Laura Paavolainen

Nitrogen transformations in boreal forest soils in response to extreme manipulation treatments

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Supervisor: Docent Aino Smolander
Finnish Forest Research Institute
Vantaa Research Centre

Reviewers: Professor Pertti Martikainen
University of Kuopio, Finland
Department of Environmental Sciences
and
National Public Health Institute, Finland
Laboratory of Environmental Microbiology

Docent Michael Starr
Finnish Forest Research Institute
Vantaa Research Centre

Opponent: Professor Tryggve Persson
Swedish University of Agricultural Sciences, Uppsala
Department of Ecology and Environmental Research

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Preface

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Original publications

The thesis is based on the following articles, which in the text will be referred to by their Roman numerals.

I Smolander A., Priha O., Paavolainen L., Steer J. and Mälkönen E. 1998. Nitrogen and carbon transformations before and after clear-cutting in repeatedly N-fertilized and limed forest soil. Soil Biology & Biochemistry 30, 477-490.

II Paavolainen L., Smolander A., Lindroos A.-J., Derome J. and Helmisaari H.-S. Nitrogen transformations and losses in forest soil subjected to sprinkling infiltration. (submitted manuscript).

III Paavolainen L. and Smolander A. 1998. Nitrification and denitrification in soil from a clear-cut Norway spruce (*Picea abies*) stand. Soil Biology & Biochemistry 30, 775-781.

IV Paavolainen L., Kitunen V. and Smolander A. 1998. Inhibition of nitrification in forest soil by monoterpenes. Plant and Soil 205, 147-154.

V Paavolainen L., Fox M. and Smolander A. 1999. Nitrification and denitrification in forest soil subjected to sprinkling infiltration. Soil Biology & Biochemistry (in press).

The author's contribution

Paper I

Laura Paavolainen has performed part of the experimental work and part of the calculation and interpretation of the results. She has participated in the preparation of the manuscript.

Papers II - V

Laura Paavolainen is the corresponding author. She has planned the experimental setup together with the co-authors and performed part of the experimental work. She is responsible for writing and interpretation of the results.

1. Introduction

1.1. N cycle processes in boreal forest soils

Nitrogen (N) is one of the nutrients essential to living organisms. In boreal forest ecosystems available nitrogen in the soil is the nutrient most strongly limiting the growth of trees (Aaltonen, 1926; Kukkola and Saramäki, 1983; Mälkönen, 1990; Nilsson and Wiklund, 1995). Although boreal forest soils contain large amounts of organically bound nitrogen (Viro, 1969), the rate of decomposition is relatively slow and the amount of mineralized nitrogen is low (*e.g.* Nômmik, 1982). It is generally accepted that N mineralization plays a decisive role in supplying nitrogen to plants. However, it has recently been demonstrated that conifers such as *Pinus sylvestris* and *Picea abies* can take up some organic forms of N via mycorrhiza (Näsholm *et al.*, 1998). Thus, N may be transferred to plants without having to be converted into mineral forms and so by "short-circuiting" the N cycle (Chapin III, 1995; Northup *et al.*, 1995).

The N cycle in undisturbed boreal coniferous forests is relatively closed, most of the nitrogen being recycled within the soil-microbe-plant system (Nômmik, 1982) (Figure 1). The total input of N to the soil from atmospheric deposition and nitrogen fixation is usually small, but it usually exceeds the N output through leaching and denitrification resulting in a net accumulation of N in the soil (Nômmik, 1982). Clear-cutting, liming, prescribed burning and an increased nitrogen input (via deposition or fertilization) can disrupt the nitrogen cycle (Aarnio and Martikainen, 1992; Martikainen *et al.*, 1993; Pietikäinen and Fritze, 1995; Priha and Smolander, 1995; Smolander *et al.*, 1995; Kubin, 1998). This may result in an increased leaching of nitrogen (particularly nitrate) from the forest floor, indicating that the N cycle has changed from a tight cycle to an open one. This may increase the risk of nitrate pollution of surface- and groundwater.

The atmospheric input of N to forests in Europe has increased during the recent decades due to the emissions of NO_x from combustion processes and of NH_3 from agricultural activities (Dise and Wright, 1995). In central and western Europe, the annual deposition of mineral N in the 1990's has exceeded 50 kg ha^{-1} (Dise et al, 1998). In southernmost Finland the mean bulk deposition of mineral nitrogen in open area during 1988-1996 was about 6 kg ha^{-1} yr^{-1} (Kulmala *et al.*, 1998). In Finland nitrogen deposition is approximately 30% organic nitrogen and 70% mineral nitrogen, of which nitrate and ammonium are present in equal proportions (Järvinen and Vänni, 1990).

In regions with low nitrogen deposition, this input can act as a fertilizer. In forest ecosystems with limited nitrogen availability there is an increase in growth and productivity. Increased nitrogen deposition may, however, eventually lead to "nitrogen saturation" of previously nitrogen-limited systems, *i.e.* nitrogen availability exceeds the capacity of the plants and soil microbes to assimilate all the nitrogen (Aber *et al.*, 1989). This may result in harmful effects on forest growth (McNulty *et al.*, 1996; reviewed by Rasmussen, 1998) and increase the leaching of nitrate (Gundersen *et al.*, 1998). Nitrogen saturation may only have

occurred in southern Scandinavia, but the situation may change in the future because nitrogen deposition is exceeding the critical load in many parts of northern Europe (Lepistö, 1996; Nilsson *et al.*, 1998). In Finland, losses of nitrate from the forest soil due to nitrogen saturation are expected mainly from the most fertile forests (MT and OMT site type) in southern and central parts of the country where N deposition is also highest (Lepistö, 1996).

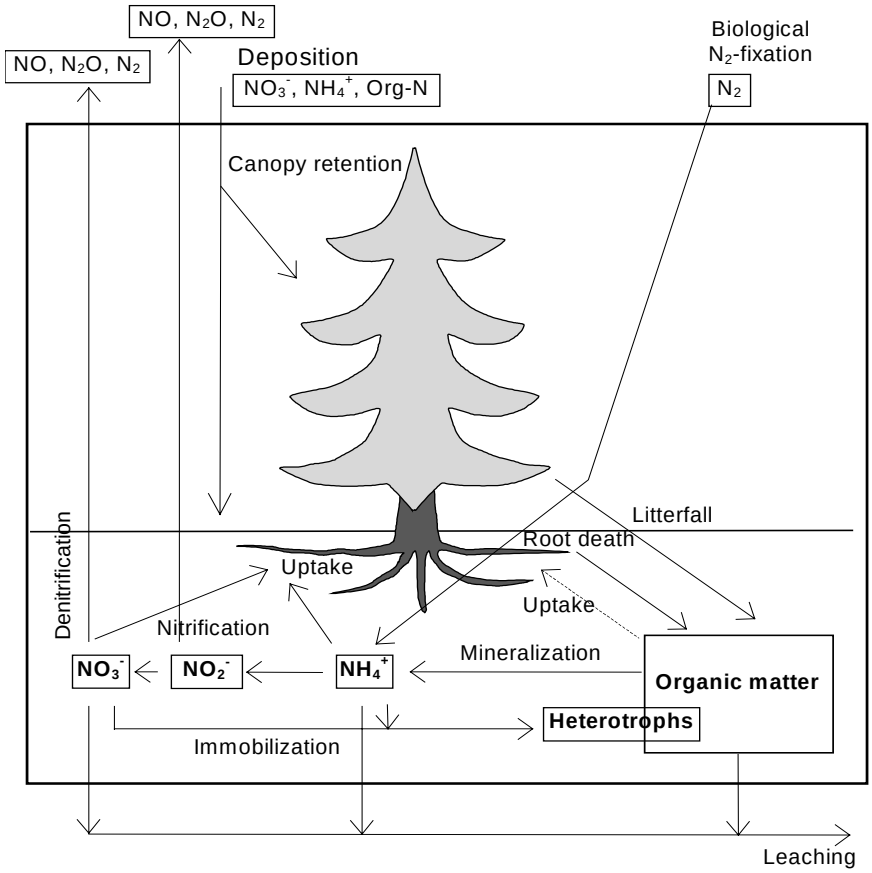

Figure 1. N cycling in a boreal forest ecosystem (modified from van Miegroet and Johnson, 1993)

1.1.1. Mineralization/immobilization of nitrogen

Nitrogen mineralization is usually slow in boreal forest soils due to low soil pH, low temperature and poor litter quality. In Norway spruce forest soil (litter, humus and mineral soil layer to a depth of 50 cm) in Sweden annual net N mineralization was estimated to be 0.5 – 5.0% of the total amount of N, *i.e.* 35-105 kg N ha^{-1} (Persson and Wirén, 1995). The organic horizons (litter and humus layer) accounted for 32-74% of the annual mineralization. The ammonium released during mineralization is competed for by most components of the soil biota, but

particularly by plant roots and soil microbes. In the humus layer of Finnish coniferous forest soils, the nitrogen in the microbial biomass accounts for about 4-6 % of the total amount of nitrogen (Martikainen and Palojärvi, 1990; Smolander *et al.*, 1994).

1.1.2. Nitrification

Gram-negative bacteria of the family *Nitrobacteraceae* are responsible for autotrophic nitrification (Bock *et al.*, 1992). Ammonium is oxidized to nitrite by NH_4 oxidizers and nitrite further oxidized to nitrate by NO_2 oxidizers. Gaseous nitrogen compounds (NO, N_2O, N_2) can be produced as a by-product of nitrification (see section 1.1.3).

$$NH_4^+ + O_2 + H^+ \rightarrow NH_2OH + H_2O \rightarrow NO_2^- + 5H^+$$
$$NO_2^- + H_2O \rightarrow NO_3^- + 2H^+$$

The NH_4 oxidizers found in the soil belong to the genera *Nitrosospira*, *Nitrosomonas*, *Nitrosolobus*, *Nitrosovibrio* and *Nitrosococcus*, while the genus *Nitrobacter* is regarded as the dominant NO_2 oxidizer (Watson *et al.*, 1981; Laanbroek and Woldendorp, 1995). However, Head *et al.* (1993) proposed, on the basis of the analysis of 16S rRNA gene sequences, that *Nitrosolobus*, *Nitrosovibrio* and *Nitrosospira* strains should be classified as a single genus.

Nitrosospira species tend to dominate in acidic soils (Prosser, 1989), and they have been found in coniferous forest soils in Finland and Sweden (Martikainen and Nurmiaho-Lassila, 1985; Klemedtsson *et al.*, 1999). Autotrophic nitrifiers obtain their energy for growth from the oxidation of ammonium or nitrite and assimilate carbon from carbon dioxide. However, *Nitrosomonas europeae* can grow mixotrophically with ammonium and organic compounds (*e.g.* Stüven *et al.*, 1992) and *Nitrobacter* can grow heterotrophically, *i.e.* use organic compounds for both carbon and energy (Bock *et al.*, 1976, 1992).

A much more heterogeneous group of bacteria and fungi are involved in heterotrophic nitrification (Kuenen and Robertson, 1988). As heterotrophic nitrification does not appear to yield energy for growth, these organisms must have other reasons for carrying out the reactions. Focht and Verstraete (1977) suggested that heterotrophic nitrifiers could utilize certain intermediates of nitrogen oxidation as growth factors or as biocidal factors to assist in their competition and survival.

Nitrification by heterotrophic microorganisms *in vitro* is well documented, but the ecological significance of such a process in nature is uncertain. It has been generally considered that heterotrophic nitrifiers are unimportant in the formation of nitrate in soils. However, they may be of significance in acidic forest soils, where their large numbers or high biomass might compensate for their relative inefficiency. In some acidic coniferous soils, heterotrophic nitrifiers have in fact been considered to be responsible for nitrification (Killham, 1987,1990; Duggin

1991; Papen and von Berg, 1998). One reason why heterotrophic nitrification may be important in these soils is that autotrophic nitrification requires a higher pH than that which prevails in acidic forest soils (Kuenen and Robertson, 1988). However, recent studies have shown that autotrophic nitrification does occur in acidic coniferous forest soils and that heterotrophic nitrification does not play an important role (De Boer *et al.*, 1992; Martikainen *et al.*, 1993; Rudebeck and Persson, 1998).

1.1.3. Denitrification

In denitrification nitrate is converted to gaseous nitrogen in the absence of oxygen. The final product of denitrification is N_2, but N_2O and NO are also released. Nitrite, N_2O and NO are intermediate products in the reaction chain.

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

Denitrification is carried out by facultative anaerobes, predominantly heterotrophic bacteria, the most common being species of the genera *Pseudomonas* and *Alcaligenes* (Focht and Verstraete, 1977). Most of denitrifying bacteria require anaerobic conditions, but some species continue to denitrify at varying levels of dissolved oxygen (Lloyd et al, 1987; Jetten et al, 1997).

It has been shown that some nitrifiers also can denitrify. *Nitrobacter* cells are able to grow by denitrification under anaerobic environments (Bock *et al.*, 1988), and *Nitrosomonas europaea* has been shown to reduce nitrite to gaseous nitrogen compounds (NO, N_2O, N_2) under conditions of oxygen stress by simultaneous oxidation of ammonium (Poth and Focht, 1985; Bock *et al.*, 1992). In addition to the production of N_2O by nitrite reduction, it has been thought that N_2O is also produced directly in ammonia oxidation (Yoshida and Alexander, 1970; reviewed by Prosser, 1989). However, Poth and Focht (1985), using isotopic techniques and kinetic analysis of labeled substrates and products, showed nitrite reduction to be the sole source of N_2O in *Nitrosomonas europaea*. The authors suggest that the process functions to: (i) conserve oxygen for use by the ammonia monooxygenase, (ii) reduce production of nitrite (which may accumulate to toxic levels), and (iii) decrease competition for oxygen by nitrite oxidizers, by denying them the source of substrate.

In addition to denitrification and autotrophic nitrification, also heterotrophic nitrifiers and fungi have been suggested to participate in N_2O production in acidic forest soils (Robetson and Tiedje, 1987). Thus, the microbial community able to produce nitrogen gases in forest soil seems to be very complex (Martikainen, 1996)

1.2. What controls soil nitrogen transformations?

Nitrogen transformations in forest soils are generally controlled by: (i) climatic conditions (mainly temperature and moisture), (ii) chemical composition of the litter, (iii) soil pH and C:N ratio, (iv) plant-produced inhibitors (v) soil animals and (vi) the availability of nutrients, substrate and energy sources (reviewed by Gundersen and Rasmussen, 1990; van Miegroet and Johnson, 1993 and Huhta *et al.*, 1998). In the following sections only the effects of nitrogen input, soil pH, soil moisture and aeration and allelopathy on nitrogen transformations are examined as these are the factors that are the most relevant to this study.

1.2.1. Nitrogen input

It is difficult to draw conclusions about the effects of nitrogen on sites receiving an increased atmospheric deposition because deposition contains many other chemical components in addition to nitrogen. It has been suggested that the effects of extra nitrogen input on soil properties could be evaluated from experiments were N deposition is simulated by N fertilization (*e.g.* Gundersen *et al.*, 1998) and from long-term N fertilization experiments (Mälkönen, 1990).

Increased nitrogen inputs may lead to increased N mineralization in forest soils. N deposition was experimentally increased by nitrogen additions (NH_4NO_3) in coniferous forest stands in Sweden, Denmark and UK (from 12-18 to 47-53 kg N ha^{-1} yr^{-1}) whereas deposition was decreased by roofs constructed in the forest in the Netherlands (from 40-46 to 4 kg N ha^{-1} yr^{-1}), (Gundersen *et al.*, 1998). N addition at the low deposition sites resulted in increased net N mineralization, whereas N removal at the high deposition sites resulted in decreased net N mineralization.

The effect of increased N inputs via fertilization can depend on number of factors, including the type of fertilizer used. Ureaformaldehyde $(NH_2CONHCH_2NHCONH_2)_n$, a slow-release N fertilizer, was shown to increase the net formation of mineral N in laboratory incubations of Scots pine forest soils, while fast-release urea and ammonium nitrate had no significant effect on soil mineral N formation (Martikainen *et al.*, 1989). In long-term N fertilization experiments in Norway spruce stands, however, fertilization with fast-release N fertilizers (ammonium sulfate, urea and ammonium nitrate) alone or together with liming increased the net formation of soil mineral N both in laboratory (Priha and Smolander, 1995; Smolander *et al.*, 1995) and in field incubations (Smolander *et al.*, 1995).

In long-term N fertilization experiments, N fertilization initiated net nitrification in humus layer samples of Norway spruce stands both in laboratory (Priha and Smolander, 1995; Smolander *et al.*, 1995) and in field incubations (Smolander *et al.*, 1995). However, at these sites liming was also occasionally needed to initiate nitrate production. Nitrification is usually stimulated more by the addition of urea, which increases soil pH, than by the addition of mineral nitrogen compounds (Martikainen, 1984). The addition of ammonium salts can even inhibit

nitrification in coniferous forest soils, presumably due to the resulting decrease in soil pH (Martikainen, 1985a). Thus the addition of N fertilizers can both initiate and enhance nitrification, unless some other factor, such as pH, is limiting (see section 1.2.2).

The possible stimulation of nitrification also depends on the amount of nitrogen added and on the characteristics of the soil. A single application of urea was found not to increase net nitrification activity in boreal coniferous forest soils (Aarnio and Martikainen, 1992). In another study, *in situ* net nitrification did not show a relationship to simulated increased nitrogen deposition during a 2.5-year experiment (Emmett *et al.*, 1995). However, intensive nitrification was measured in Dutch forest soils exposed to high nitrogen deposition over four decades (Tietema *et al.*, 1993). Changes in soil organic matter quality, and especially changes in the C:N ratio, may be necessary before changes in net nitrification can be observed (Emmett *et al.*, 1995; 1998). Nitrification potentials have been found to be related to the C:N ratio of the forest floor so that soils having a C:N ratio more than 25-30 have been reported to have minimal nitrification ability (Gundersen and Rasmussen, 1990).

The N_2O emissions from forest soil are likely to increase as a result of nitrogen addition (Brumme and Beese, 1992; Sitaula and Bakken, 1993; Klemedtsson *et al.*, 1997; Gundersen *et al.*, 1998). However, as N_2O production is dependent on the availability of nitrate, soils subjected to intensive nitrogen fertilization did not produce N_2O unless the soils also nitrified (Priha and Smolander, 1995). The effect of N fertilization on denitrification may depend on the fertilizer used. Urea fertilizers generally stimulate denitrification more than mineral nitrogen fertilizers (Pluth and Nômmik, 1981). The greater response to urea may be because: (i) urea increases nitrification, thus providing source of nitrate, (ii) urea increases soil pH, and (iii) hydrolysis of urea results in the transformation of C-containing compounds into soluble forms, which in turn provide energy sources for denitrifiers (Foster, 1985; reviewed by Martikainen, 1996). Mineral nitrogen fertilizers and resulting increase in salinity may even result in osmotic stress that inhibits the activity of heterotrophic microbes (Martikainen, 1996).

1.2.2. Soil pH

In Finnish liming experiments in Norway spruce stands, the soil pH of the humus layer increased from about 4.1 to 4.4 (Derome *et al.*, 1986) but the effect on net N mineralization was negligible (Smolander *et al.*, 1995). In other studies on acidic forest soils, increasing the soil pH by liming has either increased (Persson et al, 1989; Persson *et al.*, 1990/91) or decreased net N mineralization (Popovic, 1984; Persson *et al.*, 1990/91; de Boer *et al.*, 1993). In a Swedish coniferous forest soil, while liming decreased the release of nitrogen from the litter layer, the effect in the humus and mineral soil layers depended on the C:N ratio. Liming did not seem to have any significant effect on nitrogen release when the soil C:N ratios were 27 to 37, but it was increased when the soil C:N ratios were 24 to 27 (Persson *et al.*,

1990/91). It could be that in the soils with high C:N ratio, raising the pH increases the immobilization of N more than in the soils with low C:N ratio.

Nitrification has long been considered to be restricted to soils with a neutral or slightly alkaline pH. However, the existence of nitrification in acidic soils has now been demonstrated (*e.g.* De Boer *et al.*, 1992; Martikainen *et al.*, 1993). Nitrate production in acidic forest soils could be due to heterotrophic nitrifiers (section 1.1.2), or to autotrophic nitrifiers active in microsites with higher pH values than the bulk soil pH (Overrein, 1967) or adapted to acidic conditions (De Boer *et al.*, 1992; Martikainen *et al.*, 1993). Nitrification in acidic soils is probably limited by the characteristics of the NH_4 oxidizers since NO_2 oxidizers can live in acidic conditions (Hankinson and Schmidt, 1988; Laanbroek and Woldendorp, 1995). Due to the acid tolerance of the NO_2 oxidizing bacteria, the accumulation of nitrite is hardly to be expected in acidic soils (Laanbroek and Woldendorp, 1995). De Boer *et al.* (1990) classified nitrifiers as acid-sensitive or acid-tolerant on the basis of nitrate production in ammonium-enriched soil suspensions at pH 6 and 4. They recognized four patterns of nitrification: (i) no nitrate production at either pH, (ii) acid-sensitive nitrate production (production at pH 6 but not at 4), (iii) acid-tolerant, pH dependent nitrate production (production at both pH 4 and 6, with the production at pH 6 being at least 1.5 times faster than at pH 4), and (iv) acid-tolerant, pH independent nitrate production (production at both pH 6 and 4, with the production at both pH values being almost equal). In Finland, acid-tolerant nitrification was found in a forest soil receiving high ammonium deposition from a nearby mink farm (Martikainen *et al.*, 1993).

In spite of the existence of nitrifiers adapted to acidic soil, low pH seems to control nitrification in many forest soils. Low soil pH restricted nitrification in the humus layer of forest stands in Sweden and Norway (Persson and Wirén, 1995). In Finnish forest soils, liming alone or together with nitrogen addition was needed to initiate nitrification (Priha and Smolander, 1995; Smolander *et al.*, 1995), implying that the absence of nitrification was due to the low soil pH. Although soil pH may locally be an important regulator of nitrification, it is not generally a good predictor of regional differences (Robertson, 1982). This may be related to shifts, at different pH values, in the relative significance of different types of nitrifiers, acid-sensitive versus acid-tolerant or heterotrophic versus autotrophic nitrifiers (Berg *et al.*, 1997).

The pH dependency of nitrate production may be different in the different layers of boreal coniferous forest soil. Nitrification in the humus layer and upper mineral soil was not affected by pH, whereas in the litter layer increasing the soil pH stimulated nitrification (Martikainen *et al.*, 1993). Rudebeck and Persson (1998) showed that nitrification was more pH dependent in the humus layer than in the mineral soil. This shows that generalizations about nitrification in forest soil cannot be made on the basis of studies on the humus layer alone.

The optimum soil pH usually given for denitrification is in the neutral range, pH 6-8 (Paul and Clark, 1989). Finnish forest soils are naturally acidic (Starr and Tamminen, 1992), and therefore denitrification occurs either at a reduced rate (Müller *et al.*, 1980) or requires the soil pH to be raised, *e.g.* through liming (Priha

and Smolander, 1995). The low denitrification activity measured in acidic soils could be due to small populations of denitrifiers protected in microsites with a neutral pH or due to denitrifiers with a low pH optima (Nägele and Conrad, 1990). Parkin *et al.* (1985) showed that an acid-tolerant denitrifying population had been selected in an agricultural soil over a 20-year period of low pH.

Increasing forest soil pH by liming has been shown to decrease N_2O emissions (Brumme and Beese, 1992). As low soil pH is known to favor N_2O production in denitrification and thus increase the N_2O/N_2 ratio (Focht and Verstraete, 1977), total denitrification may not have been reduced but rather the N_2O/N_2 ratio decreased in response to increased pH of the forest soil. This also explains why N_2O is usually the main product of denitrification in acidic forest soils (*e.g.* Nägele and Conrad, 1990; Kester *et al.*, 1997). In addition, there is evidence to show that acidity favors the production of N_2O associated with the activity of acid-tolerant nitrifiers in both boreal and temperate coniferous forest soils (Martikainen, 1985b; Martikainen *et al.*, 1993; Martikainen and De Boer, 1993). In acidic forest soils both nitrification and denitrification have been suggested to be an important source of N_2O (Robertson and Tiedje, 1984; Sitaula and Bakken, 1993; Martikainen and De Boer, 1993; Ambus, 1998).

1.2.3. Soil moisture and aeration

Microbial activities are affected by soil moisture and the control it has on soil aeration. In a Scots pine forest in Sweden soil moisture seemed to be the main factor in determining the dynamics of the soil bacterial populations (Lundgren and Söderström, 1983). Both excess and too little moisture may limit microbial activity. If soil moisture becomes too high, anaerobic conditions may develop and aerobic processes such as N mineralization and nitrification decrease (Ohte *et al.*, 1997). In addition to regulating the oxygen content of the soil, moisture also partly regulates the availability and movement of nutrients to the microbes. Net N mineralization and nitrification in laboratory incubations of samples from the humus layer of a coniferous forest stand were strongly related to moisture (Tietema *et al.*, 1992). Stark and Firestone (1995) showed that diffusional limitation of the substrate supply and adverse physiological effects associated with cell hydration can explain the decline in the activity of nitrifiers at low moisture content.

In addition to moisture as such, soil nitrogen dynamics are also sensitive to soil wetting and drying cycles (reviewed by van Miegroet and Johnson, 1993; Pulleman and Tietema, 1999). Rewetting a dry soil is usually accompanied by an N mineralization flush and a concomitant increase in nitrification (Birch, 1959). Lamersdorf *et al.* (1998) studied whether N mineralization and nitrification in forest soils were enhanced by summer droughts followed by rewetting periods. In general, no marked nitrification pulses were found after rewetting, except for some small areas. In a similar experiment, Ryan *et al.* (1998) reported signs of increased N mineralization due to rewetting.

For a particular soil, denitrification rates usually increase as the moisture contents increases and the amount of air-filled pores decreases (Davidson and Swank, 1986; Sitaula and Bakken, 1993; Jordan *et al.*, 1998). In addition, soil moisture affects the N_2O/N_2 production ratio in denitrification; with increasing anoxic conditions, the proportion of N_2O in the denitrification products decreases (*e.g.* Firestone *et al.*, 1979). The N_2O production in nitrification increases at lower oxygen concentrations (Goreau *et al.*, 1980). The contribution of nitrification as N_2O source should be the highest under microaerophilic conditions, when N_2O reduction in the denitrification process is inhibited by oxygen and when nitrifiers, limited in their use of oxygen as an electron acceptor, also form N_2O.

1.2.4. Allelopathy

Allelopathy can be defined as any direct or indirect harmful or stimulatory effect exerted by one organism on another through the production and release of chemical compounds (Rice, 1984). Recent literature on allelopathy reflects wide interest in hypotheses that plants and plant residues release allelopathic chemicals that inhibit nitrification in soil (Bremner and McCarty, 1996). In allelopathic inhibition the inhibitor compounds must have a direct effect on cell physiology. For example, a carbon compound which suppresses nitrification by supporting the growth of heterotrophic microbes and thus enhancing N immobilization, is not an allelochemical.

Rice and Pancholy (1972, 1973) suggested that in some climax ecosystems nitrification is inhibited by allelopathic phenolic compounds produced by the vegetation. According to these authors, plants that inhibit nitrification have a competitive advantage over other plants because the oxidation of ammonium to nitrate leads to the conversion of non-leachable forms of nitrogen into leachable forms, and plants cannot utilize nitrate without expending energy to reduce it to ammonium. Thus inhibition of nitrification results in the conservation of both energy and nitrogen. Rice and Pancholy (1972) also hypothesized that nitrification decreases in the course of succession due to increasingly effective inhibition of nitrifying bacteria by later successional vegetation. There is evidence that late-successional species, such as conifers, prefer ammonium over nitrate as a nitrogen source (*e.g.* Kronzucker *et al.*, 1997). Thus for these species, the inhibition of nitrification would make good biological sense.

The most recent allelopathic hypothesis is that put forward by White (1986, 1991, 1994), who proposed that the vegetation in Ponderosa pine ecosystems inhibits nitrification in the soil by releasing volatile organic compounds, monoterpenes. White (1991) showed that different monoterpenes from needle resin possessed variable inhibition of net nitrification, and that inhibition was a function of the monoterpene concentration.

Bremner and McCarthy (1988, 1996), among others, have criticized these allelopathic hypotheses and showed, for example, that the addition of monoterpenes resulted only in nitrogen immobilization and there was no inhibition

of ammonium oxidation. They also state that to adequately prove the existance of allelopathic interactions it is necessary to demonstrate that the postulated allelochemicals occur in soils associated with the ecosystems under study and that they exert allelopathic effects when they are added to these soils at concentrations at which they have been detected (Bremner and McCarthy, 1996).

1.3. Potential environmental consequences of nitrification and denitrification

Nitrification is an important process in determining the potential leaching losses from forest soils. In northern coniferous forest ecosystems, nitrate leaching has been observed after disturbance, *e.g.* clear-cutting (*e.g.* Tamm *et al.*, 1974; Lepistö *et al.*, 1995; Kubin, 1998). Excess nitrate leached from the soil often ends up in lakes and streams where it has been implicated in: (i) excess growth of plants and algae (eutrophication), (ii) health problems such as infant and animal methemoglobinemia, and (iii) the formation of carcinogenic nitrosamines by reaction with other nitrogenous compounds (Paul and Clark, 1989). Nitrification may also be a source of acidification in some forest soils where N is in excess of plant and microbial demand (reviewed by Gundersen and Rasmussen, 1990).

N_2O is produced both in nitrification and denitrification. In acidic forest soils N_2O has been shown to be the main product of denitrification (see section 1.2.2). N_2O is a greenhouse gas participating in the warming of the climate, and it is also involved in the destruction of stratospheric ozone, which protects living organisms from ultraviolet radiation (Crutzen, 1981).

2. Aims of the study

Because of the tight cycling of nitrogen usual in boreal forests, it is difficult to ascertain the role of the various nitrogen transformation processes involved and the factors affecting them. The aim of the study was to investigate the response of nitrogen transformations in coniferous forest soils to extreme manipulation treatments so as to accentuate the nitrogen transformation processes and thereby gain a better understanding. Most attention was paid to the factors regulating nitrification, since this is an important process determining the potential nitrogen losses from the soil. Nitrogen transformations were studied both with laboratory and field experiments.

The research was carried out at two sites in southern Finland. At one of the study sites the risk of nitrogen mobilization was maximized. A Norway spruce stand growing on a fertile site had been manipulated earlier through long-term N fertilization and pH increase (by liming) followed by clear-cutting (I, III). At this study site, besides studying N transformations, the possible allelopathic inhibition of nitrification was also studied (IV).

At the other study site, the effect of irrigation on soil N transformations was studied (II, V), as part of a project to evaluate the use of sprinkling infiltration to artificially recharge groundwater reserves. Groundwater will be used to an ever-increasing extent by urban water utilities in Finland in the near future. The development of new forms of artificial recharging the groundwater which have a low environmental impact, but provide water of high quality, is thus important. One such new method is sprinkling infiltration. In this method, untreated surface water (2000 times the annual rainfall) is sprinkled directly onto the forest soil via a network of pipes, and therefore does not cause as much direct disturbance to the vegetation and soil surface as *e.g.* basin recharge. This provided a unique opportunity to study the effect of extreme irrigation on soil N transformations. In this experiment the leaching of nitrate was of special interest.

3. Materials and Methods

A brief summary of the methods used is given here. More detailed information can be found in the original publications I-V and in the references cited therein.

3.1. Experimental sites

The results presented in this study come from two experimental sites, one in Patasalo and one in Ahvenisto (Figure 2).

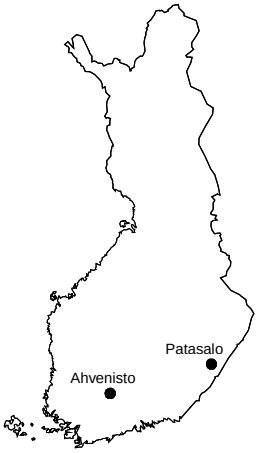

Figure 2. Location of the experimental sites.

The experimental site in studies I, III and IV was a 60-year old Norway spruce (*Picea abies* L.) stand growing on mineral soil in the commune of Patasalo, Kerimäki, in south-east Finland (Table 1, Figures 2 and 3). Factorial fertilization experiments have been carried out in the stand (Smolander *et al.*, 1994). The treatments were liming (Ca), nitrogen fertilization (N), liming and nitrogen fertilization (CaN), and a control (0). In the Ca treatment, finely-ground limestone was applied twice, in 1958 and 1980, totaling 6000 kg ha^{-1}. In the N treatment, the plot had received nitrogen fertilization 7 times, first as ammonium sulfate (2 times), then as urea (3 times) and later as ammonium nitrate with dolomite (2 times), totaling 860 kg N ha^{-1}. The last application was made in 1986. The stand was clear-cut in January 1993 and the stems removed. The logging residues (branches and needles) were spread evenly over the surface of each clear-cut plot. In addition to the control plot (0) mentioned above, which was subjected to clear-cutting, there was also a forested reference plot (0(for)) which was not clear-cut.

The sprinkling infiltration study (II, V) was carried out in the Ahvenisto esker area, near Hämeenlinna in southern Finland (Table 1, Figures 2 and 4). The esker formation is an important groundwater area for drinking water supply. Artificial recharging of the groundwater in Ahvenisto was started in 1976 using infiltration

basins. The quality of the artificial groundwater produced by basin recharge has been good, apart from the high iron concentrations. Experimental sprinkling infiltration was started to improve the oxidizing conditions and water purification efficiency in the infiltration area. Due to sprinkling infiltration, the iron concentrations have generally been below the limit value set by the Finnish Ministry of Health for household water *i.e.* 0.2 mg l^{-1}. Sprinkling infiltration was performed on a relatively steep slope (about 20-25º sloping to the east). The stand was a mixture of 110-160-year old Scots pine *(Pinus sylvestris* L.) and 110-120-year old Norway spruce (*Picea abies*). Surface water from a near-by lake was pumped to the plots via a network of pipes. Water was sprinkled directly onto the forest floor from two lines of holes (hole dia 4-5 mm) in the irrigation pipes at 20-cm intervals (Figure 1 in II). The study area was divided into 6 plots representing 2 controls, and the following infiltration treatments: continuous and periodical infiltration (one month's periods) during the summertime, and continuous infiltration during the wintertime. In addition, the recovery of the soil after cessation of infiltration was studied on one of the plots. Each plot was further divided into 2-3 subplots (Figure 1 in II). The amount of irrigation water supplied to the site was more than 2000 times the annual precipitation. The amounts of irrigation water are given in Table 1 in II.

Table 1. General characteristics of the experimental sites. Meteorological data are from years 1961-1990

	Patasalo	Ahvenisto
Forest site type[1]	OMT	OMaT
Geographical location (longitude/latitude)	61º51'N/29º22'E	61º01'N/24º47'E
Altitude a.s.l. (m)	85	100
Mean annual temperature (ºC)	4.2	4.5
Mean annual rainfall (mm)	590	630
N deposition[2]	3	4
Soil type	Haplic podzol	Carbic podzol
Soil texture	Fine sand till	Sandy till[3]
Humus type	Mor	Moder
pH(H_2O)[4]	4.2	5.0
C:N ratio[4]	28	26

[1]Forest site type classification by Cajander (1949)

[2]Mean bulk deposition of mineral nitrogen in the area measured in the open area in 1988 –1996 (kg ha^{-1} yr^{-1}) (Kulmala et al., 1998)

[3]Spatial mixture with some areas of sandy till and some of gravel

[4]In Patasalo experiment the average value from years 1992 -1995 (I)
In Ahvenisto experiment the average value for the two control plots from years 1996 - 1998 (II, V)

a

b

*Figure 3. The Patasalo N-fertilization, liming and clear-cutting experiment: (**a**) forested reference plot (0(for)), (**b**) nitrogen fertilized plot (N) on the third summer after clear-cutting.*

a

b

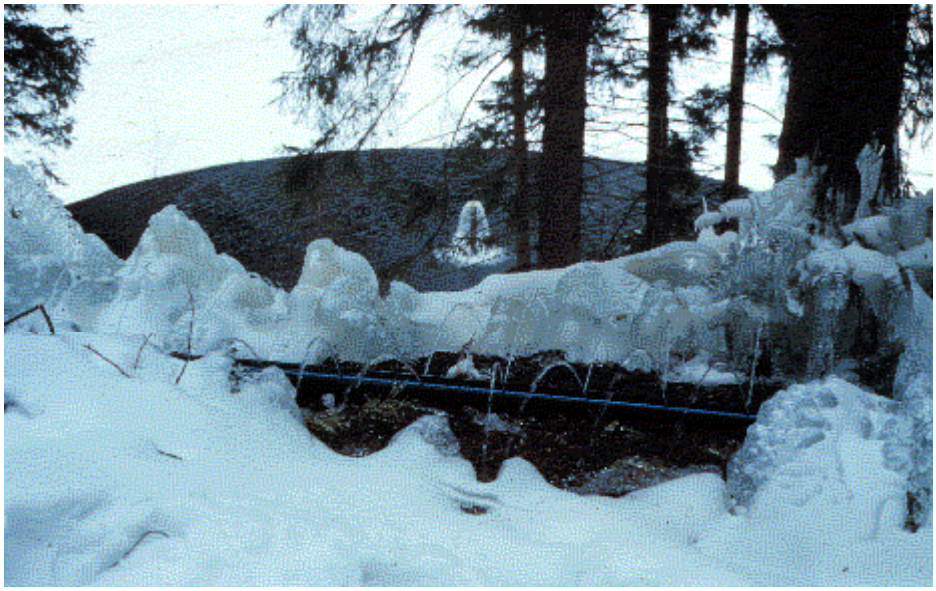

*Figure 4. The Ahvenisto sprinkling infiltration experiment: (**a**) continuous infiltration during the summertime, (**b**) continuous infiltration during the wintertime.*

3.2. Soil sampling and chemical analyses

Soil samples were taken from the humus layer (F+H layers) (I–V), and from the upper mineral soil (the uppermost 0-10 cm) (V). 20-30 cores (core diameter 25 mm in II, V and 50 mm in I, III, IV) were taken systematically from each study plot or subplot and pooled to give a representative sample for the plot or subplot. Green plant material was removed and the fresh samples were sieved through a 2.8 mm (humus) or a 2 mm (mineral soil) sieve.

Fresh soil samples were used in all the analysis, except in determining total C and N (I-V). Organic matter content was measured as loss in weight after ignition at 550°C (I-V). Soil pH was measured in a suspension of soil in H_2O (I-V) or 10 mM $CaCl_2$ (I) (3:5 v:v). Total C and N were determined from air-dried samples on a CHN analyzer (CHN-600, LECO) (I, V).

3.3. Determination of microbial biomass C and N, and C mineralization

Microbial biomass N and C were determined using the fumigation-extraction (FE) method (Brookes *et al.*, 1985; Vance *et al.*, 1987) (I), and microbial biomass C also using the substrate-induced respiration (SIR) method (Anderson and Domsch, 1978; West and Sparling, 1986) (I). CO_2-C production at constant temperature (14°C) and moisture (60% of the water-holding capacity, WHC) was measured in order to evaluate aerobic C mineralization (I).

3.4. Studies on nitrogen transformations

Nitrogen transformations were studied in aerobic incubation experiments in the laboratory at constant temperature (14°C, except in IV in which the temperature was 22-24°C) and moisture (60% of the WHC) (I-V). Before and after incubation, NH_4-N and (NO_2+NO_3)-N were extracted in 40 ml 1 M KCl, and measured with a flow injection analyzer (FIA Star 5020, Tecator). Net nitrification and the formation of mineral N were calculated by subtracting the initial soil NH_4-N and (NO_2+NO_3)-N concentrations from the final (post-incubation) concentrations. The effect of a pH increase on nitrogen transformations was studied by increasing the pH of the soil by adding $CaCO_3$ in the laboratory (V).

The nitrification potential of the soil samples was studied in ammonium-enriched soil suspensions with continuous shaking (De Boer *et al.*, 1992). The pH of the soil suspensions was kept at their original pH (IV) or adjusted to pH 4 and 6 (III) or to a pH gradient from 4.4 to 6.2 (III) or 4.7 to 6.7 (V).

The nature of nitrification (autotrophic or heterotrophic) was studied by incubation with C_2H_2 at a partial pressure of 2.5 Pa (III, V).

The most probable number (MPN) method, described by Martikainen (1985c), was used to determine the numbers of autotrophic NH_4 and NO_2 oxidizers in the soil (III, V).

N_2O production was studied in laboratory incubations at constant temperature (14ºC) and moisture (100% of the WHC) with either no acetylene or with acetylene at a partial pressure of 10 kPa (III, V). In order to determine the contribution of autotrophic nitrification the samples were also treated with 2.5 Pa of acetylene (V).

In the denitrification enzyme activity (DEA) measurements (Luo *et al.*, 1996), solutions of KNO_3 and glucose were added and the moisture-content of the soils adjusted so that they were water-logged (V). The air in the bottles was replaced with N_2 and acetylene added to give a partial pressure of 10 kPa. The samples were incubated for 5 h with continuous shaking at 22°C.

3.5. Studies on allelopathy

Passive diffusive samplers were used to collect volatile organic compounds (VOC) from the soil in the field and in the laboratory (IV). VOCs were analyzed by gas chromatography-mass spectrometry. The effect of monoterpenes on carbon and nitrogen transformations was studied by exposing the soils to vaporized monoterpenes or by adding a monoterpene solution to the samples (IV).

3.6. Measurements of nitrogen losses

Concentrations of NH_4-N, (NO_2+NO_3)-N and total N were determined from sprinkling infiltration water, percolation soil water and groundwater (II). Percolation soil water was collected below humus layer using plate lysimeters and by means of suction-cup lysimeters at depths of 40 and 100 cm below the ground surface. Groundwater was sampled from an observation pipe located within the infiltration area. Organic nitrogen was calculated as the difference between total nitrogen and mineral nitrogen.

Fluxes of N_2O from the soil were measured in the field by the static chamber method as described by Martikainen *et al.* (1995) and Nieminen (1998) (II). Cylinder-shaped chambers (volume 20 l, height 35 cm) with an open bottom were pushed against the soil surface so that the lower edge of the chamber sank about 5 cm below the soil surface. N_2O emissions from the soil to the chamber were measured by sucking gas samples from the chambers with polypropylene syringes (50 ml) at 3, 15 and 30 minutes after the chambers had been installed in the soil.

3.7. Statistical analyses

In study I Pearson's correlation coefficients were used to determine whether there were any linear relationships between the measured properties. In study IV the *t*-test (2 means) or ANOVA (more than 2 means) was used to compare the means of different treatments. In ANOVA the differences between means were tested using Dunnett's or Tukey's test. ANOVA (V) and ANOVA for repeated measures (II) were used for instance to determine the overall effect of infiltration on soil properties. Differences between the means were considered statistically significant when $p < 0.05$. The data were log-transformed when necessary.

4. Results and discussion

4.1. Response of limed and N fertilized forest soil to clear-cutting

Nitrogen and carbon transformations in samples from the humus layer were investigated in a Norway spruce stand at Patasalo one year before and for three years after clear-cutting (I, III).

Clear-cutting increased soil microbial biomass C and N, and C mineralization in all the plots. However, this effect was evident only during the first summer (I). Clear-cutting changes the microclimate, and soil temperature and moisture are increased (Heiskanen, 1989), which in turn can stimulate mineralization (Matson and Vitousek, 1981). There is also an increase in the amount of decomposable organic material in the form of dead roots and logging residues but, at the same time, a decrease in litter production, root exudates and mycorrhizas. When plant debris decomposes after cutting, the nutrients released may be taken up by soil microbes and the developing vegetation. Vitousek and Matson (1984) considered microbial immobilization of N to be even more important than N retention by the developing vegetation in preventing N losses after clear-cutting. Before conclusions can be drawn about the significance of microbial biomass in retaining N in the ecosystem in Patasalo, the turn over rate of the microbial biomass should be known. In any case, the role of microbial biomass in retaining nitrogen should have been important before vegetation had developed, *i.e.* in the first summer after cutting, and in the early summers after that.

The effect of clear-cutting on soil microbial biomass and numbers and carbon transformations depends on the time elapsed since cutting. An increase soon after cutting has often been observed, followed by a decline to the control level or even lower (Sundman *et al.*, 1978; Bååth, 1980; Lundgren, 1982; Bauhus and Barthel, 1995; Pietikäinen and Fritze, 1995). No decrease in microbial biomass and C mineralization due to cutting was observed in this 3-year study (I). The response may differ between sites depending on site characteristics, on the amount and quality of the logging residues left after clear-cutting, on the development and species composition of the ground vegetation, and on the nitrogen content of the forest soil. In this study no consistent and profound differences were observed between the fertilization treatments (I). For example, the reduced microbial biomass in the N treated plot measured before cutting was also still evident after cutting.

In the samples taken before clear-cutting, the net formation of mineral N was highest in the soil samples from the N fertilized plots, and notable net nitrification occurred only in the samples from the CaN plot (I) (Table 2). Clear-cutting increased net formation of mineral N in all except the CaN plot. In addition, clear-cutting initiated net nitrification in all the plots. This was also seen in the number of NH_4 oxidizers. Two years after clear-cutting less than 10 NH_4 oxidizers cm^{-3} soil were found in the forest soil ((0(for) plot)), whereas in the clear-cut plots the

number was above 30 000 (III) (Table 2). Stimulatory effects of clear-cutting on nitrification have been observed in several other forest ecosystems (Smith *et al.*, 1968; Tamm *et al.*, 1974; Matson and Vitousek, 1981; Fisk and Fahey, 1990; Duggin *et al.*, 1991).

The stimulating effect of clear-cutting on the net formation of mineral N and net nitrification lasted throughout the study period in all except the CaN plot (I). Attiwill and Adams (1993) suggested that clear-cutting increases N mineralization for a relatively short period, followed by a longer period in which net N mineralization decreases. Within 1-2 years of clear-cutting N mineralization and the pools of inorganic nitrogen in the soil are similar to those before clear-cutting. Fisk and Fahey (1990) found that the nitrification potential is also depressed from 2 to 6 years after clear-cutting in northern hardwood forests. However, in some forests the effect of increased nitrification and nitrate leaching lasted for almost 10 years after clear-cutting (Matson and Vitousek, 1981; Kubin, 1998). As with microbial biomass and C mineralization, the response of N mineralization and nitrification may differ between sites depending on site characteristics such as the nitrogen content of the soil. In this study, however, previous N fertilization did not affect nitrification and the net formation of mineral N after clear-cutting, or the effect was even suppressive (I).

Table 2. Numbers of nitrifiers, net formation of mineral N and net nitrification in the soils from the humus layer

Experimental site and treatment	Numbers of NH_4 oxidizers[1] MPN cm^{-3} soil	Numbers of NO_2 oxidizers[1] MPN cm^{-3} soil	Net formation of mineral N[2] µg g $o.m.^{-1}$ 40 d^{-1}	Net nitrification[2] µg g $o.m.^{-1}$ 40 d^{-1}
Patasalo[3]				
before clear-cutting				
0(for)	nd	nd	150 (100 - 200)	1 (0 - 4)
0	nd	nd	100 (50 - 150)	0
Ca	nd	nd	120 (50 - 200)	10 (1 - 30)
N	nd	nd	280 (250 - 300)	5 (1 - 10)
CaN	nd	nd	390 (300 - 600)	540 (400 - 700)
after clear-cutting				
0(for)	10	100	170 (50 - 300)	0
0	110000	170000	500 (200 - 900)	580 (0 - 900)
Ca	450000	160000	340 (100 - 500)	450 (200 - 700)
N	37200	40000	470 (200 - 700)	420 (50 - 800)
CaN	220000	290000	260 (10 - 600)	340 (50 - 600)
Ahvenisto				
Control	1000	900	70 (-20 - 200)	6 (0 - 50)
Infiltration	350000	550000	240 (50 - 600)	360 (100 - 900)

[1] The results are means from year 1995 (Patasalo) (III) and 1998 (Ahvenisto) (V)

[2] The results are means (lowest and highest values in parentheses) from years 1992 (Patasalo before clear-cutting), 1993-1995 (Patasalo after clear-cutting) (I) and 1996-1998 (Ahvenisto) (II)

[3] Treatment symbols: 0(for) = forested reference, 0 = control, Ca = liming, N = N fertilization, CaN = liming and N fertilization, nd = not determined

N_2O production was studied in laboratory experiments. Before clear-cutting, denitrification occurred only in soil samples from the CaN plot (Priha and Smolander, 1995). After clear-cutting (2-years), denitrification was observed in samples from all the clear-cut plots (III). Denitrification is dependent on nitrification and therefore clear-cutting, which promoted nitrate production, made denitrification possible. Martikainen in his literature review (1996) also reported increased N_2O production after clear-cutting. Of the clear-cut plots, the rate of denitrification was highest in soil samples from the limed plots (III).

4.2. Effects of sprinkling infiltration on soil nitrogen transformations

The effect of sprinkling infiltration on soil nitrogen transformations was studied in Ahvenisto esker. The studies primarily focused on the humus layer (II, V), but the underlying mineral soil was also studied during the third summer of infiltration (V, see section 4.6).

The response of soil nitrogen transformations to infiltration was similar in all the plots, irrespective of the infiltration treatment. Soil NH_4-N concentrations tended to be higher and the net formation of mineral N was significantly higher in the soils that had been treated with infiltration (infiltration soils) than in the control soils (II) (Table 2). (NO_2+NO_3)-N was present only in the infiltration soils. This was explained by net nitrification and by the fact that the numbers of nitrifiers were about 500 times higher in the infiltration than in the control soils (II, V) (Table 2). A population of about 1000 nitrifiers cm^{-3} soil was present in the control soils (V). The presence of nitrifiers in the untreated soils enabled the quick response of nitrate production after sprinkling infiltration. Net nitrification was already intensive in the soil from the continuous summertime infiltration plot after about one month of infiltration (II).

After cessation of infiltration, the net production of nitrate in the laboratory incubation experiments declined with time (Table 3 in II). This would suggest that the activity or numbers of nitrifiers had declined due to the cessation of infiltration. In spite of the cessation of infiltration, the pH of the humus layer of this plot had not decreased with time (Helmisaari *et al.*, 1999). Thus the soil will continue to produce nitrate after the cessation of infiltration, but probably at a decreased rate without the continuous input of ammonium in the infiltration water and because of the lower soil moisture.

Both denitrification enzyme activity (DEA) and the rate of denitrification were measured in the laboratory from soil samples taken during the third summer of infiltration (V). With only a short incubation time, DEA is dependent on pre-existing denitrifying enzymes, whereas in denitrification measurements the longer incubation time allows the synthesis of new enzymes (Luo *et al.*, 1996). Without the addition of substrate, N_2O was produced only in the infiltration soils (V). DEA was significantly higher in both the humus and mineral soil layers of the

infiltration plots than in the control plots (V). The DEA values measured from the infiltration soils were about 3 times higher than those reported by Priha and Smolander (1999) for Scots pine and Norway spruce forests in Finland.

4.3. Nitrogen transformations in untreated soils

Nitrogen transformations were studied in the laboratory using sieved fresh samples. Measurements of nitrogen mineralization in controlled laboratory conditions provide an estimate of the pools of mineralizable nitrogen present at the time of sample collection, but there may be an overestimation if sieving stimulates mineralization (Raison *et al.*, 1987). Core incubations in the field are considered to give a better estimate of *in situ* net nitrogen transformations (Binkley and Hart, 1989). However, at the Patasalo experiment before clear-cutting, patterns of nitrogen transformations (net N mineralization and nitrification) were similar in field and laboratory incubations (Smolander *et al.*, 1995).

Net formation of mineral N in laboratory incubations varied from about -20 – 200 and 50 – 300 µg N g $o.m^{-1}$ 40 d^{-1} in the humus layer of the untreated soils (control plots) in the Ahvenisto and Patasalo experiments, respectively (I, II) (Table 2). This is of the same order of magnitude as in Scots pine and Norway spruce stands of different fertility in Finland reported by Martikainen *et al.* (1989) and Priha and Smolander (1999).

Hardly any net nitrification occurs in the acidic coniferous forest soils of Finland as shown by the results from both laboratory and field incubations (Martikainen, 1984; Aarnio and Martikainen 1992; Priha and Smolander, 1995; Smolander *et al.*, 1995), except in some forests growing on a fertile site (Aaltonen, 1926; Priha and Smolander, 1999). Aaltonen (1926) found low nitrification activity in soils from CT, VT, MT and OMT type forest sites (in order of fertility, for the Finnish classification see Cajander, 1949), whereas in more fertile OMaT forest sites nitrate production was considerably higher. Priha and Smolander (1999) studied soils from Scots pine, Norway spruce and birch OMT and VT sites, and reported appreciable net nitrification only in the OMT Scots pine site. The forests in this study were growing on fertile sites (at Patasalo on OMT and at Ahvenisto on OMaT site) but still net nitrification was negligible in the humus layer of the control plots (I, II) (Table 2).

Net nitrification determined in incubation experiments is a reasonable approach in describing nitrification capacity, since the nitrifiers are provided with more optimal conditions (such as moisture) and the competition for nutrients from plant roots is eliminated. However, if no nitrate accumulates, we cannot conclude that the soil has no nitrification activity; the absence of nitrate may be due to active consumption by the soil microorganisms (Stark and Hart, 1997).

Additional knowledge about nitrification in the humus layer was obtained by enumerating the nitrifiers (MPN method) and by measuring net nitrification in ammonium-enriched soil suspensions (III, V). These measure the nitrification potential of the soil, as the amount of ammonium does not inhibit nitrification. The

number of NH_4 oxidizers rather than the number of NO_2 oxidizers better reflects the changes in the potential nitrification activity of the soil (Martikainen, 1985c, Aarnio and Martikainen, 1995). NO_2 oxidizers have been shown to live in acidic conditions (Hankinson and Schmidt, 1988), and it can therefore be assumed that a reasonably large and functioning population of NO_2 oxidizers is continuously present in acidic forest soil (Aarnio and Martikainen, 1995). The number of NH_4 oxidizers in the soil samples from the control plots in the Ahvenisto and Patasalo experiments were about 1000 and 10 cm^{-3} soil, respectively (III, V) (Table 2). The difference in the number of NH_4 oxidizers was reflected in nitrate production in the ammonium-enriched soil suspensions kept at high pH (about 6). Production was detected in the samples from the Ahvenisto experiment but not in those from Patasalo (III, V). The formation of aggregates by nitrifying bacteria can distort the numbers obtained by MPN counts (De Boer *et al.*, 1989). Thus, the soil suspension method is probably more reliable for measuring the nitrification potential of a specific soil (Priha and Smolander, 1999). In other studies on Finnish coniferous soils, Martikainen (1985c) and Aarnio and Martikainen (1995) found negligible number of NH_4 oxidizers in CT and MT sites, whereas Priha and Smolander (1999) found approx. 1000 cm^{-3} soil or no NH_4 oxidizers in OMT and VT sites, respectively.

The reason for the negligible net nitrification observed in the untreated soils at both sites may be different. In the Patasalo experiment the absence of nitrate accumulation was probably due to the low number of NH_4 oxidizers which in turn is attributable to other factors that have kept the natural population originally low. Conversely, in the Ahvenisto experiment the nitrifiers present were perhaps unable to express their potential or then immobilization of nitrate was so high that net nitrification could not be detected. The nitrate concentrations in soil percolate water were negligible in the untreated soils at both sites (II, and for the Patasalo experiment see Smolander *et al.*, 1995). The N_2O emissions were also very low, as discussed below (II, and for the Patasalo experiment see Smolander *et al.* 1998), indicating that if nitrate was produced it was immediately immobilized.

Most nitrification studies have been carried out on the humus layer. However, considerable nitrification potential has been found in both the litter (De Boer *et al.*, 1992; Martikainen *et al.*, 1993) and in the mineral soil (Persson and Wirén, 1995). In the Patasalo experiment, the soil suspension experiments were performed with unsieved soil that also included the litter layer, but in this case nitrate production was also negligible (results not presented). The net nitrification of samples from the upper mineral soil layers of the control plots in both experiments was also negligible (V, and for the Patasalo experiment see Smolander *et al.*, 1995).

Due to the negligible amount of nitrate in the soils, the control soils did not produce N_2O in the laboratory without addition of nitrate (III, V). The DEA in samples taken from the humus and mineral soil layers of the control plots in the Ahvenisto experiment was about 100 and 50 ng cm^{-3} h^{-1} (V), which is similar to that for the soil in a Norway spruce OMT site in Finland (Priha and Smolander, 1999). In the field measurements, the mean N_2O emission during the growing season from the control soils in the Ahvenisto experiment was about 0.03 mg N m^{-}

^{2} day^{-1} (II). This is very close to that measured in a Norway spruce forest in Sweden (Klemedtsson *et al.*, 1997) and in the Patasalo experiment ((0(for) plot)) (Smolander *et al.*, 1998).

4.4. Why are there changes in nitrogen transformations?

The effects of combined liming and N fertilization (CaN plot before clear-cutting), clear-cutting (in all except the CaN plot), and the sprinkling infiltration on nitrogen transformations were remarkably similar. All the treatments increased the net formation of mineral N and initiated net nitrification, and the net formation of mineral N and net nitrification were of the same order of magnitude after the treatments (I, II) (Table 2). After clear-cutting and initiation of the sprinkling infiltration treatment the numbers of nitrifiers were 30 000 - 600 000 cm^{-3} soil and the nitrifiers were acid-sensitive and autotrophic (III, V) (Table 2). The reasons for these responses, however, are probably different between the studied treatments.

4.4.1. Net formation of mineral N

The increase in the net formation of mineral N before clear-cutting as a result of N fertilization (I) (Table 2) has been reported earlier (Priha and Smolander, 1995; Smolander *et al.*, 1995). After clear-cutting, however, the net formation of mineral N was on the same level or even lower in the N fertilized plots than in the unfertilized, clear-cut control plot (0) (I) (Table 2). It can only be speculated what were the reasons for this even suppressive effect of previous N fertilization after clear-cutting. One reason could be greater immobilization of mineral N by the soil heterotrophic community in the N fertilized plots compared to the other plots during the 40-day incubation.

The increased net formation of mineral N after clear-cutting can be explained by the same factors as for the increase in C mineralization, *i.e.* the change in microclimate (increased moisture and temperature), even though C and N mineralization were not correlated (I). In the Ahvenisto sprinkling infiltration experiment, the net formation of mineral N in the infiltration soils was probably also stimulated by enhanced moisture, as reported also by Tietema *et al.* (1992). Accordingly, in Scots pine forest in Sweden the soil bacterial populations were related to soil moisture content and rainfall (Lundgren and Söderström, 1983). N mineralization can also be enhanced by soil wetting/drying cycles (van Miegroet and Johnson, 1993; Pulleman and Tietema, 1999). Despite this, no clear differences in the net formation of mineral N in the plots receiving summertime continuous infiltration (plot 2) and summertime periodical infiltration (plot 3, infiltration in about one month's periods) were observed (II).

Results concerning the pH dependence of N mineralization are contradictory. Liming, used to counteract the acidification of forest soil (Derome *et al.* 1986), is

known to either increase or decrease net N mineralization (see section 1.2.2). In the Patasalo experiment, the net formation of mineral N correlated positively with pH within the lower pH range (pH 3.9-4.9), but negatively within the higher pH range (pH 4.9-6.9) (I). In the Ahvenisto infiltration experiment, the net formation of mineral N was enhanced by adding $CaCO_3$ in the laboratory to increase soil pH. Increasing the pH of the control soils to 6.7 increased the net formation of mineral N (V). The results of the Ahvenisto and Patasalo experiments, however, are not directly comparable, as the addition of lime to the soil in the laboratory is very different from that in the long-term field liming experiments.

In the experiment in which lime was added in the laboratory to the soils from the Ahvenisto infiltration experiment, net nitrate production appeared to stimulate the net formation of mineral N (Figure 1 in V). Moreover, the net formation of mineral N was about double in samples not treated with acetylene (*i.e.* nitrate production not inhibited) compared to the samples in which nitrate production was inhibited by 2.5 Pa of acetylene (results not presented). Acetylene may have stimulated the immobilization of mineral N but, due to the low concentration, it would not explain the difference. It has been reported that in forest soil ammonium is immobilized at higher rate than nitrate (Overrein, 1967; Pang, 1985), and thus net nitrogen mineralization could be higher in soils with nitrate production. However, another explanation could be that ammonium production was controlled by the kinetics of ammonium oxidation.

4.4.2. Nitrification

The availability of ammonium is an important factor controlling nitrification in forest soil (Robertson, 1982). This was also observed in the Patasalo experiment before clear-cutting where nitrification occurred only in the soils fertilized with N (ammonium sulfate, urea and ammonium nitrate), although not without liming (I) (Table 2). The total amount of N added to the N plots in the Patasalo experiment during the 30-year period was 860 kg ha^{-1}, which would average about 30 kg ha^{-1} y^{-1}. This is a very large N addition compared to the average bulk mineral N deposition of about 3 kg ha^{-1} y^{-1} measured in this area (Table 1). The liming of forest soils has been used in Europe to counteract the acidifying effects of nitrogen and sulfur deposition (Derome *et al.*, 1986; Kreutzer, 1995). However, the results from the Patasalo experiment indicate that if soil pH in boreal forests subjected to heavy nitrogen deposition is increased by liming, there is a high risk for excess nitrate production and its subsequent leaching.

As with the net formation of mineral N, previous N fertilization did not affect net nitrification after clear-cutting or the effect was even suppressive (I) (Table 2). It can only be speculated what were the reasons for the suppressive effect of the previous N fertilization in our study. One reason could be greater immobilization of mineral N by the soil heterotrophic community in the N fertilized plots compared to the other plots, as suggested earlier in section 4.4.1. In addition, the pH of the soil on the N plot was slightly lower than that on the unfertilized, clear-

cut control plot (0), and therefore this lowering in pH could be enough to partly inhibit the activity of nitrifiers that were already very close to the lowest pH level they could tolerate.

Clear-cutting and the sprinkling infiltration treatment increased the net formation of mineral N and thus the availability of ammonium in soils (I, II) (Table 2). Before clear-cutting (particularly in the O and Ca plots) and before the initiation of the sprinkling infiltration treatment, it might be considered that the availability of ammonium restricted nitrification. However, when samples from the untreated plots ((0(for) plot in the Patasalo experiment and control plots in the Ahvenisto experiment)) were incubated in ammonium-enriched suspensions at a pH close to their natural pH, no production of nitrate was detected (III, V). This suggests that the availability of ammonium was probably not the main reason for the restriction of nitrification before the treatments. However, once nitrate production had started after the treatments, the availability of ammonium appeared to control the activity of the nitrifiers. In the CaN plot at the Patasalo experiment, net nitrification was shown to be restricted by the availability of ammonium (I, III) and in the Ahvenisto experiment, after cessation of infiltration, the decreased availability of ammonium reduced net nitrification (II).

Acidity is generally considered to be the factor inhibiting nitrification in coniferous forest soils (Tietema *et al.*, 1992). The pH of the humus layer was increased by about 0.6-0.7 pH units at Patasalo in the first summer after clear-cutting (in unlimed soils from about 4.2 to 4.9 and limed soils 5.3 to 6.0) (I) and by about 1.5 units after the initiation of the sprinkling infiltration treatment at Ahvenisto (from about 5 to 6.5) (II). Incubating the soils in aerobic suspensions in which no microsites with a higher pH can occur, allows the determination of a true pH dependency. The soils from the N and CaN plots after clear-cutting and the infiltration and control soils in the Ahvenisto experiment showed a clear and consistent response to a pH gradient in the soil suspension, but differed in their sensitivity (III, V). The soil samples from the Patasalo experiment (N and CaN plot) produced nitrate at pH 5.2, whereas in the soil samples from the Ahvenisto experiment nitrate production was negligible at pH 5.3. Thus, even though the nitrifiers in the soils from both sites were acid-sensitive according to the classification of De Boer *et al.* (1990), the minimum pH values allowing net nitrification seemed to be higher in the Ahvenisto experiment than in the Patasalo experiment.

When the pH of the soil suspension was kept relatively high (about pH 6), the control soils from the Ahvenisto infiltration experiment produced nitrate, whereas no production was detected in the samples from the forested reference plot in the Patasalo experiment (III, V). In the soil samples from the forested reference plot at Patasalo, the low number of NH_4 oxidizers probably did not have time to respond to the increase in pH during the 3-week incubation (III), or else other factors were inhibiting nitrate production. In the Ahvenisto infiltration experiment, net nitrification in the control soils was also initiated by increasing the pH of soil samples with lime, without any addition of ammonium (V).

For the reasons outlined above, the increase in soil pH in the Ahvenisto experiment as a result of infiltration treatment was considered to be the main reason for the initiation of nitrification. In the unlimed plots in the Patasalo experiment, the increase in pH might partly explain the initiation of nitrification after clear-cutting, since net nitrification rate and pH correlated within the lower pH range (pH ≤ 4.9) (I). An increase in soil pH cannot, however, be the main reason for the initiation of nitrification, because before clear-cutting the soil pH in the limed plots (Ca and CaN) was similar but net nitrification was only detected in the CaN plot (I). Furthermore, net nitrification in the Ca plot after clear-cutting continued throughout the study period, even though the soil pH returned to its original level.

It is concluded that either a low pH (O and N plots) or the availability of ammonium (O and Ca plots) restricted the nitrifiers before clear-cutting. The initiation of nitrification after clear-cutting was probably the result of several factors, including the increase in pH and N mineralization rate, and also the decrease in allelochemical inhibitors produced by spruce may have had an effect (see section 4.5).

4.4.3. N_2O production

In the laboratory measurements, N_2O production without substrate additions was appreciable only in those samples from the humus (III, V) and mineral soil layers (V) which also exhibited net nitrification. In addition to the increased availability of nitrate, the sprinkling infiltration treatment stimulated denitrification probably as a result of the increase in the soil moisture content and pH, both of which are known to increase the production of N_2O in forest soil (*e.g.* Müller *et al.*, 1980; Davidson and Swank, 1986; Henrich and Haselwandter, 1997; Jordan *et al.*, 1998). In the clear-cut plots at Patasalo experiment, denitrification was highest in the soil with the highest pH (CaN plot), both before and after addition of nitrate in the laboratory (III).

High pH is known to decrease the N_2O/N_2 production ratio in denitrification (Focht and Verstraete, 1977). In the soil samples from the clear-cut plots in Patasalo experiment, especially the limed ones (pH 5.5-6.1), and in the infiltration soils in Ahvenisto experiment (pH about 6.5), N_2 was the main product of denitrification (III, V). In contrast, N_2O was the main product after the addition of substrate in the forested reference plot in the Patasalo experiment (pH about 4) (III). In other studies, N_2O is also considered to be the main product of denitrification in acidic forest soils (*e.g.* Nägele and Conrad, 1990; Kester *et al.*, 1997).

At both study sites, N_2O production by nitrification was only minor (III, V). High soil moisture contents can favor N_2O production by denitrification (Inubushi *et al.*, 1996; Bollmann and Conrad, 1998). Therefore the relevance of the laboratory measurements of N_2O production, which are made on samples after adjusting the moisture content to 100% of the WHC, to field conditions is limited.

In any case, the soil in the Ahvenisto infiltration experiment can become saturated during infiltration, and the N_2O under these conditions therefore probably originates mainly from denitrification.

4.5. The role of allelopathy

In the Patasalo experiment, monoterpenes (mostly α- and β-pinenes), measured using soil microair diffusive samplers in the field, were detected in considerable concentrations in the soil microair of the forest plot ((0(for)), but not of the clear-cut plot (0 plot) (IV). Net nitrification in the humus layer in both the aerobic incubation experiments and in the ammonium-enriched soil suspensions was inhibited by exposure to vaporized monoterpenes at similar concentrations at which they had been detected at the forest plot. This indicates direct inhibition of nitrification by monoterpenes. Monoterpenes have been reported to inhibit nitrification in other studies, too. Monoterpenes from needle resins inhibited nitrification in soil collected from a Ponderosa pine ecosystem (White, 1986, 1991), and monoterpenes from redwood forests inhibited the growth of *Nitrosomonas europeae* in batch cultures (Ward *et al.*, 1997).

Monoterpenes can exhibit two kinds of inhibitory effect: specific inhibition of ammonia monooxygenase (AMO, the primary enzyme in ammonia oxidation) by competitive or noncompetative inhibition at low concentrations, and a general toxicity at high concentrations (White, 1988; Ward *et al.*, 1997). The degree of inhibition can differ depending on the molecular structure of the monoterpenes. The most inhibitory monoterpenes in soil bioassays and batch cultures have been proved to be limonene and α-pinene (White, 1991; Ward *et al.*, 1997). Accordingly, in our study α-pinene was found to inhibit nitrification (IV). β-pinene, however, was found not to significantly inhibit the growth of *Nitrosomonas europeae* (Ward et al, 1997), whereas in our study β-pinene inhibited nitrification in soil suspension (IV). The different inhibition patterns may be due to differences in the NH_4 oxidizing populations since, according to 16S rDNA based studies, only *Nitrosospira* was found in soil from the Patasalo experiment (Aarnio *et al.*, unpublished data).

Exposure to monoterpenes increased the respiration activity of the soil (IV), as has also been reported by Amaral and Knowles (1998). Bremner and McCarty (1988) suggested that the apparent inhibition of nitrification observed when soils are exposed to vapours of terpene is due to immobilization of ammonium by microbial activity stimulated by the organic C from these vapours. This indirect inhibition of nitrification cannot be excluded in our experiments either. Monoterpenes can be used as an energy source by a portion of the soil microbial population (Misra *et al.* 1996). The stimulated respiration activity by the vapours from the mixture of terpenes could point to this. It can be concluded that monoterpenes may partly explain the negligible nitrification observed in the forest soil in the Patasalo experiment and that the inhibition effect could be both direct and indirect.

In Patasalo the terpenes were probably mainly emitted by the roots of Norway spruce and not so much by the forest soil (IV). There is no clear consensus of the purpose of such hydrocarbon production and emission by vegetation (Benjamin *et al.*, 1996). Monoterpenes appear to be produced by the plants as a result of environmental stress and as a defense against plant pathogens and herbivores (rewieved by Paine *et al.*, 1997), but may perhaps also indirectly affect plant nutrition. Norway spruce prefers ammonium over nitrate as a nitrogen source (Kronzucker *et al.*, 1997). It may be possible that Norway spruce produces monoterpenes also to influence the rates of nitrification and nitrate leaching, which, in turn, influences the amounts of ammonium and nitrate available for uptake.

Because nitrate was produced in the humus layer of the CaN plot before clear-cutting, monoterpenes obviously did not completely inhibit nitrification in this plot (I) (Table 2). According to White (1994), inhibition of nitrification by monoterpenes can only be expressed in available carbon-rich or nitrogen-limited soils. In such soils, the addition of available carbon in the form of monoterpenes does not result in the total consumption of the monoterpenes by the heterotrophic community and, therefore, monoterpenes will persist in the soil. As mentioned above, the introduction of monoterpenes increased soil respiration, which indicates that the soil heterotrophs in Patasalo experiment could use monoterpenes as a substrate. Before clear-cutting, SIR-derived microbial biomass was generally the highest in the soil from the CaN plot (in contrast to FE-derived microbial biomass C or C mineralization) (I). In SIR method respiration response is measured from soil amended with glucose, and thus it is thought to measure the active part of microbial biomass. The results from the SIR measurements indicate that the soil from the CaN plot responded effectively to carbon addition (glucose), implying that the soil was carbon limited. This further implies that the consumption of monoterpenes could have been high in the CaN plot, thus reducing the persistence of monoterpenes in the soil. Because monoterpene emissions were not measured before clear-cutting, nothing is known about possible differences in the production of monoterpenes between the plots. However, the consumption of monoterpenes on the CaN plot may have exceeded their production.

4.6. Nitrogen transformations: humus layer vs. mineral soil

In the Ahvenisto experiment the net formation of mineral N in both treated and control plots was higher in samples from the mineral soil than the humus layer, both when expressed volumetrically or gravimetrically (the soil volume was determined in the laboratory with sieved fresh soils) (V). In contrast, the net formation of mineral N (per cm^{-3} soil) in the Patasalo experiment was usually higher in the samples from the humus layer than from the mineral soil both before (Smolander *et al.*, 1995) and after clear-cutting (Smolander *et al.*, 1999). In

Norway spruce sites in Finland, the net formation of mineral N (per cm^{-3} soil) was higher in samples from the humus layer than from the mineral soil, whereas in Scots pine sites the opposite was true (Priha and Smolander, 1999).

Considerable nitrification has been found in the mineral soil layers of boreal forest soils, and the soil layers below the humus layer may therefore contribute substantially to nitrate leaching from forest soil (Persson and Wirén, 1995; Rudebeck and Persson, 1998). In the samples from the uppermost mineral soil layer of the infiltration plots, net nitrification was similar or higher than that in the humus layer, and the (NO_2+NO_3)-N concentrations were about double those in the humus layer (per cm^{-3} soil) (V). This shows that the mineral soil provided a suitable habitat for nitrifiers. The difference in (NO_2+NO_3)-N concentrations between the humus and mineral soil layers could also be explained by the increased biomass of grasses on the infiltration plots (Helmisaari *et al.*, 1998, 1999). Grasses take up nitrate in preference to ammonium (Falkengren-Grerup and Lakkenborg-Kristensen, 1994). Most of the grass roots will be present in the humus layer, leaving the nitrate in the mineral soil beyond the uptake of grasses. Conversely, in the Patasalo experiment the net production of nitrate was always lower in the mineral soil both before (CaN plot, Smolander *et al.*, 1995) and after clear-cutting (Smolander *et al.*, 1999).

Rudebeck and Persson (1998) showed that nitrification was more pH dependent in the humus layer than in the mineral soil. In our study we only examined the pH dependency of nitrification in the humus layer (III, V). However, in the Ahvenisto experiment, nitrate was not detected in the mineral soil before the initiation of infiltration treatment (results not presented) nor in the control plots (V), but after soil pH had increased to about 6.5 due to infiltration, nitrate production was initiated. This suggests that the pH dependency was similar in mineral soil or, at least, that acid-tolerant nitrifiers were not abundant in the mineral soil either.

In the Ahvenisto infiltration experiment both denitrification enzyme activity (DEA) and the rate of denitrification were higher in the humus layer than in the mineral soil (per cm^{-3} soil) in both treated and control plots (V). Henrich and Haselwandter (1997) found denitrification to be considerably higher in the humus layer of an acidic Norway spruce forest stand than in the mineral soil (per g dry weight), and they attributed this to the higher nitrate concentration of the humus layer. In our study, however, the nitrate concentrations were higher in the mineral soil. In terms of substrate availability, denitrification would be therefore expected to occur more freely. The difference in N_2O production between the layers is probably explained by the greater availability of organic carbon in the humus layer than in the mineral soil, as also suggested by Regina *et al.* (1998a).

4.7. Nitrogen losses

4.7.1. Nitrogen leaching

Nitrate concentrations are usually extremely low (< 0.2 mg NO_3-N l^{-1}) in the soil solution of undisturbed forests in Finland (Soveri and Ahlberg, 1990; Lindroos *et al*., 1995; Starr *et al*., 1995; Piirainen *et al*., 1998; Derome *et al*., 1999). Nitrate leaches more readily to groundwater than ammonium because the anion absorption capacity of forest soils is low and nitrate has a low affinity for anion exchange sites. High nitrate concentrations in the groundwater used for drinking water can pose a threat to human health. The Finnish Ministry of Health in 1994 uses a critical limit for nitrate-N in household drinking water of 6 mg l^{-1}.

Before clear-cutting in Patasalo experiment, increased nitrification was reflected as increased nitrate concentrations in the soil percolation water collected under the humus layer (Smolander *et al.*, 1995). The highest nitrate-N concentrations in percolation water collected under humus layer, summer average about 8 mg l^{-1}, were observed when nitrogen and lime were added together. The ability to nitrify is an important characteristic related to nitrate leaching, even though some of the leached nitrate may originate from deposition, especially in N saturated forests (Gundersen *et al.*, 1998). Dise and Wright (1995) reported that nearly 70% of the variation in the output of N in European forests was explained by the deposition of N in throughfall. In forest soils in southwest Sweden Nohrstedt *et al.* (1996) found that leaching was related more to soil conditions than to nitrogen deposition; elevated leaching occurred at the site having the highest nitrification potential and a low C:N ratio. According to Gundersen et al (1998) the reason why N input and output are coupled at some sites and uncoupled at others is the difference in the N status of the forests (low N status meaning that the forests are N limited and high N status that the forests are N saturated by the deposition). Nitrate leaching will occur at high N status even with moderate N deposition but at low N status high N deposition may still be retained, at least for several years.

Because of the initiation of nitrification in Patasalo experiment (I) (Table 2) clear-cutting also resulted in appreciable nitrate leaching in the unfertilized soils (Smolander *et al.*, 1998, 1999). Nitrate losses from clear-cut areas have been reported in several forest ecosystems (*e.g.* Tamm *et al.*, 1974; Lepistö *et al.*, 1995; Kubin, 1998).

In the Ahvenisto infiltration experiment the mean (NO_2+NO_3)-N concentration in percolation water (below humus layer and at a depth of 40 cm and 100 cm) during infiltration was close to that of the infiltration water (about 0.2 mg l^{-1}), but during breaks in infiltration the concentrations generally exceeded 10 mg l^{-1} (II). During infiltration the nitrate produced by the infiltration soils was diluted by the large amounts of infiltration water. Therefore the risk of nitrate leaching seems to be at it highest during breaks in infiltration.

Infiltration continued throughout the year on some of the plots and thus the concentrations in groundwater represent conditions during infiltration. The groundwater (NO_2+NO_3)-N concentration remained very low and close to the

average values for groundwater in Finland (mean 0.2 mg l^{-1}) (Lahermo *et al.*, 1990; Soveri and Ahlberg, 1990). As stated above, the high (NO_2+NO_3)-N concentrations produced by the infiltration soils was diluted by the large amounts of infiltration water. Thus it would appear that the leaching of nitrate does not pose a threat to the quality of groundwater at least as long as infiltration is continued in the irrigation area. However, this conclusion is based on a 3-year experiment, and the long-term effect of infiltration is not yet known. Moreover, if the soil pH remains at a relatively high level after the cessation of infiltration, nitrate will be produced to some extent and there will be a high potential risk of nitrate leaching from the soil in percolation water. The possible risk this poses to groundwater quality depends on the size of the infiltration area in relation to the whole aquifer.

Organic nitrogen can play an important role in the leaching of nitrogen from forest soil (Rosén and Lundmark-Thelin, 1987; Stevens and Wannop, 1987; Piirainen *et al*., 1998). Soluble organic matter in percolation water can include organic compounds derived from rainwater and throughfall, root exudates and the products of litter decomposition. About 80% of the nitrogen in percolation water collected from below the humus layer was in an organic form in the control plots in the Ahvenisto infiltration experiment (II). This was also observed in the Patasalo experiment before clear-cutting, particularly in the plots not given nitrogen (Smolander *et al.*, 1995). During breaks in infiltration at Ahvenisto experiment, the organic N concentration in percolation water below humus layer was at the same level in the infiltration and control plots. This suggests that the concentration of organic N leached from the infiltration plots during natural recharge (*i.e.* due to rainfall during the breaks in infiltration) was not higher than that from the control plots. Moreover, the organic N concentration in the groundwater was about half that in the infiltration water, implying that the esker retained organic N. In the Patasalo experiment, clear-cutting increased the leaching of organic nitrogen (Smolander *et al.*, 1995, 1999) as has also been observed in other studies (Sollins and McCorison, 1981; Vitousek and Mellilo, 1979).

4.7.2. N_2O fluxes

The increased production of N_2O in the groundwater recharge area of the Ahvenisto (II), even though it is a hazardous greenhouse gas, can be considered locally beneficial as it decreases nitrate concentration in the soil and, therefore, the risk of nitrate leaching into groundwater. However, the flux of N_2O-N to the atmosphere from the infiltration plots during one summer, approx. 0.02 g m^{-2}, was very small compared to the amount of nitrate added with the infiltration water ((approx. 200 g m^{-2} of (NO_2+NO_3)-N during one summer)) (II). Thus, during infiltration, N_2O production seemed to have only a very small effect on the N losses via leaching (II).

The mean daily flux of N_2O from the infiltration soils measured in the field at Ahvenisto during the growing season was 0.2 mg N m^{-2} day^{-1} (varying from 0.02 to 0.6) (II). This is similar to the daily N_2O-N flux measured during the growing

season from the clear-cut plots in the Patasalo experiment (Smolander *et al.*, 1998) but about 5 times higher than from a poorly drained Norway spruce forest in Sweden (Klemedtsson *et al.*, 1997) and about 10 times lower than that from a forested peatland in Finland (Regina *et al.*, 1998b).

N_2O may also dissolve in the water and thus be transported away by runoff water (Bowden and Bormann, 1986; Nieminen, 1998). This suggests that part of the produced N_2O may have been transported downward with the infiltration water. It was confirmed, however, that the lake water used for infiltration did not markedly contribute to the N_2O emissions measured from the soil.

As stated above (section 4.4.3), N_2O production in the Ahvenisto experiment was primarily derived from denitrification. N_2 was the main product of denitrification with only about 25% of the denitrification products being released as N_2O (V). Denitrification thus reduced the amount of nitrate in the soil primarily as N_2, *i.e.* in a form that is not harmful to the environment. Based on this data, the daily nitrogen losses due to denitrification (N_2 and N_2O) during the growing season in the areas subjected to infiltration can be roughly estimated to be about 1 mg N m^{-2} day^{-1}. However, one should be cautious in extrapolating results obtained from short-term laboratory incubations to field conditions.

5. Summary

Because of the tight cycling of nitrogen usual in boreal forests, it is difficult to ascertain the role of the various nitrogen transformation processes involved and the factors affecting them. The aim of the study was to investigate the response of nitrogen transformations in coniferous forest soils to extreme manipulation treatments so as to accentuate the nitrogen transformation processes and thereby gain a better understanding. One of the study sites, a 60-year old Norway spruce stand, was subjected to clear-cutting. During the 30 years before clear-cutting the stand had been repeatedly limed, fertilized with N, and given both treatments combined. At the other study site, dominated by Norway spruce, groundwater reserves were recharged artificially by sprinkling infiltration, *i.e.* sprinkling lake water (2000 times the annual rainfall) directly onto the forest soil. Most attention was paid to the factors regulating nitrification, since this is an important process determining the potential nitrogen losses from the soil.

Nitrogen fertilization alone or together with liming, clear-cutting and the sprinkling infiltration treatment all enhanced the net formation of mineral N, initiated net nitrification and increased N_2O production in the soil. After clear-cutting, however, previous N fertilization had no effect on net nitrification or even suppressed it. Thus although there is a risk of nitrogen mobilization after clear-cutting, it is not necessarily higher in soils also subjected to increased nitrogen inputs. The above results suggest that coniferous soils in Finland have a high capacity to retain added nitrogen even under such extreme conditions.

Nitrification was shown to be controlled by several factors. The main reason for the initiation of nitrification in soils subjected to the sprinkling infiltration treatment was the increase in soil pH. The initiation of nitrification after clear-cutting was probably due to the increase in soil pH and ammonium availability. The reduction in allelopathic inhibitors, monoterpenes, probably also played a role as nitrification was shown to be inhibited by exposure to monoterpenes at concentrations similar to those detected in the forest soil microair. Increased nitrate concentrations, soil pH and moisture, and the availability of organic matter all stimulated N_2O production, which was mainly derived from denitrification. At the sprinkling infiltration site denitrification was considered to be a positive phenomenon; it reduced the amount of nitrate in the soil primarily as N_2, *i.e.* in a form that is not harmful to the environment.

Mineral nitrogen concentrations in the groundwater at the sprinkling infiltration treatment site were very low. The nitrate produced in the soil was diluted by the large amounts of infiltration water. Therefore, continuous infiltration could be used to recharge groundwater without the risk of increasing groundwater nitrate concentrations to harmful levels. However, after the cessation of infiltration nitrate production continued, which may cause a high potential risk of nitrate leaching from the soil in percolation water. The possible risk this poses to groundwater quality depends on the size of the infiltration area in relation to the whole aquifer.

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