significant needle mass loss and extensive lignin degradation. *L. pinastri* colonizes the needles when they are still attached to the tree and hence is already present when the needles reach the forest floor. Possibly, the ecological strategy of *L. pinastri* may be to rapidly assimilate resources needed to fruit, since the fungus has been found to rapidly disappear in community studies in the field (Lindahl *et al.*, 2007).

In systems where the fungi were unable to cause any apparent lignin losses the lignin fraction, as shown by the proximate analysis, was increased by 7 to 18%. The reason for this increase is unknown but possibly lignin-like substances or other recalcitrant compounds are formed during decomposition. The chitin produced by the growing fungus should end up in the ADF-soluble fraction according to Fioretto *et al.* (2005). However, the fate of other fungal products, such as melanin, is not known. Interestingly, the lignin concentration of the needles prior to the incubation was about 18% compared to the 23% found in the control needles after the experiment was completed. Possibly the treatment of freeze drying and autoclaving could have increased the fraction of recalcitrant compounds in the needles.

As expected, *M. androsaceus* and *M. epipterygia*, which were included as reference species, caused the highest needle mass loss, 64 and 52%, respectively. Interestingly, *M. epipterygia* caused two-fold higher loss of lignin than of cellulose, suggesting preferential lignin degradation compared with *M. androsaceus*, which caused similar cellulose and lignin loss. Possibly, this difference can be related to their ecological strategies. *M. androsaceus,* which grows rapidly and causes high mass loss of newly shed pine needles (paper IV), is mainly found in the uppermost litter layer (Lindahl *et al.*, 2007; Holmer & Stenlid, 1991). *Mycena* species often colonize litter at slightly later stages of decomposition (Lindahl *et al.*, 2007; Frankland, 1998), which is consistent with the preferential lignin degradation observed in paper III. The third basidiomycete included, a strain belonging to the clade *Clavulina/Sistotrema* which appeared to be limited to cellulose degradation and similar taxa, have been found in association with mosses.

Neither of the taxa belonging to the Helotiales and Dothideomycetes strains here had any apparent lignin degrading capacity and the ability to depolymerize cellulose varied. The ecological strategies remain unclear for most of the tested taxa and it is striking that we know so little, in fact, close to nothing, about most of the fungal species found in decomposing litter.

6   General discussion and conclusions

Although care should be taken when extrapolating results from laboratory microcosms, using only two species of litter decomposing fungi, to field conditions involving many species, these studies demonstrate major potential effects of fungal translocation on litter decomposition. The importance of translocation of carbohydrates and nutrients in ectomycorrhizal fungi in connection to C turnover in boreal forests is well established (Heinemeyer *et al.*, 2007). This thesis shows that translocation of resources between substrates of different quality by litter decomposing fungi may also have a decisive influence on C and N turnover in boreal forests.

In accordance with the overall tightly regulated N cycle found in the boreal forest (Tamm, 1991), the litter decomposing fungi tested here appear to have developed a strategy to effectively maintain assimilated N in order to increase their activity and growth. As shown in the thesis, the meager amount of N in recently abscised pine needles limits the metabolism as well as growth and decomposition activity of *M. androsaceus* and *M. epipterygia*. Litter decomposing fungi have been found to be limited to the uppermost litter layer (<4 years old) and are thereby presumably not able to access the humus layer, where a major part of the soil organic N is found. In field studies, the net gain in total N observed in early litter decomposition is generally followed by a decrease of N during the second or third year of decomposition (Gebauer *et al.*, 2000; Melillo *et al.*, 1989; Berg & Staaf, 1981). This net decrease of N is likely to reflect fungal redistribution from the well decomposed litter directed to support mycelial colonization and utilization of new substrates. Presumably, litter decomposing fungi maintain an N pool in the upper litter layer larger than that expected based on the initial N contents of the litter in order to facilitate efficient assimilation of resources and subsequent growth. Both *M. androsaceus* and *M. epipterygia* translocated assimilated N when colonizing new needle litter leading to a net

gain in the total N pool. This way, both fungi were able to increase their growth in the substrate.

In addition, both *M. androsaceus* and *M. epipterygia*, were observed to readily translocate C within their mycelia, effectively reduce local C deficiency and subsequently restrict N-mineralization. In undisturbed boreal forest ecosystems, coniferous litter decomposes over a long time period, maintaining a continuous supply of C. Due to the slow turnover rates, relatively fresh litter resources will always be present in the system, ensuring that C is available for mycelial translocation. Thus, the ability of litter fungi to connect substrates of different quality together with a well-developed capacity to translocate C could possibly explain the low N-mineralization rates observed in fungal dominated ecosystems. In this way, excess N loss may be reduced and N may instead be allocated to support colonization of new substrates, again stressing the significance of resource translocation for the function of these fungi. Consequently, when able to connect substrates of different quality, both *M. androsaceus* and *M. epipterygia* increased their efficiency; *i.e.* allocated a larger proportion of assimilated C to biomass production. The changes in C-use efficiency observed here, depending on the availability of carbohydrates and N, have direct implications for the global C cycle, since they affect the balance between C storage and release.

The main N losses from the fungal-needle complex occurred in organic form corresponding to the overall patterns of N mobilization in boreal forests (Persson *et al.*, 2000; Northup *et al.*, 1995). However, the very low actual loss of N as either DON or NH_4-N observed here, in spite of the very advanced decomposition stage of the old litter, emphasizes the extremely conservative N dynamics displayed by these fungi. Consequently, litter decomposing fungi do not appear to be involved in the release of nutrients available for direct plant uptake as is normally perceived to be a principle function of saprotrophic microorganisms in ecosystems (Berg, 2000). As the major executors of organic matter depolymerization, litter decomposing fungi (at least those with ligninolytic capacity) are nevertheless essential in N cycling. N appears to be mobilized to support primary production mainly in deeper soil horizons through the activity of mycorrhizal fungi (Lindahl *et al.*, 2007; Lindahl *et al.*, 2002; Melillo *et al.*, 1989). In fact, most ectomycorrhizal fungi have the potential to produce enzymes such as proteases (Nygren *et al.*, 2007), chitinases (Lindahl & Taylor, 2004) and peroxidases (Bödeker *et al.*, 2009), presumably in order to access nutrients bound in complex organic forms (Read & Perez-Moreno, 2003). The results obtained here support the view put forward by Lindahl *et al.* (2007) that at an ecosystem level, saprotrophic fungi mainly mineralize C

from the upper litter layer whereas N is mainly mobilized and support primary production through the activity of mycorrhizal fungi in the humus layer. Although N losses from the litter layer appear low, the amount of N transferred to deeper soil layers must balance the output of N from this pool to support primary production, or even exceed outputs since N generally accumulates in the humus layer. Presumably, a major part of the N entering deeper soil horizons is derived from microbial constituents, such as cell wall components. Active nutrient transfer between the different soil layers may also occur as a result of competitive interactions between saprotrophic and mycorrhizal fungi (Lindahl *et al.*, 1999).

This thesis emphasizes the importance of acknowledging the fundamental difference in physiology of different groups of decomposer microorganisms. Assuming that filamentous fungi and unicellular organisms, such as yeast fungi and prokaryotes, exhibit similar functional properties may constrain our understanding of C and nutrient cycling. Recognizing the functional differences between different groups of litter decomposing fungi is also important. Still, our knowledge about most of the fungal taxa found in decomposing litter is still limited. This thesis shows that the capacity of litter decomposing fungi to translocate resources may influence processes affecting both the global C cycle and ecosystem N turnover. Understanding the mechanisms affecting litter decomposition in boreal forests is important, especially in light of globally changing environmental conditions.

7 Future prospects

The potential mechanisms observed in these studies, which regulate C and N turnover in decomposing needle litter, should be tested on more species and under field conditions before general conclusions on functional significance of litter fungi in forest ecosystems can be made. Especially in the light of the potential influence of interspecific interaction on these processes, such interactions should also be included. The knowledge obtained from laboratory studies could form a basis for designing relevant field experiments. The potential importance of fungal C translocation for limiting N-mineralization in boreal forest, for example, would be interesting to try and investigate in a field experiment. The challenge is to investigate this under field conditions without disturbing the systems by severing the delicate mycelial structures and restricting translocation.

Another challenge is to include the mechanisms driving fungal decomposition in models of C and N cycling. For example, the possibility of interactions between decomposing substrates of different quality should be incorporated into the models.

In addition, the connection between C and N allocation and the responses seen in fungal growth and needle decomposition could be studied in more detail by targeting specific degrading enzymes. The possible connection between the active enzymes involved and the release of DON would be interesting to investigate further, since DON is the major form of mobile N in the boreal forest.

It is striking that our knowledge of the ecological strategy of the ascomycetous litter fungi, which constitute a large part of the fungal community in litter, is so limited. Linking ecologically relevant taxa to function, both qualitatively and quantitatively, within this group of fungi is a challenge for future research.

An increased knowledge and understanding of mechanism and driving forces influencing litter decomposing fungi could potentially aid in interpreting responses to changing environmental conditions such as N deposition, fertilization, elevated CO_2 and increased temperatures.

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