

*Oili Tarvainen*

SCOTS PINE AND ITS  
ECTOMYCORRHIZAL  
SYMBIONTS UNDER  
CHRONIC LOW-LEVEL  
URBAN POLLUTION—  
RESPONSES AND  
RESTORATION

FACULTY OF SCIENCE,  
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*OILI TARVAINEN*

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RESPONSES AND RESTORATION**

Academic dissertation to be presented with the assent of the Faculty of Science of the University of Oulu for public defence in Kuusamonsali (Auditorium YB210), Linnanmaa, on 18 December 2009, at 12 noon

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*Abstract*

Boreal urban forests are becoming more and more fragmented and, at the same time, are exposed to low-level but long-term nitrogen and sulphur deposition. Natural mid-boreal forests are dominated by few tree and shrub species, while herbs and grasses are rare. Soils in mid-boreal forests are rich in ectomycorrhizal (ECM) fungi, forming a symbiotic relationship with forest trees, which is important for the nutrient cycle especially in nutrient-poor ecosystems.

Aims of this thesis were 1) to study differences between mid-boreal urban and rural forests in composition of macrofungi and structure of plant communities, 2) to explore whether responses of Scots pine seedlings to excess N are mediated via soil or via air, and 3) to study if partial removal of vegetation and humus layer alleviates the adverse effects of excess N on plant and fungal communities, and promotes performance and regeneration of Scots pine in urban forests. The growth responses of seedlings and their mycorrhizal colonization were studied in both field and greenhouse experiments. Peroxidase (POD) activity was used as a root stress indicator.

Urban forests were poor in number of fruiting ECM fungal species, but rich in herbs and grasses in the field layer, as compared to rural forests. These differences were thought to result from changes in soil properties. ECM fungal colonization in Scots pine roots did not respond significantly to high nitrogen load in soil, but high root POD activity indicated changes in root physiology.

ECM macrofungal diversity in urban forests was not markedly increased during a five-year survey after a partial humus removal treatment. On the other hand, fruiting of *Cortinarius semisanguineus* is a positive response to the treatment. Scots pine seedling emergence was poor due to rapid revegetation at the urban sites, but the treatment promoted both root and shoot growth of planted Scots pine seedlings in urban forests. Decreased root POD activity in Scots pine seedlings in the humus removal treatment possibly indicates lowered root stress.

Soil manipulations may result in a risk of nutrient leakage, and a risk of invasion by non-typical plants. Also, small stand size with high recreation pressure causes a risk for tree regeneration in urban forest stands. These risks need to be considered when planning management of urban coniferous forests.

*Keywords:* boreal forests, ectomycorrhizae, environmental pollution, environmental restoration, fungi, humus, nitrogen deposition, phosphorus, *Pinus sylvestris*, seedlings, soil, sporocarp production, sulphur deposition, urban environments, vegetation changes



*Ei se ole tärkeää, kuinka pitkälle olemme päässeet, vaan se kuinka lähelle olemme tulleet toisiamme. — H. Hesse*





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## Abbreviations

ANOVA	Analysis of variance
C:N	Carbon-to-nitrogen (ratio)
DON	Dissolved organic nitrogen
ECM	Ectomycorrhiza
HPLC	High-performance liquid chromatography
OM	Organic matter
PCA	Principal component analysis
PC-ORD	Software for ordination analysis
POD	Peroxidase
sp.	Species
spp.	Several species belonging to the same genus
SPSS	Software for statistics
TDN	Total dissolved nitrogen
TDP	Total dissolved phosphorus



## List of original articles

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:

- I Tarvainen O, Markkola AM & Strömmer R (2003) Diversity of macrofungi and plants in Scots pine forests along an urban pollution gradient. *Basic and Applied Ecology* 4: 547–556. DOI: 10.1078/1439-1791-00156.
- II Markkola AM, Tarvainen O, Ahonen-Jonnarth U & Strömmer R (2002) Urban polluted forest soils induce elevated root peroxidase activity in Scots pine (*Pinus sylvestris* L.) seedlings. – *Environmental Pollution* 116: 273–278. DOI: 10.1016/S0269-7491(01)00126-9.
- III Tarvainen O, Markkola AM, Ahonen-Jonnarth U, Jumpponen A & Strömmer R (2004) Changes in ectomycorrhizal colonization and root peroxidase activity in *Pinus sylvestris* nursery seedlings planted in forest humus. *Scandinavian Journal of Forest Research* 19: 400–408. DOI: 10.1080/02827580410030181.
- IV Tarvainen O, Ohenoja E, Strömmer R & Markkola AM (2009) Responses of fungal and plant communities to partial humus removal in mid-boreal N-enriched forests. Manuscript.
- V Tarvainen O, Strömmer R & Markkola AM (2009) Responses of Scots pine seedlings to partial humus removal in mid-boreal N-enriched forests. Manuscript.
- VI Tarvainen O, Leal Riesco I & Markkola AM (2009) Management of humus layer for restoration of urban mid-boreal forests: a greenhouse experiment. Manuscript.

*Author's contribution:* O. Tarvainen participated in fungal surveys (I), establishing the experiments (II), and was responsible for statistical analyses (I & III). She planned the field experiment (IV & V) together with supervisors A.M. Markkola and R. Strömmer and the greenhouse experiment (VI) by herself. She was responsible for the laboratory work and statistical analyses interpreted in papers IV, V and VI. She was the corresponding author for papers I and III-VI, and participated in the writing of paper II.



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# 1 Introduction

Soils in boreal forests are typically fungal-dominated systems characterized by the closed nutrient cycle, slow processes and low productivity (van der Heijden *et al.* 2008). The communities of ectomycorrhizal (ECM) fungal symbionts associated with forest trees are highly diverse (Allen *et al.* 1995, Väre *et al.* 1996, Taylor *et al.* 2000). It is estimated that several hundreds of ECM fungal species can be found in boreal climatic zone (Dahlberg 2002). In contrast to the rich ECM fungal community, the understory vegetation tends to be low in diversity and have a relatively extensive, uniform structure in pristine forests in Finland (Kokko *et al.* 2002). Typically, boreal forests are rich in slowly growing dwarf shrubs, many of which are evergreen species.

Plant species composition affects both the amount and quality of litter. Biochemical composition of litter, decomposition processes, and soil organic matter formation have important roles in the nutrient cycle (Nilsson *et al.* 1999, Prescott *et al.* 2000, Vestgarden 2001). Litter in boreal forests consists mostly of needles of coniferous trees, which are decomposed slowly (Prescott *et al.* 2000). This leads to a situation where the forest humus layer acts as a long-term sink of organically bound nitrogen (Berg & Dise 2004, Vestgarden *et al.* 2004). Thus, it is not surprising that most common plant species in boreal forests, such as ectomycorrhizal trees, Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L.) H. Karst.), and ericoid mycorrhizal shrubs, *e.g.* blueberry (*Vaccinium myrtillus* L.), are capable of bypassing nitrogen mineralization and taking up organic nitrogen by their mycorrhizal roots (Näsholm 1998, Näsholm *et al.* 1998, Persson & Näsholm 2001). This is because some ECM and ericoid fungal symbionts generally are capable of using organic N sources in the forest humus (Smith & Read 2008).

Ectomycorrhizal symbiosis is more than necessary for the nutrient uptake of trees in a nutrient poor environment, but the relationship is also costly, since mycorrhizal fungi consume considerable amounts of carbon assimilated by foliage. Godbold *et al.* (2006) suggest that the external mycelium of mycorrhizal fungi is the dominant pathway through which carbon enters the soil organic pool, exceeding the input via leaf litter and fine root turnover. Furthermore, the role of mycorrhizal fungi in the functioning of ecosystems is irreplaceable, as they are responsible for not only carbon input but also the major part of the nitrogen and phosphorus cycle in the soil (van der Heijden *et al.* 2008). Almost 100% of fine root tips in boreal trees are colonized by ECM fungi (Taylor *et al.* 2000), and

several ECM fungal species inhabit roots of an individual tree (Saari *et al.* 2005). Due to their relatively short life span, fine roots and mycorrhizae of forest trees are distinctly affected by chemical characteristics of the soil (Rennenberg *et al.* 1998, Persson & Ahlström 2002, Hagerberg *et al.* 2003). High N availability in the soil is generally assumed to decrease allocation to below-ground plant parts, as plants allocate resources to organs they use to acquire limiting resources (Bloom *et al.* 1985). Thus, decreased below-ground allocation in temperate forests (Nadelhoffer 2000) might be a result of increased foliar uptake of N (Sievering *et al.* 2007, Tomaszewski & Sievering 2007, Sparks 2009). Also, changed availability of other nutrients in the soil may affect allocation to roots (Corbin *et al.* 2003, Hagerberg *et al.* 2003, Dighton *et al.* 2004).

Scots pine seedlings of natural origin are colonized by ECM fungi immediately as their roots start to grow. Nursery seedlings commonly used in forest regeneration are mainly colonized by mycorrhizal fungi which are typical only in the nursery soils (Lehto 1989, Kernaghan *et al.* 2003, Iwański *et al.* 2006). When the nursery seedlings are planted in the forest, these fungal symbionts are replaced by indigenous ECM fungi originating from the forest soil (Dahlberg 1990, Pennanen *et al.* 2005). Differences in environmental growth conditions in the soil cause the changes in the ECM fungal community structure observed in roots of outplanted seedlings (Jones *et al.* 2003, Heinonsalo 2004, Cudlin *et al.* 2007). Also the genotype of an individual tree may affect the ECM fungal community colonizing roots (Korkama *et al.* 2006). Moreover, Korkama *et al.* (2007) suggest that different tree clones directly or indirectly affect understory vegetation and soil microbes. Thus, performance of Scots pine is dependent on its own genotype as well as indigenous ECM fungal inocula in soil, and is affected by environmental conditions and stress factors.

### **1.1 Root peroxidase activity as an indicator for stress**

Stress reactions in plants are often studied by measuring biomass and nutrient allocation into different plant parts, but also by using enzyme activity levels as indicators. For example, peroxidase (POD) activity has been widely used as a stress indicator (Passardi *et al.* 2005). Non-mycorrhizal pine seedlings maintain higher POD activity than mycorrhizal ones (Mitchell *et al.* 1986, Ahonen *et al.* 1989). On the other hand, some ECM fungi may increase POD levels in the ECM roots of Scots pine seedlings (Günther *et al.* 1998). It is suggested that auxins produced by the ECM fungi reduce POD-catalyzed cross-linking of cell-wall

constituents (Salzer & Hager 1993, Charvet-Candela *et al.* 2002). This mechanism may make the cell walls in the roots less rigid and allow colonization of the intercellular space by symbiotic fungi (Tarkka *et al.* 2001). During early colonization, mycorrhizal fungi may temporarily increase POD activity in roots (Salzer & Hager 1993), but the activity decreases when the mycorrhiza formation is established (Spanu & Bonfante-Fasolo 1988, Münzenberger *et al.* 1997). Furthermore, exposure of roots to certain saprophytic and pathogenic fungi leads to a two- to four-fold increase in root POD activity (Asiegbu *et al.* 1999). Also, ECM fungi can occur along a biotrophy-saprotrophy continuum (Jones & Smith 2004, Koide *et al.* 2008), which may also affect changes in root POD activity.

## **1.2 N deposition effects on boreal forests**

Anthropogenic N deposition has resulted in enhanced N availability in many areas. Forests in these areas show a clear response to an increased N load (Bobbink *et al.* 1998, Gundersen *et al.* 1998, Andersson *et al.* 2002, Dighton *et al.* 2004, Nordin *et al.* 2005, Högberg *et al.* 2006). Forest soils generally display great biological and structural complexity. Chronic N deposition decreases spatial heterogeneity of soil N processes, which may have strong implications for forest field layer plant species (Gilliam 2006). Composition and structure of plant communities change due to various interactive processes, such as competitive exclusion (Hutchings *et al.* 2003). Excess N favors plants with high relative growth rates (Tilman 1996). In boreal forests, N enrichment to a level at which it is no more a limiting factor for plant growth often leads to a switch from the shrub-dominated field layer to dominance of herbs and grasses (Fangmeier *et al.* 1994, Strengbom *et al.* 2001, 2003). Slow-growing shrub species are adapted to low N levels and decline with increasing competition with more fast-growing plant species.

Responses of forest field layer species to excess N may arise from nitrogen mediated changes in interactions between plants and their mycorrhizal symbionts (Gilliam 2006, van der Heijden *et al.* 2008). While N deposition may increase the productivity of forest trees and number of understory herbs and grasses in boreal forests, enrichment of N in forest soils may be unfavorable to the ECM fungi (Ruotsalainen & Kozlov 2006). Nitrogen deposition in the coniferous forests of Europe has decreased the number of ectomycorrhizal species producing sporocarps (*e.g.* Arnolds 1991, Brandrud 1995, Kårén 1997, Lilleskov *et al.* 2001). Sporocarp inventories are used in evaluating the responses of ECM fungal

communities to disturbances, even though sporocarp production alone is not sufficient for determining the diversity of fungal communities. Not all ECM fungi produce hypogeous sporocarps which could be recorded in macrofungal inventories. According to Kårén (1997) and Brandrud & Timmermann (1998), excess N affects sporocarp production of ECM fungal species more than the total number of ECM fungal species in a community. Thus, the fruiting of fungal species can be used to find disturbance indicator species for N deposition effects.

The direct and indirect effects of N enrichment on biological processes are difficult to assess. Pathways of N incorporation into an ecosystem vary across environments (Sparks 2009). The availability of N and, thus, the effects of N deposition on the ecosystem are modified by the differences in soil organic matter, clay, and moisture content (Vestgarden & Kjønaas 2003); climatic and meteorological conditions (de Vries *et al.* 2003); and soil pH (Stevens *et al.* 2004). A critical N load level for significant harmful effects in forest ecosystems is suggested to be much lower for acid soils than for more neutral soils (Falkengren-Grerup & Diekmann 2003). The critical load estimates for boreal forests in Scandinavia varies from 1.3 kg (Rosén *et al.* 1992) to 6 kg N ha<sup>-1</sup> year<sup>-1</sup> (Nordin *et al.* 2005). These levels are much lower than critical N load levels in the more southern forest areas (de Vries *et al.* 2000, Posch *et al.* 2001). As compared to these estimates, the nitrogen deposition level in the area of this study is relatively low (approx. 2 kg NO<sub>3</sub>-N ha<sup>-1</sup> year<sup>-1</sup>; Oulun seudun ympäristövirasto 2005), although higher than the lowest estimate for critical load. Moreover, effects of N enrichment on the biota persist for substantially longer periods than direct effects of excess N on soil chemical properties. This situation should be considered when assessing critical loads of N deposition for ecosystems (Strengbom *et al.* 2001). Moreover, the amount and quality of other nutrients, especially phosphorus (P), in boreal forest soils may also become limiting factors due to N deposition (Hagerberg *et al.* 2003).

The interplay of nutrients at the ecosystem level is challenging in studies where the nutrient levels are manipulated. Total removal of vegetation and the humus layer (sod-cutting) is used in mid-Europe as a method for reducing the N load in N-enriched coniferous forests and to promote ECM fungi (de Vries *et al.* 1995, Baar & ter Braak 1996, Baar 1997, Baar & Kuyper 1998, Smit *et al.* 2003, Boxman & Roelofs 2006). According to these experiments (Table 1), sod-cutting increases soil pH and decreases concentrations of exchangeable N and P compounds in both mineral and newly accumulated humus/litter layers. Sod-cutting also increases the number of ECM fungal species producing sporocarps.

In contrast, repeated litter removal increased fruiting of some *Russula* species but decreased fruiting of *Lactarius subdulcis* Bull. ex. Fr. and many saprophytic fungi in a Swedish experiment (Tyler 1991; Table 1). In order to see whether the negative effects of excess N can also be successfully diminished in mid-boreal urban forests, responses of fungal as well as plant species need to be studied.

### 1.3 Urban forests

Urban forests represent patches of biodiversity within otherwise degraded landscapes (Vidra *et al.* 2007). Compact city structure is admired as being resource efficient and economically sustainable, but people also desire contact with nature (van den Berg *et al.* 2007). Natural areas within cities can be considered as a design option that promotes urban sustainability (van den Berg *et al.* 2007, Pickett & Cadenasso 2008). Sustainability means improving the local residents' quality of life through environmental planning. Fragmentation of natural areas within cities often leads to a situation where species diversity of urban forests can differ greatly from that in more natural forests. Not only does the chronic deposition of pollutants affect the urban forest ecosystems, but also the increased recreational pressure causes changes in these forests.

The main consequence of urbanization is biotic homogenization of plant communities (Alvey 2006). This means that urban biota will become more uniform in species composition by an increase in the abundance and range expansion of common native or non-typical species, and by a decrease in the abundance or even extinction of the originally rare species (McKinney 2006, Kühn & Klotz 2006). These events are related to disturbance frequency, isolation of patches from each other, habitat requirements of plant species, and plant dispersal ability (Niemelä 1999). Relatively small changes in local land use are linked to changes in vegetation (Borgmann & Rodewald 2005, Vidra & Shear 2008). Changes in species composition and structure also vary geographically because of differences in growing season length, temperature, precipitation and soil nutrient levels.

**Table 1. Responses of ectomycorrhizal (ECM) fungi, available N and P compounds and soil pH to soil manipulation in various experiments. Sod-cut = total removal of vegetation and humus layer. Sod-addition = addition of sod-cut humus over existing humus layer. No<sub>sp</sub>= number of ECM fungal species, No<sub>sporo</sub>=number of ECM sporocarps ↑=higher/increase, ↓=lower/decrease, 0=no difference/change in comparison to control, ?=no clear result, -=not measured.**

Tree species	Stand age	Treatment year	Evaluation year	Treatment	ECM fungi		Humus / Litter			Mineral soil			Reference
					No <sub>sp</sub>	No <sub>sporo</sub>	N	P	pH	N	P	pH	
<i>Fagus sylvatica</i>	110–140	1988	1989–1990	repeated litter-removal	?	↑↓	-	-	-	-	-	-	Tyler 1991
<i>Pinus sylvestris</i>	40–80	1985	1986–1989	sod-cut	↑	↑0	-	-	-	-	-	-	de Vries <i>et al.</i> 1995
<i>Pinus sylvestris</i>	6–69	1990	1993	sod-cut	↑	↑	↓	↓	↑	↓	↓	↑	Baar & ter Braak 1996
<i>Pinus sylvestris</i>	15–20	1990	1993	sod-cut	-	-	-	-	-	0	0	0	Baar 1997
<i>Pinus sylvestris</i>	16	1990	1990–1993	sod-cut	0	0	-	-	-	-	-	-	Baar & Kuypers 1998
<i>Pinus sylvestris</i>	40	1990	1990–1993	sod-cut	↑	0	-	-	-	-	-	-	Baar & Kuypers 1998
<i>Pinus sylvestris</i>	69	1990	1990–1993	sod-cut	↑	↑	-	-	-	-	-	-	Baar & Kuypers 1998
<i>Pinus sylvestris</i>	70	1996	2001	sod-cut	↑	↑	↓	↓	↑	-	-	-	Smit <i>et al.</i> 2003
<i>Pinus sylvestris</i>	60	2001	2001	sod-cut	-	-	-	-	-	-	-	↑	Boxman & Roelofs 2006
<i>Fagus sylvatica</i>	110–140	1988	1989–1990	repeated litter-addition	?	↑↓	-	-	-	-	-	-	Tyler 1991
<i>Pinus sylvestris</i>	6–69	1990	1993	sod-addition	0	0	0	0	0	0	0	0	Baar & ter Braak 1996
<i>Pinus sylvestris</i>	15–20	1990	1993	sod-addition	-	-	-	-	-	-	↑	0	Baar 1997

Both species diversity and tolerance of individual species to disturbance (Niemelä 1999, Byers & Noonburg 2003, Zvereva *et al.* 2008) affect responses of a plant community to urbanization. Tolerance of plant communities to recreational use parallels the productivity of the forest stand (Malmivaara-Lämsä 2008) and the size of forest fragments (Hamberg *et al.* 2008). Forest fragmentation leads to an increase in forest edges, which may function as ‘collectors’ of wind-carried nutrients and pollutants both affecting ecological processes near edges (Weathers *et al.* 2001). Also changing light conditions in the forest patches (Coomes and Grubb 2000, Gilliam 2006, Hamberg *et al.* 2008) and trampling intensity (Malmivaara *et al.* 2002) are important factors affecting understory vegetation in urban forests.

Fungi in urban areas are studied less than those in more pristine forests. Many studies report the harmful effects of pathogenic fungi on urban trees (*e.g.* Annesi *et al.* 2003, Terho *et al.* 2007, Schubert *et al.* 2008). Studies describing mycorrhizal fungal communities in urban areas are few (Markkola *et al.* 1995, Baxter *et al.* 1999, Egerton-Warburton & Allen 2000, Cousins *et al.* 2003, Dighton *et al.* 2004, Ochimaru & Fukuda 2007). Sporocarp production of ECM fungi decreases along rural-urban gradients in pine (Markkola *et al.* 1995) and oak forests (Ochimaru & Fukuda 2007). Similar decreases are observed in ECM morphotype diversity along rural-urban N pollution gradients in mature oak (Baxter *et al.* 1999) and pine stands (Dighton *et al.* 2004), and also in the diversity of arbuscular mycorrhizal fungi in shrublands (Egerton-Warburton & Allen 2000). Most of these studies are conducted in the temperate climatic zone, whereas only one study has been done in mid-boreal forests (Markkola *et al.* 1995). The response of ECM fungi is similar in all of these studies, but no special indicator species or group can be named because of differences in species composition in different areas.

#### **1.4 Restoration of urban forests**

The main focus of many restoration projects in urban areas has involved eradicating non-typical plant species (*e.g.* Byers *et al.* 2002, Borgmann & Rodewald 2005, Ruiz-Jaén & Aide 2006, Vidra *et al.* 2007). Recovery of urban ecosystems can also be stimulated by planting woody species (Ruiz-Jaén & Aide 2006) or by re-introducing native plants with repeated removal of non-typical species (Vidra *et al.* 2007). However, it is assumed that the risk of reinvasion by non-typical plant species is rather constant because of continuing external

disturbances (Byers *et al.* 2002, Vidra *et al.* 2007). Urbanized areas themselves may serve as the main source from which non-typical species can further spread into an area (Tait *et al.* 2005). Thus, restoration of urban forests needs to be planned and examined well before any large-scale operations.

Manipulation of the vegetation and forest soil as a restoration method in urban forest areas also includes environmental risks. Intensity of soil manipulation (Gomez *et al.* 2002) and even size of manipulated gaps (Prescott *et al.* 2003) affect rates of nutrient mineralization, which may promote changes in plant growth (Baar 1997, Gomez *et al.* 2002), and soil biota (Setälä *et al.* 2000), and often leads to leaching of nutrients from the system (Kubin 1998, Spangenberg & Kölling 2004, Piirainen *et al.* 2007). On the other hand, removing soil N load should enhance root growth, as lower N availability in the soil generally increases biomass allocation to belowground plant parts (Baar 1997, Nadelhoffer 2000). Increased biomass growth, especially allocation into root biomass, could possibly be a sign of decreased risk of nutrient leakage.

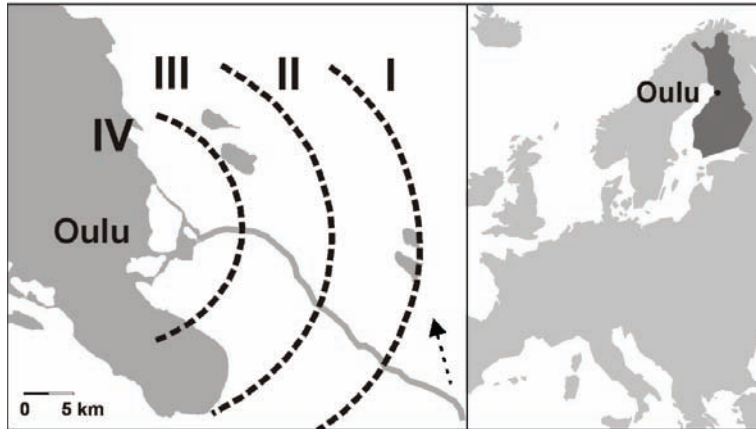


## 2 Aims of the research

Soil ecosystems in Scots pine forests on an anthropogenic pollution gradient around the industrialized city of Oulu, northern Finland, have been studied since 1987 (*e.g.* Ohtonen *et al.* 1989). The urban forests differ from the rural forests in many soil properties, such as a thicker humus layer, lower microbial activity (*e.g.* Ohtonen 1994) and lower fungal biomass allocation to ECM sporocarps (Markkola *et al.* 1995). The differences are assumed to be a result of long-term low-level N and S deposition in urban forests, which increases the number and biomass of herbaceous species and thus also change the quality and accumulation of soil organic matter. Accumulated N and S compounds in urban soil could possibly elevate POD activity in Scots pine roots (Markkola *et al.* 1990), the increase of which is assumed to indicate stress in plant tissues (*e.g.* Katainen *et al.* 1984, Roitto *et al.* 1999, 2001, 2003, Passardi *et al.* 2005).

There were three main aims in this thesis:

1. to study how mid-boreal urban Scots pine forests differ from rural ones in terms of composition of macrofungi and structure of the plant community (I, IV). It was hypothesized that the species richness of ECM macrofungi decreases along the urban pollution gradient, whereas diversity of plant species increases, and plant species with high growth rates become more abundant, towards pollution sources. The ECM fungal species were evaluated in order to find specific indicator species which reflect the pollution gradient.
2. to study whether the effects of N and S compounds in Scots pine and its ECM fungal symbionts are mediated via soil (II, V, VI) or via air (III). It was hypothesized that addition of N and S compounds and usage of N-enriched soil of a natural origin would decrease ECM fungal colonization in the roots and increase root POD activity in seedlings. Also, indirect effects of gaseous ozone as well as gaseous N and S compounds were investigated, all of which were expected to negatively affect root condition and ECM fungal colonization of seedlings indirectly via their effects on shoots.
3. to study if partial removal of vegetation and humus layer alleviates the adverse effects of excess N on plant and fungal communities (IV), and promotes performance and regeneration of Scots pine in urban forests (IV, V).



**Fig. 1.** Location of study zones around the city of Oulu. Arrow indicates the main wind direction, as approximately 40% of wind blows from south and south-east (I).

## 3 Materials and Methods

### 3.1 Study sites

The study area (Fig. 1), the city of Oulu and its surroundings, is located within the middle boreal vegetation zone (Ahti *et al.* 1968) in northern Finland (65°00'N, 25°30'E). The forests are characterized by nutrient-poor soils and Scots pine as a dominant tree species. The principal emissions in the area have decreased during the last decades. The NO<sub>x</sub> emissions have decreased to one third, while SO<sub>2</sub> emissions have decreased to half of the late-1980s level. The main pollution sources are located within the urban area, and pollution decreases gradually with distance from the city center.

In total, 20 study sites (I) were chosen in mature Scots pine stands. Five sites were selected for each of the four pollution zones (Fig. 1), which were determined by sulphur concentration in Scots pine needles at various distances from the city center (Karhu 1986). Total nitrogen concentrations in the forest humus layer were on average 14, 17, 19 and 20 mg g<sup>-1</sup> OM, and total sulphur concentrations 2.1, 2.6, 3.1 and 3.6 µg g<sup>-1</sup> OM in zones I, II, III and IV, respectively (Markkola *et al.* 1995).

Fifteen mature Scots pine forest sites were chosen for the restoration study (IV, V). Seven urban sites were located in the city of Oulu and eight rural sites 40 km southeast of the city in the county of Muhos (64°45'N, 26°00'E). Four of the urban sites were the same as in our earlier investigation in zone IV (I).

Average annual precipitation for the years 1971–2000 was 518 and 517 mm, and mean temperature +2.2 and +2.1°C, in Oulu and Muhos, respectively (Drebs *et al.* 2002). The monthly precipitation and snow cover follow similar trends in both areas.

### 3.2 Greenhouse and chamber experiments

In 1992 (II), axenically grown 12-week-old Scots pine seedlings were planted in 280-cm<sup>3</sup> pots with forest humus. In the first experiment (A), the seedlings were grown in humus originating from three non-polluted or three polluted forests (20 seedlings in each). In the second experiment (B), the seedlings grown in non-polluted humus were treated with five levels of ammonium or potassium sulphate (10 seedlings in each). After seven months, half of the seedlings in experiment

(A) and all of those in experiment (B) were harvested. The other half of the seedlings in experiment (A) were subjected to winter acclimation conditions for another six weeks.

In the third experiment (III), one-year-old containerized Scots pine seedlings were planted in a mixture of natural forest humus and sand in 500-cm<sup>3</sup> pots and placed in outdoor open-top-chambers (30 seedlings in each). Chambers were fumigated using combinations of ambient or elevated levels of gaseous O<sub>3</sub>, SO<sub>2</sub> and NO<sub>x</sub>. Ten seedlings were harvested from each chamber at 4, 8 and 16 weeks after planting in July, August and October 1992.

For the fourth experiment (VI), the soil was sampled in June 2001 from three urban sites and from three rural sites. Eight soil cores, 12 cm in depth and 6.8 cm in diameter, were taken within a 5-m radius from the central point of a randomly chosen sampling plot at each study site. The entire humus layer was removed from four of the soil cores. The rest of the soil core, *i.e.* the upper mineral layer with a minor amount of humus on the top, was used as a group simulating restoration management with an intensive removal of the humus layer (total humus removal treatment). The humus layer, which was only removed from the core used for the total humus removal treatment, was in turn used in comparisons as a group simulating slight disturbance (humus disturbance treatment). The four intact soil cores were used as controls. The sown-origin seedlings were harvested four months after establishing the experiment.

### **3.3 Restoration experiment in the field**

A restoration experiment with partial removal of the vegetation and humus layer (partial humus removal treatment) was established in June 2001 (IV, V). A round study plot was randomly established at each of the seven urban and eight rural study sites, and an experimental square of 2 m × 2 m was placed in the middle of each plot. One-half of the square was treated as a partial humus removal treatment by removing all above-ground parts of the vegetation and the upper half of the humus layer, and the other half was left as a control treatment. Both treatments were further divided into two subsquares, one of them used for vegetation surveys (IV), and the other for monitoring the development of one-year-old Scots pine nursery seedlings (21 in each subsquare; V). Seedlings were harvested in September 2003, three growing seasons after planting.

### **3.4 Fungal and plant community studies**

The structure of fungal and plant communities was studied along the urban pollution gradient (I). Species composition of macrofungi was inventoried at each site within a 50-m radius in 1987–1990 and again in 1997. The sporocarp production of ECM and saprotrophic fungi was studied by collecting all of them along a 50-m<sup>2</sup> transect, one per site. In 1987, five soil samples from each site were taken for ECM morphotyping (see 3.6). Coverages of higher plants, bryophytes, and lichens were estimated from ten randomly selected 1-m<sup>2</sup> quadrats per site in June–August 1989.

The effects of the restoration treatment on plant and fungal communities were studied at urban and rural forest sites (IV). The presence/absence of the ECM and saprophytic macrofungal species was inventoried 4–6 times at two-week intervals each autumn during 2001–2005. All sporocarps in the control and partial humus removal subsquares were collected. To obtain a representative estimate for the whole ECM fungal community at each site, sporocarps of ECM fungal species found from the entire study plot were also recorded.

Vegetation cover analyses were conducted before establishing the restoration treatments (IV) in June 2001 from a 0.25-m<sup>2</sup> subsquare in both halves of the experimental square. The visual estimation method was used to obtain a percentage of the total area covered by vegetation (Jalonen *et al.* 1998). This was repeated in July 2003. The percent cover of exposed soil, litter and plant species in the partial humus removal treatment was estimated in October 2001 and 2002 by recording the frequency of each species at 100 random points within a 0.25-m<sup>2</sup> subsquare (Stampfli 1991).

### **3.5 Shoot and root growth parameters**

The plant growth parameters were measured from above- and below-ground parts of the seedlings (II, III, V, VI). Needles and stems were sampled by keeping each year-class of the shoot separate. Needle length was measured from ten current-year needles per seedling (V, VI). Roots were divided into coarse (> 1 mm in diameter) and fine (< 1 mm) roots. Fine roots were cut into pieces and mixed, and sub-samples were randomly collected for dry weight, fungal biomass and root POD activity analyses. Biomass of the needles, lateral shoots, stems and coarse roots and the C:N ratio of the current-year needles were determined (V, VI).

### **3.6 Mycorrhizal colonization and peroxidase activity in the roots**

The percentage of ectomycorrhizae was calculated as the number of root tips on which ectomycorrhizae were observed per the number of all root tips in the subsamples using a dissection microscope (I, II, III, VI). Mycorrhizal short roots were classified into morphotypes based on their color and morphological features, and morphotype frequencies were recorded (I, II, III).

Fungal biomass in the fine roots (II, III, V, VI) was estimated from freeze-dried, ground roots using a modified ergosterol assay (Nylund & Wallander 1992). Ergosterol was quantified with high-performance liquid chromatography (HPLC). The estimate for total fungal biomass per seedling was obtained by multiplying the root ergosterol concentration by the total fine root biomass (III, V, VI).

Root POD activity and specific POD activity per root protein content (II, III, V, VI) were measured in frozen, homogenized fine roots of the pine seedlings (Roitto *et al.* 1999, 2001).

### **3.7 Soil analyses**

In the field experiments, the soil nutrient status was measured in the beginning (I, IV) and at the end of the experiment (V). Soil analyses were also done at the end of the greenhouse experiment (VI). Soil subsamples (I, IV) were analyzed for total N and P concentration. The soil acidity was measured in water extracts (IV, V, VI). The homogenized fresh or defrosted soil samples were analyzed for gravimetric moisture and organic matter (OM) content. Subsamples extracted with 0.5 M K<sub>2</sub>SO<sub>4</sub> were used for analyses of dissolved nutrients: ammonium N, nitrate N, total dissolved N (TDN), total dissolved P (TDP) and phosphate P. Dissolved organic N (DON) was calculated by subtracting the inorganic N from the total dissolved N.

The soil C:N ratio was studied in 2002 in the field restoration experiment. Soil subsamples were dried at room temperature, ground, and analyzed for C and N concentration the same way as the foliar C:N ratio.

Biological soil analyses included living fungal biomass (IV, VI) and soil respiration (IV). Fungal biomass was estimated as ergosterol concentration ( $\mu\text{g g}^{-1}$  soil OM) using a modified ergosterol assay (Nylund & Wallander 1992). Soil respiration, an index of microbial activity, was analyzed as described by Ohtonen (1994).

### **3.8 Data analysis**

Statistical analyses were performed using mean values for each treatment, site or subsquare. The differences in plant and fungal community structure and in soil properties between the urban and rural sites were analysed using one-way ANOVA or non-parametric tests (I, IV), indicator species and principal component analyses (PCA, I). Impacts of soil origin and addition of N and S on Scots pine seedling growth, root POD activity, and mycorrhizal parameters were analysed with two-way ANOVA (II). Repeated measures ANOVA was used in analysing effects of gaseous pollutants and time of season on Scots pine seedlings (III). Effects of soil origin and restoration treatments on soil properties, fungal and plant species, and Scots pine seedlings were tested using linear mixed models (IV, V, VI). The tests were performed either with SPSS or PC-ORD packages for Windows. More detailed descriptions for the tests can be found in corresponding original papers.





## 4 Results

### 4.1 Comparison of urban and rural forests

#### 4.1.1 Macrofungal and plant communities in urban and rural forests

Fruiting of fungi and plant communities both showed differences between sites of differing anthropogenic influence (Table 2; I, IV). The number of fruiting ECM fungal species, especially those of *Cortinarius* spp., decreased towards the N and S emission sources. The diversity of ECM macrofungi was lowest at the most polluted urban sites, whereas diversity among ECM morphotypes in pine roots did not differ between the pollution zones. Saprophytic macrofungal communities were quite similar at all sites.

The fungal diversity recorded in 1987–1990 in the 50-m radius plots consisted of 168 taxa (I). In an additional survey in 1997, 25 new species were found, most of them at urban sites. Eight fruiting fungal species reflected the differences between the pollution zones and showed distinctly contrasting strategies in their responses. Most of them were declining in relation to increasing pollution (PCA with fungal indicator species; I). These species were almost all ectomycorrhizal: *Chroogomphus rutilus* (Schaeff.:Fr.) O.K.Mill., *Cortinarius anomalus* (Fr.:Fr.) Fr. coll., *Cortinarius brunneus* (Pers.:Fr.) Fr. coll., *Cortinarius gentilis* (Fr.:Fr.) Fr., *Cortinarius semisanguineus* (Fr.) Gillet, and *Suillus variegatus* (Sw.:Fr.) Kuntze., with only one species, *Cantharellula umbonata* (J.F.Gmel.:Fr.) Singer, considered as saprotrophic. One indicator species, *Paxillus involutus* (Batsch:Fr.) Fr., showed quite the opposite trend and proved to be pollution tolerant.

**Table 2. Responses of macrofungi and vegetation to N load and to restoration by partial removal of humus layer. ↑=higher/increase, ↓=lower/decrease, 0=no difference/change in comparison to control.**

Parameter	N enriched soil	Partial humus removal
	I & IV	IV
Fruiting macrofungi		
All ectomycorrhizal fungi	↓	↑
<i>Chroogomphus rutilus</i>	↓	0
<i>Cortinarius anomalus</i>	↓	0
<i>Cortinarius brunneus</i>	↓	0
<i>Cortinarius gentilis</i>	↓	0
<i>Cortinarius semisanguineus</i>	↓	↑
<i>Suillus variegatus</i>	↓	0
<i>Paxillus involutus</i>	↑	0
All saprophytic fungi	0	↓
<i>Cantharellula umbonata</i>	↓	0
Vegetation		
Mosses	↓	↓
Lichens	↓	↓
Herbs	↑	0
Graminoids	↑	0
Shrubs	↓0	0
Woody species	0	0

Within smaller 5-m radius plots, 90 fruiting ECM fungal species were found during 2001–2005 (IV). In this survey, differences in ECM fungal communities remained similar between the urban and rural study sites, as was observed in earlier surveys. The rural sites were rich in *Cortinarius* spp., among which *C. gentilis*, *C. obtusus* Fr.:Fr. and *C. paragaudis* Fr. coll. occurred solely at the rural sites (IV). Also, *Suillus variegatus* and *Cantharellula umbonata*, which were indicator species in the earlier survey, were typical for the rural sites. *Paxillus involutus* was present at all urban sites, but was not found at the rural sites.

In contrast to the ECM fungal community, the diversity of plant species on the forest floor increased towards the N and S emission sources. Dwarf shrubs, *Vaccinium myrtillus* and *V. vitis-idaea* L. were dominant species in the field layer at all sites (I, IV). Close to the emission sources, slowly growing plant species were displaced by species typical of more nutrient-rich forests. Thus, herbs and grasses became more abundant while the number of bryophyte species diminished and lichens were absent. *Deschampsia flexuosa* (L.) Trin., *Maianthemum bifolium* (L.)

F.W.Schmidt, *Trientalis europaea* L., *Rubus idaeus* L. and *Sorbus aucuparia* L. were typical plant species at the urban forest sites.

#### **4.1.2 Properties of urban and rural forest soils**

Differences in the soil parameters (Table 3) between the pollution zones were similar when comparing parameters measured in 1987 (I), in 2000 (I), and when the restoration experiment was started in 2001 (IV). The thickness of the humus layer and its total N concentration increased towards the pollution sources. Acidity and ammonium N concentration in the humus layer did not differ significantly, although ammonium N showed large variation and was on average 3–4 times higher at the polluted urban sites than at the most rural sites (I). In addition, at the urban sites of the restoration experiment, the humus layer was thicker and its pH, total N, ammonium N, and total P were higher as compared to the rural sites (IV). In contrast, dissolved organic N, total dissolved P, and phosphate P in the humus layer were lower at the urban than at the rural sites. Basal respiration and soil ergosterol concentration were lower in the urban than in the rural soils (IV).

#### **4.2 Responses of Scots pine seedlings to excess N**

The effects of N pollution on Scots pine seedlings (Table 3) were mediated via soil (II, V, VI), whereas aerial pollution did not affect the seedlings during the first growing season after planting (III). While root POD activity increased only slightly after addition of N and S compounds to the soil (II), using the urban soil as a growing medium resulted in increased root POD activity (II, V). Urban soil did not affect root POD activity in the seedlings grown four months in the greenhouse (VI).

Neither the intensity of mycorrhizal colonization nor the relative proportions of ECM morphotypes were affected by the origin of soil used as a growth media of pine seedlings (II). The number of ECM morphotypes in both greenhouse experiments was low (II, VI data not shown). In the field experiment, soil origin did not affect fungal biomass in the seedlings, which was estimated as ergosterol concentration in the fine roots (V). In the greenhouse experiment, concentration and total content of ergosterol in the pine roots both were at a higher level in the urban than in the rural intact soil (VI).

Only slight differences were found in the growth of planted Scots pine nursery seedlings between urban and rural areas. At the urban sites the N concentration in current-year-needles was higher, while the needles were shorter, than those at the rural sites (V). Similarly, neither above- nor below-ground seedling biomasses were affected by the origin of the soil in a seven-month greenhouse experiment (II). On the contrary, sown-origin Scots pine seedlings generally grew larger in the urban soils than in the rural soils in the four-month greenhouse experiment (VI).

Seasonal differences were detected in responses of Scots pine seedlings (II, III). Needle and stem biomass increased during the winter acclimation period in the greenhouse experiment (II), whereas in the open-top chamber experiment, above-ground and below-ground seedling biomass both increased during the growing season (III). Root-to-shoot ratios of Scots pine seedlings increased evenly during the experimental period parallel to increased fine-root biomass (III). Mycorrhizal colonization increased in both experiments towards the end of the growing season. On the other hand, fungal biomass in fine roots peaked in August, 8 wks after planting (III). Root POD activity increased in six weeks in 9-month-old seedlings during winter acclimation in the greenhouse (II), while POD activity decreased during the growing season in 18-month-old seedlings in open-top chambers (III).

**Table 3. Responses of Scots pine seedlings and soil properties to excess soil N, N addition, and management of humus layer of urban forest soil. ↑=higher/increase, ↓=lower/decrease, 0=no difference/change in relation to control/intact soil, -=not measured.**

N enriched soil	N addition via soil	N addition via air	Parameter	Humus disturbance	Partial humus removal	Total humus removal
I, II, V & VI	II	III		VI	V	VI
<b>Scots pine seedlings</b>						
0	-	-	Height growth	0	0	0
0	0	0	Shoot biomass	0	↑	↓
0↑	-	0	Needle biomass	0	↑	↓
↓↑	-	-	Needle length	0	↑	0
↑	-	-	Needle N %	0	0	↓
0↑	-	0	Root biomass	0	↑	↓
↑0	0	0	POD activity in roots	↑	↓	↓
0	-	0	Fungal biomass in roots	0	0	0
0	-	0	Number of ECM morphotypes	0	-	0
<b>Soil parameters</b>						
↑	-	-	pH	0	↑	↑
↑	-	-	Total N	-	-	-
↑	-	-	Ammonium N	↑	↑	0
↑	-	-	Total P	-	-	-
↓	-	-	Phosphate P	↑	0	0
↓	-	-	C to N ratio	-	0	-
↓	-	-	Fungal biomass	0	-	0
↓	-	-	Microbial activity	-	-	-

### 4.3 Reduction of N enrichment in urban forests

#### 4.3.1 Responses of fungal and plant species to restoration

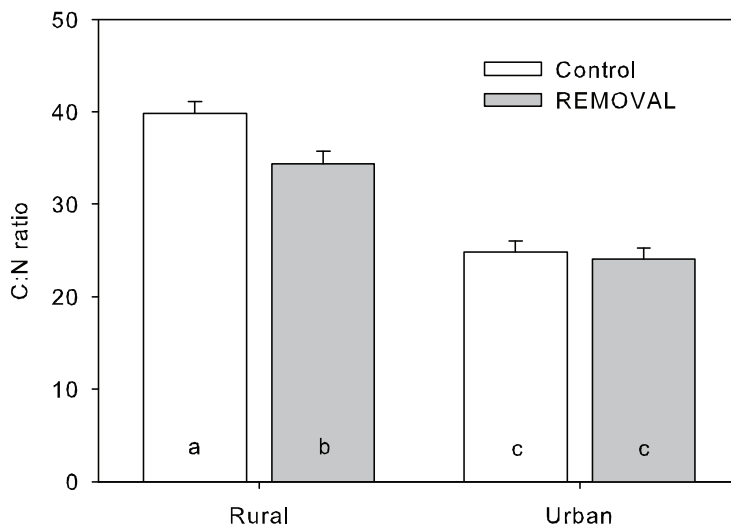
The partial humus removal treatment affected ECM fungal species, which produced more sporocarps during the study period as compared to the control, whereas saprophytic fungi showed a decreasing trend (Table 2; IV). However, the change in the fruiting ECM fungal community was slow during the first three years (2001–2003), and the stimulating effect of the partial humus removal to sporocarp production was more obvious only in the last study year, 2005. At the urban sites, effects were generally weaker than at the rural sites. The partial

humus removal treatment resulted in three fruiting ECM fungal species in 2005, whereas during the whole survey period, only five ECM fungal species were found in the urban subsquares: *i.e.* *Cortinarius croceus* (Schaeff.) Gray, *C. semisanguineus*, *Inocybe sp.*, *Lactarius rufus* (Scop.:Fr.) Fr. and *L. turpis* (Weinm.) Fr..

The plant species number did not differ between the partial humus removal and control treatments (IV). After the third growing season in 2003, the plant cover in the partial humus removal treatment of the urban sites had reached the initial level recorded in June 2001 (Table 2). *Deschampsia flexuosa*, *Sorbus aucuparia* and *Vaccinium* spp. recovered well, showing equal coverage in 2003 to that before establishing the experiment. In contrast, *Empetrum nigrum* L. suffered from the treatment and did not return to the urban sites during the study period. Mosses recovered very poorly, and lichens were absent even after the partial humus removal treatment.

#### **4.3.2 Soil nutrient availability**

The thickness of the humus layer was reduced to approximately 60% of that of the control after partial humus removal treatment in the field (V). The C:N ratio in the humus layer did not differ between the control and partial humus removal treatments at urban sites, whereas at rural sites the C:N ratio was higher than at urban sites and decreased after the partial humus removal treatment (Fig. 1). The partial humus removal treatment at urban sites resulted in higher levels of soil ammonium and nitrate N as well as DON as compared to the control. In the greenhouse soil manipulation experiment (VI), the levels of ammonium N were high in the humus disturbance treatment, whereas the levels of nitrate N were high in the total humus removal treatment. The treatments did not differ in soil pH, DON, TDN or soluble P compound levels in the urban soils, whereas the disturbance treatment increased soil fungal biomass in both urban and rural soils as compared to the other treatments (VI).



**Fig. 2. Carbon-to-nitrogen ratio (mean C:N ratio + standard deviation) in rural and urban forest humus layer in 2002, two growing seasons after partial removal of vegetation and humus layer (REMOVAL). Significant differences are expressed with different letters.**

#### **4.3.3 Responses of Scots pine to restoration**

Soil manipulations resulted in different responses in the field and in the greenhouse experiments. In the field experiment, after the partial humus removal treatment the growth of the pine seedlings was generally facilitated (V). At urban sites, the partial humus removal treatment resulted in higher needle and fine-root biomass and a slight decrease in the root-to-shoot ratio as compared to the control. A slight decrease in foliar N concentration and root POD activity were found at urban sites. Fungal biomass in the roots, estimated as root ergosterol concentration, did not differ between the treatments. Emergence of Scots pine seedlings was poor during the study period, and new seedlings were found only at two of the urban sites after partial removal of the vegetation and humus layer (IV).

In the greenhouse experiment, both the humus disturbance and total humus removal treatment decreased pine seedling growth in the urban soils (VI). Root to shoot ratio did not vary among the soil manipulation treatments. A slight decrease

in foliar N concentration as well as root POD activity were found after total humus removal in seedlings grown in the urban soils. On the other hand, root POD activity was at the highest level in the humus disturbance treatment with an increase in soluble N compounds in the soil. Fungal biomass in the roots, measured as root ergosterol concentration, showed no specific response to treatments.



## 5 Discussion

### 5.1 Fungi in urban coniferous forests

The number of ECM fungal species producing sporocarps decreased along the urban pollution gradient. Especially, species belonging to *Cortinarius* spp. were rare at urban N-enriched sites. This is parallel to earlier findings that ECM morphotype diversity and ECM sporocarp production decrease along rural-urban pollution gradients (Baxter *et al.* 1999, Dighton *et al.* 2004, Ochimaru & Fukuda 2007). The high N load in the soil is reflected by a declining number of fruiting ECM fungal species in more natural coniferous forests (Ohenoja 1994, Brandrud 1995, Kårén & Nylund 1997, Jonsson *et al.* 2000, Peter *et al.* 2001, Lilleskov *et al.* 2001), while excess N does not seem to affect the species richness of saprophytes (Ohenoja 1994, Peter *et al.* 2001). Similarly saprophytic fungi did not differ along the urban pollution gradient.

Brandrud (1995) divides ECM fungal species into stress-tolerant and stress-sensitive taxa. *Paxillus involutus* and *Lactarius rufus* belong to stress-tolerant fungi, dominating the macrofungal community under acidification (SO<sub>4</sub>; Shaw *et al.* 1992, Såstad & Jenssen 1993) and N-fertilization treatments (Ohenoja 1994). On the other hand, Lilleskov *et al.* (2001) assess *L. rufus* as a nitrophobic species, because its sporocarp production decreases due to long-term N addition. While *Paxillus involutus* is a species with a wide range of tolerance (Chalot *et al.* 1996, Courbot *et al.* 2004, Fransson *et al.* 2005, Langenfeld-Heyser *et al.* 2007), *Cortinarius* spp. as well as *Russula* spp. are among the most sensitive and the first to suffer when N input increases (Brandrud 1995, Brandrud & Timmermann 1998, Strengbom *et al.* 2001). One indicator species in this study, *Cantharellula umbonata*, is mainly presumed to be saprophytic, but Ohenoja (1995) suggests that yearly weather conditions and especially winters affect its fruiting in a similar way as the fruiting of ECM fungi. In the present study, *C. umbonata* decreased along the urban pollution gradient, as did the ECM fungal indicator species.

Though the present and several other studies show that ECM fungi fruit less under excess N, the proximal mechanisms for the changes are unclear (Brandrud & Timmermann 1998, Lilleskov & Bruns 2001, Hobbie *et al.* 2005, Carfrae *et al.* 2006, Lilleskov *et al.* 2008). The decrease in sporocarp production of ECM fungi may be related to a reduction in the number of species forming sporocarps and to factors that disturb the development of sporocarps (Kårén & Nylund 1997).

Increased nitrogen assimilation of ECM fungi under excess N consumes more C than growth of extramatrical mycelium and sporocarp development (Carfrae *et al.* 2006, Sims *et al.* 2007). Also, the nutrient status of the host tree affects fungal growth (Nilsson & Wallander 2003, Nilsson *et al.* 2005). It is hypothesized that most ECM fungal species can survive periods of excess N supply, but decreasing vitality indicates that they may disappear with time if N availability remains high (Brandrud 1995), and a decline in ECM fungal diversity can be seen long after termination of N exposure (Strengbom *et al.* 2001). However, studies based on sporocarp inventories may not give a sufficient picture, as sporocarp production often varies due to differences in weather conditions (Ohenoja 1993, Jonsson *et al.* 2000). Moreover, not all ECM fungal species produce above-ground sporocarps that could be surveyed.

Excess N has been reported to affect the formation of mycorrhizae less than sporocarp production (Wallander 1995, Kårén & Nylund 1997, Brandrud & Timmermann 1998, Jonsson *et al.* 2000, Peter *et al.* 2001). Peter *et al.* (2001) found that the impact of nitrogen on below-ground ECM fungal diversity is slight, but the change in the ECM fungal community can be seen at both above- and below-ground levels. Also dispersal abilities of ECM fungi are low, as the main portion of a fungal biomass is allocated in external mycelium (Wallander *et al.* 2001). Furthermore, the chances of successful establishment are weak even though an individual ECM fungal propagule can travel long distances (Peay *et al.* 2007). Responses of the ECM fungal community in urban forests to excess N may partially depend on a variety of other environmental factors (Lilleskov *et al.* 2001, Jones *et al.* 2003, Heinonsalo 2004). Trampling, which is also an urban disturbance factor, has not been found to negatively affect sporocarp production of fungi (Egli *et al.* 2006). However, trampling can cause damage in mycorrhizal fine roots of beech seedlings but not in roots of mature trees (Waltert *et al.* 2002). Thus, when studying the community structure of ECM fungi, all stages of the fungal life cycle (mycelia, mycorrhizal root tip, sporocarp) and also age of the tree symbionts should be taken into consideration.

One probable mechanism for reduced fruiting of ECM fungi is that increased N availability stimulates shoot growth of the host plant instead of C allocation to the fungal symbionts (Wallander *et al.* 1994, Markkola *et al.* 1995, Nilsson & Wallander 2003), resulting in decreased C flow to soil. Carbohydrate support to ECM fungi is generally linked to plant nutrition (Nehls *et al.* 2007). Foliar N uptake increases under high N deposition levels (Sparks 2009). The increase in foliar uptake means a lowered demand for root uptake. Thus, the resource

allocation to roots and the necessity of mycorrhizal symbiosis decreases. It is likely that the different benefits and costs of symbiosis with different fungal species may play an important role in shaping ECM fungal communities (Tuomi *et al.* 2001). It has been suggested that the host plant may even be able to choose a symbiont with low C-cost (Saikkonen *et al.* 1999, Kuikka *et al.* 2003, Markkola *et al.* 2004, Bever *et al.* 2009). Under excess N, the host plant may allocate less C to those ECM fungi with abundant external hyphal structures that are costly to the host plant. Such species as, *e.g.* *Cortinarius* and *Suillus*, were found to decrease towards the urban end of the gradient. This may result in less C-demanding smooth mycorrhizal morphotypes becoming more common at the root level.

However, the picture is not simple. It is suggested that the fungus itself, rather than the host plant, modifies the carbon allocation patterns to fit with the N supply. This ability varies among ECM fungal species depending on their capacity to assimilate N and their sensitivity to harmful levels of N (Wallander 1995, Gorissen & Kuyper 2000). Moreover, carbon allocation to external ECM mycelium is reduced in the presence of a wood-decomposer fungus (Leake *et al.* 2001) which raises the question of a possible increase in competition for carbon between ECM and saprotrophic fungi at sites with a high N load. In addition, Lindahl *et al.* (2007) propose that interactions between decomposers and mycorrhizal fungi are antagonistic. Some mycorrhizal fungi evidently also have decomposing ability which affects soil C balance (Talbot *et al.* 2009). High soil N could mediate changes in ECM fungal communities, which may have further long-term ecological significance by affecting the capacity of their plant symbionts, *i.e.* long-living trees, to grow in changing forest ecosystems. However, present knowledge about these mechanisms is ambiguous to date.

## 5.2 Plant diversity in urban coniferous forests

A distinctly higher number of herb and grass species was observed at the urban end of the pollution gradient, whereas the number and cover of mosses and lichens were diminished at urban sites (I, IV). Also, the number of herb and grass species increased from 1989 to 2003 at the urban sites. In a 1960's forest inventory (Estama 1967), the urban study sites were still classified into the dryish *Empetrum-Vaccinium* forest type with fewer understory species. The increase in plant species diversity was evident. During recent decades, urbanization and low-level N and S deposition have affected the urban forest sites. In more nutrient-rich forests, N enrichment decreases plant species richness (Bobbink *et al.* 1998,

Vanha-Majamaa & Reinikainen 2000), having a direct effect on competitive abilities of various plant species (Hutchings *et al.* 2003, Gilliam 2006). In particular, bryophytes are sensitive to increases in N input (Mäkipää 1994, Strengbom *et al.* 2001, Nordin *et al.* 2005). As Falkengren-Grerup and Diekmann (2003) state, even a slight increase in soil pH affects N processes in the soil, the plant life history traits and plant response to N deposition. Species indicating high N (Diekmann & Falkengren-Grerup 1998) were more typical at urban sites with higher pH and available N levels than at rural sites with lower pH and available N levels.

Plant species recorded only at the urban sites were *Deschampsia flexuosa*, *Luzula pilosa* (L.) Willd., *Maianthemum bifolium*, *Trientalis europaea*, *Rubus idaeus* and *Sorbus aucuparia*, which are typical for mesic boreal forests with Norway spruce as the dominating tree species (Kokko *et al.* 2002, Hamberg *et al.* 2008). Furthermore, the herb and grass species found at urban sites are more typical of open forest edges than of a closed forest (Hamberg *et al.* 2008). Bryophytes declining at urban sites are sensitive to increased light (Hamberg *et al.* 2008) and to trampling (Malmivaara *et al.* 2002, Törn *et al.* 2006, 2009, Hamberg *et al.* 2008). Thus, many plant species found at urban sites are generally uncommon species in mid-boreal pine dominated forests.

Successful invasions by non-typical species are more common in human-modified habitats than in less disturbed and pristine habitats (Niemelä 1999, McKinney 2005, Alvey 2006). In the present study, the level of recreation increased towards the city center, which could have contributed to reduced cover of ground layer mosses and lichens and to the increased number of herb and grass species. The urban sites were relatively small patches with a fairly open canopy, except the most eutrophic sites characterized by a high *Sorbus aucuparia* density. Increased nutrient and light availability at urban sites could be expected to favor plants with high growth rates. *Deschampsia flexuosa* is often found as a dominating species with increasing N (Rosén *et al.* 1992, Kellner & Redbo-Torstensson 1995, Strengbom *et al.* 2001), but also with increasing light availability (Strengbom *et al.* 2003, Hamberg *et al.* 2008) and after silvicultural disturbances (Uotila *et al.* 2005). Strengbom *et al.* (2004) suggest that light is more important in determining *D. flexuosa* expansion than N availability, indicating more severe above-ground than below-ground competition.

However, below-ground competition may occur at the mycorrhizal level and be expressed as competition for photosynthetic C allocation. Competition can occur between ecto-, ericoid and arbuscular mycorrhizal fungi. Increased numbers

of plant species forming arbuscular mycorrhiza in coniferous forests with high N availability (Sipola 1994, Mäkipää 1994, Lilleskov *et al.* 2001, Strengbom *et al.* 2003, I, IV) support this view. Also, garden plants, *i.e.* *Impatiens glandulifera* Royle, *Humulus lupulus* L., *Glechoma hederacea* L., *Solanum dulcamara* L. etc., have spread to urban forest stands around the city of Oulu (personal observations). There is a great risk for invasion of other non-typical plant species into mid-boreal urban forests, especially due to predicted milder winters as part of global climate change.

### **5.3 Responses of Scots pine to N and S compounds**

#### **5.3.1 The effects of N and S mediated via soil**

The growth responses of Scots pine seedlings to N enrichment were apparently mediated mainly via soil (II, V, VI), whereas simulated pollution in experimental conditions as fertilizer (II) or in gaseous form (III) had no measurable effects on growth of the seedlings. Elevated gaseous pollutant concentrations have caused slight differences or negative growth responses in similar short-term studies (Edwards *et al.* 1992, Shan *et al.* 1997, Takemoto *et al.* 1997). Sparks (2009) suggests, however, that foliar uptake of deposited N could influence plant growth more readily than uptake of soil-deposited N. The N deposition level affects the magnitude of foliar uptake. Under low pollution levels, the influence of foliar N uptake might be less significant than uptake of N via soil. Consequently, the responses of seedlings grown in N-enriched urban soil, *e.g.* high root POD activity (II, V, VI) and foliar N concentration (V, VI), were obviously due to the original differences in the soil properties.

At urban sites, the dominance-shift from dwarf shrubs to herbs and grasses, and the concomitant increase in litter production together with decreased microbial activity certainly played a part in the accumulation of humus (Ohtonen 1994, IV). The thickness of the humus layer as well as total N and P concentration in the soil were higher at urban than at rural sites, indicating an higher nutrient pool. However, the levels of available N and P compounds were lower in the urban than in the rural soils (IV). The level of available P was substantially lower in the urban soils than in the rural soils, which is consistent with observations by Baxter *et al.* (2002) in a temperate rural-urban gradient.

Increased N in soil may actually create a situation where availability of P limits plant growth (Corbin *et al.* 2003, Dighton *et al.* 2004). Also, in the present study, the low amount of available P compounds in the urban soils in relation to high availability of N implies that P could become a limiting growth factor in urban N-enriched forests. The interplay between N and P availability can shape plant C allocation and affect their mycorrhizal symbionts. For example, Norway spruce responds to P limitation by increasing exploitation of P-containing minerals by ectomycorrhizal fungi (Hagerberg *et al.* 2003). *Paxillus involutus*, the species found commonly at urban sites, has been reported to be more efficient than other studied ECM fungi in P translocation to host plants (Bidartondo *et al.* 2001) and possibly to be responsible for P exploitation under P limitation (Hagerberg *et al.* 2003). Because several ECM fungal species inhabit pine seedling roots simultaneously and fine roots are short-lived, the balance between benefits and costs of symbiosis may be retained after disturbance.

Mycorrhizal colonization in Scots pine seedling roots was not affected by the origin of soil in the present experiments (II, V, VI). This was unexpected, as the inoculum potential of ECM fungi may have been declined in urban soils as suggested by earlier results obtained from mature urban trees (Markkola *et al.* 1995). This was also implied by changes in ECM macrofungal composition in the urban forests (I, IV). Mycorrhizal symbionts of mature trees may result in favoring the next generation of host plants via mycelial networks (Högberg *et al.* 1999, Simard *et al.* 2002, McGuire 2007). In addition, facilitation effect from mature plants to young seedlings varies among ECM fungal species (Nara 2005). Generally, success of Scots pine in boreal forests is related to richness of the ECM fungal community with varying abilities to uptake and translocate nutrients to their hosts together with varying abilities to adapt to environmental changes.

In boreal forests, root growth varies during the growing season, which could also be seen in the open-top chamber experiment (III). Root metabolic activity in young seedlings is slow in spring and early summer and most intense at the end of the growing season (Iivonen 2002). This is related to soil temperatures: low soil temperature decreases C allocation to roots of Scots pine seedlings, whereas root growth recovers well as soil temperature increases (Lippu 1999, Domish *et al.* 2002). The root-to-shoot ratio of 3-year-old seedlings grown at field sites (V) was at the same level as that of the seedlings grown in rural and urban soils in the greenhouse for 9 months (II), but lower than that of 4-month-old seedlings in the soil manipulation experiment (VI). The root-to-shoot ratio in one- and two-year-old Scots pine seedlings varies between 0.3–0.7 (Markkola 1996, Alberton *et al.*

2007, personal observations). The use of a cylinder to sample roots might have also affected the ratio obtained from the field experiment.

When comparing results of seedling growth responses from different climatic regions, we have to remember that resource allocation belowground may differ depending on climatic and nutritional conditions. A harsh climate with a short growing season affects C allocation in Scots pine, because there are two times more ECM root tips per unit ground area in northern Scots pine stands than in southern stands in Finland (Helmisaari *et al.* 2009). This indicates increasing C allocation to root systems in the north as compared to the south and may mirror decreasing N availability towards the north. In temperate forests, the increase in soil N availability is suggested to decrease fine-root biomass but stimulate fine-root production and turnover (Nadelhoffer 2000) and increase fine-root N concentration (Lilleskov *et al.* 2008). Moreover, intraspecific variation in growth (Korkama *et al.* 2007, Karst *et al.* 2009) and in sensitivity to pollution (Dewald *et al.* 1992, Reinert *et al.* 1996, Vanhatalo *et al.* 2002) makes it difficult to make any general conclusion about the responses of seedlings to excess N.

### **5.3.2 Root peroxidase activity**

Field observations (Markkola *et al.* 1990, IV) and present greenhouse experiments (II, VI) showed that certain factors in urban polluted forest soils elicit high POD activity in the roots of both mature trees and seedlings of Scots pine. Increased POD activity is often used as a stress and defense indicator in plant tissues (*e.g.* Mitchell *et al.* 1986, Roitto *et al.* 1999, 2001, 2003, Passardi *et al.* 2005). The difference in POD activity levels of the mycorrhizal roots is suggested to reflect differences in the ECM fungal community colonizing the roots (Albrecht *et al.* 1994, Timonen & Sen 1998). The root POD reaction in seedlings varied during the growing season, which may have resulted from changes in the composition of root fungal symbionts (II, III). High POD in roots of seedlings grown in nursery soils possibly indicates defense reactions. Ectendomycorrhizal dark septate root endophytic fungi that penetrate through the plant cell wall (Jumpponen & Trappe 1998) are typical in nursery seedlings, and they might have maintained higher POD activity in the roots at the time of planting (III). The decrease in root POD activity during the growing season in the open-top experiment was possibly due to replacement of nursery-type mycorrhizae by indigenous ECM fungi in forest humus.

Only a slight positive relation between Scots pine root POD activity and low levels of ammonium sulphate added to the soil was found in the greenhouse study (II). Also, heavy metals found in elevated concentrations in the urban soils of the study area (Ohtonen *et al.* 1990) could play a role in increased POD activity. The effect of Cu on POD activity in Scots pine roots may be minor according to previous studies (Tarvainen *et al.* 1991, Roitto *et al.* 1999, Kukkola *et al.* 2000), but it is still possible that other heavy metals such as Pb, Cr and V in urban soils acted as POD elicitors (Baycu *et al.* 2006, Brunner *et al.* 2008). Similar increases in needle POD activities were also observed (data not shown). However, use of POD activity as an indicator for N stress should be evaluated in greater detail in order to obtain a clearer picture of causal relationships.

### **5.3.3 Responses of Scots pine to restoration**

The partial humus removal treatment increased natural emergence of Scots pine seedlings, but less at the urban than at the rural sites (IV), while the treatment improved the growth of planted pine nursery seedlings (V). Disturbances caused by human activity often lead to a decline in regeneration of spruce in urban boreal forest stands (Lehvävirta *et al.* 2004). As a pioneer species, Scots pine may not be severely affected by disturbances. On the other hand, allelopathic compounds *e.g.* exudates from *Empetrum* severely decreases germination and survival of Scots pine seedlings (Nilsson *et al.* 1993). The phenolic compounds extracted from *Deschampsia flexuosa* (Baar *et al.* 1994), *Empetrum nigrum* (Nilsson *et al.* 1993) and other dwarf shrubs growing in pristine N-poor forests (Souto *et al.* 2000) are accumulated in the humus layer. However, mycorrhizal colonization of pine seedlings was not affected by different combinations of litter (Nilsson *et al.* 1999). As allelopathy in coniferous forest soils is possible at least in theory, it seems reasonable to eliminate it by soil manipulation such as partial or total humus removal.

Based on restoration experiments in mid-European coniferous forests (de Vries *et al.* 1995, Baar 1997, Smit *et al.* 2003), root growth was expected to increase due to removal of the humus layer. Resource allocation to the Scots pine seedling root system associated with ECM fungi was not as remarkable as allocation to shoots. Root growth increased, but the root-to-shoot ratio decreased after partial humus removal at the urban sites (V). In the greenhouse experiment, total humus removal decreased root growth of the seedlings grown in urban soils, but the root-to-shoot ratio did not differ from other treatments (VI). In mid-boreal



forests, availability of N compounds determines plant biomass allocation (Mäkelä *et al.* 2008), and, especially, biomass allocation to roots (Helmisaari *et al.* 2009). Thus, after restoration treatments applied to urban soil, increased N availability was incorporated into shoots (V, VI). A slight decrease in N allocation to needles after restoration could imply later growth reduction of the pine seedlings at the urban sites. As a novel finding, root POD activity decreased after partial humus removal at urban sites, which possibly predicts improved conditions for Scots pine roots.

## **5.4 Soil manipulation as a restoration method in urban forests**

### **5.4.1 Does partial removal of vegetation and humus layer improve diversity of fruiting ECM fungal species in urban forests?**

The results of the partial humus removal treatment differed from earlier studies (Table 1) conducted in mid-European Scots pine stands using sod-cutting, *i.e.* total removal of the vegetation and humus layer (de Vries *et al.* 1995, Baar & ter Braak 1996, Baar & Kuyper 1998, Smit *et al.* 2003, Boxman & Roelofs 2006) or in southern-Swedish *Fagus sylvatica* L. stands using repeated litter removal (Tyler 1991). Only the appearance of *Cortinarius semisanguineus* at the urban sites after partial humus removal treatment (IV) was promising in terms of restoration of ECM fungal communities, as this species has earlier been found to decline along an urban pollution gradient (I).

It has to be kept in mind, however, that the first years after the partial humus removal treatment represent the early stage of the restoration process. Because soil microbial processes are slower in more northerly areas compared to conditions in the temperate zone, recovery of soil after manipulation possibly needs more time. The seemingly slow response of ECM fungal species to the restoration treatment in our experiment could also partially be explained by the fact that the sporocarp production was low on average during the first years of this experiment due to dry growing seasons. Moreover, sporocarp production does not necessarily indicate what happens belowground. These results emphasize the importance of long-term studies, as sporocarp production is strongly influenced by climate and soil temperature (de Aragón *et al.* 2007) and weather conditions in previous years (Ohenoja 1993). Furthermore, according to Baar & Kuyper (1998), total humus removal enhanced fruiting of ECM fungi more in mature than in

young forest stands, which means that the response is also dependent on the age of the forest stand.

The removal of the humus layer should promote many ECM fungi over saprophytic fungi, as at least litter-decomposing saprophytic fungi are primarily found on the surface of the forest floor (Dickie *et al.* 2002, Lindahl *et al.* 2007). Thus, a relatively larger proportion of saprophytic compared to ECM fungal mycelia was removed along with the litter and humus layer, or, at least, their growth was altered. This was shown as a slight decrease in fruiting saprophytic fungi (IV), which is parallel to that in Tyler (1991). Competitive relations between saprophytic and ECM fungi could thus have changed to benefit ECM fungi. The relations between ECM fungal species could have been changed by removing most of the inocula of some ECM fungal species. Those ECM fungi that were fruiting in the urban restoration treatment are suggested to tolerate disturbance. *Cortinarius croceus* and *C. semisanguineus* produce sporocarps in soils rich in organic matter (Høiland 1992). *Cortinarius croceus* also occurs in disturbed soils, e.g. along roadsides, whereas *C. semisanguineus* is typical in nutrient-poor coniferous forests (Kytövuori *et al.* 2005). *Lactarius rufus* is a very common ECM fungal species of Scots pine forests. *Lactarius turpis* usually occurs with *Betula* or *Picea* and is common in parks (Persson *et al.* 1992). Some *Inocybe* spp. are typical also for parks and disturbed soils in Finland (Kytövuori *et al.* 2005). However, because of the small size of the treated square, the dry growing seasons and the relatively short surveying period (5 years), the present results are not adequate to make conclusions about whether a partial humus removal treatment can be used to improve conditions for ECM fungi.

#### **5.4.2 The effects of soil manipulation on vegetation and soil nutrient availability**

Restoration success may have been reduced by the rather slow recovery of the 'natural forest vegetation' after partial humus removal at urban sites, though herbs and grasses recovered rapidly. Decline and slow recovery in *Empetrum* cover confirms earlier findings that *Empetrum* suffers severely from mechanical disturbance (Tybirk *et al.* 2000). The recovery of *Vaccinium* spp. was promoted in this study by leaving the main part of the rhizomes in the soil. Also, the size of treated area was relatively small, and the surrounding vegetation remained unaffected.

It is notable that the partial humus removal treatment was not effective in reducing *D. flexuosa*, as the species recovered at four of the six urban sites. In Finland, silvicultural practices such as clear-cutting, in combination with soil preparation have been found to favor grasses, most typically *D. flexuosa* (Uotila *et al.* 2005). Thus, rapid recovery of herbs and grasses could be a sign either of a rich seed bank in the soil (Amrein *et al.* 2005) or seed dispersal from nearby seed lots (Pykälä 2004). The rapid recovery indicates that vegetation removal should be done more than once to reduce the N load in the urban forests. Also Vidra *et al.* (2007) suggest that repeated removal is required to decrease non-typical species, especially if the site is in close proximity to a propagule source. This implies that slight mechanical disturbance of the humus layer, *i.e.* raking, could be used as a restoration method if it is combined with repeated harvesting of the non-typical plants in the middle of the growing season.

Due to removal of the humus layer, part of the N and P stock in the forest soil was reduced, which was the purpose of the treatment. Disturbing N-enriched urban forest humus led, however, to increased availability of soil N, whereas there was no change (VI) or a slight increase (V) in exchangeable P levels during the first growing season after the treatment. Soluble nutrients in the soil were partly incorporated into seedling shoots, as demonstrated by high foliar N of Scots pine seedlings (V, VI). The C/N ratio in the remaining humus layer was not affected by the partial humus removal treatment at urban sites (Fig. 1). On a larger scale, soil management may lead to temporary leakages of N and P compounds to deeper soil layers, as has been observed after silvicultural practices (Kubin 1998, Piirainen *et al.* 2007). However, the leakage risk is high only if soil C/N ratio decreases to below 25 (MacDonald *et al.* 2002). They conclude that stand age, soil type, organic matter content, and soil buffering capacity affect leaching of nutrients in forest ecosystem.

In contrast to treatments disturbing the humus layer, total humus removal (VI) and similar types of experiments in mid-European forests (Baar & ter Braak 1996, Smit *et al.* 2003) resulted in decreased levels of available N and P in both the mineral and humus layer. According to Boxman & Roelofs (2006) as well, total removal of the humus layer also decreases available N levels in mineral soil. Nutrient levels in the soil are also dependent on the age of trees (Baar & ter Braak 1996) and stand origin (Baar 1997). In stands with young trees, available N concentrations in the upper 5 cm of mineral soil were higher in planted stands than in a natural control, whereas the total humus removal showed only a slight decrease in available N (Baar 1997). Also, other soil properties have a strong

influence on responses to management, as soil properties and soil management interact in affecting N dynamics (Gomez *et al.* 2002). Thus, the outcome of a restoration treatment largely depends on the size of the overall nutrient pool accumulated in the humus layer, the stand age and management intensity. These aspects should be taken into account when planning urban forest restoration.

### **5.4.3 Concluding remarks about soil manipulation methods**

According to present results, use of soil manipulation as a tool for restoration may be limited in mid-boreal urban forests. Soil manipulation treatments are time-consuming and risk nutrient leakage from forest soils and invasion of non-typical plant species. Silvicultural practices also result in similar risks, but as disturbance seldom occurs during the rotation, recovery of the natural vegetation (Uotila *et al.* 2005) and ECM fungal community (Heinonsalo 2004) is possible. Thus, as in all restoration planning, decision makers and planners should at first consider the goal for conservation and restoration projects. Is the natural mid-boreal dryish type of forest the goal, or can we accept more species rich forest types with grasses and herbs? Secondly, it is worth considering how to diminish harmful effects and pressures of recreation on the rest of the fragmented forests. Hamberg (2009) suggests that the urban spruce forests in southern Finland should be at least 3 ha in size, and as circular as possible to prevent harmful edge-effects on vegetation. Thus, risk assessment and research in improving control efforts need to be involved in practical conservation projects (Byers *et al.* 2002). The present study was the first attempt to investigate the effects of restoration in mid-boreal urban forests.

Future restoration plans should include long-term monitoring of variables that reflect important ecosystem attributes in order to determine the success of a project and to direct future management efforts (Ruiz-Jaén & Aide 2006, Vidra *et al.* 2007). As Pickett & Cadenasso (2008) emphasize, information concerning ecological processes can help to integrate research into city planning, and can be utilized in urban design projects as tool. Restoration success should be evaluated using different variables of community structure, groups of organisms, species-level responses and variables of ecosystem processes. Most of the available information considers responses of plant species to urbanization. Lichens and mosses are used as pollution indicators. Also, decomposition processes have been studied along rural-urban gradients (Ohtonen 1994, Pouyat *et al.* 1997). However, fungal diversity should be taken into account, because diversity of fruiting ECM

fungus species has decreased even in the less-urbanized mid-boreal forest stands. In boreal forests, homogenization of fungal communities is a more obvious response to urbanization than the homogenization of plant communities observed in temperate areas. Thus, fungal diversity in urban forests requires further research in order to link ecological knowledge with urban design.



## 6 Implications for management of urban coniferous forests

ECM fungal communities in urban mid-boreal forest stands had few fruiting ectomycorrhizal species after long-term low-level exposure to N and S pollution as well as human recreation, whereas plant diversity increased as slow-growing dwarf shrubs were partly replaced by herbs and grasses. Urban soils differed physically, chemically and biologically from rural soils of a similar mid-boreal forest type. The present results also imply that N and S pollution effects were mediated mainly via soil, whereas aerial pollution did not severely affect growth of Scots pine seedlings under low pollution levels. These differences in mid-boreal forests were thought to result from long-term changes in soil properties during urbanization of the area.

As expected, the adverse effects of excess N on plant and fungal communities can be alleviated by managing the soil. However, the partial humus removal treatment alone may not be sufficient for improving conditions for fungal and plant species typical in the mid-boreal forests. The fungal diversity was potentially further diminished by removing the upper layer of litter and humus. On the other hand, fruiting of *Cortinarius semisanguineus* is a positive response to the partial humus removal treatment. Emergence of new Scots pine seedlings was poor due to rapid revegetation at the urban sites, but the soil treatment promoted both root and shoot growth of planted Scots pine seedlings in the urban forests. Decreased root POD activity in Scots pine seedlings possibly indicates low root stress.

The other restoration methods, total humus removal and humus disturbance, could possibly be used as well. If the total humus removal treatment is used as a restoration method in N-rich stands, the inocula of many ECM fungi is largely removed. The total humus removal treatment is time consuming with high risk of invasion by non-typical plant species. Slight mechanical disturbance of the humus layer could be used to reduce the N load if combined with repeated harvesting of the field layer vegetation, though there is a risk of nutrient leakage.

Restoration in mid-boreal urban forests requires more research to address local needs before any large-scale operations. Maintaining urban forests close to natural ones is dependent on the size of urban forest stands and the level of recreational use. The demand to densify urban areas increases as more and more people move to cities also in northern parts of Finland. Thus, the present knowledge of urban ecology needs to be linked to urban planning.





## References

- Ahonen U, Markkola AM, Ohtonen R, Tarvainen O & Väre H (1989) Determination of peroxidase activity in some fungi and in mycorrhizal and non-mycorrhizal *Pinus sylvestris* roots. *Aquilo Series Botanica* 26: 25–30
- Ahti T, Hämet-Ahti L, Jalas J (1968) Vegetation zones and their sections in northwestern Europe. *Annales Botanici Fennici* 5: 169–211.
- Alberton O, Kuyper TW & Gorissen A (2007) Competition for nitrogen between *Pinus sylvestris* and ectomycorrhizal fungi generates potential for negative feedback under elevated CO<sub>2</sub>. *Plant and Soil* 296: 159–172.
- Albrecht C, Burgess T, Dell B & Lapeyrie F (1994) Chitinase and peroxidase activities are induced in eucalyptus roots according to aggressiveness of Australian ectomycorrhizal strains of *Pisolithus* sp. *New Phytologist* 127: 217–222.
- Allen EB, Allen MF, Helm DJ, Trappe JM, Molina R & Rincon E (1995) Patterns and regulation of mycorrhizal plant and fungal diversity. *Plant and Soil* 170: 47–62.
- Alvey AA (2006) Promoting and preserving biodiversity in the urban forest. *Urban Forestry & Urban Greening* 5: 195–201.
- Amrein D, Rusterholz H-P & Baur B (2005) Disturbance of suburban *Fagus* forests by recreational activities: Effects on soil characteristics, above-ground vegetation and seed bank. *Applied Vegetation Science* 8: 175–182.
- Andersson P, Berggren D & Nilsson I (2002) Indices for nitrogen status and nitrate leaching from Norway spruce (*Picea abies* (L.) Karst.) stands in Sweden. *Forest Ecology and Management* 157: 39–53.
- Annesi T, Coppola R & Motta E (2003) Decay and canker caused by *Inonotus rickii* spreading on more urban tree species. *Forest Pathology* 33: 405–412.
- de Aragón JM, Bonet JA, Fischer CR & Colinas C (2007) Productivity of ectomycorrhizal and selected edible saprotrophic fungi in pine forests of the pre-Pyrenees mountains, Spain: Predictive equations for forest management of mycological resources. *Forest Ecology and Management* 252: 239–256.
- Arnolds E (1991) Decline of ectomycorrhizal fungi in Europe. *Agriculture Ecosystems and Environment*, 35: 209–244.
- Asiegbu FO, Johansson M & Stenlid J (1999) Reactions of *Pinus sylvestris* (Scots pine) root tissues to the presence of mutualistic, saprotrophic and necrotrophic microorganisms. *Journal of Phytopathology* 147, 257–264.
- Baar J (1997) Ectomycorrhizal root growth in Scots pine stands in response to manipulation of litter and humus layers. *Mycorrhiza* 7: 89–94.
- Baar J & ter Braak CJF (1996) Ectomycorrhizal sporocarp occurrence as affected by manipulation of litter and humus layers in Scots pine stands of different age. *Applied Soil Ecology* 4: 61–73.
- Baar J & Kuyper ThW (1998) Restoration of aboveground ectomycorrhizal flora in stands of *Pinus sylvestris* (Scots pine) in the Netherlands by removal of litter and humus. *Restoration Ecol.* 6: 227–237.

- Baar J, Ozinga WA & Kuyper ThW (1994) Spatial distribution of *Laccaria bicolor* genets reflected by sporocarps after removal of litter and humus layers in a *Pinus sylvestris* forest. *Mycological Research* 98: 726–728.
- Baxter JW, Pickett STA, Carreiro MM & Dighton J (1999) Ectomycorrhizal diversity and community structure in oak forest stands exposed to contrasting anthropogenic impacts. *Canadian Journal of Botany* 77: 771–782.
- Baxter JW, Pickett STA, Dighton J & Carreiro MM (2002) Nitrogen and phosphorus availability in oak forest stands exposed to contrasting anthropogenic impacts. *Soil Biology & Biochemistry* 34: 623–633.
- Baycu G, Tolunay D, Özden H & Günebakan S (2006) Ecophysiological and seasonal variations in Cd, Pb, Zn, and Ni concentrations in the leaves of urban deciduous trees in Istanbul. *Environmental Pollution* 143: 545–554.
- Berg B & Dise N (2004) Calculating the long-term stable nitrogen sink in northern European forests. *Acta Oecologica* 26: 15–21.
- van den Berg AE, Hartig T, Staats H (2007) Preference for Nature in Urbanized Societies: Stress, Restoration, and the Pursuit of Sustainability. *Journal of Social Issues* 63: 79–96.
- Bever JD, Richardson SC, Lawrence BM, Holmes J & Watson M (2009) Preferential allocation to beneficial symbiont with spatial structure maintains mycorrhizal mutualism. *Ecology Letters* 12: 13–21.
- Bidartondo MI, Ek H, Wallander H & Söderström B (2001) Do nutrient additions alter carbon sink strength of ectomycorrhizal fungi? *New Phytologist* 151: 543–550.
- Bloom AJ, Chapin III FS & Mooney HA (1985) Resource limitation in plants – an economic analogy. *Annual Review of Ecology and Systematics* 16: 363–392.
- Bobbink R, Hornung M & Roelofs JGM (1998) The effects of air-borne nitrogen pollutants on species diversity in natural and seminatural European vegetation. *Journal of Ecology* 86: 717–738.
- Borgmann KL & Rodewald AD (2005) Forest restoration in urbanizing landscapes: Interactions between land uses and exotic shrubs. *Restoration Ecology* 13: 334–340.
- Boxman AW & Roelofs JGM (2006) Effects of liming, sod-cutting and fertilization at ambient and decreased nitrogen deposition on the soil solution chemistry in a Scots pine forest in the Netherlands. *Forest Ecology & Management* 237: 237–245.
- Brandrud TE (1995) The effects of experimental nitrogen addition on the ectomycorrhizal fungus flora in an oligotrophic spruce forest at Gårdsjön, Sweden. *Forest Ecology and Management* 71: 111–122.
- Brandrud TE & Timmermann V (1998) Ectomycorrhizal fungi in the NITREX site at Gårdsjön, Sweden; below and above-ground responses to experimentally-changed nitrogen inputs 1990–1995. *Forest Ecology and Management* 101: 207–214.
- Brunner I, Luster J, Günthardt-Goerg S & Frey B (2008) Heavy metal accumulation and phytostabilisation potential of tree fine roots in a contaminated soil. *Environmental Pollution* 152: 559–568.
- Byers JE & EG Noonburg (2003) Scale dependent effects of biotic resistance to biological invasion. *Ecology* 84: 1428–1433.

- Byers JE, Reichard S, Randall JM, Parker IM, Smith CS, Lonsdale WM, Atkinson IAE, Seastedt TR, Williamson M, Chornesky E & Hayes D (2002) Directing research to reduce the impacts of nonindigenous species. *Conservation Biology* 16: 630–640.
- Carfrae JA, Skene KR, Sheppard LJ, Ingleby K & Crossley A (2006) Effects of nitrogen with and without acidified sulphur on an ectomycorrhizal community in a Sitka spruce (*Picea sitchensis* Bong. Carr) forest. *Environmental Pollution* 141: 131–138.
- Chalot M, Brun A, Botton B & Söderström B (1996) Kinetics, energetics and specificity of a general amino acid transporter from the ectomycorrhizal fungus *Paxillus involutus*. *Microbiology* 142: 1749–1756.
- Charvet-Candela V, Hitchin S, Reddy MS, Cournoyer B, Marmeisse R & Gay G (2002) Characterization of a *Pinus pinaster* cDNA encoding an auxin up-regulated putative peroxidase in roots. *Tree Physiology* 22: 231–238.
- Coomes DA & Grubb PJ (2000) Impacts of root competition in forests and woodlands: A theoretical framework and review of experiments. *Ecological Monographs* 70: 171–207.
- Corbin JD, Avis PG & Wilbur RB (2003) The role of phosphorus availability in the response of soil nitrogen cycling, understory vegetation and arbuscular mycorrhizal inoculum potential to elevated nitrogen inputs. *Water, Air, and Soil Pollution* 147: 141–161.
- Courbot M, Diez L, Ruotolo R, Chalot M & Leroy P (2004) Cadmium-Responsive thiols in the ectomycorrhizal Fungus *Paxillus involutus*. *Applied and Environmental Microbiology* 70: 7413–7417.
- Cousins JR, Hope D, Gries C & Stutz JC (2003) Preliminary assessment of arbuscular mycorrhizal fungal diversity and community structure in an urban ecosystem. *Mycorrhiza* 13:319–326.
- Cudlin P, Kieliszewska-Rokicka B, Rudawska M, Grebenc T, Alberton O, Lehto T, Bakker MR, Børja I, Konôpka B, Leski T, Kraigher H & Kuyper TW (2007) Fine roots and ectomycorrhizas as indicators of environmental change. *Plant Biosystems* 141: 406–425.
- Dahlberg A (1990) Effect of soil humus cover on the establishment and development of mycorrhizae on containerized *Pinus sylvestris* L. and *Pinus contorta* ssp. *latifolia* Engelm. after outplanting. *Scandinavian Journal of Forest Research* 5: 103–112.
- Dahlberg A (2002) Effects of fire on ectomycorrhizal fungi in Fennoscandian boreal forests. *Silva Fennica* 36: 69–80.
- Dewald L, White TL & Duryea ML (1992) Growth and phenology of seedlings of four contrasting slash pine families in ten nitrogen regimes. *Tree Physiology* 11: 255–269.
- Dickie IA, Xu B & Koide RT (2002) Vertical niche differentiation of ectomycorrhizal hyphae in soil as shown by T-RFLP analysis. *New Phytologist* 156: 527–535.
- Diekmann M & Falkengren-Grerup U (1998) A new species index for forest vascular plants: development of functional indices based on mineralization rates of various forms of soil nitrogen. *Journal of ecology*, 86: 269–283.

- Dighton J, Tuininga AR, Gray DM, Huskins RE & Belton T (2004) Impacts of atmospheric deposition on New Jersey pine barrens forest soils and communities of ectomycorrhizae. *Forest Ecology and Management* 201: 131–144.
- Domish T, Finér L, Lehto T & Smolander A (2002) Effect of soil temperature on nutrient allocation and mycorrhizas in Scots pine seedlings. *Plant and Soil* 239: 173–185.
- Drebs A, Nordlund A, Karlsson P, Helminen J & Rissanen P (2002) Tilastoja Suomen ilmastosta 1971–2000 - Climatological statistics of Finland 1971–2000. *Ilmatilastoja Suomesta* 1. [In Finnish with English summary]
- Edwards GS, Friend AL, O'Neill EG, Tomlinson PT (1992) Seasonal patterns of biomass accumulation and carbon allocation in *Pinus taeda* seedlings exposed to ozone, acidic precipitation, and reduced soil Mg. *Canadian Journal of Forest Research* 22: 640–646.
- Egerton-Warburton LM & Allen EB (2000) Shifts in arbuscular mycorrhizal communities along an anthropogenic nitrogen deposition gradient. *Ecological Applications* 10: 484–496.
- Egli S, Peter M, Buser C, Stahel W & Ayer F (2006) Mushroom picking does not impair future harvests – results of a long-term study in Switzerland. *Biological Conservation* 129, 271–276.
- Estama E (1967) Oulun kaupungin metsänhoitosuunnitelma vuosille 1967–1976. Oulun kaupungin metsänhoito-osasto.
- Falkengren-Grerup U & Diekmann M (2003) Use of a gradient of N-deposition to calculate effect-related soil and vegetation measures in deciduous forests. *Forest Ecology and Management* 180: 113–124.
- Fangmeier A, Hadwiger-Fangmeier A, Van der Eerden L & Jäger H-J (1994) Effects of atmospheric ammonia on vegetation - a review. *Environmental Pollution* 86: 43–82.
- Fransson PMA, Taylor AFS & Finlay RD (2005) Mycelial production, spread and root colonization by the ectomycorrhizal fungi *Hebeloma crustuliniforme* and *Paxillus involutus* under elevated atmospheric CO<sub>2</sub>. *Mycorrhiza* 15: 25–31.
- Gilliam FS (2006) Response of the herbaceous layer of forest ecosystems to excess nitrogen deposition. *Journal of Ecology* 94: 1176–1191.
- Godbold DL, Hoosbeek MR, Lukac M, Cotrufo MF, Janssens IA, Ceulemans R, Polle A, Velthorst EJ, Scarascia-Mugnozza G, De Angelis P, Miglietta F & Peresotti A (2006) Mycorrhizal hyphal turnover as a dominant process for carbon input into soil organic matter. *Plant and Soil* 281: 15–24.
- Gomez AG, Powers RF, Singer MJ & Horwath WR (2002) N uptake and N status in ponderosa pine as affected by soil compaction and forest floor removal. *Plant and Soil* 242: 263–275.
- Gorissen A & Kuyper ThW (2000) Fungal species-specific responses of ectomycorrhizal Scots pine (*Pinus sylvestris*) to elevated [CO<sub>2</sub>]. *New Phytologist* 146: 163–168.
- Günther Th, Perner B & Gramss G (1998) Activities of phenol oxidizing enzymes of ectomycorrhizal fungi in axenic culture and in symbiosis with Scots pine (*Pinus sylvestris* L.). *Journal of Basic Microbiology* 38: 197–206.

- Gundersen P, Emmett BA, Kjønås OJ, Koopmans CJ & Tietema A (1998) Impact of nitrogen deposition on nitrogen cycling in forests: a synthesis of NITREX data. *Forest Ecology and Management* 101: 37–55.
- Hagerberg D, Thelin G & Wallander H (2003) The production of ectomycorrhizal mycelium in forests: Relation between forest nutrient status and local mineral sources. *Plant and Soil* 252: 279–290.
- Hamberg L (2009) The effects of habitat edges and trampling on vegetation in urban forests. PhD thesis, Department of Biological and Environmental Sciences, University of Helsinki. URL
- Hamberg L, Lehvävirta S, Malmivaara-Lämsä M, Rita H & Kotze DJ (2008) The effects of habitat edges and trampling on understorey vegetation in urban forests in Helsinki, Finland. *Applied Vegetation Science* 11: 83–98.
- Hanssen KH, Granhus A, Brække FH & Haveraaen O (2003) Performance of sown and naturally regenerated *Picea abies* seedlings under different scarification and harvesting regimens. *Scandinavian Journal of Forest Research* 18: 351–361.
- van der Heijden MGA, Bardgett RD & van Straalen NM (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters* 11: 296–310.
- Heinonsalo J (2004) The effects of forestry practices on ectomycorrhizal fungal communities and seedling establishment. *Dissertationes Biocentri Viikki Universitatis Helsingiensis* 9. URI: <http://ethesis.helsinki.fi/julkaisut/bio/bioja/vk/heinonsalo/theeffec.pdf>. Cited 2009/1/21.
- Helmisaari H-S, Ostonen I, Löhmus K, Derome J, Lindroos A-J, Merilä P & Nöjd P (2009) Ectomycorrhizal root tips in relation to site and stand characteristics in Norway spruce and Scots pine stands in boreal forests. *Tree Physiology* 29: 445–456.
- Hobbie EA, Jumpponen A & Trappe J (2005) Foliar and fungal  $^{15}\text{N}$ : $^{14}\text{N}$  ratios reflect development of mycorrhizae and nitrogen supply during primary succession: testing analytical models. *Oecologia* 146: 258–268.
- Högberg P, Plamboeck AH, Taylor AFS & Fransson PMA (1999) Natural  $^{13}\text{C}$  abundance reveals trophic status of fungi and host-origin of carbon in mycorrhizal fungi in mixed forests. *Proceedings of the National Academy of Sciences (USA)* 96:8534–8539.
- Högberg P, Fan H, Quist M, Binkley D & Tamm GO (2006) Tree growth and soil acidification in response to 30 years of experimental nitrogen loading on boreal forest. *Global Change Biology* 12: 489–499.
- Høiland K (1992) *Dermocybe*. In: Hansen L & Knudsen H (eds) *Nordic Macromycetes*, Vol. 2. Polyporales, Boletales, Agaricales, Russulales. Copenhagen, Nordsvamp: 280–284.
- Hutchings MJ, John EA & Wijesinghe DK (2003) Toward understanding the consequences of soil heterogeneity for plant populations and communities. *Ecology* 84: 2322–2334.
- Iivonen S (2002) Responses of Scots pine (*Pinus sylvestris* L.) seedlings to root zone temperature and nutrient supply in hydroponic culture during the second growing season. The Finnish Forest Research Institute, Research Papers 833.

- Iwański M, Rudawska M & Leski T (2006) Mycorrhizal associations of nursery grown Scots pine (*Pinus sylvestris* L.) seedlings in Poland. *Annals of Forest Science* 63: 715–723.
- Jalonen J, Vanha-Majamaa I & Tonteri T (1998) Optimal sample and plot size for inventory of field and ground layer vegetation in a mature *Myrtillus*-type boreal spruce forest. *Acta Botanica Fennica* 35: 191–196.
- Jones MS & Smith SE (2004) Exploring functional definitions of mycorrhizas: Are mycorrhizas always mutualisms? *Can. J. Bot.* 82: 1089–1109.
- Jones MD, Durall DM & Cairney JWG (2003) Ectomycorrhizal fungal communities in young forest stands regenerating after clearcut logging. *New Phytologist* 157: 399–422.
- Jonsson L, Dahlberg A & Brandrud TE (2000) Spatiotemporal distribution of an ectomycorrhizal community in an oligotrophic Swedish *Picea abies* forest subjected to experimental nitrogen addition: above- and below-ground views. *Forest Ecology and Management* 132: 143–156.
- Jumpponen A & Trappe JM (1998) Dark septate endophytes: a review of facultative biotrophic root-colonizing fungi. *New Phytologist* 140: 295–310.
- Kårén O (1997) Effects of air pollution and forest regeneration methods on the community structure of ectomycorrhizal fungi. PhD Thesis. Swedish University of Agricultural Sciences. Sweden.
- Kårén O & Nylund JE (1997) Effects of ammonium sulphate on the community structure and biomass of ectomycorrhizal fungi in a Norway spruce stand in southwestern Sweden. *Canadian Journal of Botany* 75: 1628–1642.
- Karhu M (1986) Männyn neulaset ja koivun lehdet ilman rikkikuormituksen ilmentäjinä. Phil. Lic. Thesis. Department of Botany, University of Oulu, Oulu.
- Karst J, Jones MD & Turkington R (2009) Ectomycorrhizal colonization and intraspecific variation in growth responses of lodgepole pine. *Plant Ecology* 200: 161–165.
- Katainen H-S, Karjalainen R, Mäkinen E, Jokinen J, Kellomäki S, 1984. Effects of SO<sub>2</sub> on photosynthesis and peroxidase activity in Scots pine needles. *European Journal of Forest Pathology* 14, 33–42.
- Kernaghan G, Sigler L & Khasa D (2003) Mycorrhizal and root endophytic fungi of containerized *Picea glauca* seedlings assessed by rDNA sequence analysis. *Microbial Ecology* 45: 128–136.
- Kellner O & Redbo-Torstensson P (1995) Effects of elevated nitrogen deposition on the field-layer vegetation in coniferous forests. *Ecological Bulletins* 44: 227–237.
- Koide RT, Sharda JN, Herr JR & Malcolm GM (2008) Ectomycorrhizal fungi and the biotrophy–saprotrophy continuum. *New Phytologist* 178: 230–233.
- Kokko A, Mäkelä K & Tuominen S (2002) Understorey vegetation monitoring at the Finnish Integrated Monitoring areas in 1988–1998. *Suomen ympäristö* 544. [In Finnish with English summary]
- Korkama T, Fritze H, Kiiikkilä O & Pennanen T (2007) Do same-aged but different height Norway spruce (*Picea abies*) clones affect soil microbial community? *Soil Biology & Biochemistry* 39: 2420–2423.

- Korkama T, Pakkanen A, Pennanen T 2006. Ectomycorrhizal community structure varies among Norway spruce (*Picea abies*) clones. *New Phytologist* 171: 815–824.
- Kubin E (1998) Leaching of nitrate nitrogen into the groundwater after clear felling and site preparation. *Boreal Environment Research* 3: 3–8.
- Kühn I & Klotz S 2006. Urbanization and homogenization – Comparing the floras of urban and rural areas in Germany. *Biological Conservation* 127: 292–300.
- Kuikka K, Härmä E, Markkola AM, Rautio P, Roitto M, Saikkonen K, Ahonen-Jonnarth U, Finlay R & Tuomi J (2003) Severe defoliation of Scots pine reduces reproductive investment by ectomycorrhizal symbionts. *Ecology* 84: 2051–2061.
- Kukkola E, Rautio P & Huttunen S (2000) Stress indications in copper and nickel exposed Scots pine seedlings. *Environmental and Experimental Botany* 43: 197–210.
- Kytövuori I, Nummela-Salo U, Ohenoja E, Salo P & Vauras J (2005) Helttasienten levinneisyystaulukko. In: Salo P, Niemelä T, Nummela-Salo U & Ohenoja E (eds) Suomen helttasienten ja tattien ekologia, levinneisyys ja uhanalaisuus. Suomen ympäristökeskus, Helsinki. *Suomen ympäristö* 769: 109–224.
- Lambers H, Raven JA, Shaver GR & Smith SE (2008) Plant nutrient-acquisition strategies change with soil age. *Trends in Ecology and Evolution* 23: 95–103.
- Langenfeld-Heyser R, Gao J, Ducic T, Tachd Ph, Lu CF, Fritz E, Gafur A & Polle A (2007) *Paxillus involutus* mycorrhiza attenuate NaCl-stress responses in the salt-sensitive hybrid poplar *Populus × canescens*. *Mycorrhiza* 17: 121–131.
- Leake JR, Donnelly DP, Saunders EM, Boddy L & Read DJ (2001) Rates and quantities of carbon flux to ectomycorrhizal mycelium following <sup>14</sup>C pulse labeling of *Pinus sylvestris* seedlings: effects of litter patches and interaction with a wood-decomposer fungus. *Tree Physiology* 21: 71–82.
- Lehto T (1989) Männyntaimien mykorrhitsat keskustaimitarhoilla. Mycorrhizal status of Scots pine nursery stock in Finland. *Folia Forestalia* 726.
- Lehvävirta S, Rita H & Koivula M (2004) Barriers against wear affect the spatial distribution of tree saplings in urban woodlands. *Urban Forestry & Urban Greening* 3: 3–17.
- Lilleskov EA, Bruns TD 2001. Nitrogen and ectomycorrhizal fungal communities: what we know, what we need to know. *New Phytologist* 149:156–158.
- Lilleskov EA, Fahey TJ & Lovett GM (2001) Ectomycorrhizal fungal aboveground community change over an atmospheric nitrogen deposition gradient. *Ecological Applications* 11: 397–410.
- Lilleskov EA, Wargo PM, Vogt KA & Vogt DJ (2008) Mycorrhizal fungal community relationships to nitrogen concentration over a regional atmospheric deposition gradient in the northeastern USA. *Canadian Journal of Forest Research* 38:1260–1266.
- Lindahl BD, Ihrmark K, Boberg K, Trumbore SE, Högberg P, Stenlid J & Finlay RD (2007) Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. *New Phytologist* 173: 611–620.

- Lippu J 1999. Assimilation and allocation of carbon in Scots pine seedlings during shoot elongation and as affected by soil temperature. Doctoral Thesis. University of Helsinki Department of Forest Ecology Publications 19.
- MacDonald JA, Dise NB, Matzner E, Armbruster M, Gundersen P & Forsius M (2002) Nitrogen input together with ecosystem nitrogen enrichment predict nitrate leaching from European forests. *Global Change Biology* 8: 1028–1033.
- Mäkelä A, Valentine HT & Helmisaari H-S (2008) Optimal co-allocation of carbon and nitrogen in a forest stand at steady state. *New Phytologist* 180: 114–123.
- Mäkipää R 1994. Effects of nitrogen fertilization on the humus layer and ground vegetation under closed canopy in boreal coniferous stands. *Silva Fennica* 28:81–94.
- Malmivaara-Lämsä M (2008) Effects of recreational use and fragmentation on the understorey vegetation and soil microbial communities of urban forests in southern Finland. *Dissertationes Forestales* 54. URI: <http://urn.fi/URN:ISBN:978-951-651-197-2>. Cited 2009/3/16.
- Malmivaara M, Löfström I & Vanha-Majamaa I (2002) Anthropogenic effects on understorey vegetation in *Myrtillus* type urban forests in southern Finland. *Silva Fennica* 36: 367–381.
- Markkola AM (1996) Effect of artificial defoliation on biomass allocation in ectomycorrhizal *Pinus sylvestris* seedlings. *Canadian Journal of Forest Research* 26: 899–904.
- Markkola AM, Kuikka K, Rautio P, Härmä E, Roitto M & Tuomi J (2004) Defoliation increases carbon limitation in ectomycorrhizal symbiosis of *Betula pubescens*. *Oecologia* 140: 234–240.
- Markkola AM, Ohtonen R & Tarvainen O (1990) Peroxidase activity as an indicator of pollution stress in the fine roots of *Pinus sylvestris*. *Water, Air, and Soil Pollution* 52: 149–156.
- Markkola AM, Ohtonen R, Tarvainen O & Ahonen-Jonnarh U (1995) Estimates of fungal biomass in Scots pine stands on an urban pollution gradient. *New Phytologist* 131: 139–147.
- McGuire KL (2007) Common ectomycorrhizal networks may maintain monodominance in a tropical rain forest. *Ecology* 88: 567–574.
- McKinney ML (2006) Urbanization as a major cause of biotic homogenization. *Biological Conservation* 127: 247–260.
- McKinney ML (2005) Species introduced from nearby sources have a more homogenizing effect than species from distant sources: evidence from plants and fishes in the USA. *Diversity and Distributions* 1: 367–374.
- Mitchell RJ, Garrett HE, Cox GS & Atalay A (1986) Boron and ectomycorrhizal influences on indole-3-acetic acid levels and indole-3-acetic acid oxidase and peroxidase activities of *Pinus echinata* Mill. roots. *Tree Physiology* 1: 1–8.
- Münzenberger B, Otter T, Wüstrich D & Polle A (1997) Peroxidase and laccase activities in mycorrhizal and non-mycorrhizal fine roots of Norway spruce (*Picea abies*) and larch (*Larix decidua*). *Canadian Journal of Botany* 75: 932–938.



- Nadelhoffer KJ (2000) The potential effects of nitrogen deposition on fine-root production in forest ecosystems. *New Phytologist* 147: 131–139.
- Nara K (2005) Ectomycorrhizal networks and seedling establishment during early primary succession. *New Phytologist* 169: 169–178.
- Näsholm T (1998) Qualitative and quantitative changes in plant nitrogen acquisition induced by anthropogenic nitrogen deposition. *New Phytol* 139: 87–90.
- Näsholm T & Persson J (2001) Plant acquisition of organic nitrogen in boreal forests. *Physiologia Plantarum* 111: 419–426.
- Näsholm T, Ekblad A, Nordin A, Giesler R, Högberg M & Högberg P (1998) Boreal forest plants take up organic nitrogen. *Nature* 392: 914–916.
- Nehls U, Grunze N, Willmann M, Reich M & Küster H (2007) Sugar for my honey: Carbohydrate partitioning in ectomycorrhizal symbiosis. *Phytochemistry* 68: 82–91.
- Niemelä J (1999) Ecology and urban planning. *Biodiversity and Conservation* 8: 119–131.
- Nilsson LO & Wallander H (2003) Production of external mycelium by ectomycorrhizal fungi in a norway spruce forest was reduced in response to nitrogen fertilization. *New Phytologist* 158: 409–416.
- Nilsson LO, Giesler R, Bååth E & Wallander H (2005) Growth and biomass of mycorrhizal mycelia in coniferous forests along short natural nutrient gradients. *New Phytologist* 165: 613–622.
- Nilsson M-C, Högberg P, Zackrisson O & Fengyou W (1993) Allelopathic effects by *Empetrum hermaphroditum* on development and nitrogen uptake by roots and mycorrhizae of *Pinus silvestris*. *Canadian Journal of Botany* 71: 620–628.
- Nilsson M-C, Wardle DA & Dahlberg A (1999) Effects of plant litter species composition and diversity on the boreal forest plant-soil system. *Oikos* 86: 16–26.
- Nordin A, Strengbom J, Witzell J, Näsholm T & Ericson L (2005) Nitrogen Deposition and the Biodiversity of Boreal Forests: Implications for the Nitrogen Critical Load. *Ambio* 34: 20–24.
- Nylund J-E, Wallander H (1992) Ergosterol analysis as a means of quantifying mycorrhizal biomass. *Methods in Microbiology* 24: 77–88.
- Ochimaru T & Fukuda K (2007) Changes in fungal communities in evergreen broad-leaved forests across a gradient of urban to rural areas in Japan. *Canadian Journal of Forest Research* 37: 247–258.
- Ohenoja E (1993) Effect of weather conditions on the larger fungi at different forest sites in northern Finland in 1976–1988. *Acta Universitatis Ouluensis A* 243.
- Ohenoja E (1994) Forest fertilization and the fruiting body production of larger fungi. In: Martikainen P (ed) Effect of Fertilization on Forest Ecosystem. *Biological Research Reports from the University of Jyväskylä* 38: 140–155.
- Ohenoja E 1995. Effect of winter conditions on the fruit body production of larger fungi. *Acta Universitatis Ups. Symb. Bot. Ups.* XXX:3, 163–168.
- Ohtonen R (1994) Accumulation of organic matter along a pollution gradient - application of Odum's theory on ecosystem energetics. *Microbial Ecology* 27: 43–55.
- Ohtonen R, Markkola AM, Torvela H (1989) Total sulfur content in the humus layer of urban polluted forest soils. *Water, Air, and Soil Pollution* 44: 135–141.

- Ohtonen R, Markkola AM, Heinonen-Tanski H & Fritze H (1990) Soil biological parameters as indicators of changes in Scots pine forests (*Pinus sylvestris* L.) caused by air pollution. In: Kauppi P, Anttila P & Kenttämies K (eds) *Acidification in Finland*. Berlin, Springer-Verlag: 373–393.
- Ohtonen R, Munson A & Brand D (1992) Soil microbial community response to silvicultural intervention in coniferous plantation ecosystems. *Ecological Applications* 2: 363–375.
- Ohtonen R, Ohtonen A, Luotonen H, Markkola AM (1992) Enchytraeid and nematode numbers in urban, polluted Scots pine (*Pinus sylvestris* L.) stands in relation to other soil biological parameters. *Biology and Fertility of Soils* 13: 50–54.
- Oulun seudun ympäristövirasto (2005) Oulun ympäristön tila 2005. URI: <http://www.ouka.fi/ymparisto/pdf/Ymptila%202005.pdf>. Cited 2009/2/20.
- Passardi F, Cosio C, Penel C & Dunand C (2005) Peroxidases have more functions than a Swiss army knife. *Plant Cell Reports* 24: 255–265.
- Peay KG, Bruns TD, Kennedy PG, Bergemann SE & Garbelotto M (2007) A strong species–area relationship for eukaryotic soil microbes: island size matters for ectomycorrhizal fungi. *Ecology Letters* 10: 470–480.
- Pennanen T, Heiskanen J & Korkama T (2005) Dynamics of ectomycorrhizal fungi and growth of Norway spruce seedlings after planting on a mounded forest clearcut. *Forest Ecology and Management* 213: 243–252.
- Persson H & Ahlström K (2002) Fine-root response to nitrogen supply in nitrogen manipulated Norway spruce catchment areas. *Forest Ecology and Management* 168: 29–41.
- Persson J & Näsholm T (2001) Amino acid uptake: a widespread ability among boreal forest plants. *Ecology Letters* 4: 434–438.
- Persson O, Printz P & Stordal J (1992) *Lactarius*. In: Hansen L & Knudsen H (eds) *Nordic Macromycetes, Vol. 2. Polyporales, Boletales, Agaricales, Russulales*. Copenhagen, Nordsvamp: 360–374.
- Peter M, Ayer F & Egli S (2001) Nitrogen addition in a Norway spruce stand altered macromycete sporocarp production and below-ground ectomycorrhizal species composition. *New Phytologist* 149: 311–325.
- Pickett STA & Cadenasso ML (2008) Linking ecological and built components of urban mosaics: an open cycle of ecological design. *Journal of Ecology* 96: 8–12.
- Piirainen S, Finér L, Mannerkoski H & Starr M (2007) Carbon, nitrogen and phosphorus leaching after site preparation at a boreal forest clear-cut area. *Forest Ecology and Management* 243: 10–18.
- Posch M, Hettelingh J-P & de Smet PAM (2001) Characterization of critical load exceedances in Europe. *Water, Air, and Soil pollution* 130: 1139–1144
- Pouyat RV, McDonnell MJ & Pickett STA (1997) Litter decomposition and nitrogen mineralization in oak stands along an urban-rural land use gradient. *Urban Ecosystems* 1: 117–131.
- Prescott CE, Maynard DG & Laiho R (2000) Humus in northern forests: friend or foe? *Forest Ecology and Management* 133: 23–36.

- Prescott CE, Hope GD & Blevins LL (2003) Effect of gap size on litter decomposition and soil nitrate concentrations in a high-elevation spruce–fir forest. *Canadian Journal of Forest Research* 33: 2210–2220.
- Pykälä J (2004) Immediate increase in plant species richness after clear-cutting of boreal herb-rich forests. *Applied Vegetation Science* 7: 29–34.
- Reinert RA, Shafer SR, Eason G, Schoeneberger MM & Horton SJ (1996) Responses of loblolly pine to ozone and simulated acidic rain. *Canadian Journal of Forest Research* 26: 1715–1723.
- Rennenberg H, Kreutzer K, Papen H & Weber P (1998) Consequences of high loads of nitrogen for spruce (*Picea abies*) and beech (*Fagus sylvatica*) forests. *New Phytologist* 139: 71–86.
- Roitto M, Ahonen-Jonnarth U, Lamppu J, Huttunen S, 1999. Apoplastic and total peroxidase activity in Scots pine needles at subarctic polluted sites. *European Journal of Forest Pathology* 29, 399–410.
- Roitto M, Strömmer R, Ahonen-Jonnarth U, Hyvärinen M & Markkola AM (2001) Does the lichen mat alleviate the effects of wet deposited nickel on soil microorganisms and Scots pine (*Pinus sylvestris* L.) seedlings? *Plant and Soil* 230: 267–277.
- Roitto M, Markkola AM, Julkunen-Tiitto R, Sarjala T, Rautio P, Kuikka K & Tuomi J (2003) Defoliation-induced responses in peroxidases, phenolics and polyamines in Scots pine (*Pinus sylvestris* L.) needles. *Journal of Chemical Ecology* 29: 1905–1918.
- Rosén K, Gundersen P, Tegnhammar L, Johansson M & Frogner T (1992) Nitrogen enrichment of Nordic forest ecosystems. The concept of critical loads. *Ambio* 21: 364–368.
- Ruiz-Jaén MC & Aide TM (2006) An integrated approach for measuring urban forest restoration. *Urban Forestry & Urban Greening* 4: 55–68.
- Ruotsalainen AL & Kozlov MV (2006) Fungi and air pollution: Is there a general pattern? In: Rhodes D (ed) *New topics in environmental research*: 57–103.
- Saari SK, Campbell CD, Russell J, Alexander IJ & Anderson IC (2005) Pine microsatellite markers allow roots and ectomycorrhizas to be linked to individual trees. *New Phytologist* 165: 295–304.
- Saikkonen K, Ahonen-Jonnarth U, Markkola AM, Helander M, Tuomi J, Roitto M & Ranta H (1999) Defoliation and mycorrhizal symbiosis: a functional balance between carbon sources and belowground sinks. *Ecology Letters* 2: 19–26.
- Salzer P & Hager A (1993) Effect of auxins and ectomycorrhizal elicitors on wall-bound proteins and enzymes of spruce (*Picea abies* (L.) Karst.) cells. *Trees* 8, 49–55.
- Schubert M, Fink S & Schwarze FW (2008) Evaluation of *Trichoderma* spp. as a biocontrol agent against wood decay fungi in urban trees. *Biological Control* 45: 111–123.
- Setälä H, Haimi J & Siira-Pietikäinen A (2000) Sensitivity of soil processes in northern forest soils: are management practices a threat? *Forest Ecology and Management* 133: 5–11.

- Shan Y, Izuta T, Aoki M & Totsuka T (1997) Effects of O<sub>3</sub> and soil acidification, alone and in combination, on growth, gas exchange rate and chlorophyll content of red pine seedlings. *Water, Air, and Soil Pollution* 97: 355–366.
- Shaw PJA, J, Poskitt J & McLeod AR (1992) The effects of sulphur dioxide and ozone on the mycorrhizas of Scots pine and Norway spruce in a field fumigation system. *Mycological Research* 96: 785–791.
- Sievering H, Tomaszewski T & Torizzo J (2007) Canopy uptake of atmospheric N deposition at a conifer forest: part I -canopy N budget, photosynthetic efficiency and net ecosystem exchange. *Tellus* 59B: 483–492.
- Simard SW, Jones MD & Durall DM (2002) Carbon and nutrient fluxes within and between mycorrhizal plants. In: van der Heijden MGA & Sanders IR (eds) *Mycorrhizal ecology*. Berlin, Springer-Verlag: 33–74.
- Sims SE, Hendricks JJ, Mitchell RJ, Kuehn KA & Pecot SD (2007) Nitrogen decreases and precipitation increases ectomycorrhizal extramatrical mycelia production in a longleaf pine forest. *Mycorrhiza* 17: 299–309.
- Sipola K (1994) Effects of forest fertilization on the ground vegetation of coniferous forests on mineral soil sites in North Finland. In: Martikainen P. (ed.) *Effect of Fertilization on Forest Ecosystem*. Biological Research Reports from the University of Jyväskylä 38: 98–111.
- Smit E, Veenman C & Baar J (2003) Molecular analysis of ectomycorrhizal basidiomycete communities in a *Pinus sylvestris* L. stand reveals long-term increased diversity after removal of litter and humus layers. *FEMS Microbiology Ecology* 45: 49–57.
- Smith SE & Read DJ (2008) *Mycorrhizal symbiosis*. 3<sup>rd</sup> edn. Academic Press.
- Souto XC, Chiapusio G & Pellisier F (2000) Relationship between phenolics and soil microorganisms in spruce forests: significance for natural regeneration. *Journal of Chemical Ecology* 26: 2025–2034.
- Spangenberg A & Kölling C (2004) Nitrogen deposition and nitrate leaching at forest edges exposed to high ammonia emissions in Southern Bavaria. *Water, Air, and Soil Pollution* 152: 233–255.
- Spanu P & Bonfante-Fasolo P (1988) Cell-wall-bound peroxidase activity in roots of mycorrhizal *Allium porrum*. *New Phytologist* 109: 119–124.
- Sparks JP (2009) Ecological ramifications of the direct foliar uptake of nitrogen. *Oecologia* 159: 1–13.
- Stampfli A (1991) Accurate determination of vegetational change in meadows. *Vegetatio* 96: 185–194.
- Stevens CJ, Dise NB, Mountford JO & Gowing DJ (2004) Impact of nitrogen deposition on the species richness of grasslands. *Science* 303: 1876–1879.
- Strengbom J, Nordin A, Näsholm T & Ericson L (2001) Slow recovery of boreal forest ecosystem following decreased nitrogen input. *Functional Ecology* 15: 451–457.
- Strengbom J, Walheim M, Näsholm T & Ericson L (2003) Regional differences in the occurrence of understorey species reflect nitrogen deposition in Swedish forests. *Ambio* 32: 91–97.

- Strengbom J, Näsholm T & Ericson L (2004) Light, not nitrogen, limits growth of the grass *Deschampsia flexuosa* in boreal forests. *Canadian Journal of Botany* 82: 430–435.
- Såstad SM & Jenssen HB (1993) Interpretation of regional differences in the fungal biota as effects of atmospheric pollution. *Mycological Research* 97: 1451–1458.
- Tait CJ, Daniels CB & Hill RS (2005) Changes in species assemblages within the Adelaide metropolitan area, Australia, 1836–2002. *Ecological Applications* 15: 346–359.
- Takemoto BK, Bytnerowicz A, Dawson PJ, Morrison CL & Temple PJ (1997) Effects of ozone on *Pinus ponderosa* seedlings: comparison of responses in the first and second growing season of exposure. *Canadian Journal of Forest Research* 27: 23–30.
- Talbot JM, Allison SD & Treseder KK (2008) Belowground responses to climate change. Decomposers in disguise: mycorrhizal fungi as regulators of soil C dynamics in ecosystems under global change. *Functional Ecology* 22: 955–963.
- Tarkka MT, Nyman TA, Kalkkinen N & Raudaskoski M (2001) Scots pine expresses short-root-specific peroxidases during development. *European Journal of Biochemistry* 268: 86–92.
- Tarvainen O, Ahonen-Jonnarth U, Markkola AM & Väre H (1991) The influence of Al, Cu and Ni on peroxidase activity in seedlings of *Pinus sylvestris* and mycelia of *Suillus variegatus*. In: Lobarzewski J, Greppin H, Penel C & Gaspar Th. (eds.), *Biochemical, Molecular and Physiological Aspects of Plant Peroxidases*. Proceedings of the 2nd International Symposium. Lublin, Poland, 1990.
- Taylor AES, Martin F & Read DJ (2000) Fungal diversity in ectomycorrhizal communities of Norway spruce (*Picea abies* (L.) Karst.) and beech (*Fagus sylvatica* L.) along north-south transects in Europe. In: Schulze E-D (ed) *Carbon and nitrogen cycling in European forest ecosystems*. *Ecological studies* 142: 343–365.
- Terho M, Hantula J & Hallaksela AM (2007) Occurrence and decay patterns of common wood-decay fungi in hazardous trees felled in the Helsinki City. *Forest Pathology* 37: 420–432.
- Tilman D (1996) Biodiversity: Population versus ecosystem stability. *Ecology* 77: 350–363.
- Timonen S & Sen R (1998) Heterogeneity of fungal and plant enzyme expression in intact Scots pine – *Suillus bovinus* and – *Paxillus involutus* mycorrhizospheres developed in natural forest humus. *New Phytologist* 138: 355–366.
- Tomaszewski T & Sievering H (2007) Canopy uptake of atmospheric N deposition at a conifer forest: part II – response of chlorophyll fluorescence and gas exchange parameters. *Tellus* 59B: 493–501.
- Törn A, Rautio J, Norokorpi Y, Tolvanen A (2006) Revegetation after short-term trampling at subalpine heath vegetation. *Annales Botanici Fennici* 43: 129–138.
- Törn A, Tolvanen A, Norokorpi Y, Tervo R & Siikamäki P (2009) Comparing the impacts of hiking, skiing and horse riding on trail and vegetation in different types of forest. *Journal of Environmental Management* 90: 1427–1434.
- Tuomi J, Kytöviita MM, Härdling R (2001) Cost efficiency of nutrient acquisition and the advantage of mycorrhizal symbiosis for the host plant. *Oikos* 92: 62–70.

- Tybirk K, Nilsson M-C, Michelsen A, Lakkenborg Kristensen H, Shevtsova A, Tune Strandberg M, Johansson M, Knud Erik Nielsen, Riis-Nielsen T, Strandberg B & Johnsen I (2000) Nordic Empetrum Dominated Ecosystems: Function and Susceptibility to Environmental Changes. *Ambio* 29: 90–97.
- Tyler G (1991) Effects of litter treatments on the sporophore production of beech forest macrofungi. *Mycological Research* 95: 1137–1139.
- Uotila A, Hotanen J-P & Kouki J (2005) Succession of understory vegetation in managed and seminatural Scots pine forests in eastern Finland and Russian Karelia. *Canadian Journal of Forest Research* 35: 1422–1441.
- Vanha-Majamaa I & Reinikainen A (2000) Muuttuvan maankäytön vaikutus kasvillisuuteen. In: Reinikainen A, Mäkipää R, Vanha-Majamaa I & Hotanen J-P (eds) *Kasvit muuttuvassa metsäluonnossa*. Kustannusosakeyhtiö Tammi, Helsinki. 302–317.
- Vanhatalo M, Bäck J & Huttunen S (2002) Differential impacts of long-term (CO<sub>2</sub>) and O<sub>3</sub> exposure on growth of northern conifer and deciduous tree species. *Trees* 15: 304–313.
- Väre H, Ohenoja E & Ohtonen R (1996) Macrofungi of oligotrophic Scots pine forests in northern Finland. *Karstenia* 36: 1–18.
- Vestgarden LS (2001) Carbon and nitrogen turnover in the early stage of Scots pine (*Pinus sylvestris* L.) needle litter decomposition: effects of internal and external nitrogen. *Soil Biology & Biochemistry* 33: 465–474.
- Vestgarden LS & Kjønaas OJ (2003) Potential nitrogen transformations in mineral soils of two coniferous forests exposed to different N inputs. *Forest Ecology and Management* 174: 191–202.
- Vestgarden LS, Nilsen P & Abrahamsen G (2004) Nitrogen cycling in *Pinus sylvestris* stands exposed to different nitrogen inputs. *Scandinavian Journal of Forest Research* 19: 38–47.
- Vidra RL & Shear TH (2008) Thinking locally for urban forest restoration: A simple method links exotic species invasion to local landscape structure. *Restoration Ecology* 16: 217–220.
- Vidra RL, Shear TH & Stucky JM (2007) Effects of vegetation removal on native understory recovery in an exotic-rich urban forest. *The Journal of the Torrey Botanical Society* 134: 410–419.
- de Vries BWL, Jansen E, Van Dobben HF & Kuyper ThW (1995) Partial restoration of fungal and plant species diversity by removal of litter and humus layers in stands of Scots pine in the Netherlands. *Biodiversity and Conservation* 4: 156–164.
- de Vries W, Reinds GJ, Klap JM, Van Leeuwen EP & Erisman JW (2000) Effects of environmental stress on forest crown condition in Europe. Part III: Estimation of critical deposition and concentration levels and their exceedances. *Water, Air, and Soil pollution* 119: 363–386.
- de Vries W, Reinds GJ & Vel E (2003) Intensive monitoring of forest ecosystems in Europe. 2: Atmospheric deposition and its impacts on soil solution chemistry. *Forest Ecology and Management* 174: 97–115.

- Wallander H (1995) A new hypothesis to explain allocation of dry matter between mycorrhizal fungi and pine seedlings in relation to nutrient supply. *Plant and Soil* 168–169: 243–248.
- Wallander H, Nylund J-E & Sundberg B (1994) The influence of IAA, carbohydrate and mineral concentration in host tissue on ectomycorrhizal development on *Pinus sylvestris* L. in relation to nutrient supply. *New Phytologist* 127: 521–528.
- Wallander H, Nilsson LO, Hagerberg D & Bååth E (2001) Estimation of the biomass and seasonal growth of external mycelium of ectomycorrhizal fungi in the field. *New Phytologist* 151: 753–760.
- Waltert B, Viemken V, Rusterholz H-P, Boller T & Baur B (2002) Disturbance of forest by trampling: Effects on mycorrhizal roots of seedlings and mature trees of *Fagus sylvatica*. *Plant and Soil* 243: 143–154.
- Weathers KC, Cadenasso ML & Pickett STA (2001) Forest edges as nutrient and pollutant concentrators: potential synergisms between fragmentation, forest canopies and the atmosphere. *Conservation biology* 15: 1506–1514.
- Zvereva EI, Toivonen E & Kozlov MV (2008) Changes in species richness of vascular plants under the impact of air pollution: a global perspective. *Global Ecology and Biogeography* 17: 305–319.





## Original articles

- I Tarvainen O, Markkola AM & Strömmer R (2003) Diversity of macrofungi and plants in Scots pine forests along an urban pollution gradient. *Basic and Applied Ecology* 4: 547–556. DOI: 10.1078/1439-1791-00156.
- II Markkola AM, Tarvainen O, Ahonen-Jonnarth U & Strömmer R (2002) Urban polluted forest soils induce elevated root peroxidase activity in Scots pine (*Pinus sylvestris* L.) seedlings. – *Environmental Pollution* 116: 273–278. DOI: 10.1016/S0269-7491(01)00126-9.
- III Tarvainen O, Markkola AM, Ahonen-Jonnarth U, Jumpponen A & Strömmer R (2004) Changes in ectomycorrhizal colonization and root peroxidase activity in *Pinus sylvestris* nursery seedlings planted in forest humus. *Scandinavian Journal of Forest Research* 19: 400–408. DOI: 10.1080/02827580410030181.
- IV Tarvainen O, Ohenoja E, Strömmer R & Markkola AM (2009) Responses of fungal and plant communities to partial humus removal in mid-boreal N-enriched forests. Manuscript.
- V Tarvainen O, Strömmer R & Markkola AM (2009) Responses of Scots pine seedlings to partial humus removal in mid-boreal N-enriched forests. Manuscript.
- VI Tarvainen O, Leal Riesco I & Markkola AM (2009) Management of humus layer for restoration of urban mid-boreal forests: a greenhouse experiment. Manuscript.

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