Nitrogen in Soil Water of Coniferous Forests

Effects of Anthropogenic Disturbances

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Abstract

In boreal and temperate forests, long-term elevated nitrogen (N) load may eventually saturate forest ecosystems with N, i.e. total N ecosystem input exceed ecosystem sinks for N, and N losses via soil water transport may then increase and negatively impact environmental quality.

This thesis is based upon four studies (reported in papers I-IV), and the overall aims were to assess and analyse effects on soil water N in coniferous forests of two types of anthropogenic disturbance: "chemical disturbance" (long-term experimental N addition and N deposition), and "physical disturbance" (clear-cutting and subsequent soil scarification). Effects of these disturbances were addressed in both field experiments and process-based ecosystem modelling. In the field experiments, soil water N was collected from both organic (O) horizons and mineral soil, at 0.5 m depth, during several growing seasons to assess temporal variation in the N concentration (Paper I). In addition, microbial variables in soil samples of the O-horizon were analysed in the laboratory to assess responses of the soil microbial community to long-term N addition in forest experiments and along a N deposition gradient (Papers II and IV). In the modelling, a process-based ecosystem carbon and N model (CoupModel) was calibrated to measurements obtained during the regeneration phase of a Scots pine (*Pinus sylvestris* L.) forest in an N fertilization experiment where soil scarification was applied (Paper III).

The results showed that long-term N addition to a boreal Norway spruce (*Picea abies* (L.) Karst) forest can alter the quantity and seasonal dynamics of dissolved organic nitrogen (DON) concentrations in soil water collected from the O-horizon. However, DON concentrations were low in soil water collected from mineral soil under all N treatments and probably only contributed to small net N losses in this forest. Although microbial variables of the O-horizon were affected by N loading they were similar under N loading that resulted in the leaching of small amounts of nitrate (<2 kg ha^{-1} $year^{-1}$ of NO_3-N) and those that resulted in the leaching of large amounts (>15 kg ha^{-1} $year^{-1}$ of NO_3-N). Further, soil scarification increased soil water N leaching from a Scots pine forest, as calculated with the CoupModel, during the regeneration phase, particularly in previously N-fertilized pine stands.

Keywords: nitrogen, leaching, boreal, temperate, N loading, clear-cutting, soil scarification

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Contents

List of Publications

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

I Rappe George, M. O., Gärdenäs, A. I., Kleja, D. B. (2013). The impact of four decades of annual nitrogen addition on dissolved organic matter in a boreal forest soil. *Biogeosciences* 10, 1365-1377.

II Rappe George, M. O., Choma, M., Čapek, P., Börjesson, G., Kaštovská, E., Šantrůčková, H., Gärdenäs, A. I. (Manuscript). Effects of long-term nitrogen addition and deposition on microbial variables in the organic horizon of Norway spruce forest soils.

III Rappe George, M. O., Hansson, L. I., Ring, E., Jansson, P.-E., Gärdenäs, A. I. (Manuscript). Nitrogen leaching following clear-cutting and soil scarification from a forest regeneration area in Sweden - a modelling study based upon a fertilization experiment.

IV Choma, M., Rappe George, M. O., Čapek, P., Bartá, J., Kaštovská, E., Gärdenäs, A. I., Šantrůčková, H. (Manuscript). Recovery of the ectomycorrhizal community after termination of long-term nitrogen fertilization of a boreal Norway spruce forest.

The contribution of Martin Rappe George to the papers included in this thesis were as follows:

I Took part in planning of the study. Performed the soil sampling and sample preparation for carbon and nitrogen stock estimation. Performed the data analysis and writing, with assistance of co-authors.

II Planned and performed the study together with co-authors. Performed the soil sampling together with co-authors. Performed the phospholipid fatty acid analysis. Performed the data analysis and writing, with some assistance of co-authors.

III Took part in planning of the study. Performed the soil sampling. Performed the modelling, data analysis and writing, with some assistance of co-authors.

IV Planned the study together with co-authors. Performed the soil sampling with co-authors. Assisted in data analysis and writing.

Abbreviations

C_{mic}	Total carbon content in microbial cytoplasm
C/N ratio	The ratio of carbon to nitrogen (on a mass basis) in any given material
DOC	Dissolved organic carbon: total organic carbon in water samples after passage through a 0.2 μm filter
DOM	Dissolved organic matter: total organic matter in water samples after passage through a 0.2 μm filter
DON	Dissolved organic nitrogen: total organic nitrogen in water samples after passage through a 0.2 μm filter
N_{mic}	Total nitrogen content in microbial cytoplasm
PLFA	Phospholipid fatty acid
PCA	Principal component analysis
SOM	Soil organic matter: Total amount of organic matter in soil.

1 Introduction

Forest ecosystems are important for global biodiversity because they host vast ranges of niches and species. Human societies are dependent on forests for provision of wood-, fibre-, and fuel-production, clean water and carbon (C) sequestration. N availability typically limits primary production of temperate and boreal forests (Tamm, 1991; Vitousek & Howarth, 1991; LeBauer & Treseder, 2008) and forest ecosystems are strongly influenced by nitrogen (N) inputs, status and turnover. This is a cause of concern because human activity has roughly doubled rates of production of easily available forms of N since the late 1960s, and N deposition has increased globally (Vitousek et al., 1997; Fowler et al., 2013). Although N deposition rates have likely declined in Europe as a whole 1990-2009, there is large temporal and spatial variability (Tørseth et al., 2012). Furthermore, projections of future global N deposition do not indicate that levels will be decreasing in the near future (Galloway et al., 2004). For instance, data from the Swedish throughfall monitoring network indicate that inorganic N concentrations in throughfall did not change between 1996 and 2008, although N emissions decreased (Pihl Karlsson et al., 2011).

The N status of forest ecosystems is an important parameter that affects numerous processes and variables, among others, eutrophication, acidification, long-term site fertility and ecosystem C balance. Variations in temperature sensitivity of decomposition of SOM fractions and its interactions with soil N availability influence both heterotrophic respiration and net ecosystem productivity and hence play a pivotal role for soil-climate interactions (Gärdenäs et al., 2011). Thus, changes in the N cycling of temperate and boreal forests are likely to affect processes beyond those directly involved in terrestrial ecosystem N cycling. Widespread N limitation in temperate and boreal forest ecosystems imply that N availability is low in relation to ecosystem demands in these biomes, and soil water N leaching losses should be negligible under such conditions. However, long-term elevated N load may

eventually lead to N saturation, i.e. total N ecosystems inputs exceeding ecosystem sinks for N (Aber et al., 1989; Aber et al., 1998), and N losses via water transport and in gaseous form may then increase. Nitrate (NO_3^-) leaching is pivotal in this context as NO_3^- is a mobile anion. Furthermore, NO_3^- leaching is connected to acidification of soils (van Bremen et al., 1983). As demands of forests for provision of timber, fibre and fuel production will likely increase in the future (Harrison et al., 2010), harvest intensities may increase, which may also increase soil acidification. However, in N limited forests, such as typically found in boreal and temperate zones, the duration required to saturate forests with N at N loading rates similar to N deposition in Europe may be long (Aber et al., 1998; Fenn et al., 1998; Wright et al., 2001). Long-term N addition experiments, thus, offer the possibility to study the effects of N loading in originally N-limited boreal and temperate forests. However, the importance of dissolved organic N (DON) for soil water N speciation and forest ecosystem N retention under long-term elevated N load has received less attention than inorganic forms of N. Moreover, the response of the soil microbial community to elevated N loading and the link to forest ecosystem N retention is in need of further research.

Forest management operations are routinely carried out across forest landscapes. Clear-cutting is known to increase soil water NO_3^- concentrations, thereby increasing risks of N losses (Gundersen et al., 2006). Moreover, mechanical site preparation is commonly undertaken to improve seedling survival and growth in some countries. In Sweden, for instance, site prepartion is performed on >80% of the clear-felled area annually (Swedish Forest Agency, 2014). Mechanical site preparation may increase soil water N concentrations, although there are conflicting reports on its effects in this respect (Piirainen et al., 2007; Nohrstedt, 2000; Ring et al., 2013). Clear-cutting and site preparation of forest stands previously fertilized with N, or of elevated N availability for other reasons, may increase risks of undesirable N leaching. However, there is a scarcity of studies on this topic.

2 Aims

The studies this thesis is based upon addressed effects of two types of disturbance on organic and inorganic forms of N in soil water in coniferous forests: "chemical disturbance" (changes in N availability due to long-term N addition and N deposition), and "physical disturbance" (clear-cutting and soil scarification). The effect of the combined effect of the two types of disturbance was studied as well. Empirically, responses of soil microbial variables and abiotic variables to varying levels of historic N load were studied in both N fertilization experiments and along a N deposition gradient. Thus, the study sites represent wide ranges of ambient and historic N loads, and management intensities. In a modelling exercise, a process-based ecosystem C and N model (CoupModel) was calibrated against field measurements obtained during the regeneration phase of a Scots pine (*Pinus sylvestris* L.) forest in an N fertilization experiment where disc trenching was applied. The aims were:

- To determine the effect of long-term N addition, and recovery from N addition, on soil water chemistry in a boreal Norway spruce (*Picea abies* L. Karst.) forest, particularly the quantity and quality of dissolved organic matter. (Paper I).

- To analyse effects of increased N availability on soil O horizon chemistry and microbial variables in long-term N addition experiments and along a N deposition gradient across Norway spruce forests in Sweden and the Czech Republic. (Papers II and IV).

- To quantitatively evaluate effects of clear-cutting, soil scarification and previous N fertilization on rates of soil water N leaching at a Scots pine regeneration area in central Sweden. (Paper III).

3 Background

Globally, the largest pool of N is found in rocks and mineral, $197*10^{15}$ Mg (Walker, 1977). The second largest pool of N is found in the atmosphere (mainly in the form of dinitrogen gas, N_2, $3.9*10^{15}$ Mg of N), followed by oceans ($2.3*10^{13}$ Mg of N) and terrestrial ecosystems ($1.6*10^{11}$ Mg of N) (Rosswall, 1983). In terrestrial ecosystems, a large part of the total N (commonly more than 85%) is found in the soil (Cole & Rapp, 1981). Of the N present in soil organic matter (SOM), Schulten and Schnitzer (1998) estimated that proteins and protein derived N typically make up 40%, amino sugars 5-6%, heterocyclic N compounds 35% and NH_3-N 19% of which 1/4 is fixed as NH_4^+ on clay minerals. Knicker (2004) argued, based on a literature review, that amide N in peptide-like structures were the dominating form of soil organic N, and heterocyclic forms of N were of large abundance only in fire affected soils. DON consists of a diverse mixture of organic N compounds of plant, SOM and microbial origin, and contributes significantly to forest ecosystem N budgets (Qualls & Haines, 1991). Inorganic N in soils is present mainly in the form of ammonium (NH_4^+) and nitrate (NO_3^-) in soil solution and at exchange sites.

Much of the N in soil is not available for plant N uptake. The production of available forms of N is governed by the processes of decomposition, ammonification and nitrification, by which low-molecular weight organic N, NH_4^+ and NO_3^- are produced and subsequently taken up by plants or microorganisms (Schimel & Bennett, 2004). N can be nitrified from available NH_4^+ (autotrophic) and organic N sources (heterotrophic). Apart from substrate availability, nitrification is also dependent (inter alia) on the abundance of nitrifiers, O_2 availability and pH (Sylvia et al., 2005). Although low soil pH likely limits autotrophic nitrification, nitrification is observed in acid soils (de Boer & Kowalchuk, 2001; Šantrůčková et al., 2009; Kaňa et al., 2015). The N taken up by vegetation and other organisms is eventually returned to the soil as

litter, to form soil organic N. N may be lost from the ecosystem by soil water movements (in organic and inorganic forms) or harvest practices. Gaseous losses from the ecosystem consist of N_2 and N_xO, produced during denitrification and nitrification.

N is mineralized and immobilized by a broad spectra of soil organisms. Although some pools of N in a terrestrial ecosystem are small compared to others (e.g. the N pool in SOM is much larger than the soil NO_3^- and NH_4^+ pools), they may be highly important for overall ecosystem N cycling. The flux through a pool, e.g. NH_4^+ in soil solution, is proportional to its turnover rate. Thus, the flux through small soil inorganic N pools (especially NH_4^+ and NO_3^-) can be large if production and consumption processes are rapid. For instance, Davidsson et al. (1992) found that net N mineralization rates were higher in a middle-aged forest than an old growth forest, but the gross N mineralization rate was three times higher in the old growth forest soil. High turnover of forest soil NH_4^+ and NO_3^- pools and uncoupled net and gross N mineralization in forests have been subsequently demonstrated in numerous studies, e.g. Stark & Hart (1997) and Blaško et al. (2013). Plants have historically been regarded as poor competitors for available N in soil. However, net N immobilization estimates over growing seasons (Nadelhoffer et al., 1994), and increasing recognition of the significance of both plants' uptake of organic N (Näsholm et al., 1998) and trees' mycorrhizal associations, have prompted shifts in views. In the long-term, at spatial scales commensurate with the overall root zone (including extramatrical mycelium extensions of root surfaces), plants are now regarded as rather good competitors for N (Schimel & Bennett, 2004).

Leaching of N from N-limited forests should theoretically be low as plant and microbial demands relative to N availability are high. Accordingly, low soil water N concentrations in temperate and boreal forests have been reported, especially in forests with low ambient N deposition (Gundersen et al., 2006; Nilsson et al., 1998; Pihl-Karlsson et al., 2011). Inorganic N concentrations in stream water are also low (and the main forms of N are organic) in undisturbed, unpolluted forests with low N availability in South American highlands (Hedin et al., 1995). However, elevated NO_3^- concentrations in soil water is frequently observed after long-term N addition (Aber et al., 1998) or in forests in regions with elevated N deposition (Pihl-Karlsson et al., 2011), clear-cutting (Gundersen et al., 2006) and other types of disturbance, e.g. windthrow (Hellsten et al., 2015).

3.1 Effects of N addition

The effects of N loading (via experimental N addition, N fertilization and/or N deposition) to boreal and temperate forests have been the topic of extensive research. Increasing N loading of temperate and boreal forests can potentially alleviate N limitation and thus increase tree growth. Forest fertilization with N (a single shot application of 150 kg ha^{-1} $year^{-1}$ of N) increases stem-wood growth, according to Swedish experience, by approximately 15 m^3 ha^{-1} $year^{-1}$ for up to seven years after application (Pettersson, 1994; Nohrstedt, 2001; Pettersson & Högbom, 2004). However, long-term N loading can also lead to saturation of forest ecosystems with N, accompanied by increased N losses in the form of soil N leaching, most notably in the form of NO_3^-, and gaseous losses (N_2, N_xO) (Aber et al., 1989; Aber et al. 1998). Lovett & Goodale (2011) used an N mass balance model to distinguish "kinetic" from "capacity" N saturation of forest ecosystems. Kinetic N saturation can be found in forests where losses of N are elevated, but some N is still retained in the ecosystem. Capacity N saturation can be found in ecosystems where the net sink for N is 0, and N losses equal inputs. The authors stress the importance of accounting for multiple sources and sinks for N of the ecosystem for understanding N saturation of forest ecosystems. The leaching of NO_3^- is pivotal to ecosystem N retention since it sorbs weakly to the soil and is thus a mobile anion. Soil NO_3^- leaching is also connected to acidification of soils. The acidifying impact of N fluxes on a forest ecosystem can be simplified by a mass balance equation (van Bremen et al., 1983) describing changes in soil acid neutralizing capacity (ANC_{Soil}) on an annual basis:

$$-\delta ANC_{Soil} = (NH_{4\,in}^{+} - NH_{4\,out}^{+}) - (NO_{3\,in}^{-} - NO_{3\,out}^{-})$$

where *in* = the flux to the forest ecosystem above the forest canopy and *out* = losses below the root zone (i.e. leaching losses from the system). The equation is only valid if rates of conversion of NH_3 (gas) into NH_4^+ (aqueous) in the atmosphere can be neglected. The relationship was developed during a period of heavy acid deposition loading in central and western Europe in the 1980s, and indirectly states that one mole of acidity is generated for every mole of NO_3^- leached from the terrestrial system. Accordingly, Bergholm et al. (2003) showed that under experimental loading with ammonium sulphate ($(NH_4)_2SO_4$) for ten years of a middle-aged Norway spruce forest in southern Sweden soil N cycling significantly contributed to the soil proton load. Initially tree NH_4^+ uptake and, in subsequent years, nitrification and NO_3^- leaching were the main cause for the increased soil proton load in plots treated with ammonium sulphate. There is evidence that the type of N added to a forest influences the nitrification and hence, NO_3^- leaching (Tamm & Popovic, 1995).

For example, Grip (1982) found that urea ($CO(NH_2)_2$) application (which reduces soil acidity) increased NO_3^- leaching in surface water more than NH_4NO_3 additions.

Fenn et al. (1998) concluded that high N deposition, high soil N stores, low soil C/N ratios, short growing periods and short water residence times increase mature forest ecosystems' susceptibility to N saturation and (hence) elevated N leaching rates. Gundersen et al. (1998) found a negative correlation between soil O-horizon C/N ratio and soil water NO_3^- in European forests, with O-horizon C/N ratios < 25 at sites where excessive NO_3^- leaching was observed. Binkley & Högberg (1997) concluded from an extensive review of fertilization experiments in Sweden, where forests typically have low N status, that historic N loads had not affected the overall health and productivity of Swedish forests, since NO_3^- leaching rates were generally low following experimental N addition. However, at local scales, Pihl-Karlsson et al. (2011) found that some tree stands, typically in south-western parts of Sweden with high ambient N deposition, showed signs of elevated N availability, with NO_3^- in mineral soil water >0.5 mg NO_3-N l^{-1}. Accordingly, at Gårdsjön experimental catchment, south-western Sweden, Moldan et al. (2006) observed elevated NO_3^- in draining surface water after two years of weekly N additions (corresponding to 35 kg ha^{-1} $year^{-1}$ of N) supplied by a sprinkler system. Nevertheless, over 13 years of N addition, only 5% of the N load was leached as NO_3^-, 44% was incorporated in trees and vegetation, and 51% was incorporated into SOM.

Results from field experiments has shown there is considerable potential for added N to be retained in forest ecosystems and N leaching losses seldom exceed N loadings (Aber et al., 1998; Johnson, 1992; Binkley & Högberg, 1997). At the Klosterhede N addition experiment, high N retention was observed without increased tree growth in response to N addition (Gundersen, 1998). Andersson et al. (2001) concluded a, initially N limited, Norway spruce forest at the optimum nutrition experiment Stråsan displayed high retention of added N (35 – 108 kg N ha^{-1} $year^{-1}$) for 30 years. From the same forest experiment Blaško et al. (2013) found gross N mineralization approximately an order of magnitude higher in N treatments compared to control after an additional 14 years of annual N addition at 30 kg N ha^{-1} $year^{-1}$. However, net N mineralization was similar between N treatments, as gross microbial NH_4^+ consumption increased as well (Blaško et al., 2013). A large part of the added N is typically retained in the soil (Fenn et al., 1998; Johnson, 1992). In a ^{15}N tracer experiment Melin et al. (1983) investigated in what proportions added N was retained in soil and vegetation in an old pine forest stand two growing

seasons after N fertilization with a single-shot application of 100 kg ha^{-1} of N (NH_4NO_3). The soil was initially poor in N, with C/N ratio of O horizon of 42 g g^{-1}. The estimated soil N retention was between 40-60% after two growing seasons, which supports the results from other studies (reviewed by Johnson, 1992). Under N fertilization regimes employed in Swedish forestry (single-shot application of 150 kg ha^{-1} of N), 5 - 10% of added N is estimated to be leached to surface waters (Edlund, 1994, Ring, 2007), but leaching of up to 14% has been reported during the first year following fertilization of 150 kg N ha^{-1} from a catchment in central Sweden (Lundin & Nilsson, 2014).

The high N retention generally observed in forest ecosystems has been attributed to three main causes (Aber et al., 1998). One is direct chemical fixation of inorganic N into SOM under high levels of NH_4 and high pH. While this mechanism is plausible, it does not appear to occur at high rates compared to N immobilization (Aber et al., 1998), and it should be quantitatively less important under acid conditions with low NH_4^+ availability. The others are increased N immobilization by free-living saprotrophs under elevated N availability and mycorrhizal N assimilation and exudation of extracellular enzymes which react with humus to form stable organic N compounds (Aber et al., 1998). Högberg et al. (2003) hypothesized that trees allocate more C belowground to fine roots and associated mycorrhiza under low N availability, thus fuelling gross N immobilization and preventing net N mineralization. Indeed, lower flux of belowground allocation of recent photosynthate C (determined by ^{13}C tracer) after N addition (100 kg ha^{-1} of N) was reported for a Scots pine stand in northern Sweden (Högberg et al., 2010). Bahr et al. (2013) found lower growth of extramatrical mycelium (EMM) under elevated N deposition at sites in the Swedish throughfall monitoring network, but could not distinguish between effects of reductions in EMM production and increases in N deposition on soil water NO_3^- concentrations. In a Norway spruce forest in southern Sweden soil water inorganic N increased and EMM production decreased with N fertilization (Bahr et al., 2015). However, in the aforementioned study N + phosphorus (NP) fertilization reduced soil water inorganic N, whilst EMM production decreased further, which suggest that not only the effects of N addition on mycorrhiza, but the whole microbial community and N immobilization need consideration. The response of the soil microbial community to elevated N and how this is connected to ecosystem N retention is thus in need of further research, as addressed in papers II and IV in this thesis.

While effects of N addition to forest ecosystems on inorganic N soil water forms have been addressed in many studies, the consequences for DOM leaching have received less attention. Since DOM is produced during decomposition, its mobilization to soil solution may be influenced by processes that affect decomposition. Addition of N affects decomposition, as reflected in decreased mass loss rates of low-quality litter (Knorr et al., 2005) and in late stages of decomposition (Berg & Matzner, 1997). Fog (1988) concluded from a review of studies on decomposition that high availability of N is linked to an increase in the formation of water soluble, partially decomposed lignin degradation products. Increased soil water DOC flux and indications of higher abundance of incomplete lignin degradation derivatives was found under high S and N deposition in German spruce forests (Guggenberger, 1994), and deciduous forests in North America (Pregitzer et al., 2004). However, there are studies which have shown no such effect as well (Currie et al., 1996; Raastad & Mulder, 1999). It has been suggested that increases in DOC concentrations in mineral soil under long-term N loading are the result of processes occurring in the soil organic horizon (Zak et al., 2006). DOC leaching depend on the amount of substrate organic matter, such as leaf and woody litter (Park et al., 2002), but solubility of DOC is also controlled by dissociation of functional groups and presence of polyvalent cations. Indeed, the effect of N addition on DOC may be explained by the different effects of N addition on soil acidity (Evans et al., 2008). How the abovementioned controls on forest soil water DOM are affected by long-term N addition is not well known. DON accounts for a majority of total N dissolved in soil water (Kranabetter et al., 2007) and surface waters of boreal forests (Sponseller et al., 2014). Therefore there is need of long-term studies of the effects of N addition on soil water DON in boreal forest ecosystems, as addressed in paper I in this thesis.

3.2 Effects of clear-cutting and soil scarification

Thirty years ago, Vitousek and Matson (1985) showed that intensive forestry operations could increase mineralization of N, nitrification and N losses in a field experiment in south-eastern USA. Many studies have subsequently confirmed these findings, and clear-cutting is a forest management practice generally considered to contribute to N leaching from forestland in Sweden (Stendahl and Hjerpe, 2007, Ring, 2007). Futter et al. (2010) estimated the N leaching from regeneration areas in Sweden to contribute 3% of the total Swedish N load to the Baltic.

Vegetation N demand decreases dramatically after clear-cutting of a forest stand, while soil net N mineralization and nitrification often increase (Holmes & Zak, 1999; Prescott, 1997; Fisk & Fahey, 1990; Paavolainen & Smolander, 1998). Microbial immobilization of N has been suggested to influence mineral N availability following harvest (Vitousek & Matson, 1985; Prescott, 1997; Bergholm et al., 2015). Furthermore, initial N immobilization during decomposition of litter and harvest residues of low N content may be responsible for delays in increases in soil water N concentrations following clear-cutting sometimes observed at low-fertility sites (Gundersen et al., 2006). Increased soil gross nitrification following clear-cutting have been reported (Pedersen et al., 1999). Consequently, NH_4^+ and NO_3^- soil water concentrations typically increase following clear-cutting (Dahlgren & Driscoll, 1994; Futter et al., 2010; Hedwall et al., 2013; Bergholm et al., 2015). The growing vegetation (ground and field vegetation, and seedlings, naturally regenerated or planted) also constitutes a sink for N on clear cuts (Emmett et al., 1991; Hedwall et al., 2015).

Clear-cutting has also been shown to affect the water balance, with increased runoff resulting from reductions in evapotranspiration (Hornbeck et al., 1993). Thus, NO_3^- concentrations in soil water and leaching is likely to increase following clear-cutting, as confirmed by observations at both plot level (Futter et al., 2010), and in stream water (Rosén et al., 1996). Elevated NO_3^- concentrations in groundwater following final felling have been observed for up to 4 years at Söderåsen, SW Sweden (Wiklander et al., 1991) and up to 10 years in a Finnish study (Kubin, 1998). In addition, N leaching following clear-cutting may be positively correlated with site fertility (Gundersen et al., 2006) and, at least at sites in southern Sweden examined by Akselsson et al. (2004), ambient N deposition rates. Similarly, Berdén et al. (1997) reported a positive correlation between previous N load (long-term annual N addition) and NO_3^- leaching after clear-cutting of forest stands at the optimum nutrition experiment site Stråsan in central Sweden. However, lower N fertilization rates than those applied in the previously discussed studies, at doses less than 450 kg ha^{-1} of N, have not resulted in increases in soil water NO_3^- concentrations after clear-cutting (Ring, 1996; Ring et al., 2013).

Site preparation is commonly applied in Sweden, >80% of the clear-felled area is subjected to site preparation annually (Swedish Forest Agency, 2014), as a measure to improve plant seedling survival and growth (Örlander et al., 1990; Johansson et al., 2012). Soil scarification techniques include both continuous (e.g. disc trenching) and intermittent (e.g. mounding) methods. However, a common feature of all soil scarification methods is the physical

disturbance to the soil. Disc trenching creates ridges and furrows. Between two ridges or two furrows, the O-horizon is largely intact. Soil scarification may affect SOM turnover, as litter mass loss rates are higher in mounds and ridges, than rates of mass loss of litter deposited on the soil surface (Johansson, 1994; Lundmark-Thelin & Johansson, 1997). However, rates of soil net N mineralization per unit SOM do not increase (Smolander et al., 2000) and soil CO_2 fluxes may increase (Malik & Hu, 1997) or decrease (Mjöfors et al., 2015) following mounding or disc trenching. Moreover, soil water NO_3^- concentrations increased in mineral soil under ridges as compared to undisturbed soil in a Finnish (Piirainen et al., 2007) and in ridges compared to furrows at two Swedish forests (Ring et al., 2013). Nohrstedt (2000) found that although mounds generated by soil scarification were associated with higher KCl-extractable inorganic N pools, the furrows were associated with lower pools of inorganic N, leading to no compound effect on total soil inorganic N. In a study at the same regeneration area, Ring (1996) found no significant increase in soil solution NO_3^- concentrations during the first four years following a simulated soil scarification treatment. Ring et al. (2013) investigated the effects of disc trenching in previously N fertilized Scots pine forest on soil water NO_3^- during the first six years of the regeneration phase. It was found that previous N fertilization did not affect soil water NO_3^-, but that soil water NO_3^- concentrations were higher below ridges than furrows created by disc trenching. However, since post-harvest N leaching losses may be affected by ambient N deposition (Akselsson et al., 2004) or previous N fertilizer load (Berdén et al., 1997), if soil scarification methods alleviate, or exacerbate, the effects of previous N fertilization is in need of further research, as addressed in paper III in this thesis.

4 Materials and methods

4.1 Study sites and experimental design

Stråsan

Stråsan refers to the site of a forest optimum nutrition experiment, designated Stråsan E26A, in central Sweden (WGS84: 60°92'N, 16°01'E), approximately 40 km northeast of the city Falun (Tamm et al., 1974) (Table 1). The site is located 360 meters above sea level (m. a. s. l.). The climate is cold temperate: the long-term (1961-1990) annual mean temperature in the area is 3.2°C and annual mean precipitation 740 mm. The N deposition in the area is 3.2 kg N ha^{-1} $year^{-1}$. The previous Norway spruce stand was clear-felled in 1956 and the site was subjected to burning in 1957. The current Norway spruce stand was planted in 1958. Field vegetation was sparse during sampling in 2010-2013, dominated by blueberry (*Vaccinium myrtillus)*. Ground vegetation is dominated by mosses, with some lichens. The soil is classified as a haplic podzol with parent material consisting of glacial till, dominated by medium and fine sand (Tamm et al., 1974). Soil pH is in the acidic range (pH in 1:10 soil:H_2O w:v is 4.9 in O-horizon, and 5.0 in the mineral soil), and base saturation in the mineral soil is approximately 8%.

The experiment has a randomized block design with two blocks, and each treatment replicated once within each block. The experimental plots measure 30 m × 30 m. Treatments consist of addition of various mineral nutrients (most notably N, P, K alone and in combination) of which some have been ongoing since 1967. However, this thesis only considers effects of the control (N0) and two N treatments, N1 and N2, in which fertilizer N was added as ammonium nitrate (NH_4NO_3), as detailed in Table 2. In 2010 the standing stem volume was on average 286, 425 and 442 m^3 ha^{-1} in the N0, N1 and N2 plots, respectively.

Hagfors

Hagfors refers to another fertilization experiment, designated 165 Hagfors, initiated in 1981 in a Scots pine stand near the municipality of Hagfors (WGS84: 59°99'N, 13°71'E), south-central Sweden (Table 1). The climate is cold temperate: the long-term (1961-1990) annual mean temperature in the area is 3.5°C and the annual mean precipitation is 671 mm (Alexandersson & Karlström, 2001). The N deposition in the area is 5.9 kg N ha^{-1} $year^{-1}$. The study site is situated 190 m. a. s. l. on a well-drained, podzolized sandy-silty till soil and has a site quality class of 5.9 m^3 ha^{-1} yr^{-1} (Ring et al. 2011). The field layer was dominated by blueberry (*Vaccinium myrtillus)* prior to clear-cutting in 2006 and the ground vegetation was classified as a "lichen-rich" type (Nohrstedt, 1998) according to the Swedish site classification system (Hägglund & Lundmark, 1982). The experiment at the site had a split-plot design with seven levels of N fertilizer application as main factor replicated in three blocks and soil scarification as sub-plot factor. The experimental N treatments considered in this thesis consist of two levels: a control (0N: 0 kg ha^{-1} of N) and fertilization equivalent to 150 kg ha^{-1} of N, in three applications at 8-year intervals beginning in 1981, giving a total dose of 450 kg ha^{-1} of N (450N). The fertilizer N was NH_4NO_3 in the first two applications, and NH_4NO_3 supplemented with dolomite chalk in the last application (Table 3).

The 0N and 450N plots were clear-cut (harvesting stems, tops and branches) in March 2006. Following clear-cutting, every experimental plot was split into two subplots (measuring 15×30 m), assigned to receive the treatments no scarification (no DT) or scarification (DT). In May 2006, the subplots assigned to scarification were disc-trenched with a Bracke disc trencher (with two rotating discs at the rear) carried by a Timberjack 1710D forwarder. The disc trencher created furrows with average heights and widths of 0.17 and 0.7 m, respectively, ridges with average heights and widths of 0.21 and 0.63 m, respectively, and 0.72 m wide areas in-between these features (Ring et al., 2013). Thus, the area-weighted proportions of the created furrows, ridges and areas between them were 0.413, 0.373 and 0.213, respectively. Scots pine seedlings (1.5 year-old) were planted in the furrows during May and June 2006 at 2 m intervals (Johansson et al., 2013).

Skogaby

The experimental spruce forest site Skogaby is situated in southern Sweden (WGS84: 56°55'N, 13°21'E), ca. 15 km from the city Laholm (Table 1). The site is located 95 m a. s. l. The soil type is poorly developed podzol (haplic podzol) characterized as a loamy sand with clay content 4 – 7% on a bedrock

of gneiss (Bergholm et al., 1995). Historically, the site was a heather (*Calluna vulgaris* L.) heathland used for cattle grazing and afforested in 1913 with Scots pine. The current Norway spruce stand was planted with seedlings of Polish provenance in 1966 (Bergholm et al., 1995) and control (N0) plots had an average standing stem volume of 244 m^3 ha^{-1} in 2010 (pers. comm. U. Johansson, SLU). There is very sparse field layer with some grasses, and ground vegetation is dominated by mosses.

The experiment was initiated in 1988 in a randomized block design, with 45 m × 45 m experimental plots. At the start of the experiment there were four blocks, however, trees in two blocks were windthrown in a winter storm during 2005. Some treatments at Skogaby involve additions of water and several nutrients to the forest, but this thesis only considers effects of the control (N0) and another treatment designated NS, involving addition of ammonium sulphate, $(NH_4)_2SO_4$ (Table 4).

Čertovo

The study site Čertovo is situated in the watershed of Čertovo lake in the Šumava Mountains (WGS84: 49°16'N, 13°19'E), Czech Republic (Table 1) at an altitude of 1057 m a. s. l. The soil type is a haplic podzol developed on a bedrock of gneiss with granite intrusions (Kopáček et al., 2002). The area of the study site is covered by a mature (~150 years old) Norway spruce forest, with a minor components (ca. 3%) of beech (*Fagus sylvatica* L.) and fir (*Abies alba* Mill.). This area has been largely dominated by Norway spruce forest since the preboreal era (ca. 10 000 years B.P.) (Jankovská, 2006). The field layer vegetation is dominated by blueberry (*Vaccinium myrtillus* L.). No experimental treatment has been applied in this forest, but it has a history of long-term high acidic deposition (amounting to ca. 1170 kg ha^{-1} of N from 1950 to 2010), and currently ambient N deposition is 14.6 kg ha^{-1} $year^{-1}$ of N (Kopáček & Hruška, 2010).

Table 1. *Some basic characteristics of the sites considered in this thesis.*

	Unit	Stråsan	Skogaby	Hagfors	Čertovo
Geographic coordinates	WGS84	60°92'N, 16°01'E	56°55'N, 13°21'E	59°99'N, 13°71'E	49°16'N, 13°19'E
Mean annual temperature	°C	4.3	7.6	3.5	5.4
Mean annual precipitation	mm yr^{-1}	620	1187	671	1413
Current nitrogen deposition	kg ha^{-1} $year^{-1}$ of N	3.2[f]	14.8 [a]	5.9	14.6[b]
Nitrogen deposition, 1950-2010	kg ha^{-1} of N	93[f]	723[c]	NA†	1168[b]
Dominant tree species	-	Norway Spruce	Norway Spruce	Scots pine	Norway Spruce
Stand age (in 2013)	years	55	47	7	150
Ground vegetation	-	Mosses and lichens	Mosses	Lichens	Mosses, lichens and blueberry
Soil type	FAO	Haplic Podzol	Haplic Podzol	Haplic Podzol	Haplic Podzol
Soil base saturation (~0.3 m depth)	%	8[d]	8[c]	NA†	9
Experimental treatments		N0	N0	N0	None
		N1	NS	N450	
		N2		Disc trenching (DT)	
				No disc trenching (no DT)	
Main reference		Tamm et al. (1974)	Bergholm et al. (1995)	Ring et al. (2011)	Kopáček et al. (2002)

[a] Olsson et al. (2013), [b] Kopáček & Hruška (2010), [c] Bergholm et al., (2003), Olsson et al. (2013), Hansen et al. (2013), [d] Eriksson et al. (1996), [f]Estimated from inorganic N in throughfall (Swedish Environmental Institute, IVL) and precipitation records (Swedish Meteorological and Hydrological Institute , SMHI) from nearby monitoring stations, †NA=not available.

Table 2. *Nitrogen (N) addition treatments at Stråsan E26A (all values are in the unit kg ha^{-1} year^{-1} of N). Ambient N deposition in the area amounts to ca. 3 kg ha^{-1} year^{-1} of N (2005-2012). N was applied as NH_4NO_3 (s).*

Year	N0	N1	N2
1967-1969	0	60	120
1970-1976	0	40	80
1977-1990	0	30	60
1991-2013	0	30	0
Sum 1967-2013	0	1570	1760

Table 3. *Nitrogen (N) addition treatments at Hagfors 165 (all values are in the unit kg ha^{-1} year^{-1} of N). Ambient N deposition in the area amounts to ca. 6 kg ha^{-1} year^{-1} of N (2003-2012). N was applied as NH_4NO_3 (s) in 1981 and 1989, and NH_4NO_3 (s) supplemented with dolomite chalk in 1997.*

	N0	N450
1981	0	150
1989	0	150
1997	0	150
Sum 1981-1997	0	450

Table 4. *Experimental treatments at Skogaby (all values are in the unit kg ha^{-1} year^{-1} of N). Ambient N deposition in the area amounts to ca. 15 kg ha^{-1} year^{-1} of N (2005-2013). N was applied as $(NH_4)_2SO_4$ (s) until 2001.*

	N0	NS
1988-2001	0	100 kg N, 114 kg S ha^{-1} year^{-1}
2002-2013	0	0
Sum 1988-2013	0	1400

4.2 Soil chemistry (Papers I, II, III and IV)

The O horizon C and N stock estimates reported in Papers I and III were calculated from analyses of soil samples collected from a grid along five equally spaced lines across each experimental plot at Stråsan and Hagfors. Samples were pooled to obtain one composite sample per plot. Soil samples were stored cool during transport. Samples were sieved (4 mm) at laboratory to remove roots and homogenize the samples. Dry weights were determined by drying to constant weight at 105 °C. Total C and N contents of each sample were determined with a CNS-2000 elemental analyzer (LECO Instruments, USA).

Soil samples for determination of soil chemistry (Papers II and IV) were collected from the experimental plots by digging with a spade and gathering 0.3 × 0.3 m samples of O horizon material from two points, along two randomly chosen perpendicular sides of each experimental plot at Stråsan and Skogaby, resulting in eight samples per treatment (four samples × two blocks). Samples were taken at fixed distances along the side of the experimental plot, at 10 and 20 m from the corner. In Čertovo, four sampling points were selected in the long-term monitoring plot with similar spacing as in Stråsan and Skogaby. Samples were stored on dry ice during transport. Samples were sorted to remove roots (> 1 mm diameter) and passed through a 4 mm sieve. Their dry weights were determined after drying in oven at 105 °C to constant weight. Total N contents were measured using a Vario Micro cube analyser (Elementar GmbH, Germany). N extractable with 0.5 M potassium sulphate solution (N_{K2SO4}) at a 1:4 ratio (w/v) was analysed using a LiquiTOC II TOC/TN analyser (Elementar GmbH, Germany). Water-extractable C and N were extracted with deionised water (1:30, v/w), then NH_4-N and NO_3-N were analysed using a QC8500 Flow Injection Analyzer (Lachat Instruments, USA) and both dissolved organic C (DOC) and dissolved N (DN) using a LiquiTOC II TOC/TN analyzer (Elementar GmbH, Germany). Ultraviolet (UV) absorbance of water extracts at 254 nm was measured in a 1 cm cuvette using a UV-1800 spectrophotometer (Shimadzu Corporation, Japan), and distilled water as a blank. Specific UV-absorbance (SUVA; mg C^{-1} m^{-1}) was then calculated by dividing the UV absorbance at 254 nm (m^{-1}) by the DOC concentration of each water extract (mg C l^{-1}).

4.3 Soil water chemistry (papers I and II)

Soil water was sampled at the optimum nutrition field experiment site Stråsan during the 1995, 2009 and 2013 growing seasons. Sampling dates were evenly

spaced at approximately two-week intervals, from 1995-06-20, 2009-06-08 and 2013-06-07 to 1995-11-21, 2009-11-12 and 2013-11-04, respectively. Soil water was collected from two types of lysimeters: zero-tension lysimeters (sampling the O horizon soil water) and prenart suction cups (sampling mineral soil B horizon soil water).

The zero-tension lysimeters were each constructed of a plexiglass trough (0.3 × 0.3 m) with two layers of polyethylene nets on top. The O horizon material rested on top of the polyethylene net. The trough was connected by silicone tubing to a borosilicate glass bottle. The mineral soil water was collected by samplers consisting of a porous (polytetrafluorethylene) cup with a pore size of 4 μm (Prenart Equipment Aps, Frederiksberg, Denmark) connected by silicone tubing to a borosilicate glass bottle. Borosilicate glass bottles collecting O horizon and mineral B horizon soil water were installed at the bottom of 0.5 m deep soil pits with a Styrofoam lid on top. The zero-tension lysimeters sampled water moving freely under gravity whilst prenart teflon samplers sampled soil water via application of suction at an initial pressure of -70 kPa. Six zero-tension lysimeters and four suction samplers were installed in each plot placed at the outer projection of tree canopies to ensure similar effects of throughfall. All lysimeters and mineral soil water samplers were installed one year before the first sampling. Water samples collected in 2009 and 2013 from individual lysimeters of each type were pooled per plot and sampling occasion. Water samples were shipped to the laboratory on the day of sampling.

Soil water samples were stored at +2°C prior analysis, and laboratory analysis were usually performed within 1 week of sampling. Water samples were filtered through a 0.2 μm filter (Acrodisc PF, Gelman Sciences, MI) and analysed for pH, DOC, NO_3-N (aq.), NH_4-N (aq.) and total nitrogen (TN) in sampling years 1995 and 2009. In 1995 and 2009 the samples' contents of seven metals (Al, Fe, Mn, Ca, K, Mg, Na) and four anions (Br, Cl, PO_4^{3-}, SO_4^{2-}) were also determined in soil water extracts. A portion of each filtered sample was acidified to pH 3 with HCl, then subjected to total organic carbon (TOC) analysis, using a TOC-500 Analyzer (Shimadzu Corporation, Kyoto, Japan) in 1995, and a Shimadzu TOC-VCPH Analyzer in 2009. In 1995, TN samples were subjected to persulphate oxidation by mixing them with equal volumes of a solution consisting of 10 g $K_2S_2O_8$ in 1 L of 0.15 M NaOH and boiling under pressure (14 kPa) for 25 min. Before analysis of NO_3 (aq.), by Flow Injection Analysis (FIA), 0.25 mL of 1.44 M H_2SO_4 was added. Total N (TN) was determined with a TNM-1 TN Analyzer (Shimadzu Corporation, Kyoto, Japan)

in 2009. Anions (Br^-, Cl^-, PO_4^{3-}, SO_4^{2-}, and NO_3^-) were analysed by ion chromatography using a Dionex 2000i/SP column in 1995 and a Metrosep A Supp 5 column in 2009. In 2013, only NO_3^- was determined in soil water samples using FIA (FIAstar 5000 analyzer, FOSS). Dissolved organic nitrogen (DON) was calculated by subtracting the N content in inorganic N species (NO_3-N and NH_4-N) from the TN concentration. The limit of detection for NO_3-N and NH_4-N were 0.01 mg l^{-1}. Values below limit of detection were set to 0.005 mg l^{-1}.

4.4 Soil physics (paper II and III)

Soil physical properties were determined from steel cylinder (ø = 7 cm) samples taken at Hagfors for the study reported in paper III. Samples were taken from the wall of a ditch dug at the centre of the regeneration area, but outside the experimental plots. Disc trenching had been applied perpendicular to the ditch, creating ridge, furrow and between-furrow microsites. The cylinders for the soil analysis were taken in 2006 directly below undisturbed soil and between-furrow microsites (n=4), at six depths from the upper surface of the mineral soil down to 0.45 m. Steel cylinder samples were also taken in the autumn of 2011 at two or three depths down to 0.45 m directly below the ridges, furrows and between-furrow microsites (n=9) and the undisturbed soil (n=2). Particle size distribution, bulk density, porosity, gravimetric moisture content, water holding capacity at six water tensions and saturated hydraulic conductivity were determined on these samples (ISO11274, 1998; ISO11277, 2009). At Hagfors, 30 Time-Domain-Reflectometry (TDR) probes (CS616, Campbell Scientific Ltd., UK) were installed in a ditch at the center of the regeneration area, eight in each microenvironment (ridge, furrow, between-furrow) and six in the undisturbed soil. In each microenvironment TDR-probes were installed at two depths: 0.2 and 0.45 m from the upper surface of the mineral soil. Soil temperature was monitored by 105T temperature probes (Campbell Scientific Ltd., UK), installed in the ditch close to the TDR probes. Hourly measurements of soil temperature and volumetric water content (VWC) started on 22 June, 2006, a few weeks after installation, and continued until 29 September, 2011. Volumetric water content was estimated from the TDR-probe readings, after accounting for variations in soil temperature and using standard calibration of the probes. At Hagfors, the following local weather variables were also measured, hourly during 2006 – 2011, at the centre of the ditch: air temperature and humidity (using a Hygroclip probe, Rotronic AG, Switzerland), global radiation (using a 200SZ pyranometer, Li-Cor Inc., USA)

and wind speed at 1.9 m height, and precipitation (using an ARG100 tipping bucket) at ground level.

For the study reported in paper II, soil samples were taken during autumn 2012 at three depths (0.1, 0.2 and 0.5 m) from opposite sides of two 1-m-deep pits dug in each N0 and N1 plot at Stråsan. Three samples were taken per depth using steel cylinders (ø = 7 cm), giving 72 samples in total. Particle size distribution, bulk density, porosity, gravimetric moisture content, water holding capacity at six water tensions and saturated hydraulic conductivity were determined from these samples (ISO11274, 1998; ISO11277, 2009). Soil temperature and moisture at Stråsan were monitored by TDR and temperature probes connected to a datalogger (Campbell Scientific Ltd., UK) for data storage. TDR and temperature probes were installed at 0.1, 0.2 and 0.5 m depths. The TDR and temperature probes were installed in the outer 5 m of the N0 and N1 plots. Volumetric water contents were estimated from the TDR-probe readings (during 2012-11-30 to 2015-08-25) after accounting for variations in soil temperature and using standard calibration of the probes.

4.5 Soil microbiology (paper II and IV)

Microbial biomass C, N and P and enzyme assays

Soil samples were collected for determination of microbial variables as described in section 4.2. Microbial carbon (C_{mic}) and nitrogen (N_{mic}) were determined using the chloroform fumigation-extraction method according to Vance et al. (1987) and Cabrera & Beare (1993). C_{mic} and N_{mic} were calculated as the differences between C and N contents in fumigated and non-fumigated soils using extraction coefficients of 0.45 (Vance et al., 1987) and 0.54 (Brookes et al., 1985), respectively. The phosphorus content in microbial biomass (P_{mic}) was determined according to Brookes et al. (1982) and Kalčík & Macháček (1995). Microbial P content was calculated using an extraction coefficient of 0.4 (Brookes et al., 1982). Basal soil respiration (BR) was measured as the increase in CO_2 concentration during 7 days of soil incubation at 15°C in bottles sealed with rubber covers, using an Agilent 7820A gas chromatograph (Agilent Technologies, USA).

Phospholipid fatty acids (PLFA)

Extraction of PLFAs was performed on O-horizon soil samples equivalent to 0.3 g freeze-dried soil, according to Bligh and Dyer (1959), modified by White et al. (1979). The fatty acid methyl ester (FAME) methylnonadecanoate (19:0; Larodan, Malmö, Sweden) was used as internal standard and added to samples

prior to mild alkaline methanolysis. The resulting FAMEs were analyzed on a gas chromatograph (Hewlett Packard 6890) with a flame-ionisation detector (GC-FID) as described by Steger et al. (2003). Individual FAMEs were determined by comparing retention times with FAME standards (FAME 37-47885-U; Supelco, Bellefonte, US). Chemicals used were of analytical grade and all glassware was burnt (500°C, 12 hrs) before use.

In total, 30 PLFAs were identified in each soil sample, then specific PLFAs (or combinations thereof) were assigned to certain functional groups of organisms, cautiously as some PLFAs occur in several distinct groups (Frostegård & Bååth, 2011). The PLFA 18:2ω6,9 was interpreted as indicative of eukaryotic cell membranes, assumed in the sampled forests soils to represent fungal cell membranes (Frostegård and Bååth, 1996; Kaiser et al., 2010). 18:2ω6,9 and 18:1ω9 (another fungal PLFA biomarker) also showed strong positive correlations across our dataset ($p<0.01$; $R=0.72$), corroborating the assumption (Erwin, 1972; Frostegård & Bååth, 2011). The sum of PLFAs i15:0, a15:0, 15:0, i16:0, 16:1ω9, 16:1ω7, i17:0, 17:1ω8, 17:0, cy17:0, 18:1ω7 and cy19:0 was used as signature set of PLFA biomarkers for bacterial biomass (Frostegård & Bååth, 1996). i15:0, i16:0 and i17:0 are common in Gram-positive bacteria, and their sum was used as a signature set of PLFA biomarkers for them (Zelles, 1999). Similarly, the sum of 16:1ω7, 18:1ω7 and cy19:0 was calculated as an indicator of the abundance of Gram-negative bacteria and the sum of 10Me16:0, 10Me17:0 and 10Me18:0 as an indicator of the abundance of actinobacteria (Zelles, 1999; Dungait et al., 2011). The ratio of cyclopropyl/precursor PLFA (Cy/Pre), here interpreted as indicating metabolic stress of the microbial community, was calculated as the sum of cy17:0 and cy19:0 divided by the sum of 16:1ω7 and 18:1ω7 (Bossio & Scow, 1998).

Fungal community composition

To probe the composition of the fungal communities in the samples, the ITS2 region in their DNA contents was extracted, amplified by polymerase chain reaction (PCR), and sequenced. All steps in these analyses, including sample preparation, were undertaken by LGC Genomics GmbH (Berlin, Germany). The PCR mixtures included about 5 ng of DNA extract, 15 pmol of each forward primer, ITS7 (Ihrmark et al., 2012), and reverse primer, ITS4 (White et al., 1990) in 20 µL of MyTaq buffer containing 1.5 units of MyTaq DNA polymerase (Bioline, USA) and 2 µl of BioStabII PCR Enhancer (Sigma Aldrich, USA). The forward and reverse primers used to amplify each sample, had the same 8-nt barcode sequence. The PCR thermal program consisted of 2

min denaturation at 96°C, followed by 30 cycles of 96°C at 15 s, 50°C at 30 s, and 72°C at 60 s. DNA concentrations of the amplicons of interest were determined by gel electrophoresis. About 20 ng portions of amplicon DNA from each of up to 48 samples carrying different barcodes were pooled. DNA samples for which PCR initially failed were diluted 10-fold and the PCR reaction was repeated. The amplicon pools were purified with one volume Agencourt AMPure XP beads (Beckman Coulter, USA) to remove primer dimers and other small mispriming products, then subjected to additional purification using MinElute columns (Qiagen, Germany). About 100 ng of each purified amplicon pool of DNA and the Ovation Rapid DR Multiplex System 1-96 (NuGEN, UK) were used to construct Illumina libraries, which were pooled, size-selected by preparative gel electrophoresis then sequenced on an Illumina MiSeq platform using V3 Chemistry (Illumina, USA).

For further analyses, only the most abundant genera (with >0.5% relative representation in >10% of samples) were extracted from the total fungal community. A lifestyle was assigned to each of these genera according to lists compiled by Tedersoo et al. (2014), and an exploration type was assigned to each ectomyccorhizal (ECM) genus. For *Russula* and *Lactarius*, exploration types vary among species, therefore the exploration type was determined at species level, if possible. Only contact, short-distance and medium-fringe ECM types were designated, other types were not considered.

4.6 Ecosystem C and N modelling (paper II and III)

4.6.1 Model description

CoupModel (Jansson and Karlberg, 2004, Jansson, 2012) is a process-oriented ecosystem model that calculates water, heat, C and N balances of terrestrial ecosystems. The functional unit in the model represents a 1 m^2 soil pedon with growing vegetation of one or more plant types. The model runs on daily time steps, and requires driving data in the form of weather and N deposition: precipitation, global radiation, relative humidity, temperature, wind speed and N concentration in precipitation and dry deposition of N.

The soil in the model is defined as a series of layers, between which flows of water, heat, C and N are calculated. Soil water fluxes are numerically solved with Darcy's law as generalized for unsaturated conditions by Richards (1931). Soil evaporation is calculated with the Penman-Monteith equation (Monteith, 1965). Rates of biological processes, such as SOM decomposition, N mineralization and root water uptake, are dependent on soil temperature and

moisture. Soil C and N are present in three pools of SOM (Litter1, Litter2 and humus) and species in soil water (DOC, DON, NH_4^+, NO_3^-) and two root biomass pools (coarse and fine roots). Litterfall enters the two litter pools and is decomposed by first order rate equations. Litter2 decomposes more slowly than Litter1, and humus decomposes even slower. Microbial biomass is implicitly considered part of the SOM pools and has fixed C/N stoichiometry and carbon use efficiency (CUE), except for mycorrhiza which are implicitly represented as part of the fine root biomass. N mineralization is calculated from the decomposition rate, CUE of microbial biomass and C/N ratios of substrate and microbial biomass. Nitrification is dependent on the nitrification rate coefficient, soil water NH_4^+ and the biomass of nitrifiers in the specific soil layer. Soil nitrifier biomass is calculated in the model with growth functions that respond to soil water, temperature, and soil water content of DOC and NO_3^-. Solutes in soil water (NO_3^-, NH_4^+, DOC and DON) are passively transported between soil layers via water movements. NO_3^- and NH_4^+ can be taken up by roots (and associated mycorrhizae) or immobilized by free-living saprotrophic decomposers. NH_4^+ and DON in soil water are in chemical equilibrium with the soil solid phase as specified by their respective adsorption coefficients.

The plants are described in terms of their abiotic characteristics and storage of C and N in their tissues (stem, needles, coarse roots and fine roots including mycorrhiza). C assimilation in plants is calculated with a light use efficiency approach (Monteith, 1977), where C is assimilated proportionally to the intercepted global radiation and limited by unfavourable temperature, moisture and N availability. Specified plant tissues' maintenance respiration rates are calculated by a Q_{10}-approach and litterfall rates are specified for each plant tissue. The N in litterfall is proportional to the N content of the litter source, i.e. plant tissue (needles, stem, coarse roots or fine roots). Plant N uptake is a function of plant N demand, which is calculated from the C allocated to different plant structures along with a predefined minimum C/N ratio of the plant tissue, as proposed by Ingestad & Ågren (1988). If plant N demand is not met by mineral N uptake then organic N uptake is activated: soil N is withdrawn in organic form to meet the remaining N demand in proportion to the organic uptake rate coefficient (Eckersten & Beier, 1998; Gärdenäs et al., 2003).

4.6.2 Model application

In the study reported in Paper III, the ridges created by disc trenching were defined in the model as a double humus layer with no pine seedlings but growing field vegetation. The furrows were defined as mineral soil with no overlying humus layer but with both growing pine seedlings and field vegetation. The area between two furrows was defined as undisturbed soil without planted pine seedlings but with growing field vegetation. The compound disc trenching effect, that is the combined effects of creating these three kinds of microsites, was calculated by area-weighting states or fluxes across them (which respectively covered 37.3, 41.3 and 21.3% of the total area of the disc trenched plots). The soil C and N stocks in the model layers were based on soil C and N stock estimates performed in 2005, before clear-cutting of the previous Scots pine forest stand (Ring et al., 2011). The O horizon C and N stocks were similar in both 0N and 450N treatments, although the O horizon C/N ratio in the 450N treatment was lower (37) than in the 0N treatment (39) (Ring et al., 2011).

CoupModel was calibrated against experimental data from the Hagfors field experiment using Generalized Likelihood Uncertainty Estimation (GLUE) methodology (Beven & Binley, 1992; Beven, 2006). Thirty-five parameters were randomly sampled within predefined ranges and the calibration was performed on 14 target variables measured at the Hagfors site (Table 5). For each treatment 24 000 randomly distributed model parameter sets were generated, and those that reproduced observations of all 14 measured variables within the limits of acceptance listed in Table 5 were retained. For variables with low frequency sampling — soil stocks of C and N, plant seedling aboveground C and N, field layer vegetation aboveground C and N, and snow mass in mm equivalents (mm) — 95% confidence intervals (C.I.) for the three blocks at the site were calculated and used as limits within which a parameter set could be accepted as producing descriptive, or behavioral, renditions of the ecosystem. Variables with medium and high sampling frequencies — soil water chemistry variables (NO_3-N, NH_4-N, DON), time series of VWC and soil temperatures — were treated using a combination of mean error (ME) and coefficients of determination (r^2) of the model output, and mean values for the three blocks, to identify parameter sets that could be accepted.

Table 5. *Field observations at the Hagfors regeneration area used as calibration variables for the estimation of the posterior parameter sets. The limit for acceptance of posterior model parameter set and details on field measurements are shown. ME = mean error, r^2 = coefficient of determination. Data are from Ring et al. (2011), Ring et al. (2013), Johansson et al. (2013) and Paper III.*

Variable	**Unit**	**Limit for acceptance**	**Number of time points; Measurement years**
Plant and soil			
Soil carbon, mor layer	Mg C ha^{-1}	ME =12 (0N), 6 (450N)	2; 2005, 2012
Soil nitrogen, mor layer	Mg N ha^{-1}	ME = 0.5 (0N), 0.3 (450N)	2; 2005, 2012
Pine seedling aboveground carbon	kg C ha^{-1}	ME = 168 (0N), 204 (450N)	1; 2011
Pine seedling aboveground nitrogen	kg N ha^{-1}	ME = 5.7 (0N), 1.9 (450N)	1; 2011
Field layer vegetation aboveground carbon	kg C ha^{-1}	ME = 926 (0N), 158 (450N)	1; 2011
Field layer vegetation aboveground nitrogen	kg N ha^{-1}	ME = 17.4 (0N), 5.33 (450N)	1; 2011
Soil physics			
Soil temperature, 0.2 m	°C	ME = 2, r^2>0.9	1921; 2006 – 2011
Soil temperature, 0.45 m	°C	ME = 2, r^2>0.9	1921; 2006 – 2011
Soil volumetric water content, 0.2 m	%	ME = 4, r^2>0.1	1072; 2006 – 2011
Soil volumetric water content, 0.45 m	%	ME = 4, r^2>0.1	1072; 2006 – 2011
Snow cover	mm equivalents	ME = 8.6	2; 2009, 2010
Soil water chemistry			
Soil water ammonium nitrogen (NH_4-N)	mg NH_4-N l^{-1}	ME = 0.4, r^2 >0.1	28; 2005 – 2011
Soil water nitrate nitrogen (NO_3-N)	mg NO_3-N l^{-1}	ME = 0.4, r^2 >0.1	28; 2005 – 2011
Soil water dissolved organic nitrogen	mg N l^{-1}	ME = 0.4, r^2 >0.1	28; 2005 – 2011

In the study reported in Paper II, the hydrological submodel of CoupModel was used to estimate water flows at 0.5 m depth at the optimum nutrition experiment site Stråsan during 2009 and 2013. The major components of the water balance were calculated, including evapotranspiration given by the Penman-Monteith combination equation. Brooks & Corey (1964) model parameters were estimated from laboratory measurements performed on steel cylinder soil samples as detailed in 4.4. Model calculations were calibrated manually, varying the canopy resistance, to fit soil temperature and moisture at 0.1, 0.2 and 0.5 m depths measured at Stråsan. Calculated runoff with the model was compared against runoff at a nearby catchment monitored by the Swedish Meteorological and Hydrological Institute (SMHI, 2015) to evaluate the magnitude and difference between years (2009 and 2013) in annual runoff. Leaching fluxes of N species were calculated by multiplying calculated water fluxes (at 0.5 m soil depth) with measured soil water NO_3^- concentrations (measured at ~0.5 m depth in 2009 and 2013). Wintertime NO_3^- concentrations were interpolated from first and last measured soil water NO_3^- concentrations in spring and autumn, respectively.

4.7 Statistical analyses (paper I, II, III, and IV)

In Paper I DOC and DON time series were analysed using a linear model including fixed effects of treatment, sampling time, year and block. Treatment effects were evaluated by analysis of variance (ANOVA). Model assumptions were checked with diagnostic plots, and log-transformation was applied, where necessary, to improve the normality of model residuals' distributions. Variables analysed on log-scale were back-transformed to obtain geometric means and associated confidence intervals. Multiple comparison tests were performed with Tukey adjustment.

In Paper II, between-treatment differences in soil chemistry, microbial biomass and activity, and signature PLFAs in the N fertilization plots and controls were analysed using a mixed linear model including a fixed effect of treatment and random effect of plot nested within block. Differences in soil chemistry, microbial biomass and activity, and signature PLFAs between sites along the N deposition gradient were evaluated using a mixed linear model including a fixed effect of site and random effect of plot. Comparisons between least squares means were adjusted for multiplicity using Tukey's method. The assumptions underlying the analysis were checked using diagnostic plots. Variables were log-transformed where necessary to satisfy model assumptions, and back-transformed to obtain geometric means and associated confidence

intervals. Patterns in the PLFA data set and sensitive microbial variables were explored by Principal Component Analysis (PCA). The PCA on PLFA profiles was fitted on the covariance matrix on mol-percentage data and the PCA of microbial variables was fitted on the correlation matrix. In each case the first two principal components were selected for further analysis. The scores and loadings of the principal components were analysed via scatterplots. All abovementioned statistical tests and data handling, except base CoupModel calculations (Paper III), were performed with R software (R Core Team, 2014).

5 Results and discussion

5.1 Soil solution chemistry and dissolved organic matter in a Norway spruce forest stand after long-term N addition (Paper I)

The results showed that four decades of annual N addition to the boreal Norway spruce forest at Stråsan had affected O-horizon soil water N concentrations and the relative abundances of N species. In control (N0) plots, DON was the dominant N form, accounting for, as a mean annual average, 76% of total N in sampled O-horizon soil water in both 1995 and 2009 (Table 6). NO_3^- and NH_4^- concentrations were frequently below detection limits (0.01 mg l^{-1}) in soil water from N0 plots sampled below the O-horizon, with no apparent difference between 1995 and 2009 (Table 7). The mean annual DON concentration was higher in N1 plots than in N0 plots in 2009. Soil water DON sampled in the O-horizon of N1 plots constituted, on average, a smaller proportion of total N, accounting for 24% (1995) and 38% (2009) of mean annual total N. Moreover, DON displayed clear seasonal trends, being highest in late spring and autumn (Figure 1, top right panel). The spring and autumn DON peaks in N1 plots were higher in 2009 than in 1995. Similarly, McDowell et al. (1998) found higher O-horizon soil water DON (with pronounced early summer peaks) in a pine forest stand in Massachusetts, USA, following long-term N addition. The more pronounced peaks in spring and autumn sampling, together with lower DOC/DON ratios, in N1 plots in 2009 than in 1995 (Figure 2) indicates progression towards a N-saturated state. Overall, the dissolved phase of organic matter displayed stronger responses and more between-year variability under long-term annual N addition than the solid phase of organic matter. This suggests that as an indicator of elevated N availability, the DOM is more likely to detect changes and sudden shifts under

Table 6. *Means (with 95% confidence intervals in parenthesis) of concentrations of dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) in O and B horizon leachates in 1995 and 2009 at Stråsan E26A (n=24 per treatment, soil water type and year). DOC and DON are in mg C l^{-1} and mg N l^{-1}, respectively. Superscripts denote significant differences at the P < 0.05 level (Tukey HSD). Within soil water type and variable, means lacking a common superscript differ. A significant Treatment * Year interaction was found for DON in O horizon soil water and mineral soil water.*

Horizon		Control	N1	N2
O	O horizon soil water (zero tension lysimeters)			
	DOC			
	1995	28.7 (19.1-43.2)^{a}	29.2 (19.4-44.1)^{a}	27.4 (18.2-41.3)^{a}
	2009	41.3 (33.5-50.9)^{b}	55.5 (45.1-68.3)^{b}	53.0 (43.0-65.4)^{b}
	DON			
	1995	0.9 (0.6-1.2)^{a}	1.1 (0.8-1.6)^{ab}	0.9 (0.6-1.3)^{a}
	2009	1.0 (0.7-1.4)^{a}	3.4 (2.4-4.9)^{c}	1.9 (1.3-2.7)^{b}
B	Mineral soil water 0.5 m depth (tension soil water samplers)			
	DOC			
	1995	1.5 (1.2-2.0)^{a}	2.1 (1.6-2.7)^{b}	3.4 (2.7-4.5)^{c}
	2009	2.3 (1.7-3.1)^{b}	3.3 (2.5-4.5)^{c}	4.7 (3.5-6.3)^{d}
	DON			
	1995	0.07 (0.05-0.09)^{a}	0.11 (0.09-0.14)^{abc}	0.14 (0.11-0.18)^{bc}
	2009	0.08 (0.06-0.12)^{ad}	0.19 (0.13-0.27)^{b}	0.11 (0.08-0.15)^{cd}

long-term N addition than the solid phase OM, since the solid phase OM is a large pool with a long turnover time. Moldan et al. (2006) reported different timescales of response of soil solid phase SOM and inorganic N species in surface water during 13 years of experimentally elevated N addition of Gårdsjön experimental catchment. Increased NO_3^- leaching was observed after two years of N addition, before any changes in the O-horizon C/N ratio could be detected. Thus onset of N leaching in surface water and changes in O-horizon C/N ratio at Gårdsjön experimental catchment were decoupled in time.

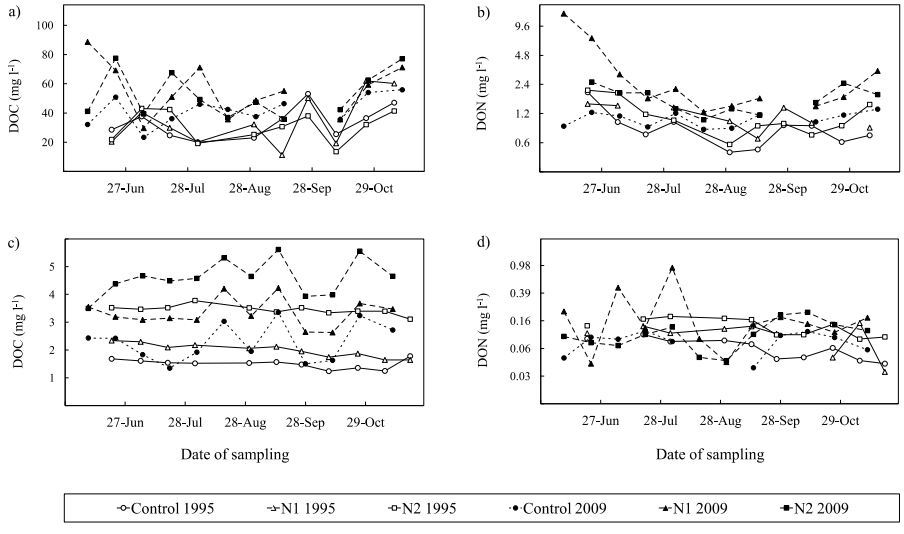

Figure 1. Concentrations of dissolved organic carbon, DOC, (a and c) and dissolved organic nitrogen, DON, (c and d) in leachates collected in O horizon Zero tension lysimeters (a and b) and mineral B horizon suction cups (c and d) during 1995 and 2009 at Stråsan E26A. Both DOC and DON are in mg l^{-1}. Note that the y-axis scales are logarithmic in (b) and (d).

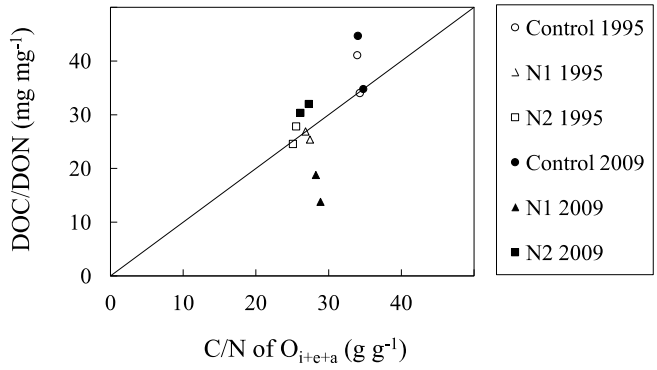

Figure 2. Dissolved organic carbon (DOC) to dissolved organic nitrogen (DON) ratios sampled using zero tension lysimeters plotted against the carbon/nitrogen-ratio (C/N ratio) in soil organic (O_{i+e+a}) horizon at the Stråsan E26A site. The black line represents the 1:1-line with intercept at y=0.

The poor correlation of DOC/DON and C/N ratio (Figure 2) and the strong seasonality suggests that DON in O-horizon soil water in N1 plots was at least partly formed from sources other than bulk O-horizon SOM, or that the soluble fraction of SOM had lower C/N ratio. Higher turnover of soil microbial N under the N treatments at Stråsan were proposed by Blaško et al. (2013), which may have contributed to O-horizon DON seasonality. This is supported by known close associations between the light fraction of DOM and both microbial N mineralization and immobilization (Cookson et al., 2005). However, studies on biodegradability of bulk DOM has shown conflicting results (Qualls & Haines, 1992; Schmidt et al., 2011). Higher relative abundance of refractory DOM with higher microbial activity has been reported for forest soils in Finland (Kiikkilä et al., 2014) and supports the view that measured water-extractable DOM represents a part of substrate which is not utilized by soil microbes. Moreover, mineralization of N from organic N sources is compound specific, depending on metabolic requirements of soil microbes (Roberts et al., 2009). However, uptake of low molecular weight organic N compounds has been suggested to be driven primarily by microbial C, rather than N, demand (Farrell et al., 2014; Schmidt et al., 2011). Andersson & Berggren (2005) found lower amino acid concentration in soil solution of a Norway spruce forest fertilized with N at a rate of 75 kg N ha^{-1} $year^{-1}$. Thus, the link between the turnover of microbial N and bulk DON in coniferous forests soils is unclear and in need of further study.

Under the ongoing N treatment N1, the mean annual NO_3^- and NH_4^+ concentrations were higher than in N0 plots, and dominated the N species in sampled soil water percolating through the O horizon in 1995 (Table 7). These effects were stronger in 2009, with the mean annual NO_3^- concentration reaching 3.7 mg NO_3-N l^{-1}. Clear signs of recovery were observed in plots receiving the treatment with terminated N addition, N2, as NO_3^- concentrations in O horizon soil water were well below those found in N1 plots, although concentrations above the detection limit were occasionally observed. The higher NH_4^+ and NO_3^- concentrations in N1 plots may have been partly due to gross N mineralization rates being higher in the O horizon under the N1 treatment, by almost an order of magnitude, than in control plots (Blaško et al., 2013) although net N mineralization was negative in all treatments at Stråsan. These gross and net N mineralization rates were determined on soil samples collected in august 2009 and point to high microbial demand for N in late summer, when O horizon soil water DON was low.

Table 7. *Nitrate-nitrogen (NO_3-N) and ammonium-nitrogen (NH_4-N) concentrations, both in mg N l^{-1}, in 1995 and 2009 at Stråsan E26A. Values are mean ± ranges (n=2). Superscripts denote significant differences at P = 0.05 level (Tukey HSD test). Within soil water type and variable, means lacking a common superscript differ*.

	Control	N1	N2
O-horizon soil water (Zero tension lysimeters)			
NO_3-N			
1995	0.02 ±<0.01[a]	1.6 ±0.51[b]	0.35 ±0.10[a]
2009	0.03 ±0.02[a]	3.7 ±0.65[b]	0.11 ±0.09[a]
NH_4-N			
1995	0.25 ±<0.02[a]	1.8 ±0.83[b]	0.50 ±0.01[a]
2009	0.30 ±0.11[a]	1.8 ±0.17[b]	0.87 ±0.31[a]
Mineral soil water 0.5 m depth (Tension soil water samplers)			
NO_3-N			
1995	<0.01 ±<0.01[a]	0.20 ±0.07[a]	<0.01 ±<0.01[a]
2009	0.01 ±<0.01[a]	0.47 ±0.28[a]	0.01 ±<0.01[a]
NH_4-N			
1995	0.01 ±<0.01[a]	0.01 ±<0.01[a]	0.01 ±<0.01[a]
2009	0.01 ±<0.01[a]	<0.01 ±<0.01[a]	<0.01 ±<0.01[a]

Mineral B horizon soil water N species were roughly an order of magnitude lower than corresponding O horizon soil water concentrations. This implies that there were considerable N consumption processes (sorption, plant N uptake and immobilization) in the upper 0.5 m of mineral soil. Mineral B horizon soil water N in N0 plots was dominated by DON, while NO_3^- and NH_4^+ concentrations were below the detection limits on all sampling occasions. This is largely consistent with the hypothesis by Hedin et al. (1995), that unpolluted, unmanaged forests in their natural state leach small amounts of inorganic N forms, and that organic N forms dominate. NO_3^- was slightly elevated in N1 plots (mean annual NO_3-N concentration, 0.47 mg l^{-1}), but the treatment effect was not statistically significant. Mean annual NH_4^+ soil water concentrations at 0.5 m depth were consistently below the detection limit in all treatments. The difference in NO_3^- concentrations between mineral and O horizon soil water was less than the corresponding differences in NH_4^+ and DON concentrations, likely because NO_3^- is a mobile anion and leaches readily, although differences in substrate utilization by plants and soil microorganisms may also have contributed. DOM is subject to considerable sorption/desorption processes as it leaches downwards in the soil profile (Fröberg et al., 2006), and DON was slightly elevated in the N1 plots (in 2009), although its mean annual concentrations were rather low (0.11 and 0.19 mg l^{-1} in 1995 and 2009, respectively). The measured inorganic soil water N concentrations imply that there was considerable N retention under the N1 treatment, during both 1995 and 2009, assuming low gaseous losses of N. The long-term mean annual runoff at Stråsan is approximately 200 - 300 mm $year^{-1}$, thus N1 plots retain a substantial proportion of the N they receive (30 kg addition and ca. 3 kg N ha^{-1} $year^{-1}$ in ambient N deposition). Moreover, soil water pH was not affected by N treatment, implying that high nitrification and subsequent NO_3^- leaching have not yet taken place in the N1 plots. Indeed, budget calculations for 1996 indicate that there was 100% retention of N in the N1 plots until 1996, and N losses had likely been low (Andersson et al., 2001).

There were no differences between N treatments in DOC quantity or quality of DOM, apart from a decreased DOC/DON ratio, in O horizon soil water (Table 6, Figure 1). However, in the soil mineral B horizon DOC was higher in plots subjected to both the ongoing N1 and the terminated N2 treatment

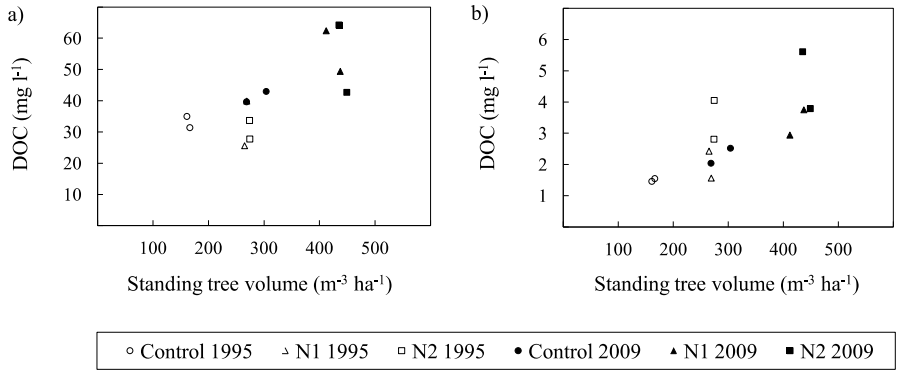

Figure 3. Mean annual soil solution dissolved organic carbon (DOC) concentrations in soil water sampled in the O horizon by zero tension lysimeters (a) and mineral B horizon by suctions samplers (b) and their relations to standing tree volumes at Stråsan E26A.

than in N0 plots. Thus, the additional DOC in mineral soil B horizon leachates likely did not leach from higher soil horizons, but originated from within the mineral soil B horizon itself.

Several explanations for the varying effects of experimental N addition on soil water DOC have been proposed, including both acidity-related mechanisms (Evans et al., 2008) and biological mechanisms (Waldrop & Zak, 2006). There were no indications that effects of the N treatments on acidity had substantially affected DOC contents in mineral soil in the study presented here. The N addition treatments considerably increased stem-wood growth (Blaško et al., 2013). Furthermore, the absolute changes in DOC in both O horizon and mineral soil water appeared to be related to the standing tree biomass, rather than soil C stocks (Figure 3). One possible explanation is that above- and below-ground litterfall and subsequent litter decomposition may have influenced DOC concentrations in soil water at Stråsan. This hypothesis is supported by previous findings that fine-root biomass (<1 mm) and fine-root length were higher under fertilization treatments similar to N1 and N2 (although P, K and micronutrients were also added) than under the control treatment at Stråsan in 1985 (Persson & Ahlström, 1990). Similarly, Fröberg et al., (2006) reported a positive correlation of soil water DOC and estimated primary productivity across a latitudinal gradient in Sweden.

5.2 Effects of long-term N addition and N deposition on soil chemistry and microbial variables (Papers II and IV)

Long-term N addition and historic N deposition were found to have had similar effects on soil chemistry variables. At Stråsan, O horizon N and inorganic N species contents (extracted in water and K_2SO_4 solution) were higher, and soil pH and O horizon C/N ratios were lower, in plots receiving N additions than in control plots, in accordance with known symptoms of long-term N loading and excess N (Paper II). At Skogaby, the patterns in differences between mean values were the same, although only the lower O horizon C/N ratio under N additions was statistically significant ($p<0.05$). Inorganic N species contents (extracted in water and K_2SO_4) increased along the N deposition gradient. The pH and C/N ratio of O-horizon also decreased along the N deposition gradient (Stråsan>Skogaby>Čertovo).

Accordingly, soil microbial variables were affected by long-term N addition (Table 8). The microbial variables that responded to N addition treatment were C_{mic}, N_{mic}, basal respiration, and the activity of extracellular enzymes, most notably leucine-aminopeptidase activity decreased under the N treatments, indicating higher N availability to microbial community. At Stråsan, C_{mic}, N_{mic} and basal respiration were all significantly lower under the N addition treatments than in control plots ($p<0.001$, 0.007 and 0.021, respectively). Reductions in respiration rates in the O horizon under the N treatments in the Stråsan experiment have been previously reported (Sjöberg et al., 2003). Effects of the NS treatment at Skogaby were similar to those of the N addition treatments at Stråsan, although fewer tested differences were statistically significant. Both N_{mic} and P_{mic} were significantly lower in NS plots than in controls ($p=0.020$ and <0.001, respectively) and C_{mic} was slightly, but not significantly, lower in NS plots. The ratio of β-glucosidase and cellobiohydrolase to leucine-aminopeptidase, C/N enzyme ratio, was higher in the on-going N1 plots at Stråsan than both N0 and N2 ($p<0.001$) and higher in the NS treatment at Skogaby compared to control ($p=0.041$). In addition, basal respiration was lower in NS plots than in controls. Similarly, there was evidence of changes in the microbial biomass and activity in response to increased N availability along the N gradient Stråsan<Skogaby<Čertovo, although fewer tested differences were statistically significant. Basal respiration was lowest at Skogaby, but microbial biomass was similar on all three sites. The activity of soil enzyme peroxidase and leucine-aminopeptidase activity was lower on Skogaby than at Čertovo. The C/N enzyme ratio differed

between sites in the N deposition gradient (p=0.001): the ratio was higher at Skogaby than Stråsan.

The total PLFAs extracted (nmol PLFA g dry soil^{-1}) confirmed the negative effect of N addition on microbial biomass at both Stråsan and Skogaby (p<0.001 and p=0.001, respectively; Table 9). This effect did not appear to be related to recovery from long-term N loading, as microbial biomass was similar in N1 and N2 plots at Stråsan (although the relative abundance of signature sets of PLFAs differed among all three treatments). The reduction in fungal to bacterial PLFA biomarkers ($F:B_{PLFA}$) under N addition at Stråsan was not significant (p=0.508). The relative abundance of PLFA biomarkers associated with Gram-positive bacteria increased (p<0.001) and Gram-negative bacteria decreased (p=0.026) in the on-going N treatment, N1, at Stråsan. The proportion of actinobacteria, as estimated from PLFA-analysis, was significantly lower in N1 plots than in controls (p<0.001). Moreover, the Cy/pre ratio increased under both N treatments at Stråsan, which may reflect stationary growth of microbial biomass due to lack of C (Bossio & Scow, 1998), although this ratio is somewhat confounded by changes in species abundances (Frostegård & Bååth, 2011). The N2 plots did not differ from controls in relative abundance of actinobacteria, gram-positive bacteria, or gram-negative bacteria, as estimated from PLFA-analysis. At Skogaby, none of the indicator PLFA variables differed between treatments, although the trends were similar to those observed at Stråsan. The relative abundance of gram-negative bacteria, gram-positive bacteria and actinobacteria were similarly affected, as estimated from PLFA biomarkers, also in the N deposition gradient.

Overall, the results were in accordance with other studies on the effect of N in similar ecosystems. Reductions in microbial biomass in response to elevated N have also been found across N fertilization experiments and N deposition gradients in several investigations (Nilsson & Wallander, 2003; Wallenstein et al., 2006; Blasko et al., 2013; Demoling et al., 2008) and reduction in soil respiration rates appears to be a general response to increases in N availability across boreal and temperate forest ecosystems (Janssens et al., 2010). The reductions in microbial biomass C associated with N fertilization might be related to reductions in abundance of ectomycorrhizal fungi (EMF) when N availability increases, and thus partly reflect a change in the microbial community composition. Reduced belowground C allocation following addition of N (at a dose of 100 kg N ha^{-1}) in a pine forest stand was reported for a site in northern Sweden (Högberg et al., 2010). Accordingly, lower

relative abundance of EMF in N1 plots than in control plots at Stråsan was observed with a loss of medium-distance exploration types, which are regarded competitive in N poor conditions (Paper IV).

In the study presented here, strong responses of soil enzyme activities were observed, notably of hydrolytic enzymes relating to C and N acquisition of microbial community. C acquiring hydrolytic enzymes increased (β-glucosidase and cellobiohydrolase) and N acquiring enzyme activity (leucine-aminopeptidase) decreased at higher N loading. This is accordance of toher previous studies (Allison et al., 2008) but stands in contrast to others (Andersson et al., 2004; Waldrop et al., 2004). The results presented here instead indicate that resource limitation increases for microbial biomass when N availability rises under long-term elevated N load. The reductions in microbial biomass C and associated declines in basal respiration under N treatments are in line with the negative effect of N addition on soil microbial growth discussed by Treseder (2008). The distinct changes in hydrolytic soil enzyme activities, suggests that the soil microbes allocated their resources towards acquisition of C substrates at higher N availability. This switch coincided with reductions in microbial biomass and respiration, together with increases in abundance of PLFA biomarkers indicative of gram-negative bacteria and ratio of cyclopropyl PLFA to precursor PLFA. Although ratios of cyclopropyl PLFA to precursor PLFAs are confounded by changes in species abundance (Frostegård et al., 2011), the increase in this ratio in response to N addition points to resource limitation of soil microbial biomass. In general, Gram-negative bacteria tend to be rapid growth, rhizosphere specialists (Schlegel, 1992) and Gram-positive slow-growing but stress tolerant (Balser, 2005). Thus, the reductions in basal respiration and microbial biomass do not seem to be connected with increases in CUE and growth as proposed by Schimel & Weintraub (2003). This study suggests that long-term N load aggravated resource limitation of soil microbial biomass, possibly of C due to reductions in belowground C allocation of trees under elevated N supply (Högberg et al., 2003; Högberg et al., 2010; Janssens et al., 2010).

Table 8. *Microbial variables of soil sampled at Stråsan and Skogaby N addition experiment sites. Values are means with 95% confidence interval in parenthesis. Within experimental site (Stråsan or Skogaby) and row variable means lacking a common superscript (^{a}, ^{b}, ^{c}) differ at p<0.05.Statistically significant treatment effects are marked in bold.*

	Stråsan			Skogaby	
Variable	N0	N1	N2	N0	NS
C_{mic}	**2790^{a} (2280 - 3300)**	**1430^{b} (921 - 1950)**	**1690^{b} (1180 - 2200)**	2380 (1620 - 3140)	1710 (952 - 2480)
N_{mic}	**532^{a} (410 - 654)**	**258^{b} (136 - 380)**	**372^{ab} (250 - 494)**	**429^{a} (348 - 510)**	**294^{b} (213 - 375)**
P_{mic}	328 (233 - 423)	221 (125 - 316)	189 (93.5 - 284)	**363^{a} (299 - 427)**	**192^{b} (128 - 256)**
C/N_{mic}	5.15 (3.88 - 6.42)	5.45 (4.17 - 6.72)	4.95 (3.68 - 6.23)	5.25 (4.24 - 6.51)	5.78 (4.67 - 7.16)
C/P_{mic}	19.3 (6.94 - 31.6)	17.1 (4.71 - 29.4)	25.1 (12.7 - 37.4)	13.6 (8.89 - 20.9)	19.7 (12.9 - 30.3)
N/P_{mic}	3.75 (2.46 - 5.04)	3.11 (1.82 - 4.40)	4.83 (3.54 - 6.12)	2.81 (2.26-3.37)	3.44 (2.88 - 3.99)
Basal respiration†	**9.26^{a} (7.12 - 12.0)**	**5.85^{b} (4.50 - 7.61)**	**5.88^{ab} (4.53 - 7.64)**	**5.99^{a} (5.19 - 6.79)**	**4.58^{b} (3.78 - 5.38)**
β-glucosidase	**245^{a} (86.5 - 404)**	**646^{b} (501 - 790)**	**226^{a} (66.8-385)**	471 (328 - 614)	528 (384 - 671)
Phosphatase	597 (228 - 967)	564 (195 - 933)	887 (518 - 1260)	536 (435 - 637)	500 (399 - 601)
Cellobiohydrolase	**37.3^{ab} (17.4 - 57.2)**	**66.3^{a} (48.2 - 84.4)**	**16.9^{b} (0 - 36.8)**	37.8 (20.3 - 55.3)	50.6 (33.1 - 68.1)
Chitinase†	160 (114 - 226)	132 (94.0 - 187)	102 (72.1 - 143)	99.5 (69.6 - 142)	95.9 (67.1 - 137)
Phenoloxidase	773 (453 - 1090)	779 (459 - 1100)	1130 (806 - 1450)	1085 (610 - 1561)	830 (355 - 1306)
Peroxidase	1450 (0 - 2930)	1150 (0 - 2630)	1770 (292 - 3250)	**694^{a} (416 - 973)**	**1160^{b} (880 - 1440)**
Leu-aminopeptidase	**47.6^{a} (42.6 - 52.6)**	**30.6^{b} (25.6-35.6)**	**26.7^{b} (21.7 - 31.7)**	**39.2^{a} (31.5 - 46.9)**	**26.7^{b} (19.0 - 34.4)**
C/N enzyme ratio	**5.97^{a} (0.00 – 12.60)**	**24.7^{b} (18.5 – 30.9)**	**9.69^{a} (3.06 – 16.3)**	**13.1^{a} (4.65 – 16.1)**	**24.6^{b} (16.1 – 33.0)**

†Values for this variable were log-transformed prior to statistical analysis, and back-transformed to obtain geometric mean and associated confidence interval.

Table 9. *Phospholipid fatty acid (PLFA) variables of soil sampled at Stråsan and Skogaby N addition experiment sites. Values are means with 95% confidence interval in parenthesis. Within row and experimental site, variable means lacking a common superscript ([a],[b], and [c]) differ at p<0.05. Statistically significant treatment effects are marked in bold.*

		Stråsan			Skogaby	
Variable	Unit	Control	N1	N2	control	NS
Total PLFA	**nmol PLFA g soil^{-1}**	**343^{a} (300 - 386)**	**234^{ab} (192 - 277)**	**229^{b} (182 - 276)**	**302^{a} (276 - 329)**	**239^{b} (212 - 265)**
F:B$_{PLFA}$	-	0.225 (0.154 - 0.296)	0.169 (0.099 - 0.241)	0.178 (0.107 - 0.249)	0.166 (0.135 - 0.197)	0.161 (0.131-0.192)
Cy-Pre	**-**	**0.695^{a} (0.550 - 0.840)**	**0.956^{b} (0.812 - 1.10)**	**0.952^{ab} (0.808 - 1.09)**	1.49 (1.22 - 1.78)	1.70 (1.42-1.98)
Actinobacteria	**Mol %**	**7.73^{a} (0.812 - 1.10)**	**11.2^{b} (9.92 - 12.4)**	**9.08^{ab} (7.83 - 10.3)**	10.2 (8.22 - 12.2)	11.9 (9.98 - 13.9)
Gram positive bacteria	**Mol %**	**18.4^{a} (17.3 - 19.5)**	**21.9^{b} (20.8 - 23.0)**	**19.4^{a} (18.4 - 20.5)**	23.9 (22.5 - 25.3)	23.9 (22.5 - 25.3)
Gram negative bacteria	**Mol %**	**18.1^{a} (15.6 - 20.6)**	**13.3^{b} (10.7 - 15.8)**	**14.8^{ab} (12.3 - 17.3)**	12.3 (10.4 - 14.1)	11.5 (9.65 - 13.4)

The data set spanned sites and treatments of historic N load that ranged approximately 93 – 2123 kg ha^{-1} of N since 1950 (table 10). Estimates of current N load spanned also a large range 3.1 – 33 kg ha^{-1} $year^{-1}$ of N for the period 2005-2013. During this period, elevated NO3-N leaching was detected in NS plots at Skogaby and at the high N-deposition Čertovo site (14.5 and 14.6 kg NO_3-N ha^{-1} $year^{-1}$, respectively). Plots subjected to all treatments at ST and the control treatment at Skogaby were leaching no, or very little, NO_3-N (less than 2 kg NO_3-N ha^{-1} $year^{-1}$). Thus, three N retention groups could be identified among the plots: i) those that currently receive small N loads and leach very little NO_3-N (low input-low output; Stråsan control and N2 plots), ii) those that currently receive relatively high N loads and leach very little NO_3-N (high input-low output; Stråsan N1 and Skogaby control plots), and iii) those that currently receive relatively high N loads and leach high amounts of NO_3-N (high input-high output; Skogaby NS and Čertovo plots).

A PCA of the microbial variables that were sensitive to N treatment separated the current low N load sites from current high N load sites (Figure 4). Principal component 1 was most strongly correlated with basal respiration (Pearson's r = -0.78), N_{mic} (Pearson's r = -0.77) and β-glucosidase activity (Pearson's r = 0.74). Principal component 2 was most strongly correlated with leucine-aminopeptidase activity (Pearson's r = 0.72) and cellobiohydrolase activity (Pearson's r = 0.64). Figure 4 indicates that soil samples within group 1 (from plots with low current N loads) had similar values of microbial variables, which differed from those of soil samples within groups 2 and 3 (from plots with high current N loads). Similarly, a PCA of the 30 identified PLFAs distinguished between current low N load and current high N load plots (Figure 4). Principal component 1 was most strongly correlated with PLFAs i16.0 (Pearson's r = 0.85), 18.1ω7 (Pearson's r = -0.84) and 10me16 (Pearson's r = 0.82). Principal component 2 was most strongly correlated with PLFAs 18:2ω6,9 (Pearson's r = -0.86), 16:00 (Pearson's r = -0.62) and 16:1ω5 (Pearson's r = -0.53). Figure 4 indicates that soil samples within group 1 (from plots with low current N loads) had similar PLFA profiles, which differed from those of soil samples in groups 2 and 3 (from plots with high current N loads). However, the important separation between group 2 (sites under high N load with low N leaching) and group 3 (sites with high N load and high N leaching) was not reflected in either the PCA of summary microbial variables nor the PCA of PLFA patterns. Thus, although the microbial variables studied here may enable identification of excess N symptoms in such forest ecosystems, they do not necessarily reflect the point of N saturation of forest ecosystems

Table 10. *Historic nitrogen (N) load (1950-2010), recent N load (2005-2013) and estimates of recent NO_3-N leaching (2005-2013) and proposed N retention class at Stråsan, Skogaby and Čertovo*

		Stråsan			Skogaby		Čertovo
Variable	Unit	Control	N1	N2	control	NS	n.a.
Historic N load (1950-2010)	kg ha^{-1} of N	93	1663	1853	723[c]	2123[c]	1168[b]
Recent N load (2005-2012)	kg ha^{-1} $year^{-1}$ of N	3.2	33.2	3.2	14.8[a]	14.8[a]	14.6[b]
Recent NO_3-N leaching	kg ha^{-1} $year^{-1}$ of N	0.05	1.97	0.05	1.00[a]	14.5[a]	15.0[e]
N retention class	-	Low in/ Low out	High in/ Low out	Low in/ Low out	High in/ Low out	High in/ High out	High in/ High out

[a] *Olsson et al. (2013),* [b] *Kopáček & Hruška (2010),* [c] *Bergholm et al., (2003), Olsson et al. (2013), Hanssen et al. (2013),* [e] *J. Kopáček, pers. comm.*

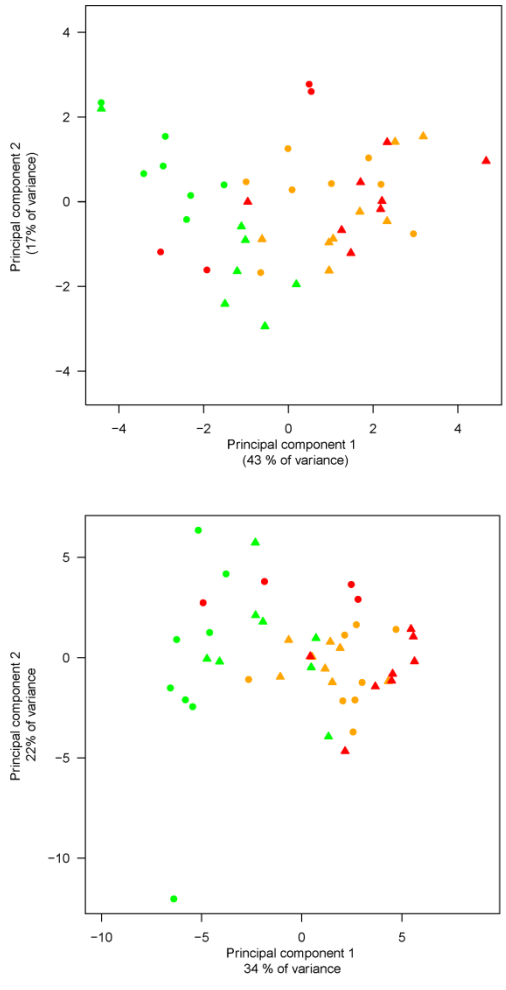

Figure 4. PCA scores of selected microbial variables (top) and profile of 30 identified PLFAs (bottom) of the sites Stråsan, Skogaby and Čertovo classified by N retention. Coloring is green = low current N load and low NO_3-N leaching (Stråsan control and Stråsan N2), yellow = high current N load but low NO_3-N leaching (Stråsan N1 and Skogaby control), and red = high current N load and high current NO_3-N leaching (Skogaby NS and Čertovo). Dots indicate sites which have not received experimental N addition, and triangles experimental plots where either NH_4NO_3 or NH_3SO_4 has been added.

where NO_3^- leaching losses are similar in magnitude to current N loading. The plots that leached NO_3^- at the highest rates (Čertovo and Skogaby NS) had high current N loads, low soil C/N ratios (<26) and high annual mean precipitation, highlighting the importance of also considering other site-specific factors in the context of N saturation of forest ecosystems (Dise et al., 1998; Gundersen et al., 1998; Fenn et al., 1998).

5.3 Nitrogen leaching after clear-cutting and soil scarification of previously N-fertilized Scots pine forest (Paper III)

Major N fluxes of the clear-cut N budget, based on posterior model simulations calibrated using data acquired from the Hagfors regeneration area, are presented in Table 11. The leaching of inorganic N (mainly NO_3-N) was higher at both N fertilization levels (0N and 450N) with disc trenching (DT) than in the corresponding treatment without it. The average leaching of DON (at 0.5 m depth) did not differ between either simulated disc trenching treatments or previous N fertilization levels. Instead, the most striking effect of the treatments was a sharp increase in mean annual inorganic N leaching; from 3.1 and 2.3 kg ha^{-1} $year^{-1}$ leaching of inorganic N under the 0N and 450N noDT treatments, respectively, to 4.6 and 6.0 kg ha^{-1} $year^{-1}$, respectively, under the 0N and 450N DT treatments. These N leaching rates are higher than those reported from the Kangasvaara catchment in eastern Finland (Piirainen et al., 2002; Lauren et al., 2005), but at the lower interval reported for this region of Sweden (Futter et al., 2010). Post-harvest N leaching losses varies considerably between sites (Gundersen et al., 2006). The N fertilization dose in our investigation (in total 450 kg N ha-1) exceeds the recommended upper limit for this region according to the guidelines given by the Swedish Forest Agency (in total 300 kg N ha-1) (Swedish Forest Agency, 2016). The differences in post-harvest N leaching losses may be connected to variations in ambient N deposition (Akselsson et al., 2004), harvest intensity (Wall, 2008) or previous N fertilization, as reported here, but more studies on this topic are needed as lower soil water NO_3^- concentrations after clear-cutting of previously urea fertilized Scots pine forest has been reported (Ring et al., 2003). Furthermore, whole-tree harvesting (WTH) was performed at Hagfors, and WTH may result in smaller post-harvest N leaching losses (Ring et al., 2015) but there are conflicting reports in this regard (Gundersen et al., 2006; Laurén et al., 2008; Wall, 2008). Average annual N accumulation in pine seedling biomass was highest, and field layer vegetation N accumulation lowest, under the DT treatments throughout the study period, in accordance with reported beneficial effects of soil scarification on plant seedling survival and growth (Johansson et al., 2012; Johansson et al., 2013).

CoupModel posterior simulations on the leaching of inorganic N from the ridge, furrow and between- furrow micro-environments provide further insights regarding the large increase in inorganic N leaching associated with the 450N DT treatment. As shown in Figure 5, the main driver for the increase under this treatment was high leaching from the ridge in the 450N treatment. The 450N

Table 11. *Average annual nitrogen (N) fluxes (kg N ha^{-1} year^{-1}) of mean (range within brackets) CoupModel posterior simulations of the Hagfors regeneration area 2006-2011. Fluxes in the disc trenched treatments (DT) were scaled up from the microenvironments ridge (R), furrow (F) and areas in between two furrows (IB) with area proportions of R=0.373, F=0.413 and IB=0.213. 0N and 450N = fertilization with N of 0 and 450 kg ha^{-1} of N respectively. noDT = no disc trenching, DON = dissolved organic nitrogen*

	0N				**450N**			
	no DT		**DT**		**no DT**		**DT**	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Leaching of DON	0.09	(0.08 – 0.12)	0.09	(0.08 – 0.11)	0.08	(0.07 – 0.09)	0.09	(0.07 – 0.10)
Leaching of inorganic N	3.1	(1.4 – 23)	4.6	(1.9 – 13)	2.3	(0.8 – 6.9)	6.0	(1.8 – 17)
Δ Pine seedling N	2.4	(0.8 – 4.1)	5.5	(3.3 – 11)	1.5	(0.7 – 2.3)	3.2	(0.2 – 15)
Δ Field vegetation N	4.5	(0.1 – 13)	2.7	(0.5 – 8.6)	5.7	(4.5 – 7.3)	1.5	(0.03 – 7.7)
Δ Soil N (0.5 m depth)	-4.1	(-25 – 2.0)	-7.0	(-18 – -2.2)	-3.6	(-8.3 – -1.3)	-4.9	(-22 – 2.1)
N deposition	5.9	(5.8 – 6.0)	5.9	(5.8 – 6.0)	5.9	(5.8 – 6.0)	5.9	(5.8 – 6.0)
Ecosystem N balance	2.7	(-17 – 4.3)	1.1	(-7.3 – 4.1)	3.5	(-1.2 – 5.1)	-0.3	(-11 – 4.1)
Number of accepted simulations	328		246		48		211	

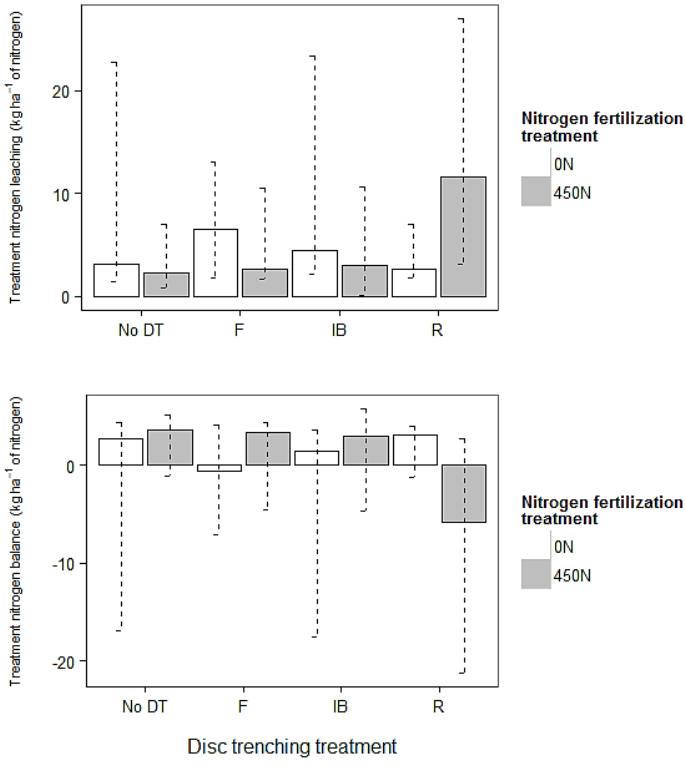

Figure 5. The mean annual mineral nitrogen (N) leaching (top) by the N treatment level (0N, 450N) and the DT treatment (control, F, IB and R) and the treatment nitrogen balance (bottom) by the N treatment level (0N, 450N) and the DT treatment (control, F, IB and R), as calculated by the CoupModel posterior simulations. The error bars denote the minimum and maximum of the posterior model simulations.

ridge treatment had both a lower soil C/N ratio, a larger SOM pool and no N uptake by pine seedlings, factors that in the model increases N mineralization and N leaching. There were only minor differences of calibrated model parameter values between treatments, especially relating to soil N mineralization and nitrification rate. The study presented in Paper III, thus, indicates that mineralization and nitrification rates did not change per unit mass of substrate, but largely mirrored the amount of SOM and its C/N ratio. This suggests a role for soil C/N ratio in determining inorganic N leaching losses

during the regeneration phase. Increases in rates of litter mass losses have been reported in mounds and ridges (Johansson, 1994; Lundmark-Thelin & Johansson, 1997), suggesting that rates of net N mineralization may be higher in them. However, in early stages the decomposition of litter with high C/N ratios typically constitutes a net sink, rather than net source, for N (Berg, 1988). Moreover, the importance of recent litter as a N sink is corroborated by reports that critical C/N ratios for net N mineralization from pine needle litter are lower in forest clear cuts than in old-growth forest (Berg & Ekbom, 1983). Moreover, Smolander et al. (2000) and Smolander & Heiskanen (2007) detected no increase in net N mineralization per unit mass of SOM in scarified soil. The 450N NoDT plots did not leach large amounts of inorganic N, possibly due to increases in vegetation N accumulation (Table 11). Since the ridge, furrow and between ridge/furrow micro-environments differed in inorganic N leaching rates, the proportions of disturbed areas will clearly influence estimates of inorganic N leaching associated with disc trenching. At Hagfors, the proportions of areas disturbed by ridges, furrows and between ridge/furrow areas were 37.3, 41.3 and 21.3%, respectively. If other weightings, perhaps more typical of contemporary forestry, were applied (25, 25 and 50%, respectively), then the leaching estimates associated with the DT treatments would be slightly lower (4.50 and 5.04 kg ha^{-1} $year^{-1}$ of inorganic N under the 0N and 450N DT treatments, respectively).

The pine seedling and field layer vegetation grew at low rates initially and vegetation N accumulation in both above- and below-ground biomass, as calculated by the model, increased notably during the third growing season (Figure 6). Vegetation biomass N per unit area by the start of the fifth growing season after clear-cutting was similar to values obtained in previous studies in Sweden (Högbom et al., 2002; Hedwall et al., 2013) and Finland (Palviainen et al., 2007). N accumulation in vegetation biomass and mineral N leaching increased simultaneously, indicating that the increases in plant N uptake were not sufficient to maintain pre-clear-cut soil mineral N concentrations and subsequent leaching of N. Since vegetation N accumulation will likely increase further beyond 2012 (the sixth growing season), the importance of the vegetation N sink for mitigating N leaching will likely increase (Emmett et al., 1991; Hedwall et al., 2015).

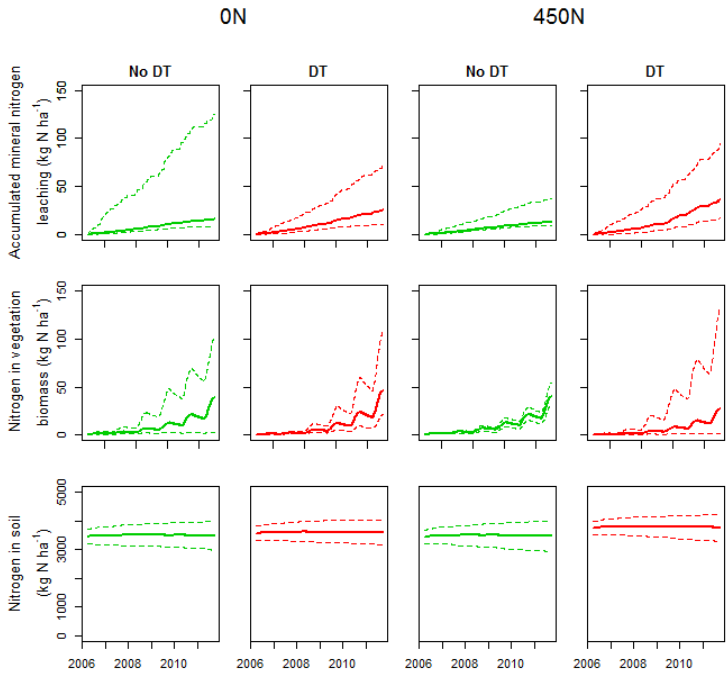

Figure 6. Accumulated inorganic nitrogen leaching (top row), accumulation in vegetation biomass nitrogen (middle row), and soil organic nitrogen down to 0.5 m depth (bottom row) under the treatments with 0 and 450 kg ha^{-1} of nitrogen (0N and 450N, respectively), and the no disc trenching and disc trenching treatments (No DT and DT, respectively) treatments at the Hagfors clear-cut, 2006-2012. Full lines indicate means, and dashed lines indicate minima and maxima of accepted model simulations.

6 Conclusions and future perspectives

Long-term annual N addition between 1967 and 2009, at an average rate of 35 kg N ha^{-1} $year^{-1}$, clearly increased the total N concentration in the O horizon soil water in the Norway spruce boreal forest examined at Stråsan, and shifted its distribution of N species towards dominance by mineral N species. While total N concentrations had increased by 2009 since 1995, the concentration and seasonality of DON increased without the O horizon C/N ratio notably changing. The increased DON in the ongoing N treatment might be related to an increased turnover of microbial N, an association in need of further study and quantification. There was considerable retention of N species in the upper 0.5 m of mineral soil, suggesting strong consumption and sorption processes. The low estimated N leaching of Stråsan N1 treatment suggest sustained high N retention of the current annual N load, assuming small gaseous losses of N, after 46 years of annual N addition at an average rate of 35 kg N ha^{-1} $year^{-1}$.

The responses of microbial variables to long-term N loading and N deposition were largely in accordance with other published studies. However, reductions in microbial biomass C and N contents after long-term N addition are indicative of aggravated resource limitation of soil microbial biomass, possible by C availability, under long-term N addition. The soil microbial variables included in the study could not elucidate in what respect high N loaded treatments that currently leach large amounts of NO_3^- (>14 kg NO_3-N ha^{-1} $year^{-1}$) differ from those that do not (<2 kg NO_3-N ha^{-1} $year^{-1}$). Indeed, the highest NO_3^- leaching rates were associated with the lowest O horizon C/N ratios and highest runoff, highlighting the importance of also considering SOM substrate properties and hydrological processes.

Leaching losses of N after clear-cutting depend on the soil scarification practices subsequently applied. The history of a site is also important to

consider when estimating N leaching potential from forest regeneration areas, as leaching was higher from disc-trenched plots that had previously received N fertilization. How this translates into effects of site fertility on N leaching losses during the regeneration phase is unclear, and needs further study, as do effects of whole tree harvesting on N leaching losses during the regeneration phase.

The observations of long-lasting effects of N addition on dissolved organic matter, previous N fertilization on post-harvest N leaching losses and estimated high N retention of the Stråsan N1 N addition treatment, all highlight the value of long-term field studies, and the importance of continued maintenance and operation of long-term field experiments in forest ecosystem research.

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