Mycorrhizal fungi and nitrogen dynamics in drained peatland

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Academic dissertation in environmental ecology

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Abstract

Fungi have a fundamental role in carbon and nutrient transformations in the acrid soils of boreal regions, such as peatlands, where high amounts of carbon (C) and nutrients are stored in peat, the pH is relatively low and the nutrient uptake of trees is highly dependent on mycorrhizae. In this thesis, the aim was to examine nitrogen (N) transformations and the availability of dissolved N compounds in forestry-drained peatlands, to compare the fungal community biomass and structure at various peat N levels, to investigate the growth of ectomycorrhizal fungi with variable P and K availability and to assess how the ectomycorrhizal fungi (ECM) affect N transformations. Both field and laboratory experiments were carried out.

The peat N concentration did not affect the soil fungal community structure within a site. Phosphorus (P) and potassium (K) deficiency of the trees as well as the degree of decomposition and dissolved organic nitrogen (DON) concentration of the peat were shown to affect the fungal community structure and biomass of ECMs, highlighting the complexity of the below ground system on drained peatlands. The biomass of extramatrical mycorrhizal mycelia (EMM) was enhanced by P and/or K deficiency of the trees, and ECM biomass in the roots was increased by P deficiency. Thus, PK deficiency in drained peatlands may increase the allocation of C by the tree to ECMs.

It was also observed that fungi can alter N mineralization processes in the rhizosphere but variously depending on fungal species and fertility level of peat. Gross N mineralization did not vary but the net N mineralization rate significantly increased along the N gradient in both field and laboratory experiments. Gross N immobilization also significantly increased when the peat N concentration increased. Nitrification was hardly detectable in either field or laboratory experiments. During the growing season, dissolved inorganic N (DIN) fluctuated much more than the relatively stable DON. Special methodological challenges associated with sampling and analysis in microbial studies on peatlands are discussed.
List of original articles


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The author’s contribution

I  Corresponding author. Performing experimental work, except for PLFA-analysis, sequencing and degree of decomposition. Responsible for writing and interpretation of the results.

II  Corresponding author. Performing experimental work. Responsible for writing and interpretation of the results.

III  Corresponding author. Performing experimental work, except for PLFA-analysis and REE-measurements. Responsible for writing and interpretation of the results.

IV  Performing Biolog-measurements. Responsible for statistical analysis and interpretation of the results.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ATP</td>
<td>Adenosine triphosphatase</td>
</tr>
<tr>
<td>CLPP</td>
<td>Community level physiological profile</td>
</tr>
<tr>
<td>Cmic</td>
<td>Microbial biomass C</td>
</tr>
<tr>
<td>DGGE</td>
<td>Denaturing gradient gel electrophoresis</td>
</tr>
<tr>
<td>DIN</td>
<td>Dissolved inorganic nitrogen</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DOC</td>
<td>Dissolved organic carbon</td>
</tr>
<tr>
<td>DON</td>
<td>Dissolved organic nitrogen</td>
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<td>ECM</td>
<td>Ectomycorrhiza</td>
</tr>
<tr>
<td>EMM</td>
<td>Extramatrical mycorrhizal mycelia</td>
</tr>
<tr>
<td>FIA</td>
<td>Flow injection analyzer</td>
</tr>
<tr>
<td>ITS</td>
<td>Internal transcribed spacer</td>
</tr>
<tr>
<td>Nmic</td>
<td>Microbial biomass N</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PLFA</td>
<td>Phospholipid fatty acid</td>
</tr>
<tr>
<td>PVPP</td>
<td>Polyvinylpolypyrrolidone</td>
</tr>
<tr>
<td>TDN</td>
<td>Total dissolved nitrogen</td>
</tr>
</tbody>
</table>
1. Introduction

1.1 Peatland

In Finland, there are about 4.7 mill. ha of drained peatlands. Peatland drainage has been practiced in Finland for over one hundred years, but more systematic drainage to enhance the growth of tree stands on peatlands began in the early 20th century, being at its height in the 1960s and 1970s (Päivänen and Paavilainen 1996). From 1950 to 1990 the volume of growing stock increased by about 22% on peatlands, mainly because of drainage (Nuutinen et al. 2000). Most of those forests are nowadays well growing stands and are gradually reaching the commercial size. During the next two decades the proportion of cuttings on forestry-drained peatlands has been estimated to reach about 20% of all cuttings (Nuutinen et al. 2000).

Peat soil consists of more or less decomposed plant residues forming a histosol soil type. Nitrogen is mainly bound in the organic compounds and therefore not readily available for uptake by plants. There is also relatively frequently an insufficiency of potassium and phosphorus. The ecohydrology of peat, i.e. the variation in the amount and quality of water, affects peat fertility by regulating the structure of decomposer communities, the pathways of decomposition and the transportation of decomposition products and water soluble nutrients.

After drainage, a growing forest affects nutrient concentrations in surface peat in two opposite ways. The uptake of nutrients by trees and binding nutrients in biomass reduce the nutrient levels in peat. On the other hand, trees filter nutrients from rain water and especially from dry deposition. These nutrients are washed to the soil by rain, thus providing a nutrient input. Several factors such as the increasing uptake of nutrients by trees, post-drainage changes in hydrology and decomposition decrease the pH of the peat (Minkkinen et al. 1999).

The N concentration in peat varies between 5 mg g\(^{-1}\) and 30 mg g\(^{-1}\) of dry matter. Thus, compared to mineral soils, the N concentration in peatlands is high. However, most of the N in peat is associated with organic compounds and released very slowly during decomposition. The P concentration in peat varies between 0.2 mg g\(^{-1}\) and 3 mg g\(^{-1}\) of dry matter. P in peat is partly in an organic form and partly in inorganic compounds together with Al and Fe. In forestry-drained peatlands the balance between the main nutrients N, P and K is often the main factor regulating tree growth (Kaunisto and Pietiläinen 2003), because P and N are released slowly and K is involved in a tight biological cycle (Laiho et al. 1999, Westman and Laiho 2003). Forestry-drained peatland can be described as poor in nutrients that are easily accessible to both plants and soil microorganisms.

1.2 Fungi in peat soil

Microorganisms, especially fungi, have a major role in carbon and nutrient transformations in soil (Lindahl et al. 2002). Fungi are the dominant decomposer organisms in the acid soils of boreal regions and are assumed to have a more dominant role than bacteria in decomposer food webs (Williams and Crawford 1983, Frostegård and Báth 1996, Andersen et al. 2006). Microbial decomposers produce degradative enzymes and exude them outside the cell. After the enzymes have broken down plant tissue structures such as cellulose into simple compounds (in this case glucose), microorganisms can assimilate these compounds to gain energy and structural carbon. Decomposer fungi have been divided to five major functional groups according their specific enzymatic profiles and preferences for specific
1.3. Ectomycorrhiza

Mycorrhizal fungi remain one of the most poorly understood components of terrestrial carbon flow and nutrient cycling dynamics. Trees are almost completely dependent on their fungal symbionts for nutrient uptake and more than 95% of the root tips in boreal forest trees are ectomycorrhizal (Fransson et al. 2000). The ability of several ectomycorrhizal fungi to utilize organic nitrogen compounds in soil (Chalot and Brun 1998, Näsholm et al. 1998, Öhlund and Näsholm 2001) enables trees to compete with saprophytic microorganisms for organic nitrogen sources. The potential importance of organic nitrogen in plant nutrition has been demonstrated in many ecosystems (Lipson and Näsholm 2001) and it is presumably also an important nitrogen source for trees in drained peatlands, where the low rate of nitrogen mineralization decreases the availability of inorganic N sources. The greater the number of different nutrients or biochemically varied compounds that are in limited supply, the greater the number of potential nutrient niches (Erland and Taylor 2003) and fungal species differing in their ability to acquire specific nutrients from peat.

Ectomycorrhizal communities in boreal forests are richer and more complex than the host plant communities. The ECM community structure generally consists of a few abundant species that colonize 50–70% of the available fine roots, and of a large number less abundant species (Erland and Taylor 2003). The soil carbon to nitrogen (C-to-N) ratio, together with acidity, has been shown to determine the soil microbial community composition (Högberg et al. 2007). The variable demands of host-dependent ectomycorrhizal species for photosynthesized C may affect the fungal community structure maintained by trees (Saikkonen et al. 1999, Gehring and Whitham 2002, Kuikka et al. 2003, Korkama et al. 2007).

Ectomycorrhiza cause costs to their host trees. It is estimated that about 10–20% (Smith and Read 1997) or up to 50% (Simard et al. 2002) of the carbon assimilated by trees is allocated to the fungal symbionts. Fungi develop a thick sheath around the terminal branches of roots and are connected to an intercellular network of hyphae called the “Hartig net” in the root cortex layer. The surrounding soil is colonized by extramatrical mycelia, which may form as much as 80% of the total ECM fungal biomass in boreal forest soil (Wallander et al. 2001). A considerable proportion of photosynthesized C allocated to the tree roots is retained in the extramatrical fungal mycelium. A recent study postulated that ECM with well developed mantles release fewer soluble exudates to the surrounding soil than those with underdeveloped mantles (Priha et al. 1999b). This C efflux to the rhizosphere affects C and N cycling as well as the numbers and biomass of microorganisms in the mycorhizosphere (Bradley and Fyles 1995).
1.4 Nitrogen cycle

A limited availability of mineral nitrogen (N) and the accumulation of organically bound nutrients characterize ecosystems in which the dominant plants form ectomycorrhizae (Smith and Read 1997). A thick organic peat layer with large reserves of unmineralized organic nitrogen characterizes peatlands, and there is also relatively frequently a deficiency of potassium and phosphorus. In forest ecosystems, mineral nutrients play a major role in all the physiological and biochemical processes. For example, nutrient deficiency can lead to decreased leaf size and in long-lived conifer needles it can reduce net primary production long after a transient nutrient shortage (Bauer et al. 2000). Nutrient uptake from soil and nutrient release via decomposition should be in balance, keeping the nutrient cycle of the ecosystem tight. Any disturbance in one or more processes of the nutrient cycle has a long-lasting effect on forest functioning.

Nitrogen is the main nutrient consumed by trees and other plants. The largest pool of N-containing compounds consists of proteins, which are structural components of the cytoplasm and nucleus. Many proteins are enzymes having a vital function by catalyzing metabolic reactions. N is also needed in amino acids, amides, alkaloids and hormones. The proportion of forest floor nitrogen contained in living fungal biomass (including sporocarps) has been estimated by Markkola et al. (1995) to be about 4%, whereas Bååth and Söderström (1979) estimated the living and dead fungal biomass to contain 15–20% of total N in a boreal forest soil. Jonasson et al. (1996) estimated fungal biomass to contain 6–7% of N in arctic ecosystems.

In soil proteins, nucleic acids and other organic N compounds are broken down by a variety of microorganisms, fungi and bacteria. This process is driven by the need of heterotrophic organisms for carbon and leads to a decrease in the C-to-N ratio of substrates and the release of nutrients as inorganic ions. The uptake of inorganic N is often regarded as covering the main N requirements of plants. However, in boreal ecosystems, where most plants form symbiotic associations with fungi, the ability of mycorrhizal fungi to utilize organic forms of N (Näsholm et al. 1998) reduces the importance of inorganic N. In peatlands the majority of N is in a bound form and not directly available for uptake by plants. A minor proportion of the N is in a soluble form, most of which consists of dissolved organic N (DON) and low concentrations of inorganic N as ammonium (NH\textsubscript{4}\textsuperscript{+}) and nitrate (NO\textsubscript{3}\textsuperscript{-}).

In peatland research, considerable attention has been paid to the production of nitrous oxide, a potent greenhouse gas, while other microbial processes in N transformations have gained less attention. Slow mineralization of soil organic N has earlier been assumed to be the main factor limiting plant growth in boreal forests and in forestry-drained peatlands. However, the uptake of amino acids in field conditions by ectomycorrhizal trees, ericaceous shrubs and herbaceous plants has been demonstrated in the field (Näsholm et al. 1998, Näsholm and Persson 2001). Thus, the availability of DON, and not only the mineralized inorganic pool, affects the overall N supply for trees, especially in peatlands. In the model presented by Lindahl et al. (2002), mineralization and plant uptake of inorganic nutrients play a less important role. According to this model, fungi have a pivotal role as decomposers and as symbiotic partners. The main flow of soil nutrients occurs between saprotrophic and mycorrhizal fungal mycelia. Decomposer fungi have a major role in taking up nutrients from the litter. The loss of nutrients from fungal mycelia is often due to competitive interactions with other mycelia or the...
Transfer of nutrients from mycorrhizal fungi to the plant host. Nutrients are thought to be released in a mineral form to the soil solution as a consequence of carbon limitation.

Root exudates and mycorrhizal symbiosis affect the bacterial community in the rhizosphere (Grayston et al. 1996, Timonen et al. 1998). However, contradictory results have been presented concerning the effects of mycorrhizal roots on N mineralization. In some studies (Menyailo et al. 2002) an influence of trees on N mineralization has been reported, whereas in others (Priha et al. 1999a, Priha et al. 1999b, Priha and Smolander 1999) no influence has been observed. Vogt et al. (1991) suggested that the lack of nitrification in boreal forest soils was attributable to the greater competitive access of ectomycorrhiza to the limited N supply by utilizing N earlier in the process of mineralization of organic material than other microorganisms, or by leaching secondary chemicals produced by plants to inhibit N transformation processes.

Heterotrophic environments such as peat, with many different microsites, have generated a range of adaptive responses among peatland microorganisms (Williams and Crawford 1983). Temperature is one of the factors regulating the microbial process rates. Significant activities are still detected at temperatures as low as 4 °C (Williams and Crawford 1983). Another important factor affecting microbial processes is low pH (Alexander 1977), which promotes fungi to dominate bacteria. The fundamental role of fungi in C and nutrient cycling is emphasized in peatlands, where high amounts of C and nutrients are stored in peat, the pH is relatively low and the nutrient uptake of trees is highly dependent on mycorrhiza because of the low mineralization rate.

2. Aims

The availability of N, P and K affect the productivity of forestry-drained peatlands. The availability of these mostly organically bound elements for trees depends on their amounts in peat and their mineralization rate. The mineralization is affected by the soil microbial community and its activity, which in turn is controlled by environmental factors like temperature, moisture and acidity. In peatlands with low pH and high C-to-N ratio fungi are assumed to be the main decomposers. On the whole fungi have a pivotal role in boreal ecosystems, as they act as decomposers and also form symbiotic associations with plants. The mycorrhizae associated with tree roots have a special role among the soil microorganisms in supplying nutrients for trees. Focusing on this important role of the fungi, the aim of this thesis was to study fungal biomass and community structure and their relation to soil N status and availability on drained peatland forests. The growth of ectomycorrhizal fungi in drained peatland forest with variable P and K availability was also investigated. In addition it was studied how the composition and activity of soil microorganisms and peat N status are related to N mineralization and concentrations of dissolved N compounds.

3. Materials and methods

3.1 Field sites

Four field sites were investigated (Fig. 1). Three of these (Alkkia, Haapua and Hepokangas), are located in Karvia, Pudasjärvi and Taivalkoski, respectively, in different temperature sum regions (Table 1). They are forestry-drained peatlands representing a natural gradient in the total N concentration (7–27 mg g⁻¹ DW in peat).
Table 1. General characteristics of the study sites.

<table>
<thead>
<tr>
<th>Site</th>
<th>Hepokangas</th>
<th>Haapua</th>
<th>Alkkia</th>
<th>Liesineva</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location</td>
<td>65° 32' N</td>
<td>65° 22' N</td>
<td>62° 9' N</td>
<td>61° 59' N</td>
</tr>
<tr>
<td></td>
<td>28° 25' E</td>
<td>27° 40' E</td>
<td>22° 52' E</td>
<td>23° 15' E</td>
</tr>
<tr>
<td>Altitude</td>
<td>240 m</td>
<td>190 m</td>
<td>175 m</td>
<td>155 m</td>
</tr>
<tr>
<td>Year of drainage</td>
<td>1962</td>
<td>1965</td>
<td>1970</td>
<td>1934</td>
</tr>
<tr>
<td>Mean temperature sum</td>
<td>850 d.d.</td>
<td>950 d.d.</td>
<td>1100 d.d.</td>
<td>1200 d.d.</td>
</tr>
<tr>
<td>Basic fertilization</td>
<td>1963; PK</td>
<td>1968; PK</td>
<td>1972; PK</td>
<td>1958; PK</td>
</tr>
<tr>
<td>Refertilization</td>
<td>1974; 0, PK</td>
<td>1978; 0, PK</td>
<td>1982; 0, PK</td>
<td>1962, 1976, 1989, 1994; 0, PK</td>
</tr>
</tbody>
</table>

1) Threshold value + 5 °C. Measured average d.d. values from 2000 – 2005.
The sites were drained for forestry in the 1960s and at the beginning of 1970s. In Karvia the peatland was planted with Scots pine (Pinus sylvestris L.). In Pudasjärvi and Taivalkoski the peatlands have natural tree stands, mainly Scots pine, but also downy birch (Betula pubescens Ehrh.) and Norway spruce (Picea abies (L.) Karst.). The fourth drained peatland, forested with Scots pine (Liesineva), is located in Parkano and represents an unbalanced PK nutrient status in the pine needles. This peatland was drained in 1934–36, PK-fertilized in 1958 and 1962 and refertilized in 1976, 1989 and 1994. The site type is a Vaccinium vitis-idaea transformed peatland site. The age of trees is 80 years (Kaunisto 1989). The study plots were selected from the area to represent four nutrient statuses of Scots pines in terms of P and K nutrition: sufficient P and sufficient K (P+K+), sufficient P and deficient K (P+K-), deficient P and sufficient K (P-K+) and deficient P and deficient K (P-K-).

3.2. Laboratory experiments

Two experiments (Experiments I and II in article IV) were performed with inoculated and non-inoculated Scots pine seedlings in five peat soils with different total nitrogen concentrations (12–27 mg g⁻¹ DW). These soils were taken from one of the field sites (Alkkia) from the acrotelm of drained peatland. In Experiment I the seedlings were inoculated with ECM isolates Lactarius rufus (Scop. ex Fr.) Fr., Suillus variegatus (Fr.) O. Kuntze and Piloderma croceum Erikss. and Hjortst., and in Experiment II three S. variegates isolates were used. Non-inoculated seedlings served as controls.

3.3 Biological and chemical analyses

Analyses and methods used in this thesis are listed in Table 2. Detailed descriptions of the procedures are provided in articles I–IV; here, only a short overview of the analyses is presented.

3.3.1 Chemical analyses

A CHN analyzer (Leco 2000) was used to determine total N and C concentrations of the peat and pine needles. The degree of decomposition of the peat was determined by the von Post method (von Post 1922) using a 10-class scale (1 = not decomposed, 10 = totally decomposed). The pH of the soil was measured after the sample had been suspended overnight in deionised water (1:5).

Dissolved N and C were determined by extracting 10 g of fresh soil sample with 100 ml of 0.5 M K₂SO₄ (2 h shaking), and filtering the suspension first through filter paper and then through a 0.45 μm membrane filter (Williams et al. 1995). Total dissolved N (TDN), NH₄-N and (NO₂⁻+NO₃⁻)-N in the extracts were determined using a flow injection analyzer (FIA Star 5020, Tecator) as described by Williams et al. (1995). DON in the extracts was obtained by subtracting the NH₄-N and (NO₂⁻+NO₃⁻)-N concentrations from TDN. Dissolved organic carbon (DOC) in the extract was analyzed with a TOC analyzer (Schimadzu 5000). The moisture contents of the samples (dried overnight at 105 °C) were also determined.

When taking the samples from peat during the growing season, the water content in the peat varies depending on the amount of water precipitation between the sampling times. This may affect the amount of dissolved N in the peat. Furthermore, we were interested in determining how much dissolved N is available in peat water without using K₂SO₄ for extraction. Therefore, in addition to the analysis described in paper II, we also analysed DIN and DON from the water in peat during the growing season of 1999. We used this data to estimate the proportion of DIN and DON in the K₂SO₄ extract compared to the water in peat and to examine whether
this proportion remains stable during the growing season. For this purpose, water was extruded from the peat samples by pressing 50 ml of peat manually in a 60 ml syringe for one hour using a self-built screwing device. The amount of extruded water was measured and diluted to 50 ml with distilled water. The suspension was treated and analyzed similarly to the $\text{K}_2\text{SO}_4$ extract.
3.3.2 Microbial biomass and community structure

The ergosterol concentration in the peat, in the root tips of Scots pine and in the mesh bags (Wallander et al. 2001) was determined by a method modified from Nylund and Wallander (1992), which consists of ethanol extraction, saponification with KOH and further extraction in pentane. This was followed by HPLC analysis with a LiCrospher 100 RP-18 column, using methanol as the eluent, and a UV-VIS detector (Merck-Hitachi) (wavelength 280 nm).

Phospholipid fatty acid (PLFA) extraction was carried out as described by Frostegård et al. (1993). Fatty acid methyl esters were detected by gas chromatography as described by Pennanen et al. (1999). The quantity of PLFAs considered to be predominantly of bacterial origin (i15:0, a15:0, 15:0, i16:0, 16:1ω9, 16:1ω7t, i17:0, a17:0, 17:0, cy17:0, 18:1ω7 and cy19:0) was used as an index of the bacterial biomass (Frostegård and Bååth, 1996), and the quantity of PLFAs 18:2ω6,9 and 18:1ω9 (Bååth 2003) served as an indicator of fungal biomass. The ratio of fungal PLFA 18:2ω6,9 (PLFAfung) to bacterial PLFA (PLFAbact) (III) or the ratio of ergosterol to bacterial PLFA (I) was used as an index of the ratio of the fungal to bacterial biomass.

Microbial biomass carbon (Cmic) and nitrogen (Nmic) were determined from fresh peat samples with the fumigation-extraction (FE) method (Brookes et al. 1985, Smolander et al. 1994). Nmic was calculated as the difference between fumigated and fresh concentrations of TDN multiplied by 0.54 (Brookes et al. 1985). Cmic was obtained by determining the difference between the DOC concentration of the fresh and fumigated peat sample using the recovery factor of 0.45 derived for peat soils by Sparling et al. (1990).

Biolog MT MicroPlates (Biolog Inc. Hayward; CA) were used for community level physiological profile (CLPP) analysis of the soil microbial community, adding 23 different organic N compounds as substrates (Campbell et al. 1997).

The structure of the fungal community in the peat was investigated by means of denaturing gradient gel electrophoresis (DGGE) and sequence analysis of the internal transcribed spacer (ITS) fragments. For peat DNA extraction, the manufacturer’s protocol for the UltraClean Soil DNA Isolation Kit, (MoBio Laboratories Inc., Carlsbad, California; USA) was used with slight modifications. The DNA was further purified using the procedure described by Vainio et al. (1998) and followed by PVPP purification as described by Pennanen et al. (2004). Fungal ITS regions were PCR-amplified using the GC-clamped ITS1F (Gardes and Bruns 1993) and ITS2 primers (White et al. 1990). Denaturing gradient gel electrophoresis (DGGE) analysis and sequencing were carried out as described by Korkama et al. (2007).

3.3.3 Nitrogen transformations

Net N transformations were measured in the field. Two replicate samples were taken to determine the initial inorganic N and TDN concentrations, and another one for field incubation and inorganic N and TDN analyses after incubation. Net ammonification and nitrification were calculated by subtracting the initial NH4+ and NO3− concentrations from the final NH4+ and NO3− concentrations at the end of the incubation period. Negative values for this difference are referred to as net immobilization (Williams 1992). The gross N mineralization and immobilization rates were measured in the laboratory and in the field using a 15N isotopic dilution technique (Davidson et al. 1991).

3.4 Statistical analyses

Statistical tests and multivariate analyses applied in this thesis are listed in Table 3.
All the statistical tests were performed using SPSS and multivariate analyses using PC-ORD.

In article I, Spearman’s correlation test was used to evaluate the effects of peat total N concentrations on the measured variables within the studied sites. The site effect was tested by ANOVA or, in the case of heterogenic variances, by the Kruskall-Wallis test followed by LSD or Dunnett’s T3 as a post hoc test. To determine the effects of peat N on the bacterial community structure, the mol percent values of individual PLFAs other than 18:2ω6,9 were subjected to principal component analysis (PCA). The differences in bacterial community structures between study sites were further tested by the Kruskall-Wallis test and the Dunnett T3 as a post hoc test. The binary matrix, which was made from DGGE gels, was analyzed by non-metric multidimensional scaling (NMS) in order to identify differences in the fungal community between the study sites.

In article II, data on seasonal fluctuation and the effects of the N gradient and fertilization on dissolved N and C concentrations, ergosterol and Cmic and Nmic were tested with analysis for repeated measures of the General Linear Model (GLM) and by linear regression analysis. In cases where the assumption of sphericity was violated (ergosterol), the Greenhouse-Geisser test was used. The N gradient was set as a covariate and fertilization as a between-subject factor when testing the differences between years, sampling dates and the interaction of year x date. The average of five subsamples from each plot was used to analyze the mineralization data. A linear regression model was used to test the influence of the N gradient on net or gross mineralization, and one-way ANOVA to test the differences between years and fertilizations.

In article III the effects of forest nutrient status (needle P and K concentration), fertilizer (sand, apatite, biotite, Rauta-PK™ or test fertilizer) and incubation period (4

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**Table 3.** Statistical tests and multivariate analyses used in the articles of this thesis.

<table>
<thead>
<tr>
<th>Data analyses</th>
<th>Article</th>
</tr>
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<tbody>
<tr>
<td>Spearman correlation test</td>
<td>I</td>
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<tr>
<td>General Linear Model (GLM); ANOVA</td>
<td>I, II, III, IV</td>
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<td>Repeated measurements ANOVA</td>
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<td>Regression analysis</td>
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<td>Greenhouse-Geisser test</td>
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<td>Mann-Whitney U-test</td>
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<td>LSD</td>
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<td>Dunnet's T3</td>
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<td>Contrast test</td>
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<td>Bonferroni test</td>
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<tr>
<td>Principal component analysis (PCA)</td>
<td>I, IV</td>
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<td>Non-metric multidimensional scaling (NMS)</td>
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</tbody>
</table>
or 16 months) on measured variables were analyzed in a full factorial ANOVA using a contrast test as a post hoc test. Interaction terms are reported when significant. Because of the heterogeneity of variances, log-transformation was used with the PLFA cy19:0, and the Kruskall-Wallis test with the PLFA fungal-to-bacterial ratio. To determine whether the nutrient status of the trees influenced the concentration of REEs, P, K and Fe in the mycorrhizal roots, one-way ANOVA was performed for each fertilizer. To ascertain whether the P or K status of the peat had any influence on the effect of fertilizer addition on EMM growth, regression analysis was performed for each fertilizer- and sand-containing mesh bag from both harvests.

In the article IV the differences between the N concentrations in the peat and between the different fungal inocula were subjected to GLM analysis and the Bonferroni test as a post hoc test. In cases with nonequal variances the non-parametric Kruskal-Wallis test and independent sample T-test were used to test differences between the means. The community level physiological profile (CLPP) was examined using PCA. The eigenvalues of the data were further analyzed by GLM.

4. Results and discussion

4.1 Fungal community structure

4.1.1 Fungal community structure on forestry-drained peatlands

The highest proportion of sequenced DGGE bands from drained peatland were most closely related to the fungi belonging to Basidiomycetes, while 14% belonged to the Ascomycetes and 10% to the Zygomycetes (I). The majority of the sequences (66%) related to the Ascomycetes were isolated from the northernmost area (I). Lower ergosterol concentrations in roots were observed in northern experimental areas than in the south (I, III), indicating a lower biomass of ECM fungi in those sites.

The sequencing results emphasized the importance of mycorrhizal fungi in the drained peatlands, because only two sequences out of sixteen matched with a saprotrophic fungus (I). According to Thormann (2006a), Ascomycetes and Zygomycetes are the fungi most frequently isolated from pristine peatland soils. A higher proportion of Ascomycetes than Basidiomycetes has been found in both moorlands (Anderson et al. 2003) and an uncut bog (Dickinson and Dooley 1967). Some other studies from boreal peatlands (Jaatinen et al. 2008) have emphasized the role of mycorrhizal fungi in biogeochemical cycles to be as important as in boreal forest ecosystems (Lindahl et al. 2002, Lindahl et al. 2007). A survey of the aboveground fruit bodies of fungi in peatlands (Salo 1980) revealed that the fungal species typical of forests on mineral soils become more common in peatlands after drainage due to improved tree growth and accelerated decomposition of the peat. The observed dominance of mycorrhizal fungi were presumed because of the afforestation of the studied sites, and the transformation of the vegetation in studied sites after drainage, more closely resembling the field layer vegetation on the mineral soils.

According to the fungal community structure assessed by PCR-DGGE, the proportion of Ascomycetes in the fungal community of the peat increased in the northernmost site. This result, together with lower ergosterol concentrations in the roots in the northern study site, suggests a decreasing abundance of ECM species, which are mainly Basidiomycetes (I). Lindahl et al. (2007) reported a shift in the fungal community from saprotrophic dominance to mycorrhizal dominance with decreasing C-to-N ratios in vertical soil profiles. These findings may reflect a
higher proportion of saprotrophic fungal species in less decomposed peat with a higher C-to-N ratio. In more decomposed peat the saprotrophs will be replaced by mycorrhizal fungi. The reasons for the differences in the role of saprophytic and mycorrhizal fungi in surface litter layer and in more decomposed organic matter in deeper soil layers reported by Lindahl et al. (2007) may be partly similar to the differences found between sites in the present study (I). Thus, in forestry-drained peatlands the state of succession or degree of decomposition in peat after drainage may determine the proportion of mycorrhizal species compared to saprotrophic species in the fungal community.

The field layer vegetation in boreal forests is mainly dominated by plants belonging to the Ericaceae family. This family forms ericoid mycorrhiza associated with fungi, which mainly belong to Ascomycetes. Thus, the higher proportion of Ascomycetes in the northernmost site may reflect an increase in dwarf shrub vegetation, which is common during succession following the drainage of these types of pine mires (Laine and Vasander 1996).

The observed lower biomass of ECM fungi in the northern site compared to the southern site (I) may reflect either a limited number of ECM fungal species that are adapted to the biotic and abiotic factors in these types of ecological niches, or the host plants may not maintain as large a fungal biomass in the northern site as in the southern sites because of the lower net photosynthesis during the growing season. However, we observed a higher richness and diversity of the ITS fragments isolated from peat at the northern site (I), which means a higher number of fungal species in this site. The fungal community structure was also different in the northern site (I). The shift in the fungal community structure from a mycorrhizal-dominated community at low C-to-N ratio sites to saprophytic dominated at high C-to-N ratio sites was found here. However, the richness and diversity index were not affected by the peat N concentration within the sites, which suggests that the differences in fungal community structure between the sites were not only due to the differences in the peat N concentrations of the sites but were probably also related to other factors, such as the decomposition degree of the peat or other temperature-related factors.

4.1.2 Effect of nitrogen concentration in peat

According to a few comprehensive reviews on the responses of ectomycorrhizal fungi to nitrogen fertilization and deposition (Wallenda and Kottke 1998, Lilleskov and Bruns 2001, Cudlin et al. 2007), an increased soil N concentration often correlates with changes in a number of ECM community attributes, such as decreased sporocarp production, lower community diversity and shifts in the relative abundance of ECM community members. In this investigation the variation in N concentration in peat was caused by natural differences in peat fertility rather than by fertilization or deposition. The observed response in N deposition studies may reflect changes in the amounts of inorganic N compounds, not in the total N concentration as such.

No clear relationship was observed between the peat N concentration and the fungal community structure assessed by PCR-DGGE, diversity or abundance of fungal ITS fragments isolated from peat samples, as expected (I), although the proportion of fungal biomass of total microbial biomass did differ between various levels of nitrogen availability in peat (I, II). It was nevertheless notable that at the northernmost site the fungal community structure differed from that in the south, responding possibly to changes in the degree of decomposition of the peat (I). The DIN-to-DON ratio was lower in the northern site, being 0.25 in Hepokangas as
compared to 0.57 in Alkkia (I). According to Lilleskov et al. (2002), N availability may be a major factor structuring ECM fungal communities. Differences in the fungal community structure which depend on the decomposition degree of the peat may also be due to differences in the carbon and nitrogen sources available for the fungi, as suggested by Lindahl et al. (2007). The different preferences of the mycorrhizal fungi for inorganic and organic forms of N (Abuzinadah and Read 1986, Sarjala 1999) may affect the frequency of the fungal species in the peatlands, and this may be one of the reasons for the different community at the northernmost site. Read and Perez-Moreno (2003) postulated that differences in the thermal optimum for the activity of the protease enzyme in the same genus favours “protein fungi” in environments where a low temperature, acidity and poor resource quality combine to inhibit N mineralization. On the other hand, differences in the photosynthate demands of morphologically and functionally variable fungal species may adjust the fungal community structure according to the carbon resources of the host plant (Saikkonen et al. 1999, Gehring and Whitham 2002), and thus changes in the fungal community may lead to less C demanding species becoming more common in the northernmost site.

4.2 Microbial biomass

4.2.1 Effect of nutrient status

The biomarkers of microbial biomass, total PLFAs, microbial biomass C and N in peat, were correlated with the peat N concentration (I, II). The direction of correlations differed between study sites and between parameters. The response of microbial biomass to the peat N level may reflect the N limitation of the microbial community. Some indications of a decrease in fungal biomass were observed at a higher peat N concentration (I, II). In the coniferous forest humus layer the relative amount of fungi has been shown to decrease and that of bacteria to increase with increasing fertility of the site (Pennanen et al. 1999). Accordingly, the relative importance of bacteria and fungi in the C and N fluxes may vary depending on the peat N concentration. Jaatinen et al. (2007) found the strongest gradient in the PLFA data to reflect changes in the site nutrient level. In their study the proportional bacterial biomass appeared to increase from an oligotrophic to a mesotrophic fen, especially in pristine plots. Although the characteristics associated with site fertility have been shown to affect the microbial biomass in both mineral soils (Pennanen et al. 1999) and in peatlands (Jaatinen et al. 2007), little has previously been known about the influence of K and P availability in the peat on soil microorganisms.

P and/or K deficiency of the trees increased PLFA 18:2ω6,9 in mesh bags, indicating that PK deficiency in drained peatlands stimulated EMM growth by increasing the allocation of C to EMM and thus enhancing the absorption area of tree roots (III). A similar trend was seen in the ergosterol concentration, although not as clear. A slight increase was also observed in the biomass of ECM in the case of P deficiency (III). The stimulating effect of K deficiency on EMM biomass was particularly noted in this study and emphasizes the importance of field experiments to obtain realistic information on the effects of extreme circumstances such as nutrient deficiency on soil microorganisms.

4.2.2 Fungal to bacteria ratio

Differences in fungal, bacterial and total microbial biomass biomarkers were found between sites as well as within sites along the N gradient (I, II). These results imply that in addition to the peat total N concentration, other factors also affect the microbial biomass. Moisture,
oxygen availability, temperature, acidity, substrate quality and the nutrient status of ecosystems have been found to affect the microbial biomass in forest soils (Williams and Crawford 1983, Pennanen et al. 1999, Högberg et al. 2007), and are probably also important factors in peatlands.

In the southern sites, with better fertility and a higher temperature sum, the total microbial biomass as well as fungal and bacterial biomass in the peat were lower than in the northern site (I). The bacterial biomass as a proportion of the total microbial biomass was still higher in the southern sites (I). This may be explained by the increasing importance of fungi in the decomposition of peat at lower pH levels, whereas the importance of bacteria is greater in more aerobic and neutral peat, such as in spruce mires and transformed drained peatlands. Scheffer et al. (2001) studied decomposition and mineralization rates in Sphagnum- and Carex-dominated peatlands and concluded that nutrient availability and adaptation of the microbial community to nutritional and other environmental conditions may be the main regulators of C and nutrient transformations in these peatlands. According to our results, peat fertility via N limitation of the microbial community and the nutritional status of trees via C allocation to roots affected the fungal biomass in peat, and thus the relative importance of bacteria and fungi in the C and N fluxes may vary depending on the peat N concentration.

4.3 N mineralization

4.3.1 Effect of the peat N concentration

Gross N mineralization did not vary, but the net N mineralization rate increased significantly along the N gradient in field (II) and in the laboratory experiments (IV). The gross N immobilization also increased significantly when the peat N concentration increased (II). Net nitrification was only observed in some cases (II, IV). The net N mineralization rate in the field and laboratory (II, IV) seemed to be at the same level or slightly higher than reported from the organic layer from mineral soils (Persson et al. 2000, Merilä et al. 2002). According to Bengtsson et al. (2003), the difference in gross N immobilization and mineralization rates between soils with different C-to-N ratios are more related to the respiration rate and ATP content, which are indices of general microbial activity, than to the C-to-N ratio of the substrates. The present results are in accordance with these, and gross N mineralization thus more closely reflects the activity of the microbial community, showing no difference at various N levels, whereas net N mineralization reflects more the quality of the decomposed substrate, and thus a greater amount of inorganic N was released at higher peat N concentrations.

A dry period decreased microbial activity and thus affected the availability of mineral N (II). Drained peatlands thus show similar periodicity to mineral soils in relation to weather conditions. Fungi did not seem to be as sensitive to drought as the bacterial community (II), which is in accordance with other studies (Lund and Goksoyr 1980). Long-living fungi can translocate nutrients and water from one part of the mycelium to another, whereas bacteria and ephemeral microfungi lack this capability, and thus they act primarily on substrates having a high C-to-N ratio and are highly dependent on moist conditions (Carlile et al. 2001).

4.3.2 Effect of ectomycorrhiza on N transformations

All the studied ECM isolates stimulated ammonification and affected DON accumulation or degradation (IV). In the release of NH$_4^+$ and DON, significant interaction between fungi and the peat N concentration was found (IV). The strongest
stimulating effect on the ammonification was observed with *Piloderma croceum*. *Lactarius rufus* and *P. croceum* had an opposite effect on DON accumulation, so with *L. rufus* the DON concentration increased during incubation, which may indicate a minor uptake of DON by this fungus, inhibition of mineralization activity compared to other studied fungi or an increase in the decomposition activity. In earlier studies an influence of trees on N mineralization has been reported (Menyailo et al. 2002), or in other cases no influence has been observed in coniferous forest (Priha et al. 1999a, Priha et al. 1999b, Priha and Smolander 1999). The effects of ECM on N transformation seen in this study may be related to changes in the bacterial community of the rhizosphere, which has been shown to be affected by root exudates and mycorrhizal symbiosis (Grayston et al. 1996, Timonen et al. 1998). ECM with a well-developed mantle release fewer soluble exudates to the rhizosphere than those with underdeveloped mantles (Priha et al. 1999b), which in this study may partly explain the differences in effects of N transformation between species differing in mantle thickness and the amount of external mycelia.

The biomass of seedlings in the laboratory experiment (IV) was smaller when the peat N concentration was over 21.7 mg g\(^{-1}\) of dry matter than under it, except when inoculated with *Piloderma croceum*. The dry mass of the roots, stems and needles varied depending on the fungal inoculum, suggesting that carbon allocation in the host plant is strongly determined by the fungal symbiont (IV). Seedlings inoculated with *Piloderma croceum* had a higher root-to-shoot ratio (1.09) than if the symbiont was *Lactarius rufus* (0.88) or *Suillus variegatus* (0.76). This effect seemed to be most clear on medium N levels. In addition to the fungal symbiont the nutritional status of trees affected their C allocation, as we observed that PK deficiency in drained peatlands may increase the allocation of C to EMM (III).

According to our laboratory experiment (IV), inoculation of pine seedlings by ECM fungi had a stronger effect than the N concentration of the peat on the growth of Scots pine seedlings. ECM fungi have two overall effects on the N cycle: they increase the supply of N to plants and reduce the supply of both N and C to saprotrophs (Leake et al. 2002). Mycorrhizas can alter N cycling and contribute to plant-litter feedback by efficiently accessing inorganic and organic N, and by actively releasing N from organic matter (Zeller et al. 2001). It is well-established that ECM fungi can utilize organic forms of nitrogen (Chalot and Brun 1998, Näsholm et al. 1998, Näsholm and Persson 2001) and now we have shown that they also affect the N mineralization processes. Utilization of organic N by ECM fungi short-circuits the conventional N cycle, reduces the supply of N to the mineralization pathway and increases the competition for mineral N between saprotrophic and mycorrhizal mycelia (Leake et al. 2002). Hence, the availability of N for tree growth is not only dependent on the mineralization rate and availability of inorganic N, but also on the fungal community structure in peat and the ECM species that occupies the peat at different fertility levels.

### 4.4 Dissolved N compounds

Dissolved N was mainly composed of NH\(_4\)\(^+\) and DON. NO\(_3\)\(^-\) concentrations were usually very low in the peat, and often no NO\(_3\)\(^-\) was detected (I, II). In laboratory incubation (IV) with peats of five different N concentrations from 12 to 27 mg g\(^{-1}\) of dry matter the NO\(_3\)\(^-\) concentrations were also usually very low or below the detection limit of the analytical equipment.

The availability of dissolved N forms in peat fluctuated during the growing season in forestry-drained peatlands, but without a consistent pattern (II).
The highest ammonium concentration was ten times higher than the lowest during the growing season (II). The NH$_4^+$ and DON concentrations in peat water (water extruded manually from the peat) fluctuated significantly between sampling dates ($p = 0.028$ and $0.022$, respectively) during the 1999 growing season (Fig. 2). They followed quite similar patterns to the K$_2$SO$_4$ extract (II), except that the DON concentrations increased in the peat water in the autumn (Fig. 2), although DON in the K$_2$SO$_4$ extract remained at a relatively constant level from June to October (II). According to Casals et al. (1995), most of the organic N in the soil leachate in pine forests originates from the freshly-fallen litter and partially decomposed litter layers, which may also hold in the peatlands, and explain the increased DON concentrations in the peat water in the autumn in our study.

The total N concentration of peat did not affect the dissolved N concentrations

![Figure 2](image-url)

**Figure 2.** Seasonal fluctuation in peat water NH$_4^+$, N and DON concentrations in experimental plots in 1999. (n = 15)
in peat water as it affected K$_2$SO$_4$ extracted TDN concentrations. The amount of dissolved N in peat water as a proportion of the total dissolved N (sum of peat water and K$_2$SO$_4$ extract) was 23%, and the maximum was 81% in October. The amount of dissolved N in peat water as a proportion of the total concentration of peat N varied between 0.2 to 0.8% (concentration of N in peat water less than 0.13 mg g$^{-1}$ dry weight peat).

In the studied plots, DON accounted for 75% of dissolved N in peat. The average figure varied between 33–100%, being higher in the north (81%) than in the south (66%). Further, extracted DON formed a greater proportion of total peat N in the north, being 0.3% in Alkkia, 0.5% in Haapua and 0.7% in Hepokangas (I). The amount of DON in soil is controlled by many processes, including atmospheric deposition (Solinger et al. 200), root exudation (Chapman et al. 200), ectomycorrhizal fungi (IV), organic matter decomposition (Persson et al. 2000), plant and microbial uptake (Näsholm et al. 1998) and its mineralization to inorganic N. The stage of decomposition of the peat has not been shown to affect the release of DON in peatlands (Kalbitz and Geyer 2002). The high proportion of DON, especially in the north, emphasizes the importance of mycorrhizal fungi in the uptake of N by plants.

4.5 Specific methodological questions challenging the microbial studies on peatlands

Peatlands imposed some challenges in sampling and analysis compared to mineral soils. The soil “layers” are not distinctive in peatlands, and the soil samples are usually divided into layers according to the distance from peat surface. However, in Finland two different datum lines have been used in forestry-drained peatlands: the line between living and dead plant material and that between “raw humus” and old peat. Raw humus is a layer composed of new fresh litter and humus developed after draining. This difference in layers often complicates the comparison of results from different studies. Another difficulty is taking a volumetric sample, especially from loose, poorly decomposed peat, which easily becomes more densely packed when taking a sample with an auger. A volumetric sample is also difficult to take from very wet peat, which will not keep its form during the process, and water also easily drains from the sample. The water content of the peat within a site also varies depending on water precipitation and the groundwater level. If one needs to know the total amounts of N in peat, including the water, the N in the lost water from a certain volume should also be known. However, in the present study the concentration of N in peat water during the growing season was rather small, being less than 1% of the total N in peat, which suggests that the water loss from the sample is not a significant source of error.

Some methods may contain sources of bias due to varying peat decomposition stages and the plant species from which the peat originates. We observed differences in results using two methods to estimate fungal biomass, ergosterol and PLFA 18:2ω6,9. In one case, when measuring fungal biomass from ingrowth mesh bags (III), the results from the two methods were parallel, but in the other case, when measured from peat (I), they were partly different. One possible reason for the difference in results was that the 18:2ω6,9 in our samples may have partly derived from the shoots and residues of Sphagnum mosses (Sundh et al. 1997), which are more abundant in peat with low H values compared to peat with high H values (Hartman et al. 2001). Zelles (1997) has reported PLFA 18:2ω6,9 from plant tissues, which suggests that poorly decomposed plant material in the peat might be a contaminating source for the PLFA biomarker.

The varying peat decomposition stage
set challenges for the methods of purifying DNA from peat samples. Although the DNA amplification in PCR was relatively successful in our study (I), most of the samples that did not amplify had high H values. Well-decomposed peat may contain more compounds inhibiting amplification, which cannot be removed by ordinary DNA purification procedures.

5. Conclusions

This thesis demonstrates the importance of fungi in N transformation in forestry-drained peatlands and N uptake under an unbalanced nutrient status. Investigations into the relationship between the peat fertility level and soil fungal community structure and biomass showed that the peat N concentration did not alone determine the community structure or biomass. Among other factors the nutritional status of trees affected the biomass of fungal symbionts and the biomass of their external mycelia. The peat N concentration did not affect the within-site soil fungal community structure or the number of ITS fragments per plot (richness). However, changes in relative abundance of fungal species along the N gradient cannot be excluded. The biomass of ectomycorrhizal fungi in relation to other soil fungi appeared to decrease in the northern site whereas the number of fungal species appeared to increase. The changes in fungal community structure corresponded with changes in the peat degree of decomposition and DON concentrations between sites, possibly reflecting the changes in the availability of different N forms for plants.

In this thesis it has been shown that besides the previously known fact that fungi can utilize organic N forms, they can also alter N mineralization processes. Nitrification was rarely observed in forestry-drained peatlands, whereas ammonification was at the same level as in coniferous forests on mineral soils. A significant interaction (fungus x peat N concentration) was found concerning NH$_4^+$ and DON transformations. The amount of DON as a proportion of both dissolved N and the peat total N concentration was higher in the northern site. In addition to ectomycorrhizal fungal species, atmospheric deposition, root exudation, organic matter decomposition, plant and microbial uptake and mineralization to inorganic N determine the overall supply of DON in soil. Thus, not only the availability of dissolved N affected tree growth, but the fungal community structure in peat and the fungal symbiont of the trees also had an impact.

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7. References


to the quantity and quality of substrate inputs. – Soil Biology & Biochemistry 36: 841–848.


CORRECTIONS

Article II, p. 1051 Figure 3. The $N_{mic}$ part of the figure was corrected.