Disturbance, Nutrient Availability and Plant Growth in Phenol-rich Plant Communities

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Abstract

Disturbances, such as fire, usually result in a higher N and nutrient availability. However, little is known regarding the role of fire-produced charcoal and other disturbance-induced effects on nutrient availability and the consequences for plant growth and ecosystem development. Late-successional boreal forests and subarctic forest-tundra ecotones are dominated by plants with high tissue-levels of phenolic compounds and have large quantities of N locked up in thick humus-layers. Factorial experiments were set up in both field and laboratory, manipulating amount of phenol-rich litter, charcoal and glycine. Nitrogen mineralization, nitrification, soluble P and phenolic compounds were measured by ionic and non-ionic resin capsules. Nitrogen-fixation was measured by acetylene reduction assays.

Removal of phenol-rich litter with the charcoal-layer intact in a recently burned stand increased growth and nutrient uptake by tree seedlings, with birch (*Betula pendula*) being the most responsive species. Litter and charcoal may function synergistically thus influencing seedling establishment after fire, especially in early-successional species like birch. Nitrification-rates increased with addition of activated carbon (AC) (as a surrogate for fire-produced charcoal) in laboratory incubations of boreal and subarctic soils and phenolic accumulation on non-ionic resin-capsules was reduced by AC amendments.

Nitrogen-fixation rates were found to increase linearly with time since fire. This increase was likely a function of the degree of colonization by cyanobacteria and site factors such as light, moisture and available N. Nitrogen addition rates of 4 Kg N ha^{-1} yr^{-1} eliminated N-fixation in late succession sites. The findings suggest that N-fixation in boreal forests becomes more important in late secondary succession were available N are low in spite of higher total N.

Subarctic birch-forests had higher soluble P and phenol concentrations compared to heath tundra. In contrast, there were no differences in available NH_4^+ or NO_3^-. The addition of glycine greatly enhanced N-mineralization rates in birch forests and heath tundra, suggesting severe N-limitation. Anthropogenic disturbances resulting in the loss of birch would greatly reduce positive influences from birch and increase soil-erosion and nutrient losses, creating both reduced N and P availability.

I conclude that a drastically altered disturbance regime in boreal and subarctic regions, both decreased (e.g. reduction of fire) and increased (e.g land-use), may have profound long-term effects on ecosystem processes.

Keyword: Charcoal, phenols, litter, N mineralization, nitrification, nitrogen fixation, fire, succession, mountain birch,

Contents

Appendix

Papers I -IV

My thesis is based on the following papers, which will be referred to by the corresponding Roman numerals in the text.

I. L.M. Berglund, T.H.DeLuca, O. Zackrisson. 2004. Activated carbon amendments to soil alters nitrification rates in Scots pine forests. (*Soil Biology & Biochemistry*, in press)

II. L.M. Berglund. The effect of charcoal and polyphenolic-rich litter on growth and nutrient acquisition of boreal tree seedlings after fire. (Manuscript)

III. O. Zackrisson, T.H. DeLuca, M-C Nilsson, A. Sellstedt, L.M Berglund. 2004. Nitrogen-fixation increase with successional age in boreal forest. (*Ecology*, in press)

IV. L.M. Berglund, T.H. DeLuca, O. Zackrisson. Soil Nitrogen and phosphorus turnover in subartic ecosystems. (Manuscript)

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Introduction

Disturbance as an ecological factor

Disturbances force ecosystems to undergo change. Natural and anthropogenic disturbances, such as fires, windstorms, landslides, flooding, logging, outbreaks of pathogens and herbivory, are all drastic events that in some way change characteristic ongoing ecosystem processes and force plants and animals to adjust to these new conditions. Since plants are not as mobile as animals, plant communities are often more directly affected. Disturbances are events concentrated to a well defined point in time and ''the mechanism which limit plant biomass by causing partial or total destruction'' (Grime 1979). Although disturbances often operate in long time intervals (up to 200 years) and often not in a regular periodicity, the impact is of such magnitude that it shapes the plant community for a long time afterwards. This makes disturbances key ecological factors in various communities and ecosystems.

Disturbance and resilience of boreal forests and subarctic ecosystems

Recurrent fires are the major form of disturbance in boreal forests (Zackrisson 1977). Boreal forests have burned frequently and although these can be stand-replacing fires, there is a great capacity for the forest to return to the pre-disturbance conditions with time (Pyne *et al.* 1996). This high resilience for drastic disturbance events also stress that some ecosystem processes most likely have evolved in response to these disturbances. In other ecosystems, such as those in the subarctic region, fire disturbances have been rare events. The cold and moist climate contributes to low fire frequencies. In general, the subarctic and arctic ecosystems are fragile and may have low resilience for disturbances due to cold temperatures and slow nutrient turnover rates (Wookey & Robinson 1997).

Fire as disturbance

Dry to mesic type boreal forests of northern Scandinavia burned on a historic mean return interval of ~100 years (Zackrisson 1977, Niklasson & Granström 2001), but for a given stand the fire intervals over time could vary from 25 years to 300 years or more. Lightning strikes (Granström 1993) and human activity are the principle fire inducing agents in these forests. The consequences of fires on different ecosystem processes (Fig. 1) are complex and both immediate and long-term. Fire effects on plant communities and soil biota vary depending on fire regime (Neary *et al.* 1999). Fire intensity is the factor that mostly influences aboveground vegetation, by direct consumption and by heat injuries to the plants, while the depth of burning during fire is most important for the belowground biota (DeBano *et al.* 1998).
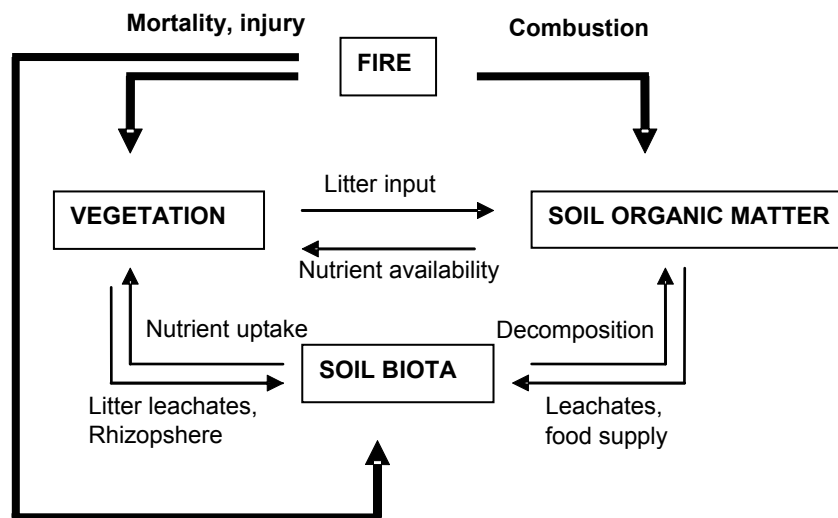
Fig. 1. A simplified schematic model of the influence of fire on various short term and long term processes in boreal forest ecosystems. Thin arrows signify secondary effects of fire such as increased flux of nutrients, and leachates, as well as increase litter production by plants and increase decomposition rates by microbes, while thick lines indicate direct effects of fire.

Combustion effects of fire

The combustion of organic fuels during a fire releases heat, a variety of gases (including CO_2) and water vapour (Pyne *et al.* 1996). After the fire has past over a given forest patch, glowing combustion (smoldering) of the humus layer can continue to cause heat flux into the forest floor and mineral soil. However, under natural conditions, there is usually incomplete combustion of the course and fine fuels and fire residuals are produced (DeBano *et al.* 1998). Depending on the extent of the combustion, fire residuals can be ash particles, charcoal particles or surface charred wood. Forest fires contribute considerable amount of charcoal to forest soils. In boreal forest soils, charcoal contents have been estimated at values up to 2000 kg ha^{-1} (Zackrisson *et al.* 1996).

Indirect effects of fire

In Scandinavian boreal forests, fires are rarely of stand-replacing intensity. Surface fires are more frequent (Schimmel & Granström 1997) and affect the ground vegetation layer, humus and smaller trees, but only scorch larger trees partly. Besides the most obvious effects of fire, such as direct consumption of plants and humus, there are several indirect effects. For plants, fires can be deadly even for those plants that are not directly consumed by the fire. Living plant material such as rhizomes and seeds can only survive temperatures up to 60 °C (Granström & Schimmel 1993) and smoke can damage the plants and increase litter production. Microorganism and soil biota can be negatively affected by fire (Pietikäinen & Fritze 1993), both as a result of direct consumption in more shallow layers of the humus, and by heat transfer down into the humus and the soil

from the fire (Choromanska & DeLuca 2002). Yet, the humus layer is an effective heat isolator, as a result the heat transfer drastically decline with increase soil depth and lethal temperatures rarely reach more than 3-5 cm down into the soil (Schimmel & Granström 1996). Moderate increase in soil temperature following fire can contribute to increased decomposition and nutrient turnover.

Ecosystem development after fire

After fire in the Scandinavian boreal forest, some vascular plants such as dwarf shrubs have the capability to rapidly recolonize the burned area by resprouting from rhizomes surviving in the ground (Schimmel & Granström 1996), while others (such as graminoids) depend on seed dispersal or seed banks to recolonize. During the first several years after fire, the bryophyte flora is dominated by acrocarpus species, then 10-20 years after fire pleurocarpous species (including *Pleurozium shreberi*) advance (Schimmel & Granström 1996). With time, the pleurocarpous moss layer accumulates and the understorey vegetation shifts to a predominance of late-successional dwarf shrubs, such as *Empetrum hermaphroditum* Hagerup, and in the overstory, the presence of Norway spruce increase (Steijlen & Zackrisson 1987). Decomposition of dead material slows down and is usually incomplete due to low litter quality, and thick humus layers starts building up (Prescott *et al.* 2000). Many of the dwarf shrubs commonly found in late-successional Swedish boreal forests are known to produce large quantities of phenolic compounds (Gallet & Lebreton, 1995; Nilsson *et al.* 1998) that may directly and indirectly influence nutrient turnover and microbial activity (Wardle *et al.* 1997). Polyphenolic compounds can form protein-tannin complexes locking-up the available N in these thick mor layers (Northup *et al.* 1995). As a result, polyphenols are suggested to alter N cycling to enhance the level of organic versus mineral N forms and reduce nutrient pool losses in infertile ecosystems (Northup *et al.* 1998).

Nitrogen in ecosystems

Plant growth depends on access to light, water and nutrients. Nitrogen, despite its global abundance, is one of the most limiting nutrients in terrestrial ecosystems. N is crucial for plant growth as it is a constituent of *e.g.* proteins, DNA and chlorophyll (Marschner 1995). Nitrogen derives originally from the atmosphere and is therefore nearly absent from newly formed soils. The atmosphere is approximately 78% N_2, but due to the oxidation state and triple bond, none of this form of N is biologically available. Certain bacteria have the capacity to biologically reduce N_2 and these can form symbiotic relationships with plants and mosses, supplying them with N (Vitousek *et al.* 2002). Decomposition of N-fixing species results in N-enrichment of soils. A smaller portion of total N-input from the atmosphere occurs via wet deposition as NH_4^+ (primarily from volcanoes and fires), and NO_3^- (produced by lightning and released during burning of fossil fuels).

Fire and Nitrogen

In forest fires a substantial amount of N is consumed through volatilization during combustion. The distribution of organic N, NH_4^+ and NO_3^- also shifts, as fires temporarily increase N mineralization and nitrification rates (Neary *et al.* 1999; DeLuca & Zouhar 2000; Choromanska & DeLuca 2001). High concentrations of NH_4^+ present in soils after fires are both produced chemically by soil heating and by increased microbial activity following fire (Choromanska & DeLuca 2002). Normally within a year after fire, pulses of NO_3^- are observed and since NO_3^- is not produced directly by heating, these pulses are commonly attributed to increased nitrification in the presence of ammonium (Wan *et al.* 2001). Nitrate is highly mobile and may be leached out of the system, also reducing total N (Wan *et al.* 2001).

Late-successional boreal forest and Nitrogen

As succession proceeds in these polyphenol-rich plant communities, the inorganic N that was released following fire is either leached or tied up in organic N cycling pathways. Late-successional boreal forests, where the N is locked-up in these thick mor layers, can be seen as severely N limited. Low N availability in an ecosystem may be due to slow turnover rates of dead material and past disturbances that have mobilized N which subsequently has been lost from the system, but low abundance of N_2 fixing organisms may also contribute to low total N (Vitousek *et al.* 2002). It is likely that in late-successional forests, N availability may be limiting for several organisms not adapted for organic N uptake or for utilizing complex-bound N. This lack of available N may create a demand for N-fixation in mid-late successional stands, and the late-successional moss *Pleurozium schreberi* (Bird) Mitt. has been found to form symbiosis with N-fixing bacteria (DeLuca *et al.* 2002b). As time pass, N is accumulated in the system at a rate of 3 kg N ha^{-1} year $^{-1}$ and total N is built up again (Wardle 1997).

Subarctic ecosystems

Subarctic ecosystems, in comparison to the boreal forest, have even lower N turnover rates and N limitation may be enhanced by the cold climate. Major disturbances are rare and fires are almost non-existent. Resilience for disturbances reducing N may be low (Wookey & Robinson 1997) and substantial amounts of mobilized N may then be potentially lost from the system. In the subarctic forest-tundra ecotone, the tree line border separating forest and arctic tundra is not a static phenomenon. Instead its location has been shown to vary through time (Sveinbjörnsson *et al.* 2002). Climatic factors have been proposed to be determining tree line position, both as direct effect on tree physiology and reduced soil nutrient availability by low soil temperature slowing down microbial activity (Sveinbjörnsson *et al.* 2002). Others have found treeline establishment more notably influences by nutrient constraints, mainly N limitation, than by climatic factors (Chapin & Shaver 1986, 1996).

Objectives

The overall aim of this thesis was to evaluate disturbance-induced effects on N availability and to describe some of the consequences for ecosystem development. Disturbances in ecosystems with low N availability can have wide rangning effects on N budgets, depending on type of disturbance and type of ecosystem. Both late-successional boreal forest stands and sites in the subarctic forest-tundra ecotone have large quantities of organic N locked up in thick humus layer and limited available inorganic N. Litter from plants in both ecosystems are also high in phenolic compounds. Disturbance in these systems usually generates an initial shift towards higher N availability. It was my intent to explore the links between disturbances, phenolic-rich litter, N cycling and the consequences for plant performance and N accumulation. In the boreal forest, fire is the most important disturbance and the system has high resilience for drastic disturbance events. I chose to concentrate on effects of fire-produced charcoal on N mineralization and plant growth in these boreal phenol-rich ecosystems. I also compared those responses in boreal forests with the response in subarctic forests and tundra which also are rich in phenols, but contrast by low importance of fires. It has been suggested that these phenol-rich arctic systems may have low resilience for disturbances. Thus, I also chose to study nutrient dynamics related to the irreversible degradation of subarctic birch forests to heath tundra. A major concern when studying N-cycling and fire, is that fire induces a substantial loss of N from the system. With time, the organic N accumulates again, however, the mechanisms behind this external input of N with time since fire are poorly understood, thus creating an intriguing set of research questions.

   More specifically, the following questions were asked (papers are referred to by roman numbers):
- How do phenol-rich litter, soil phenolic concentrations and charcoal affect N mineralization and nitrification in soils? (I, IV)
- How do phenol-rich litter and fire-produced charcoal affect growth and nutrient acquisition by tree seedlings after fire? (II)
- How does time since last fire influence N-fixation rates? (III)
- Do nutrient dynamics and soil phenolic concentrations explain disturbance-induced degradation of mountain birch forests? (IV)

Study sites

In order to answer the questions posed, field experiments were set-up in the northern boreal zone of Scandinavia (Ahti et al. 1968) and in the mountain birch forest-tundra transition zone (Haapasaari 1988), see also Fig 2. All of the boreal sites (Paper I, Paper II and Paper III) are situated within protected state nature reserves, minimizing influences of anthropogenic disturbances between the sites. All boreal sites were originally mixed Scots pine (*Pinus sylvestris* L) and Norway spruce (*Picea abies* Karst) forests of ericaceous-cladina type, prior to fire. In paper

I, each site is a late-successional stand (225-355 years since last fire), in paper II, the experiment was set up the year after a prescribe fire (< 1 years since last fire). In paper III, a chronosequence of sites were used ranging from 35 years to 355 years since last fire. In paper IV, we used subarctic heath tundra sites situated on completely treeless plateaus, situated well below the mean tree-line elevation in those areas, and dominated by ericaceous dwarf shrubs, particularly *Empetrum hermaphroditum* and lichens *Cladina* spp. Near each tundra site, a mountain birch forest was selected, with ground layer dominated by *Empetrum hermaphroditum, Vaccinium vitis-idaea* L., and *V. myrtillus* L.
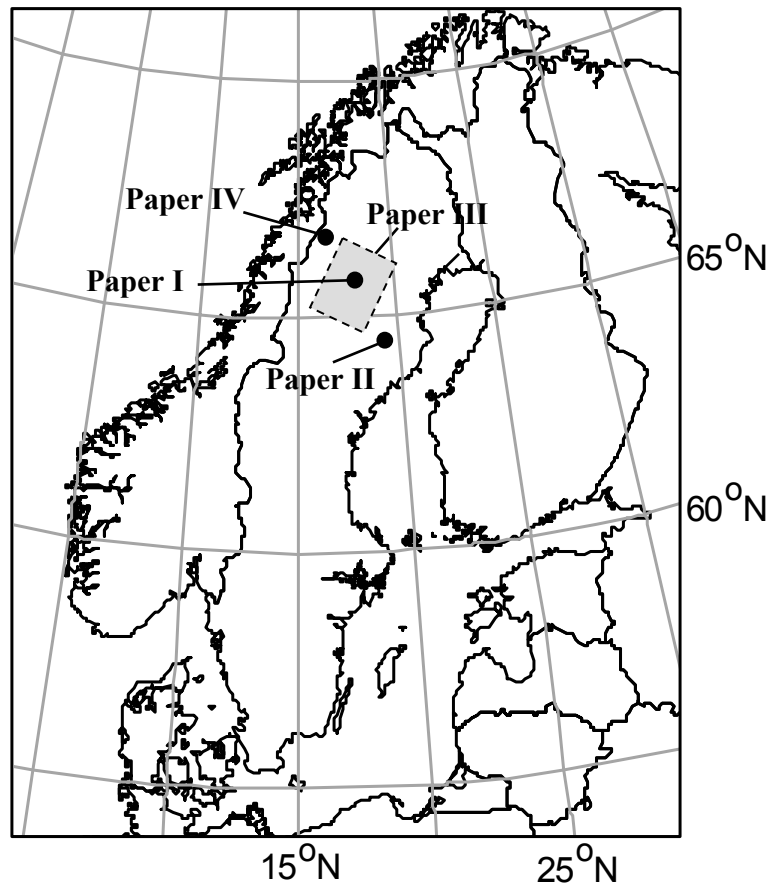

Fig. 2. Map of Scandinavia showing the location of the study areas used in each paper. In each paper, several study sites were used, however in paper I, II, IV these sites fall within a relatively small geographic area. The study sites for the chronosequence in paper III are more widely distributed (marked in grey).

Outline of papers

Paper I. Activated Carbon Amendment of Soils Alters Nitrification Rates in Scots Pine Forests.

The purpose of paper I, was to investigate the influence of charcoal on net N mineralization and nitrification rates in boreal soils. Replicated field studies were conducted at three late successional pine-dominated sites in northern Sweden and supporting laboratory incubations were conducted using soil humus collected in the field sites. Two rates of activated carbon (0 and 2000 kg AC ha^{-1}), as a surrogate for natural-occurring charcoal and two rates of glycine (0 and 100 kg N as glycine ha^{-1}) as an organic N source, were applied in factorial combination to field microplots in a randomized complete block pattern. Net nitrification, N mineralization and free phenol concentrations were measured using ionic and non-ionic resin capsules respectively. The same treatments as well as two rates of birch leaf litter (0 and 1000 kg ha^{-1}) were applied in a 8 week laboratory incubation and soils from this incubation were extracted with KCl and analyzed for NH_4^+ and NO_3^-. We found that nitrification increased with AC amendments in laboratory incubations, but this was not supported by the field study. Ammonification, as measured by NH_4^+ accumulation on ionic resins, were increased considerably by glycine applications, but some NH_4^+ was apparently lost to surface sorption to the AC. Phenolic accumulation on non-ionic resin capsules was significantly reduced by AC amendments. We concluded that charcoal affects the transformation and cycling of N in the boreal forest.

Paper II. The Effect of Charcoal and Polyphenolic Rich Litter on Growth and Nutrient Acquisition of Boreal Tree Seedlings After Fire.

The purpose of paper II was to investigate the effect of fire-produced charcoal and polyphenolic-rich litter on nutrient acquisition and growth of birch (*Betula pendula* Roth), pine (*Pinus sylvestris*) and spruce (*Picea abies*) seedlings. Previous studies have stressed that above-ground competition as well as micro-environmental differences are important factors for seedling establishment after fire, therefore we wanted to compare the effect of charcoal and litter with that of above-ground competition from field-layer and bottom layer, on seedling performance. In a recently burned site, tree seedlings of three species were planted in field plots subjected to one of the following treatments: charcoal removal, litter removal, charcoal and litter removal, removal of vascular plants, removal of bryophytes. Seedlings were later harvested (year 1 and 3) and analysed for dry weight and nutrient content. We found that the removal of litter increased the growth and nutrient uptake by all seedlings; however, birch was the most responsive species to litter removal and the presence of charcoal compared to other species. Litter removal was more important than above-ground competition from vascular plants; removal of vascular plants also increased seedling growth, but to a lesser extent than litter removal. This suggests that litter and charcoal may have important synergy functions influencing seedlings establishment after fire,

especially in early successional species like birch. With drastically reduced fire frequency in Scandinavian boreal forests due to anthropogenic influences, there is a reduction of charcoal production and this could negatively influence regeneration patterns of early successional tree seedlings.

Paper III. Nitrogen-Fixation Increases with Successional Age in Boreal Forests.

The purpose of paper III was to determine how time since last fire influences N-fixation rates in boreal forests and if N-fixations rates are a function of N and P availability. Recent evidence suggests that N-fixation by cyanobacteria associated with the common feather moss *Pleurozium schreberi* contribute a significant proportion of the total N economy. We evaluated seasonal N fixation rates on a total of 12 sites in natural forest reserves varying in time since last fire (35-355 years). In order to evaluate the influence of N availability on N-fixation three rates of N (0, 4.25 and 25.5 kg ha^{-1}) were applied once in a randomized block experiment on plots in one late-successional field sites. To test the effect of surface-applied P on N fixation rates, P was applied once at two application rates (0 and 5 kg ha^{-1}) to plots in one early- and one late-successional site. Each site was monitored for N fixation activity using a calibrated acetylene reduction assay. Nitrogen fixation rates were found to increase linearly with time since fire, and were likely a function of the degree of colonization by cyanobacteria and site factors such as available N. Even the lowest N addition rate used almost eliminated N-fixation, while P applications slightly increased N-fixation rates in the late-successional site. Our findings suggest that N-fixation in boreal forests becomes more important in late successions.

Paper IV. Soil Nitrogen and Phosphorus Turnover in Subarctic Forest and Tundra Ecosystems.

The purpose of paper IV was to investigate how N and P dynamics are influenced by organic N, phenol concentrations and litter additions in subartic forest and tundra ecosystems. It has been suggested that N availability may play a major role in determining tree-line elevation (Chapin *et al.* 1986), and in the degradation of mountain birch forest to heath tundra. Replicate field studies were conducted in three mountain birch forests and heath tundra sites in the mountains of northern Sweden. Additionally, supporting laboratory incubations were conducted in soil-humus collected from these sites. In the field, soils were amended once with two rates of glycine (0 and 100 kg N as glycine ha^{-1}) and activated C (0 and 2000 kg AC ha^{-1}), in a factorial combination on microplots. The same treatments were applied in an 8 week laboratory incubation, where also two rates of birch litter (0 and 1200 kg ha^{-1}) were added. Net nitrification, N mineralization, P mineralization and free phenol concentrations were monitored in the field using ionic and non-ionic resin capsules, and N availability was monitored in the laboratory study by measuring KCl extractable NH_4^+ and NO_3^- after 8 weeks of incubation. We found significantly higher total phenol concentrations as well as significantly higher amounts of soluble P in the birch forest compared to heath tundra. In contrast, we

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did not find any significant differences in either available NH_4^+ or NO_3^-. Glycine additions resulted in greatly enhanced ammonification both in the birch forest and the heath tundra, but in the birch forest combined addition of AC and glycine resulted in net N immobilization. Birch litter had a inhibitory influence on NO_3^- accumulation in the birch forest humus, but had no effect in heath tundra humus. Activated C significantly increased net nitrification in the laboratory incubations for both heath and birch soils, but NO_3^- accumulation on ionic resin capsules in the field were significantly decreased by charcoal amendments. Phenol accumulation in non-ionic resin capsules in the field was found to be significantly reduced by AC amendments in both the heath tundra and the birch forest. Although these subarctic ecosystems are N limited, it appears that phenol and P content, not available N, may be a major difference between the birch forest and tundra sites. There may be a positive feedback from phenol/sugar content in birch forest increasing P availability, and P limitations may play a role in the lack of birch forest re-establishment on the heath tundra.

Discussion

In the first part of this section, I address phenolic compounds and their various effects on soil processes, and I elucidate the mechanisms of phenol adsorption by charcoal. I then address the effect of charcoal and phenol-rich litter on N mineralization, as soil N availability can be an important index for plant performance. In the second part, I explicitly address the effects of charcoal and phenol-rich litter on plant responses in terms of growth and nutrient acquisition. With time since fire in these polyphenol-rich plant communities, N is tied up in organic forms that may be an unavailable source of N for many organisms. So thirdly, I addres the potential for external input of N by N-fixing symbionts in a chronosequence and how this is influenced by addition of N and P. Lastly, I address possible mechanisms behind the degradation of a polyphenol-rich plant community, in a subarctic ecosystem with inherently low resilience to disturbance.

Phenolic compounds and phenolic rich litter

In plant communities on highly acid and infertile soils, many of the dominating plant species are known to produce large quantities of secondary metabolites, such as monoterpenes and polyphenolic compounds (Northup *et al.* 1998; Hättenschwiler & Vitousek 2000; Kraus *et al.* 2003). Secondary metabolites are considered to be partly produced by plants as herbivore defence (Coley *et al.* 1985), but monoterpenes and polyphenolic compounds also have a strong effect on soil nutrient processes. Polyphenols enters the soil as leachates from plant foliage, litter and roots (Hättenschwiler & Vitousek 2000), with the main proportion probably being released from decomposing litter. In soils of both the boreal late-successional Scots pine forest (Paper I) and the subarctic birch forest-tundra ecotone (Paper IV), total phenolic concentrations are high (Fig 3). The boreal forest soils have by far the largest concentrations of soluble total phenols (Fig 3). The C:N in boreal soils is high and implies a general rich abundance of C.

In paper IV we found that the mountain birch forest soils contained larger concentrations of soluble total phenols than the heath tundra soils, although both are generally dominated by the same polyphenol-rich dwarf shrubs and contain equivalent amounts of total humus C. Mountain birch litter is high in phenols and sugar compounds, and combined with root exudates, this may explain the higher concentrations of soluble phenols.
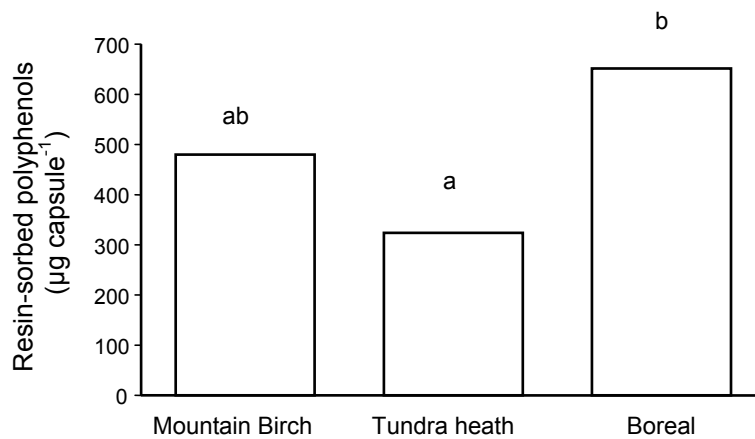

Fig. 3. Total polyphenolic compounds captured on non-ionic resins buried at the organic-mineral soil interface at study sites within boreal forest, mountain birch forest and tundra heath. Bars with dissimilar letters are significantly different at P < 0.05 (n =10).

Plant species commonly found in polyphenol-rich plant communities (such as Scots pine, birch and many ericaceous dwarf-shrubs) produce litter containing large quantities of phenolic compounds (Gallet & Lebreton 1995; Nilsson *et al.* 1998, Kainulainen & Holopainen 2002). When the polyphenol compounds have entered the soil they can either be degraded or mineralized as a carbon source by microorganisms, form complexes with proteins or metals, be adsorbed by clay particles or remain in the soil solution (Wallstedt *et al.* 2000).

Toxicity of phenols

Polyphenolic compounds may influence nutrient turnover by direct toxic effects on soil organisms involved in decomposition and N transformations (Wardle *et al.* 1997). The polyphenol toxicity may target on the metabolism of microbes and soil organisms but also inhibit and lock-up enzymes crucial for microbial activity in N turnover. In higher plants, phenolics may also directly and indirectly affect seed germination, seedling establishment and nutrition (Wallstedt *et al.* 2001).

However, polyphenols are a large and variable group of compounds and they differ in toxicity as well as in ease of degradation. Only a few compounds have been identified with regard to specific function in soil-mediated biological processes. As an example, in the boreal and arctic regions, the dwarf shrub *Empetrum hermaphroditum* is wide-spread in late successions and is well known to produce toxic phenolic compounds affecting other plants (Nilsson *et al.* 1998). The putative phytotoxic polyphenolic compound is Batatasin III (Wallstedt *et al.*

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1997, Gallet *et al.* 1999) which is not found in tissues of the closely related species *Empetrum nigrum* (Nilsson *et al.* 2000). Species-specific differences in plant tissue composition of phenol compounds, both in abundance of different types of compound and toxicity of these compounds, may therefore be important for the distinction of phenol effects on soil processes.

Carbon food source

Polyphenols are C-rich compounds that can be degraded and used as food source by some microbes (Schimel *et al.* 1998). Souto *et al.* (2000) found that in sterilized soil over 90 % of the phenolic compounds remained intact after 6 d incubation, compared to non-sterilised soil were all added phenols had been degraded, indicating that these polyphenols were acting as a C source for microbes. Phenolic compounds differ in ease of biodegradation and microorganisms may be specialized on using different groups of phenolic compounds.

Complexation of organic N

Polyphenols may form complexes with proteins and other compounds, such as heavy metals. Tannins are the largest group of water-soluble polyphenolic compounds and are primarily responsible for the protein complexation through H-bounding (Kraus *et al.* 2003). Although this can occur in leaves prior to leaf fall and during senescences, the majority of protein-tannin complexes are formed in the soil. Protein-polyphenol complexes are highly resistant to decomposition and N mineralization rates decrease substantially with increased complexation (Horner *et al.* 1988).

Polyphenols may also be adsorbed to clay particles and form sesquioxide complexes with Al and Fe, reducing the toxic effects of these compounds. Phenolic compounds can also compete with phosphate for sorption on mineral surfaces and complexation with Al and Fe (Northup *et al.* 1998; Hättenschwiler & Vitousek 2000). It has also been shown that phenolic acids are able to desorb previously fixed P (Davis 1982), and thus increase the amount of soluble P in the soil.

It has been proposed that high foliar polyphenol content may give some plant species a competitive advantage by altering nutrient cycling and in extension, altering forest dynamics (Schimel *et al.* 1996; Inderjit & Mallik 1997; Northup *et al*. 1998). As polyphenolic compounds can form protein-tannin complexes locking-up the available N in thick mor layers, polyphenols may reduce nutrient pool losses in infertile ecosystems. Species known to use organic N forms may be promoted by this enhanced level of organic versus mineral N forms (Northup *et al.* 1998). This is also highlighted by the convergent evolution of polyphenol-rich plant communities on highly acid and infertile soils (Northup *et al*. 1998).

Charcoal adsorption

Fire-produced charcoal is highly stable in soils and has been considered an inert storage form of C and other nutrients. However, fire-produced charcoal exhibits significant biological potential. Fire-produced charcoal has a highly porous structure (Fig 4) creating surfaces that have the capacity to adsorb significant amounts of polyphenolic (Zackrisson *et al.* 1996; DeLuca *et al.* 2002) and other compounds. This effect is similar to commercially produced activated carbon which is used in waste-water purification and other chemical industrial processes.
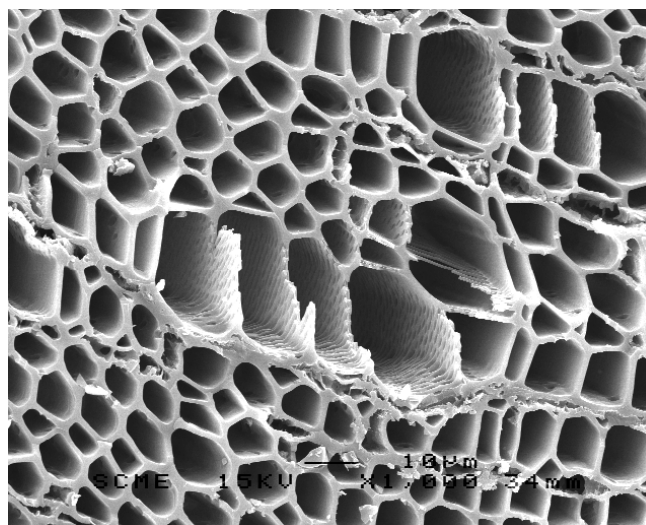

Fig. 4. Scanning electron micrograph of charcoal particles from birch (x1000). The porous structure of charcoal consists of both large and small pores, including perforated cell walls, creating a large adsorptive surface area (from Hellberg, 2004).

Adsorption of phenols

In paper I and paper IV, we found that charcoal amendments reduced total soluble phenols in soils from both the boreal forest and the subarctic mountain birch forest. This was most likely a result of phenol-adsorption by charcoal, which may result in deactivation of the phenols through chemical condensation (Piccolo 1998). This adsorption process of phenolic compound to charcoal and activated carbon depends on several factors, such as the surface area and pore size, hydrophobicity and the functional groups present on the surface of the charcoal, as well as the chemical characteristics of the phenolic compounds (Salame & Bandosz, 2003). Depending on the temperature at which the charcoal and activated carbon were formed, the surface charge may be different (Chereminisoff & Ellerbusch 1978). Charcoal and activated carbon do not substantially affect pH in soils (paper I, paper IV). However, the surface charge is pH-dependent and thus the mechanism of the adsorption will be affected by pH. At pH 3 – 6 the surface area of the AC is positively charged (Riviera-Utrilla *et al.* 2001) and phenols are

effectively adsorbed. However, with increase in pH to above 7 surface charge changes to negative and the phenolic adsorption cease (HuaWu & Pendleton 2001). Theoretically this would mean that in soils with higher pH, such as calcium-rich soils, phenol adsorption by charcoal may be minor. In paper I and paper IV, the site pH was ranging from 3.6-4.2, and therefore pH should not have affected the adsorption process. Charcoal and AC are severely hydrophobic, mainly due to the charged surface area. The hydrophobic characteristics of charcoal may contribute to the increase in soil water repellency seen after fire (DeBano *et al.* 1998). However, the charcoal adsorption function of fire-produced charcoal seams to diminish with time so that 100 years after fire, the adsorption capacity has ceased (Zackrisson *et al.* 1996).

Charcoal and microbes

Charcoal has been shown to harbor and support specific or unique microbial communities (Pietikäinen *et al*. 2000), although little is known about the functional differences of these special communities. Colonization of bacteria on AC and charcoal have been suggested to result in part from: 1) the adsorption of soluble C and nutrients acting as food source (Pietikäinen *et al.* 2000); 2) the porous structure of charcoal which is a physical shelter to microbial organisms, protecting them from soil faunal predators (Wardle *et al.* 1998); 3) detoxification of phenolic compounds detrimental for microbes (Zackrisson *et al.* 1996); 4) the type of chemical functional groups present on the charcoal surface, enhancing microbial adhesion (Rivera-Utrilla *et al.* 2001). Microbial adhesion to the charged surface areas (Zita & Hermansson 1997) of the charcoal can aggregate microbes on and around charcoal. The bacterial biofilm formed on the activated carbon can change the surface charge density, which could actually increase the adsorption capacity of some chemical compounds, such as many heavy metals (Rivera-Utrilla *et al.* 2001).

Nitrogen turnover and the effect of charcoal and litter

Nitrogen availability in soils is important for plant growth. Ammonium and NO_3^- are the most abundant inorganic forms of N in the soil. In N-limited boreal and subarctic soils, organic N is the predominant N form and inorganic N constitutes only a minor fraction. Although plants and microbes have the capacity to take up organic N forms (Näsholm *et al.* 1998), microbes and ericaceous shrubs do seem to compete more efficiently for organic N (Nordin *et al.* 2004), while other plants are depending more on inorganic N forms. Net N mineralization (the transformation of organic N to inorganic N) have been shown to decrease linearly with time since fire, and at the same time, net nitrification rates were found to decline logarithmically with time since fire, with a clear maximum in the early-successional sites (DeLuca *et al.* 2002a). In soil solutions, NH_4^+ concentrations are approximately 5-10 times larger than NO_3^- concentrations (paper I, paper IV). Nitrate concentrations in late-successional boreal forests are generally low and net nitrification is in many cases non-detectable. However, Stark & Hart (1997) found substantial gross rates of nitrification in coniferous forests, demonstrating that

turnover of NH_4^+ to NO_3^- can occur at a high rates that are masked by rapid microbial assimilation of NO_3^-.

N mineralization and charcoal and litter

Glycine is an easily degraded organic N form. In paper I and IV we found that amendments of glycine substantially increased N mineralization rates, suggesting labile N to be limiting in both boreal soils and in subarctic forest-tundra soils. Plants and microbes can use glycine without transforming it to inorganic forms (Persson *et al.* 2003), but more commonly glycine is converted into NH_4^+. Birch litter did not influence ammonification unless glycine was added (Paper IV) and then, birch litter addition resulted in some immobilization of the added N in the boreal humus and heath tundra humus, but not in the birch forest humus. The decrease in available NH_4^+ could be due to high concentrations of soluble C from newly shed birch litter that likely increased microbial activity (Pietikäinen *et al.* 2000). Carbon-rich compounds promote immobilization of inorganic N into new microbial biomass as a growing stock of microorganisms would require a source of N. Activated carbon applications combined with the glycine additions appeared to hamper the increase in NH_4^+ caused by glycine addition.

Nitrification and charcoal and litter

In Paper I and Paper IV, we found that charcoal addition to soils resulted in increased net nitrification in both incubations of boreal forest soils and subarctic mountain forest and tundra soils. Nitrification in soils depends on a range of factors such as pH and moisture. The oxidation of NH_4^+ to NO_3^- is generally thought to be substrate-limited, and many studies have shown a strong correlation between the amount of NH_4^+ in the soil and NO_3^- -conversion (DeBoer & Kowalchuk 2001). In boreal soils, we found net nitrification in the incubation experiments to be limited by a combination of ammonification rates and some other factor altered by charcoal (paper I). In the alpine forest-tundra ecotone net nitrification was not limited by the rate of ammonification (paper IV) but here too, some other factor altered by charcoal, was of great significance. The lower soil C:N ratio (~ 26) in soil from the subarctic forest-tundra ecotone compared to soils from the boreal forest (~ 40), could partly explain the differences in nitrification response to charcoal addition. The soil C:N ratio has been shown to be an important predictor of nitrification rates in soils (Currie 1999; Ste-Marie & Paré 1999).

Charcoal may alter several soil conditions and other factors important for nitrification rates. For instance, charcoal alters soil phenolic concentration via adsorption. Nitrification rates are reduced by addition of phenolics and monoterpenes (Paavolainen *et al.* 1998). Nitrifying bacteria need enzymes in order to oxidize NH_4^+ to NO_3^-, yet phenols have been suggested to form complexes with proteins and inhibit these crucial enzymes (White 1994).

Another plausible explanation of altered nitrification rates by charcoal may involve the strong hydrophobic characteristics of some charcoals. Char hydrophobicity may result in an ability to alter soil moisture conditions. When charcoal was mixed into the soil, maximum water holding capacity was reduced

(Berglund, personal observation). Nitrifiers are suggested to be more drought-tolerant than other bacteria and more resilient to repeated dry-wetting disturbances (Fierer & Schimel 2001). The hydrophobic characteristics of charcoal could also increase the microbial adhesion (Zita & Hermansson 1997) and the aggregation of bacteria, and thus produce biofilm structures with the charcoal. Nitrifiers in acid environments are proposed to have developed a mechanism by which it is possible to escape the negative effect of low pH on nitrification via biofilm formation (DeBoer *et al.* 1991).

Although nitrification is generally thought to be pH dependent, in the range of conditions studied here, pH seemed to be of minor importance. There was no increase in nitrification with glycine amendments in spite of a sharp increase in soil pH following rapid ammonification of the added glycine. Conversely, the significant increase in net nitrification when activated carbon was added was not a result of any increase in pH since addition of activated carbon alone did not significantly affect pH (paper I, paper IV).

Earlier studies have shown that birch and aspen litter promote nitrification. In a deciduous forest floor incubation nitrification was rapid, and addition of small amounts of deciduous forest floor material to a coniferous sample, significantly enhanced nitrification rates as well (Ste-Marie & Paré 1999). In paper I we found no direct impact of birch litter on nitrification in boreal soils. As birch is an early - successional species, litter from birch can be relatively abundant on certain patches in early-successional sites. Yet, litter seem to have little impact on nitrification in boreal humus in the short-term. This may also be a consequence of the lack of nitrifier colonisation of the newly shed birch litter that we used, in comparison with Ste-Mare & Paré (1999) which used forest floor material. In paper IV we found birch litter to significantly reduce net nitrification in soils from mountain birch stands, but we found no direct impact in subarctic tundra soils. In the corresponding field study we found a correlation between NO_3^- and phenol concentrations in the birch forest, but not in the heath tundra, indicating that nitrification may be more sensitive to phenol concentrations in birch humus.

Plant response

Tree seedling regeneration and growth is often enhanced after fire (Carter & Chapin 2000) as a result of changes in the micro environment, such as moisture, nutrients and in some cases, light. This is both promoted by direct fire effects (such as the increase in NH_4^+) but also by the reduced competition for from other plants and mosses in the field layer.

Effects of charcoal and litter

Wood-charcoal produced after fires are potentially an important factor influencing seedling regeneration and growth after fire (Wardle *et al.* 1998). In paper II, it was found that removal of phenol-rich pine litter while leaving the wood-charcoal layer intact in a recently burned forest, significantly increased birch, spruce and pine seedling growth and nutrition. The results indicated that there is a synergetic effect of charcoal and the reduction of phenol-rich litter, and that the increase in

seedling growth is not solely an effect of reduced phenol input from the litter. This is supported by the fact that when both litter and charcoal were removed seedling growth did not increase. However, when only charcoal was removed, plants grew equally well in those plots as in intact control plots which might be a result of the charcoal in the control plot being saturated by leached phenolic compounds. Litter input of pine needles was approximately as high as 300 g m^{-2} and year. Given that phenols (approximately 80% of the tannins) are rapidly leached from litter during the first year of decomposition (Lorentz *et al.* 2000) this might have rapidly saturated the charcoal, especially since adsorption capacity differs depending on several factors, such the temperature at which charcoal is formed and soil pH.

These effects of charcoal and litter on seedling regeneration and establishment seem to be of great importance. Previous studies have stressed above-ground competition as well as micro-environmental differences as important factors for seedling establishment after fire (Carter & Chapin 2000). In paper II, the effect of charcoal and litter was compared with the effect of above-ground competition from field-layer vascular plants on seedling performance. I found that seedlings in the litter-removal treatment showed greatest response in growth and nutrient acquisition compared to seedlings in the vascular plant removal treatment.

Plant growth and nutrition

Plant growth is limited by nutrients, light and moisture. Plant competition also influence to which extent these resources are available for use. The physiological capacity to explore these resources and the competitive ability, differ between plants species. In paper II, I found the early-successional deciduous tree species birch (*Betula pendula*) to be the most responsive species to treatments manipulating the amounts of charcoal and litter in the fire-affected forest stand. Birch grew notably better and acquired more nutrients than pine or spruce in the treatment with the overall largest seedling response (i.e. litter removal treatment). Many early-successional species have an inherently rapid growth rate and are successful on site were there is excessive light, moisture and nutrient supply. However, they are usually poor competitors when resources are limiting. Slow growing late-successional species, such as spruce, are more effective competitors. It has been suggested that late-successional species allocate more to nutrient storage than growth and therefore show relative small responses in biomass to variation in nutrient availability (Catovsky *et al.* 2002). There seems to be fundamental differences between deciduous and coniferous tree species in their plant-soil interactions. In deciduous species, such as birch, there are positive correlations between foliar N content, net mineralization and high net nitrification in deciduous forest floors (Ste-Marie *et al.* 1999). For coniferous species such relationships are not known to exist (Ollinger *et al.* 2002.).

As nitrification may be enhanced by addition of charcoal (DeLuca *et al.* 2002, paper I, paper IV), the positive growth-response of birch after litter removal with the charcoal layer intact could have been further stimulated by a charcoal-induced increase in nitrate levels. Preference for nitrate may be an important driver of seedling performance. Most plants adsorb any form of soluble N that is available in the soil, however, they differ in their relative preference and competitive ability

for different N forms (McKane *et al.* 2002.). Coniferous seedlings take up N as both organic nitrogen and ammonium (Näsholm *et al.* 1998), but to a lesser extent as nitrate. In early-successional deciduous species, such as birch, nitrate can be a preferred nitrogen source (Min *et al.* 1999).

N$_2$-fixation

As N is ultimately derived from the atmosphere, it is nearly absent from newly formed soils. Therefore, fixation of atmospheric N has normally been suggested to be most important in early-successions despite the persistence of N-limitation in late succession (Rastetter *et al.* 2001). In North America various alder species are common in early-successions and is known to fixate N$_2$ and thus contributes to the overall N budget (Uliassi & Ruess 2002). It has also been shown that N$_2$ fixation is only a minor contribution to total N supply for plant-growth in late-successional forest in these North American forests (Vitousek *et al.* 2002). However, herbaceous and woody N fixing plant species are not found in early-successional European boreal forests (DeLuca *et al.* 2002b).

Time since last fire

In paper III we found that the N-fixation rates increases with time since fire. The feather moss *Pleurozium schreberi* is a common bryophyte in boreal and alpine ecosystems and has been shown to form symbiosis with N-fixing cyanobacterium (DeLuca *et al.* 2002b). Pleurozium schreberi is not present in early successions, but starts to appear after ~20 years since fire (Schimmel & Granström 1996). Therefore primarily, N-fixation mediated by pleurozuim-cyanobacteria associations is not likely to occur in early successions because of the non-presences of the moss. In paper III, we found a linear increase in N-fixation rates in pleurozium shoots with increasing successional age of the stand. This linear increase may be due to a combination of factors that vary with time since fire and that are know to regulate N symbionts, such as changes in canopy structure and light, degree of colonisation of the cyanobacteria, and available N (Chapin *et al.* 1991; Zielke *et al.* 2002). When scaling up N fixation to forest stand level, given that *Pleurozium*-cover increases with successional age as does N fixation rates, the N contribution to the late succession forest ecosystem may be notable. This suggests that in late successional stands, the contribution of N from N-fixation can reach up to 1-2 kg ha^{-1} year^{-1}.

There is a high degree of temporal variation in N-fixation rates. In paper III, we found the highest acetylene reduction rates at the end of June and September, but very low fixation rates in the middle of the summer (end of July). Light intensity is known to influence N fixation rates (Zielke *et al.* 2002) and many cyanobacteria are depending on the host photosynthesis for carbohydrate supply. *Pleurozium schreberi* exhibit seasonal photosynthetic activity (Whitehead & Gower 2001) and this may explain the drop in N fixation rates in the middle of summer. Temporal variation in moisture availability may also regulate N fixation in cyanobacteria (Vitousek *et al.* 2002).

Nitrogen and P addition

In paper III, we found a strong negative correlation between N_2 fixation and available N. With N addition in a late-successional site with normally high rates of fixation, the N-fixation rates dropped to nearly undetectable levels. Several studies have pointed out the direct influence on N-fixations rates by soluble N concentrations (Chapin *et al.* 1991; Kitoh & Shiomi 1991). The cost of N fixation is high and if there is large amount of available N, the N fixation may be too costly and inorganic N is preferred (Rastetter *et al.* 2001).

It has been suggested that N_2 fixation is often limited by P supply (Vitousek *et al.* 2002; Uliassi & Ruess 2002). Phosphorus originates from rock weathering and the availability of P in soils is influenced by a range of factors, but most important is the geochemical constitution of the mineral soil (Bowman *et al.* 2003). Other factors such as moisture, complex-binding to Al and Fe and microbial community structure is also shaping P availability (Gielser *et al.* 2002). In early successions there are usually no P limitations, but with time P is tied-up by plants and micro-organisms or lost from the system (Wardle *et al.* 2004). In paper III, we found no clear trend in increased N fixing capacity with P addition at the early-successional site. In the late-successional site, there were some indications that P applications may have promoted N-fixation. However, this was a weak trend and further studies are necessary.

Degradation of mountain birch forest

Subartic ecosystems are highly nutrient-limited (Chapin & Shaver 1986; Schmidt *et al.* 1997; Robinson 2002) and might be of low resilience to disturbance-induced changes in nutrient cycles (Wookey & Robinson 1997; Scheffer & Carpenter 2003). Disturbance-induced alteration of forest microclimate, hydrologic cycles, and nutrient cycles are likely to play a major role in determining the fate of these systems. The mountain birch tree line areas in northern Fennoscandian mountains have been considered relatively unaffected by human exploitation. However, recent surveys have revealed past human settlements with extensive land-use. A conversion from mountain birch forest to treeless heath tundra, may have occurred around 900-1200 AD in some areas with humans settlements, and even though this human impact ceased shortly after, these areas have since remained as tundra heath lands (Hellberg et al. 2004). It has been proposed that this degradation from birch forest to heath tundra is induced by changes in biotic and abiotic factors created by this anthropogenic exploitation (Hellberg et al. 2004). These areas have not been reforested, although climate has been suitable for tree establishment, suggesting that climatic factors are not solely of importance for the conversion process (Hellberg *et al.* 2004). Chapin and Shaver (1986, 1996) found that treeline establishment in subartic Alaska was more notably influenced by nutrient constraints, mainly N limitation, than by climatic factors. This nutrient influence on treeline has also been proposed by several others (i.e Davis *et al.* 1991; Wookey & Robinson 1997; Hobbie & Chapin 1998; Sveinbjörnsson *et al.* 2002).

In paper IV, when comparing the overall N availability in previously forested arctic heath lands with mountain birch forests nearby, we found no significant

differences in either resin sorbed NH_4^+ or NO_3^-, between tundra and forest. Nor did we detect any significant differences in total N of the humus layer or the C:N ratio of both humus and mineral soil in heath tundra, compared to mountain birch forest. Addition of organic N resulted in greatly enhanced ammonification rates both in the birch forest and the heath tundra, suggesting a severe N limitation. However, the response was equal and when combined with the pattern of N availability indicates that N supply is similar for the birch forest and heath tundra. Mountain birch forest soils did, however, exhibit significantly higher soluble P concentrations and total polyphenol concentrations, compared to the heath tundra. When comparing resin-sorbed P between a mountain birch forest and an artic heath land both with similar mineral soil P contents, we found significantly higher values of P in the birch forest, indicating that the birch forest creates conditions which are favourable for P availability. Birch trees are considered to have deep root systems (Stone & Kalisz, 1991) which may successfully exploit deep mineral soils for P, and there may also be a positive feedback from phenol/sugar content in birch forest (from litter and root exudates) increasing P availability (Hättenschwiler & Vitousek 2000). Anthropogenic influences may have hampered any positive feedback effect from the birch trees as well as increased soil erosion and nutrient output. This could have inevitable shifted the nutrient balance in the soil (to be both N and P limited) and P limitations may play a role in the lack of birch forest re-establishment on the heath tundra.

Methodological considerations

Resin capsules vs. soil samples

Using ionic and non-ionic resin capsules for estimation of net nutrient flow have many advantages. This approach allows soluble NO_3^-, NH_4^+, P and polyphenols to be assessed over a specific period rather than taking a single point-in-time sample or using disruptive net N mineralization techniques. The resin-capsule technique is depending on the in-flow of soluble nutrient, but this diffusion flux rate is depending on soil moisture. Therefore, moisture variation in the field can be a significant source of variation in ion supply rates measured with resin capsules (Qian & Schoenau 2002), but variations in nutrient flux due to water flow also influences plant nutrient availability and may be seen as an inherent soil properties.

Net N mineralization assays have been widely used as standard tool for measuring plant-available N. This have been based on the assumption that microbes are more effective competitors for N and that plants can only use inorganic N that exceed microbial demand; *i.e* net mineralization (in contrast to gross mineralization). However, this opinion has recently begun to be re-evaluated (Schimel & Bennett 2004). Since plants do use organic N and in some cases also are capable of out-competing microbes for N, the net mineralization assays present more a sort of index of N turnover rather than plant available N *per se*. Yet, as an index measures of net N mineralization rates can provide valuable information.

Charcoal vs. active carbon

In paper I and paper IV activated carbon (AC) was used instead of fire-produced charcoal. The special characteristic of fire-produced charcoal is also present in activated carbon and their adsorption function is similar (Zackrisson *et al.* 1996). Activated carbon is a black powder industrially produced by combustion and several other steps, and is used in waste-water treatments and other industrial processes. Pietikäninen *et al.* (2000) compared adsorption of dissolved C between AC, wood charcoal (from *Empetrum* twigs) and humus charcoal. Activated carbon and charcoal was able to remove 51% and 42% respectively, of the dissolved C from a birch-litter water extraction. Charcoal produced from humus was not as effective adsorbent, as only 26% of the soluble C was removed. This suggests that there might be differences in adsorption capacity depending on the origin of the charcoal. However, with regard to charcoal produced from wood compared to AC there seem to be minor differences.

Conclusions

Nitrification rates in soils from both boreal, mountain birch forest and subarctic tundra were found to increase with charcoal amendments in laboratory incubations. I also found phenol concentrations in soils significantly reduced by activated carbon amendments. In the boreal humus, were the C:N ratio was high, addition of labile N was required in order to observe a nitrification response with charcoal amendments. In the subarctic forest-tundra soils, the C:N ratio was lower, indicating that microbes had less C relative to N, and here nitrification rates was not limited by N. As nitrification may be negatively influenced by the presence of phenolic compounds, I suggest that this adsorption of phenolic compounds by charcoal may partly explain the increase in nitrification. It is likely that adsorption of C sources by charcoal creates a food storage available for microbes promoting microbial activity. Birch litter (rich in phenolic compounds) had an inhibitory influence on NO_3^- accumulation in the birch forest humus, but had no effect in heath tundra humus or the boreal forest humus.

I found that phenol-rich litter and wood-charcoal does influence seedling growth. The removal of litter combined with an intact charcoal layer increased growth and nutrient acquisition in birch, spruce and pine, with birch being the most responsive species. This could be due to early-successional species attributes, such as allocation to rapid growth and response to increase in nitrate. Litter removal was more important than removal of above-ground competition from field-layer vascular plants, although removal of vascular plants also did increase seedling growth. With drastically altered presence of boreal forest fires, there is a reduction of charcoal production and this could negatively influence tree regeneration patterns, particularly of early-successional tree seedlings.

Nitrogen fixation rates were found to increase linearly with time since fire, and are likely a function of the degree of colonization by cyanobacteria and site factors such as available N. Even small amounts of added N almost entirely eliminated N-

fixation, while P applications slightly increased N-fixation rates in the late-successional site. These findings suggest that N-fixation in boreal forests becomes more important in late-successional stages.

We found significantly higher amounts of soluble P and higher total phenol concentrations in the mountain birch forest compared to the heath tundra. In contrast, we did not find any significant differences in either available NH_4^+ or NO_3^-. Addition of organic N resulted in greatly enhanced ammonification rates both in the birch forest and the heath tundra, suggesting a severe N limitation. Although these subartic ecosystems are N limited, it appears that P and phenol content, not available N, may be the major difference between the birch and tundra sites. The birch forest also seemed to have conditions of greater P availability. Disturbances could have inevitable shifted the nutrient balance in the soil (to be both N and P limited) and therefore P limitations may play a role in the lack of birch forest re-establishment on the heath tundra

In my thesis I can conclude that disturbances have broad ranging influences on nutrient availability and have important consequences for plant growth and successional development. With a drastically altered disturbance regime in boreal and subarctic regions, both decreased (such as reduction of fire) and increased (such as enhanced land-use), there may be profound long-term effects on ecosystem processes.

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