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

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Preface

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Vantaa, September, 1999

Contents

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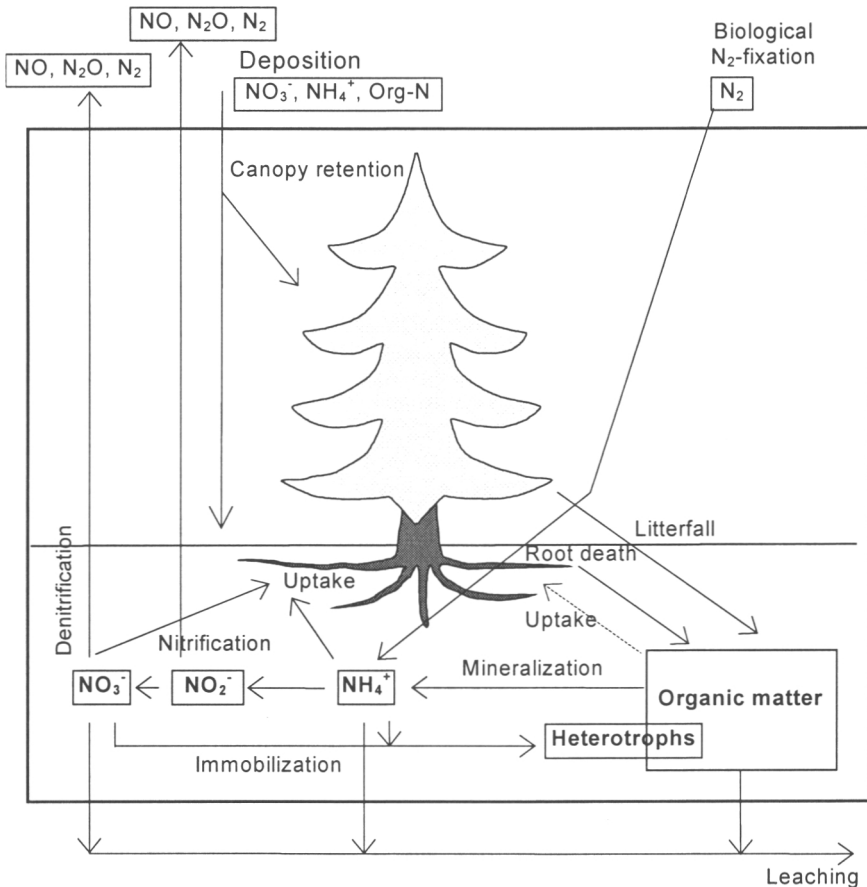
Figure 1. N cycling in a boreal forest ecosystem (modified from van Miegroet and Johnson, 1993)

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).

$$NH_4^+ + O_2 + H^+ \rightarrow NH_2OH + H_2O \rightarrow NO_2^- + 5H^+$$
$$NO_2^- + H_2O \rightarrow NO_3^- + 2H^+$$
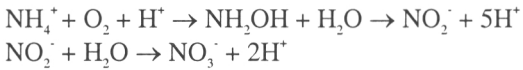
The NH_4 oxidizers found in the soil belong to the genera *Nitrosospira, Nitrosomonas, Nitrosolobus, Nitrosovibrio* and *Nitrosococcus*, while the genus *Nitrobacter* is regarded as the dominant NO_2 oxidizer (Watson *et al.*, 1981; Laanbroek and Woldendorp, 1995). However, Head *et al.* (1993) proposed, 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

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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

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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)

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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 20-25° 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)	61°51'N/29°22'E	61°01'N/24°47'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$_2$O)[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)

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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.

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 (14°C) 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

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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$_2$O 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

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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. N₂O 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

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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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Paper I

I

Pergamon

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NITROGEN AND CARBON TRANSFORMATIONS BEFORE AND AFTER CLEAR-CUTTING IN REPEATEDLY N-FERTILIZED AND LIMED FOREST SOIL

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Summary—Nitrogen and carbon transformations were monitored in a Norway spruce (*Picea abies* L.) stand in the summer before clear-cutting, and for the following three summers. During 30 y before the clear-cutting the stand had been repeatedly limed (total 6 t limestone ha^{-1}), fertilized with N (total about 900 kg N ha^{-1}), and both treatments were combined. Aerobic incubation experiments in the laboratory showed that, before clear-cutting, nitrification took place only in the soil that had been both limed and N-fertilized. Clear-cutting increased soil pH and net formation of mineral N, and initiated nitrification in all soils. These effects were observed throughout the study period. The only exception was the soil that had been both limed and N-fertilized, where the effect of clear-cutting on these N transformations was negligible or even suppressive. Generally, the greatest response in N transformations to clear-cutting was observed in the control soil. There was a small increase in microbial biomass C and N, and C mineralization in the first summer after clear-cutting. Net formation of mineral N correlated positively with pH at a lower pH range (pH 3.9–4.9) and negatively at a higher pH range (pH 4.9–6.9). C mineralization correlated positively with microbial biomass C, but there was no linear relationship between net formation of mineral N and microbial biomass N. C mineralization and net N mineralization were not correlated. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Atmospheric input of nitrogen to forest ecosystems is increasing, and an understanding of its effects is needed. Because of the many chemical components in the deposition, conclusions about the effects of nitrogen are difficult to make from sites receiving increased input of nitrogen from the atmosphere. In soils, the effects of extra nitrogen input may be evaluated from long-term N fertilization experiments (Mälkönen *et al.*, 1990; Martikainen, 1996).

The nitrogen cycle in undisturbed coniferous ecosystems is relatively closed, and the ecosystem is very efficient at retaining nitrogen (Gosz, 1981). Most of the nitrogen applied by fertilization also appears to be retained in boreal forest ecosystems (Melin and Nömmik, 1988), and losses via leaching or emissions of nitrogen oxides are small (Aarnio *et al.*, 1995; Smolander *et al.*, 1995; Martikainen, 1996). Nitrification is a crucial process which may lead to N losses, since nitrate in excess of plant

uptake is leached easily, or may be lost in the form of nitrogen oxides. The response of forest soils to nitrogen input may vary depending on soil pH. Nitrification appears to be particularly strongly pH-dependent, and liming has been shown to initiate this process (Priha and Smolander, 1995; Smolander *et al.*, 1995). There seem to be boreal forest soils receiving ammonium deposition, however, where acid-tolerant nitrification can also occur (Martikainen *et al.*, 1993).

Forest ecosystem disturbances, such as clear-cutting, may reduce plant uptake of nitrogen, and enhance nitrification and nitrogen losses. After clear-cutting the production of litter is reduced, but large amounts of logging residue and roots remain to be mineralized. In these situations, soil microbes have an even more important role than usually in nitrogen transformations, not only in nitrogen losses but also in retaining nitrogen in the ecosystem.

Our aim in this study was to maximize nitrogen mobilization: there had been a long-term increased N input, and a long-term pH increase (by liming) in a relatively fertile forest site, and the site was clear-cut. We studied nitrogen and carbon transformations before and after the clear-cutting.

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MATERIALS AND METHODS

Study site and the treatments

The study site was a 60-y-old Norway spruce (*Picea abies* L.) stand growing on mineral soil in Kerimäki, in the South-Eastern part of Finland (61°51′N/29°22′E, 85 m a.s.l.). According to the Finnish classification (Cajander, 1949), the site was *Oxalis-Myrtillus* type (OMT). The soil type was podzol, and the humus type was mor.

The stand was a subject of factorial fertilization experiment, established by the Finnish Forest Research Institute 34 y ago, before we began our study in 1992. The experimental plots were 30×30 m, but for 1994 onwards, 15×30 m. The treatments were liming (Ca), N fertilization (N), liming and N fertilization (CaN), and a control (0). In the Ca treatment, finely-ground limestone, totalling 6 t ha^{-1}, was applied twice, 34 and 12 y before this study. In the N treatment the plot had received N fertilization seven times, first as ammonium sulphate, then as urea and later as ammonium nitrate with dolomite, totalling 860 kg N ha^{-1}. The last application was 6 y before this study. Fertilization treatments have been described in more detail by Smolander *et al.* (1994).

The stand was clear-cut in January 1993. Logging residues (waste wood consisting of dead and living branches and stem tops) were evenly distributed on the surface of each clear-cut plot. In addition to the control plot (O) mentioned above, which was subjected to clear-cutting, there was a forested reference plot [O(for)] in the same stand. It was not clear-cut, and was included throughout the study.

Soil sampling and chemical analyses

Soil was sampled before clear-cutting in 1992, and after clear-cutting from 1993 to 1995. Samples were taken between May and October, three or four times during each summer. From 20 to 28 samples (core dia 50 mm) were systematically taken from the humus layer of each plot, and combined to give one composite sample. After removing green plant material, the samples were sieved (2.8 mm). Samples were stored in plastic bags at 4–6°C for not longer than 2 weeks before analysis.

Soil organic matter content was measured as loss-on-ignition at 550°C. Soil pH was measured in suspensions of soil in H_2O or 10 mM $CaCl_2$ (1/2.5, vol/vol). Total C and N were measured using an automatic CHN analyzer (CHN-600, LECO).

Determination of microbial activities and biomass

The analyses were done using three replicates of each combined soil sample.

N transformations were studied in aerobic laboratory incubation experiments at constant temperature (14°C) and moisture [60% of water holding capacity (WHC)] for 40 d, as described by Smolander *et al.* (1995). To calculate net ammonification and nitrification, initial NH_4–N and $(NO_2 + NO_3)$–N concentrations were subtracted from the corresponding post-incubation concentrations. Net formation of mineral N was estimated as the accumulation of NH_4–N and $(NO_2 + NO_3)$–N during the incubation.

C mineralization was evaluated as CO_2–C production in 2-week incubation experiments at 14°C and 60% WHC (Smolander *et al.*, 1994).

Microbial biomass N and C were determined using the fumigation-extraction method (FE), and microbial biomass C also using the substrate-induced respiration (SIR) method, as described by Smolander *et al.* (1994).

The N mineralization coefficient was estimated as the ratio of net formation of mineral N to the total soil N content (Weier and MacRae, 1993). The N efficiency factor was calculated as the ratio of net formation of mineral N to microbial biomass N (Bauhus and Barthel, 1995). Respiration-to-biomass ratio (qCO$_2$, Anderson and Domsch, 1993) was calculated as the ratio of the CO_2–C production to FE-derived microbial biomass C.

Calculation of the data

As the stand was rather fertile, different amounts of mineral soil were mixed in the humus layer, especially after the clear-cutting. Therefore the results are expressed on an organic matter (o.m.) basis.

Results are expressed as means of the three replicates at each sampling. In most cases the coefficient of variation was less than 5%. To see the effects of the treatments and clear-cutting more clearly, the mean of the three or four samplings in each summer (summer mean) was also calculated. Then the mean of forested reference plot [O(for)] was subtracted from the means of the other plots (O, Ca, N and CaN).

Pearson's correlation coefficients were used to detect if there were any linear relationships between the measured properties.

RESULTS

There were no replicate plots in the experiment and drawing conclusions should therefore be carried out with care. The stand had, however, been homogenous originally. The small or no differences in most variables between the control plot (O) and the forested reference plot O(for) before clear-cutting supported this. Only the differences between the treatments which were clearly larger than this difference (at least twice this difference) are considered here as differences. In some of the measurements there was a strong seasonal variation, which was not similar in each summer.
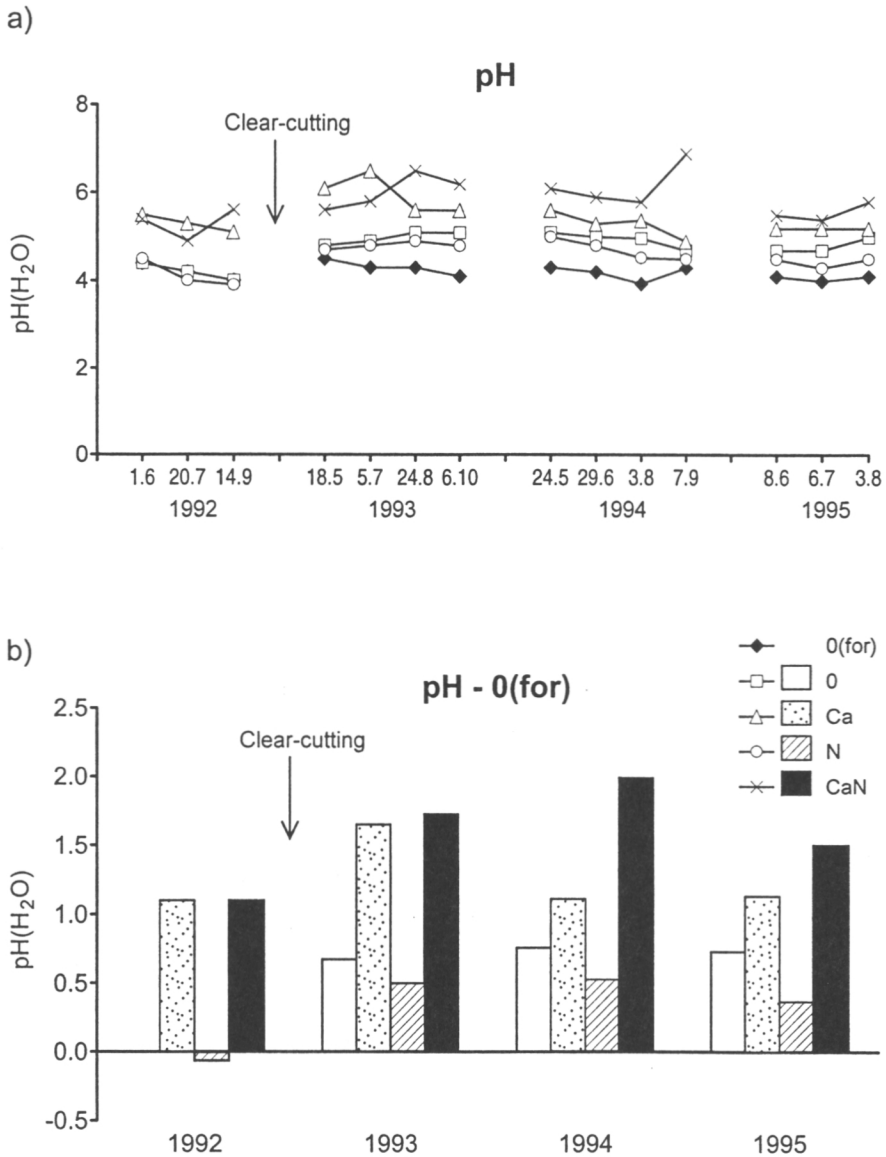
a)

b)

Fig. 1. (a) Development of soil pH(H_2O) before and after the clear-cutting. (b) The difference between the summer mean of the O, Ca, N and CaN plots and O(for) plot.

Soil pH

Before clear-cutting, pH(H_2O) was about 4.2 in unlimed soils and about one pH-unit higher in limed soils [Fig. 1(a,b)]. Clear-cutting increased the pH by 0.6–0.7 pH-units in the first summer, and pH remained slightly higher throughout the study period in all except the Ca plot. After the middle of the first summer after cutting, N fertilization had an opposite effect on pH in limed and unlimed soils: pH(O) > pH(N) but pH(Ca) < pH(CaN). Similar results were obtained with pH($CaCl_2$) except that the values were 0.4–0.9 pH-units lower than the pH(H_2O) values.

N transformations

Before the clear-cutting, there was notable net nitrification in the CaN plot only [Fig. 2(a,b)]. In general, clear-cutting increased net nitrification in all except the CaN plot, but the year in which the increase was most pronounced varied depending on the plot. In the first summer after clear-cutting, nitrification began in all clear-cut plots, first in the limed soils and later in the unlimed soils, while the forested reference plot continued without nitrification activity. In the second summer, the clear-cut plots had a much wider range of nitrification activity. Nitrification was greatest in the control plot

a)

b)
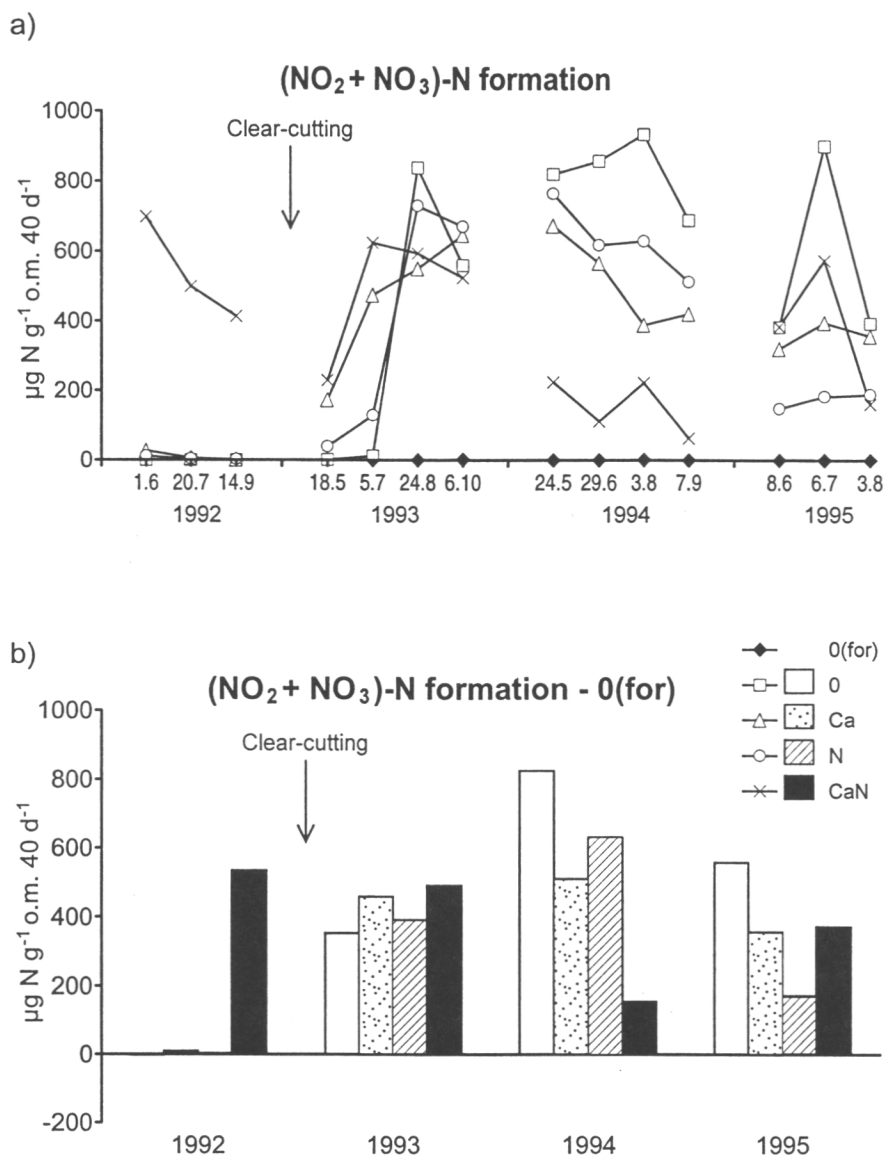
Fig. 2. (a) Development of net nitrification before and after the clear-cutting, determined in 6-week incubation experiments at constant temperature (14°C) and moisture (60% WHC). (b) The difference between the summer mean of the O, Ca, N and CaN plots and O(for) plot.

(O) and lowest in the CaN plot. During the third summer there were differences between samplings, but the O plot again showed the highest mean nitrification activity.

In 1992, net formation of mineral N was higher in the N-fertilized plots than in the others [Fig. 3(a,b)]. Clear-cutting increased net N formation in all plots except the CaN plot, and this effect lasted throughout the study, and was strongest in the O plot. In the CaN plot, net formation of mineral N did not increase after clear-cutting. In the second summer after clear-cutting, net formation of mineral N was as low in the CaN plot as

in the O(for) plot. Liming appeared to decrease the net formation of mineral N (Ca < O, CaN < N).

There was a positive correlation between net formation of mineral N and nitrification ($r = 0.75$, $P = 0.000$) [Fig. 4(a)]. There was a weak positive correlation between nitrification and pH ($r = 0.39$, $P = 0.001$, $n = 70$) [Fig. 4(b)], but, when the whole dataset was divided into half according to pH ($n = 35$), the positive correlation was much stronger at pH ≤ 4.9 ($r = 0.66$, $P = 0.000$). At pH ≥ 4.9 the correlation, if any, tended to be negative ($r = -0.30$, $P = 0.082$). For the whole dataset ($n = 70$), net formation of mineral N did not show any linear re-

a)

Net formation of mineral N
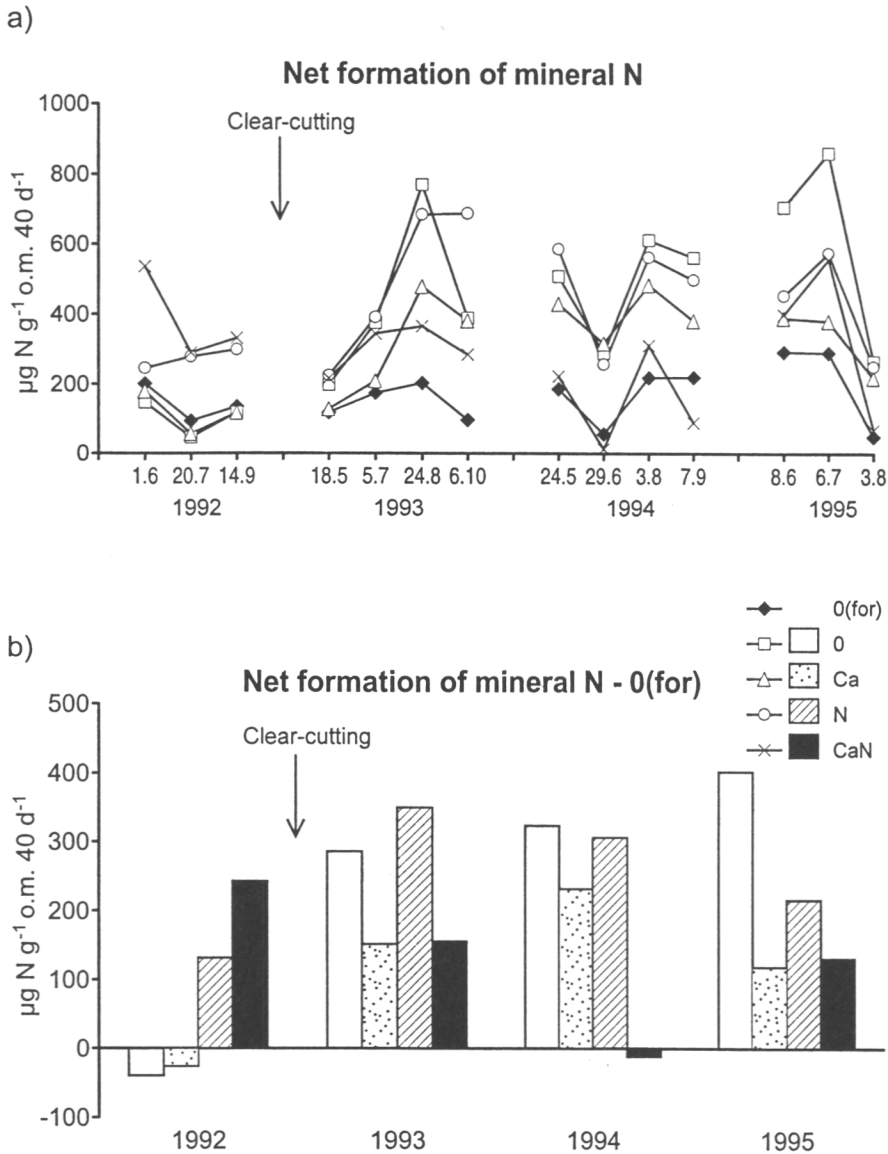

b)

Net formation of mineral N - 0(for)

Fig. 3. (a) Development of net formation of mineral N before and after the clear-cutting, determined in 6-week incubation experiments at constant temperature (14°C) and moisture (60% WHC). (b) The difference between the summer mean of the O, Ca, N and CaN plots and O(for) plot.

lationship with pH ($r = 0.05$, $P = 0.687$) [Fig. 4(c)]. When only clear-cut plots were included ($n = 44$), the correlation was negative ($r = -0.53$, $P = 0.000$). For pH ≤ 4.9, there was a positive correlation ($r = 0.50$, $P = 0.002$), and for pH ≥ 4.9 the correlation was negative ($r = -0.48$, $P = 0.003$).

At all samplings before the clear-cutting, microbial biomass N was lowest in the N plot [Fig. 5(a,b)]. Clear-cutting slightly increased the summer means of microbial biomass N in all plots in the first summer. The increase was most pronounced in the N plot. There was no relationship between net formation of mineral N and microbial biomass N ($r = -0.14$, $P = 0.249$).

Soil total N concentration and C-to-N ratio were determined for one sampling of each summer only (Table 1). C-to-N ratio was slightly lower in N-fertilized plots than in unfertilized ones every summer. Before clear-cutting, the N mineralization coefficient was highest in N-fertilized plots. Clear-cutting increased it in all but the CaN plot. Microbial biomass N accounted for 4.4 to 9% of soil total N. No clear effects of clear-cutting on this proportion could be observed.

Before clear-cutting, the N efficiency factor was highest in N-fertilized plots [Fig. 6(a,b)]. Clear-cutting increased it considerably, and the effect was seen for the whole study period. As with net for-
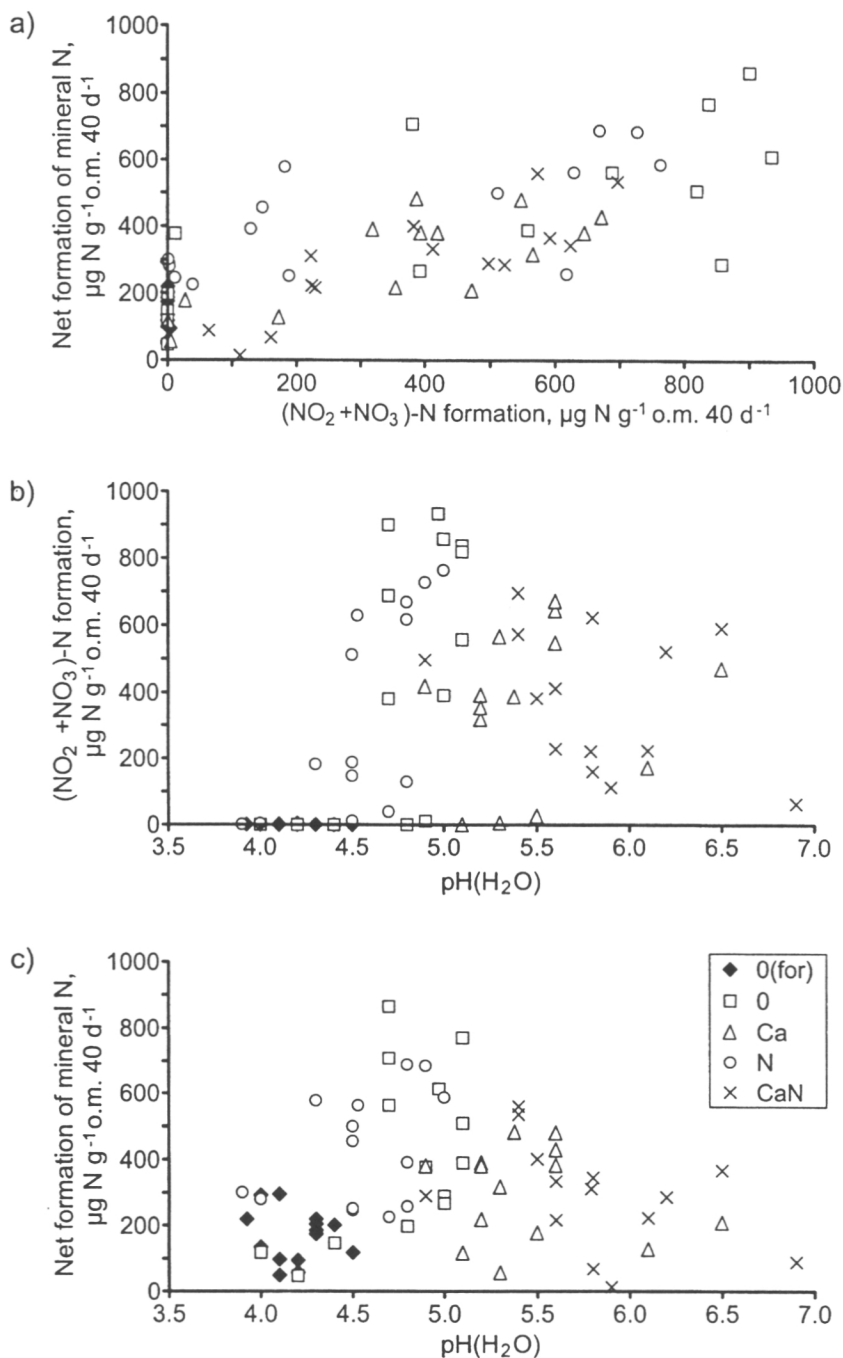
Fig. 4. (a) Relationship between net nitrification and net formation of mineral N; (b) between net nitri-
fication and pH; (c) between net formation of mineral N and pH.

mation of mineral N, the CaN plot was an excep-
tion. There was no linear relationship between N
efficiency factor and pH for the whole dataset, but
when the data were divided according to pH, the re-
lationship was similar to that between net for-
mation of mineral N and pH (at pH ≤ 4.9 $r = 0.46$,
$P = 0.005$, and at pH ≥ 4.9 $r = -0.47$, $P = 0.004$).

C transformations

For all samplings, there were no differences in C
mineralization between the treatments larger than
the difference between O and O(for) [Fig. 7(a,b)].
Clear-cutting stimulated C mineralization in all
plots, but this effect was evident only in the first
summer. In the control plot the mineralization

a)

Microbial biomass N

b)

Microbial biomass N - 0(for)
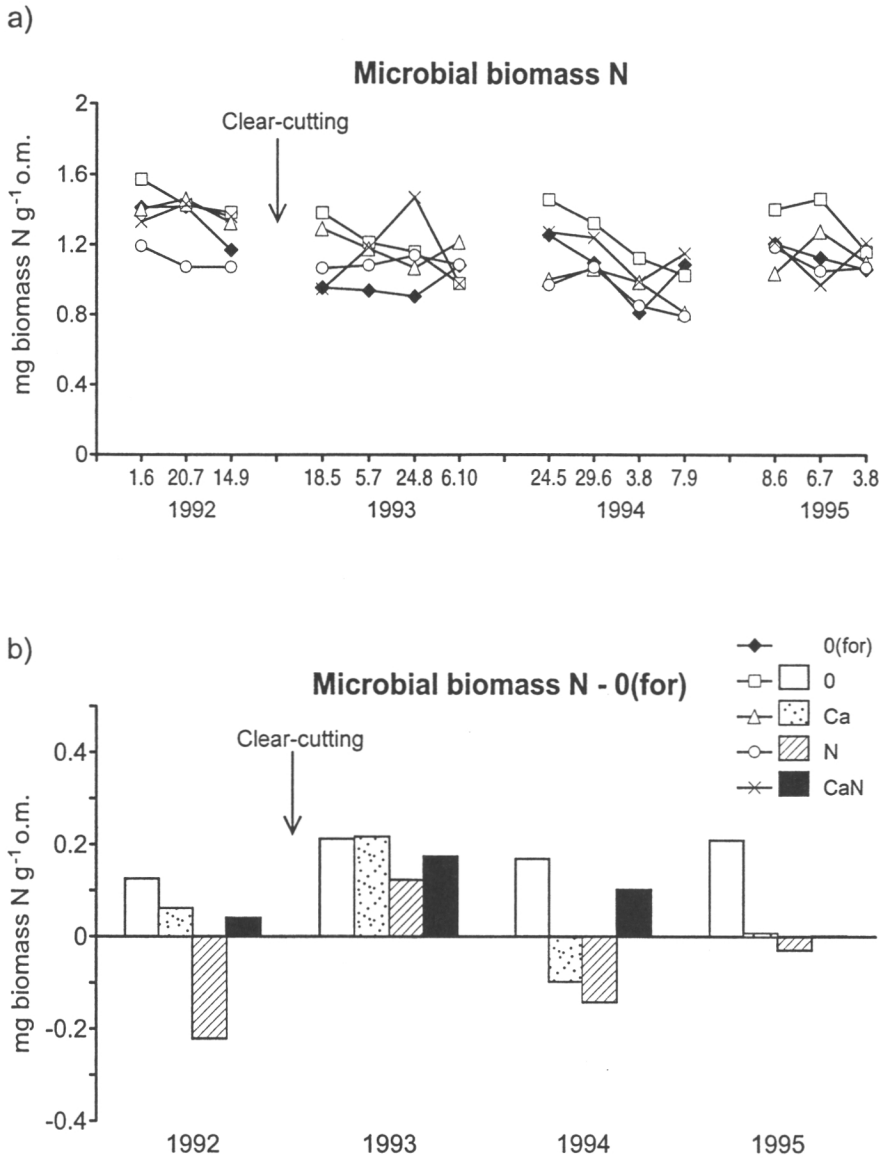
Fig. 5. (a) Development of microbial biomass N before and after the clear-cutting. (b) The difference between the summer mean of the O, Ca, N and CaN plots and O(for) plot.

almost doubled. C mineralization showed some correlation with pH ($r = 0.37$, $P = 0.001$). There was no relationship between C mineralization and net formation of mineral N ($r = -0.06$, $P = 0.645$).

No differences bigger than the difference between O and O(for) were observed in the FE-derived microbial biomass C between the treatments over the three samplings in 1992 [Fig. 8(a,b)]. There appeared to be a small increase in biomass C in the first summer after clear-cutting, but no clear response was observed after that. SIR-derived microbial biomass C was highest in limed plots and lowest in the N plot before clear-cutting [Fig. 9]. Clear-cutting increased SIR-derived biomass C during the first summer in all except the Ca plot.

The decreasing effect of N additions on biomass C was seen also after clear-cutting, and the order between plots remained the same as before cutting. Microbial biomass C correlated positively with C mineralization ($r = 0.66$, $P = 0.000$ and $r = 0.72$, $P = 0.000$, for FE- and SIR-derived microbial biomass C, respectively).

Respiration-to-biomass ratio (qCO_2), calculated from FE-derived biomass C, did not differ much between plots [Fig. 10(a,b). Clear-cutting increased it, but in the first summer only. There was a weak positive correlation between qCO_2 and pH ($r = 0.39$, $P = 0.001$).

Microbial C-to-N ratio, calculated from FE-derived microbial biomass C and N, varied from 4.5

Table 1. Organic matter (o.m.) and total N concentrations, C-to-N ratio, N mineralization coefficient, and proportion of microbial bio-mass N of total N in humus samples from different plots before (14 Sept 92) and after clear-cutting (24 Aug 93, 7 Sept 94, and 6 July 95)

Characteristic and sampling date	O(for)	O	Ca	N	CaN
O.M. (% of d.m.)					
14 Sept 92	63.7	50.9	51.1	66.9	54.8
24 Aug 93	55.2	32.9	34.9	33.3	35.2
7 Sept 94	38.3	41.3	58.7	48.7	41.1
6 July 95	21.8	15.8	25.9	17.3	28.7
Total N (mg N g^{-1} o.m.)					
14 Sept 92	18.5	17.5	18.4	19.0	17.4
24 Aug 93	18.1	18.8	19.5	21.3	18.8
7 Sept 94	15.9	15.3	17.5	18.1	17.3
6 July 95	19.7	16.5	19.7	22.0	22.0
C-to-N ratio					
14 Sept 92	29.6	28.8	27.5	26.4	25.9
24 Aug 93	27.2	27.7	24.6	24.5	23.7
7 Sept 94	28.3	30.5	26.2	25.2	25.2
6 July 95	27.8	29.1	25.3	23.8	24.8
N mineralization coefficient (μ g min N mg^{-1} total N d^{-1})					
14 Sept 92	0.18	0.17	0.16	0.39	0.48
24 Aug 93	0.28	1.02	0.62	0.80	0.49
7 Sept 94	0.35	0.92	0.54	0.69	0.13
6 July 95	0.37	1.31	0.48	0.66	0.64
Microbial biomass N (% of tot. N)					
14 Sept 92	6.30	7.90	7.18	5.63	7.82
24 Aug 93	5.00	6.16	5.50	5.35	7.85
7 Sept 94	6.89	6.67	4.66	4.43	6.62
6 July 95	5.74	8.88	6.49	4.82	4.44

Treatment symbols: O(for) = forested reference, O = control, Ca = liming, N = N fertilization, CaN = liming and N fertilization

to 8.5 (not shown). No clear trends were observed in the ratio.

DISCUSSION

Before clear-cutting, notable nitrification occurred only in the CaN plot. Thus both pH increase and N addition were necessary to initiate nitrification in this spruce stand, as was discussed by Smolander et al. (1995). After clear-cutting, nitrification began in all plots except the forested reference plot. Nitrification was originally, and remained, auto-trophic as it was inhibited by 2.5–3 Pa partial pressure of acetylene in soil samples taken before the clear-cutting (Smolander et al., 1995) and 3 y after it (Paavolainen and Smolander, unpublished data). Stimulatory effects of clear-cutting on nitrification have been observed in several forest ecosystems (Smith et al., 1968; Tamm et al., 1974; Vitousek and Matson, 1984; Duggin et al., 1991; Prescott et al., 1992; Dahlgren and Driscoll, 1994). Calculated net nitrification rates were in many cases higher than rates of net formation of mineral N, owing to nitrification of ammonium present at the start of the incubation (often a combination of relatively low ammonification rate, comparatively high initial ammonium concentration and a high nitrification potential).

The increase in nitrification after clear-cutting might result from the increase in pH and the increased availability of substrate, as net formation of mineral N increased (Fig. 3). In unlimed plots,

the increase in pH might partly explain the increase in nitrification. The positive correlation between nitrification rate and pH at the lower pH range [pH(H_2O) $\leq$ 4.9] is in accordance with other studies (Tietema et al., 1992; Persson and Wiren, 1995). In soil suspension experiments, no acid-tolerant nitri-fiers [nitrate production at pH 4 according to De Boer et al. (1990)] could be found in these soils (Paavolainen and Smolander, unpublished data), and nitrification occurred only at soil pH higher than 4.3 in the incubation experiments described here.

Effects of clear-cutting on ammonification and nitrification can differ depending on site character-istics. Clear-cutting increased net N mineralization in deciduous forest soils, while no consistent re-sponse was observed in coniferous forest soils (Van Cleve et al., 1993; Kim et al., 1995). Large and rapid nitrate losses were observed from clear-cut areas on fertile sites in Sweden, while lower quality sites had low or delayed nitrate losses (Wiklander, 1981). Unexpectedly, the previous N fertilization did not affect nitrification or net formation of min-eral N, or the effect was even suppressive as in 1995. In a Swedish study, previous fertilization with N in a pine stand increased nitrogen leaching (mainly nitrate) after clear-felling, but only when the fertilizer rate was very high (more than 1 t N ha^{-1}) (Ring, 1995). It can only be speculated what were the reasons for the even suppressive effect of the previous N additions on N transformations after clear-cutting in our study. A slightly lower pH
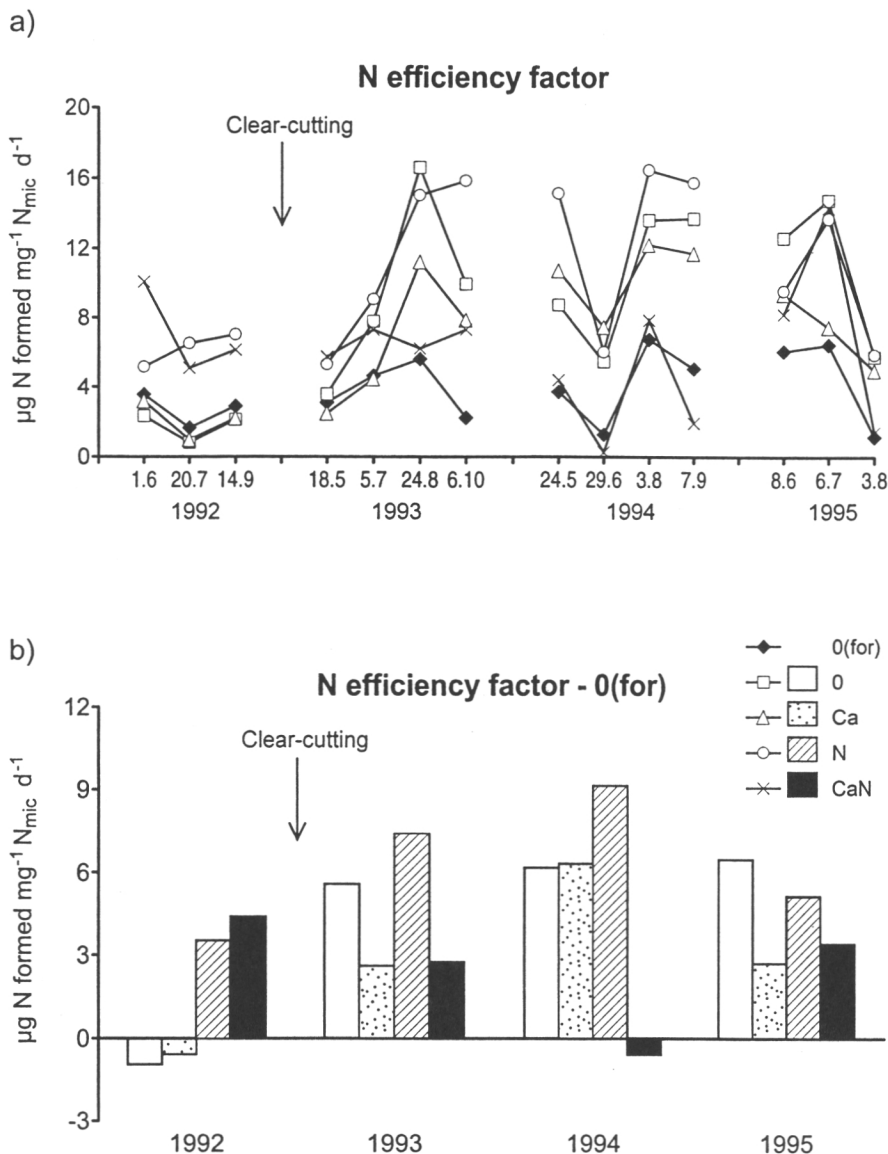
a)

b)

Fig. 6. (a) Development of N efficiency factor before and after the clear-cutting. (b) The difference between the summer mean of the O, Ca, N and CaN plots and O(for) plot.

compared to unfertilized control plot coincided with this phenomenon. At this low pH range, there was a positive correlation between pH and N transformations. Thus the drop in pH could be enough to partly inhibit the activity of nitrifiers which were already very close to the lowest pH they could tolerate. Differences in developing ground vegetation could also have an effect.

In the CaN plot where nitrification occurred already before clear-cutting, clear-cutting did not enhance net nitrification. The relatively low net nitrification rate compared to the other clear-cut plots is most probably due to the low rate in net N mineralization, i.e. ammonium availability, since there were large numbers of nitrifiers in these soils,

shown by both Most Probable Number (MPN) counts and nitrification potential experiments (Paavolainen and Smolander, unpublished data). Either ammonium was not formed fast enough during the incubations for nitrification, or mineral N (either ammonium- or nitrate-N) was immobilized. Soil pH was highest in this plot and, at this pH range, there was a negative correlation between pH and net formation of mineral N. Immobilization could be an important reason since heterotrophic microbes often out-compete nitrifying bacteria for ammonium (e.g. Robertson and Vitousek, 1982), but microbial biomass N data did not support this assumption. In the clear-cut area in general, nitrification activity correlated well with

a)

C mineralization

b)

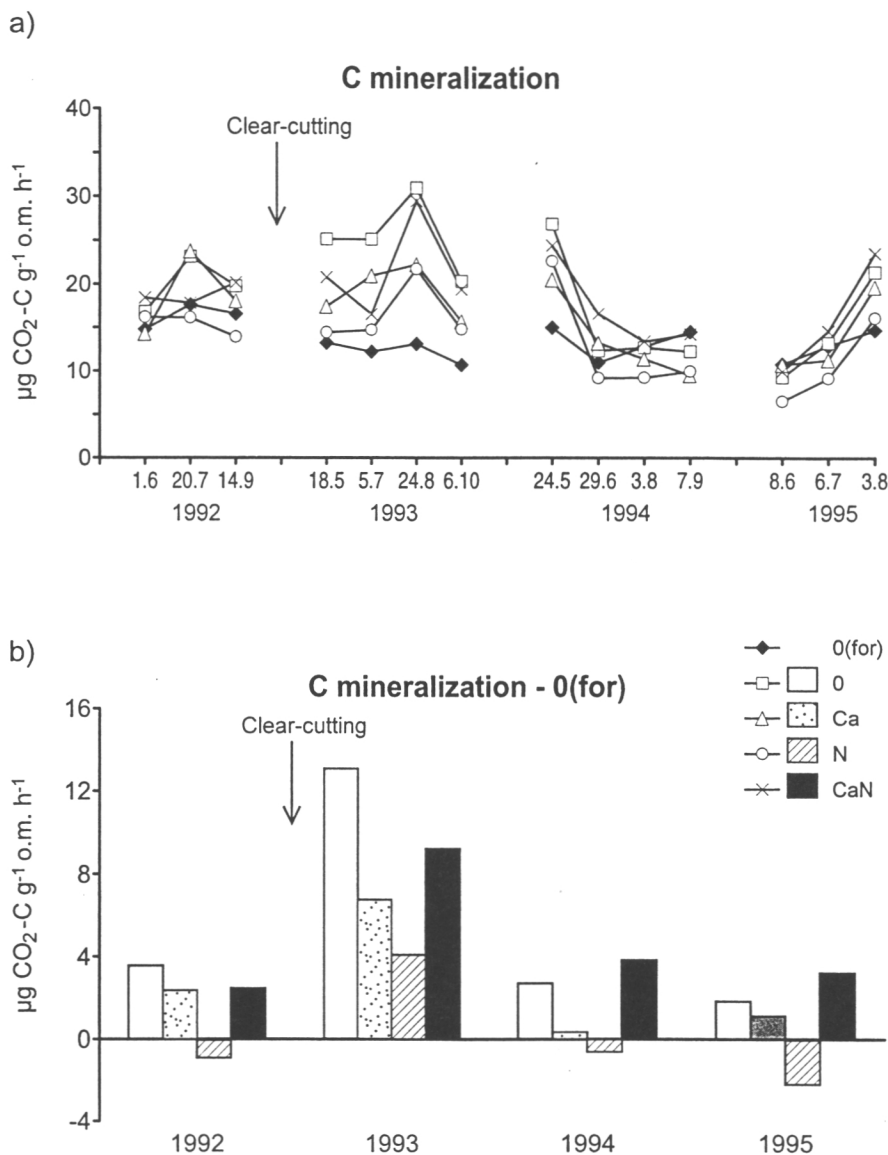## C mineralization - 0(for)

Fig. 7. (a) Development of C mineralization before and after the clear-cutting, determined in 2-week incubation experiments at constant temperature (14°C) and moisture (60% WHC). (b) The difference between the summer mean of the O, Ca, N and CaN plots and O(for) plot.

the formation of mineral N during the incubation, as observed in other sites with high nitrification rates (Tietema *et al.*, 1994). Denitrification was not the reason for the low net nitrification, since conditions in the incubation experiments did not favour it (Paavolainen and Smolander, unpublished data).

Before clear-cutting, microbial biomass N and SIR-derived biomass C were lowest in the plot which had received N addition alone. Microbial biomass and C mineralization have been observed to decrease after N addition in many forest experiments, but the reasons for the decrease are not well understood (Söderström *et al.*, 1983; Nohrstedt *et al.*, 1989; Smolander *et al.*, 1994). Liming often

causes the opposite response, and even may compensate for the suppressing effect of N addition (Smolander *et al.*, 1994). The tendency of qCO_2 to increase with increasing pH was in contrast to the observations by Anderson and Domsch (1993) who showed the ratio to correlate negatively with natural soil pH over a pH range between 3 and 7.

Clear-cutting caused an immediate increase in microbial biomass C and C mineralization. There was a change in microclimate, increased availability of decomposable organic material in the form of dead roots and logging residue but, at the same time, a decrease in root exudates and mycorrhiza. The effect of clear-cutting or tree removal on microbial
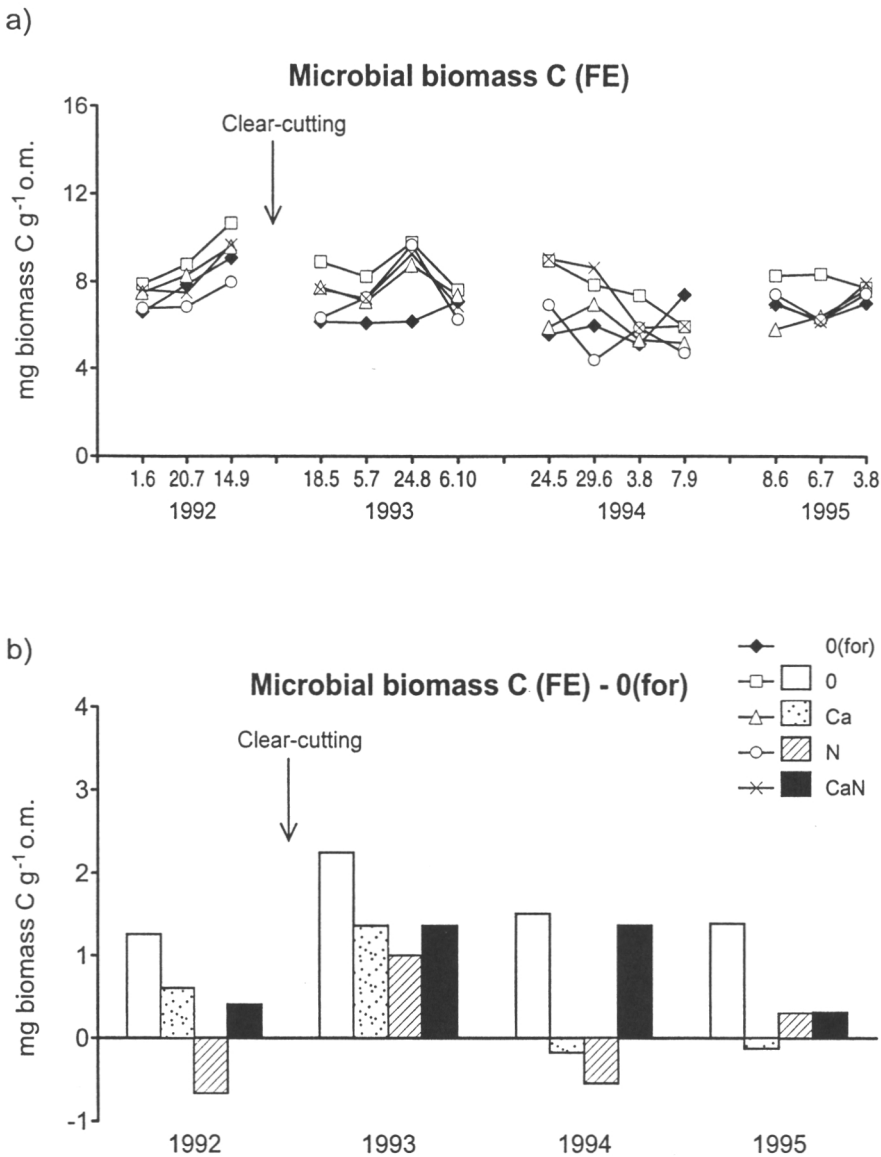
a)

b)

Fig. 8. (a) Development of FE-derived microbial biomass C before and after the clear-cutting. (b) The difference between the summer mean of the O, Ca, N and CaN plots and O(for) plot.

biomass and numbers, and C transformations appears to depend on the time elapsed since the cutting: an increase soon after the 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 due to cutting was observed during our 3-y study. As with N transformations, the response of microbial biomass to clear-cutting may differ depending on the site and its fertility. In our study, no consistent and clear differences between the treatments were observed in the response of microbial biomass and C mineralization to clear-cutting. For example, the

reduced microbial biomass in the N treated plot before cutting also was evident after it.

Microbial biomass N followed the same trends as biomass C. 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, the turnover rate of the biomass should be known. In any case, the role of microbial biomass should have been important before vegetation had developed, i.e. in the first summer after cutting, and in the early summers after that.
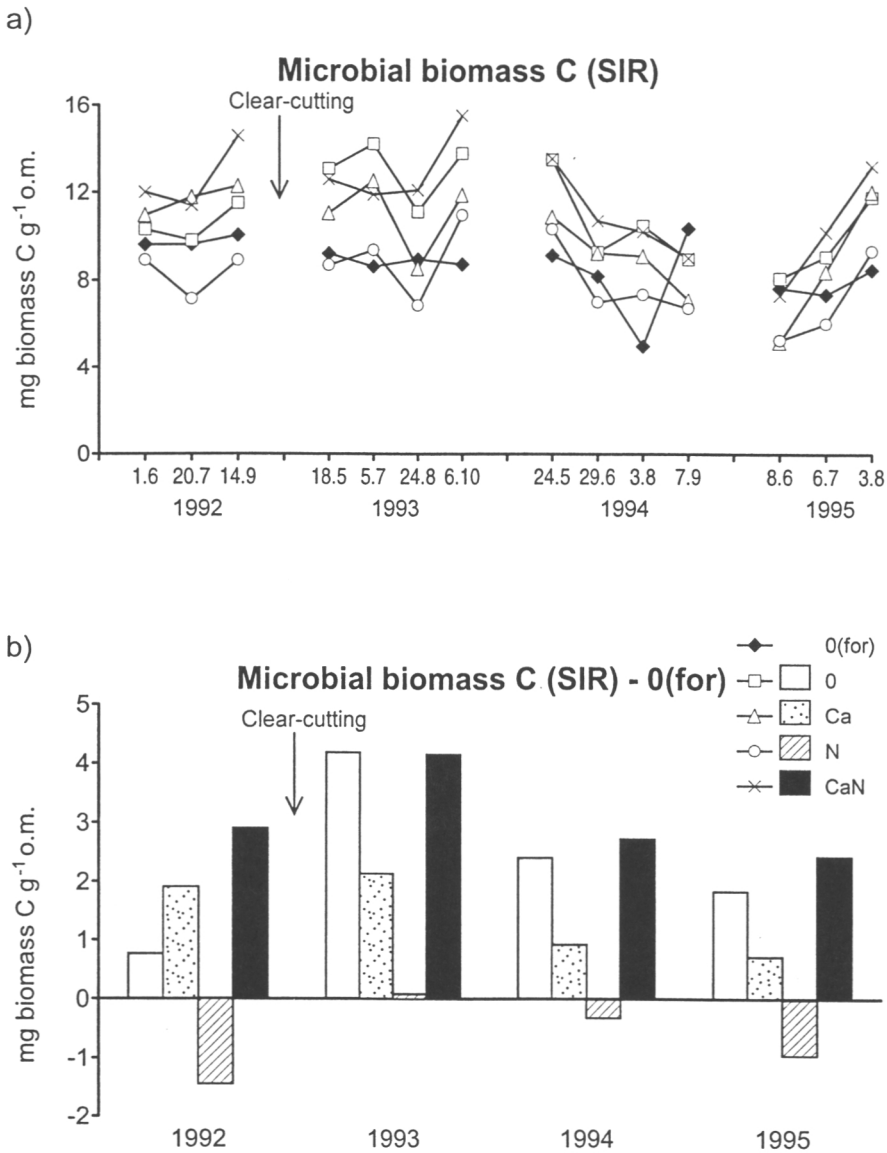
a)

b)

Fig. 9. (a) Development of SIR-derived microbial biomass C before and after the clear-cutting. (b) The difference between the summer mean of the O, Ca, N and CaN plots and O(for) plot.

Higher microbial biomass C or N corresponded with higher C mineralization. In contrast, changes in microbial biomass N or C did not explain net formation of mineral N. In accordance with the results of Bauhus and Barthel (1995), there was no significant relationship between these variables, nor did C and N mineralization correlate with each other. Accordingly, Hart *et al.* (1994) found no correlation between C mineralization and net N mineralization rates, but still found strong correlations between rates of C mineralization and gross N mineralization and immobilization.

In conclusion, clear-cutting initiated net nitrification in acid forest soil, in which it did not occur earlier unless it had been fertilized with nitrogen and limed. Previous long-term N fertilization and liming treatments did not seem to result in increased net nitrification rates after cutting. Increased net nitrification after cutting does not necessarily indicate leaching of nitrate as other processes, such as immobilization by microbial biomass and developing vegetation, can retain part of the nitrogen. In spite of this, there is a risk of nitrate leaching after clear-cutting, but it is not necessary higher in soil subjected to increased previous N input.

a)

qCO$_2$
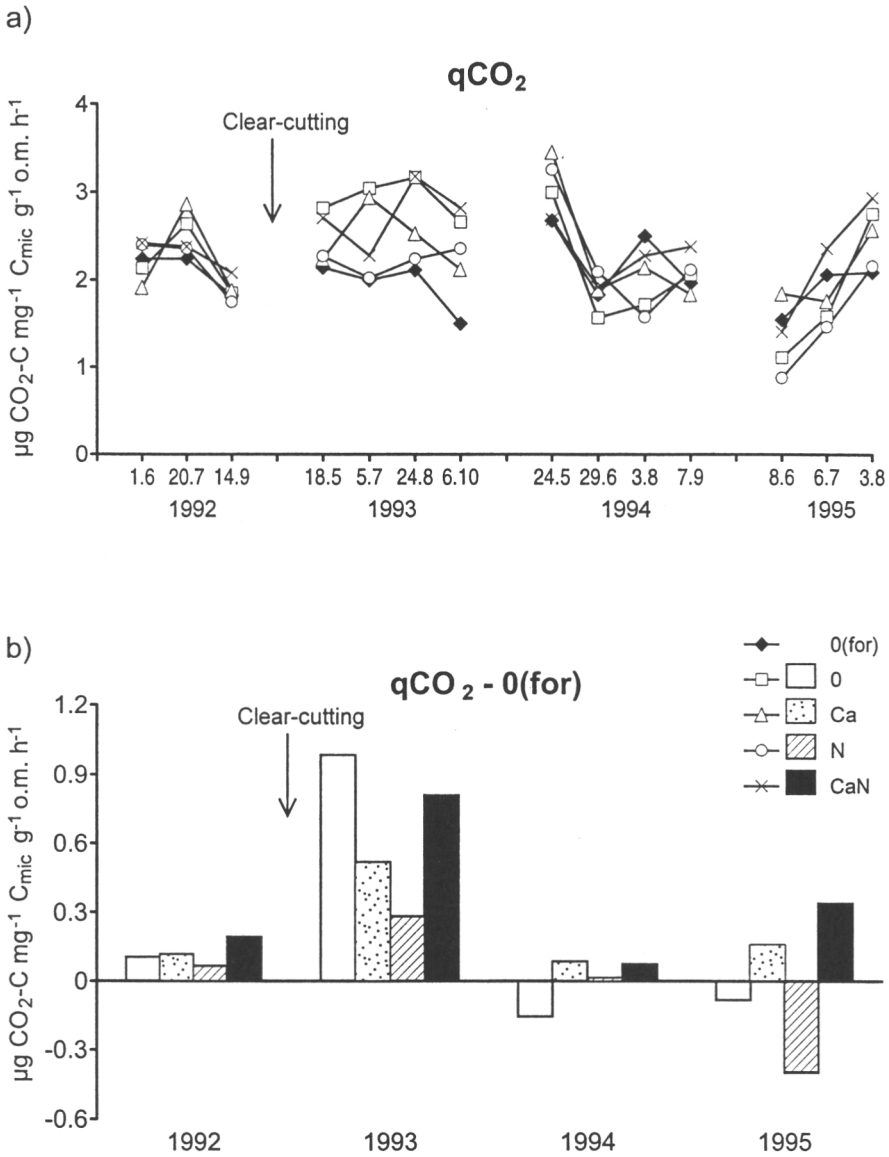

b)

qCO$_2$ - 0(for)

Fig. 10. (a) Development of qCO$_2$ before and after the clear-cutting. (b) The difference between the summer mean of the O, Ca, N and CaN plots and O(for) plot.

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Paper 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).

II

Nitrogen transformations and losses in forest soil subjected to sprinkling infiltration

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Abstract In Ahvenisto esker, southern Finland, artificial recharging of groundwater has been done by sprinkling infiltration i.e. by sprinkling lake water directly onto forest soil. The aim of the study was to investigate N transformations in the soil, especially nitrification, and N losses via leaching and gaseous emissions, during the first three years of infiltration. Already during the first year, the pH of the humus layer increased from about 5 to 6.7, and net nitrification started. Intensive nitrification continued throughout the study period. Although infiltration increased N_2O emissions from the soil, N_2O production during infiltration seemed to have only a very small effect on the N losses via leaching. The esker retained organic N. The mean (NO_2+NO_3)-N concentration in percolation water during infiltration was close to that of the infiltration water (about 0.2 mg/l) but, during breaks in infiltration, the concentrations generally exceeded 10 mg/l. The groundwater (NO_2+NO_3)-N concentration, however, remained very low (mean 0.2 mg/l) due to dilution of the nitrate, produced in the soil, by the large volume of infiltration water. The results of this three-year study show that the leaching of nitrate does not presently pose a threat to the quality of groundwater as long as infiltration is continuous. As nitrate was still being produced two years after cessation of infiltration, there is a high risk of nitrate leaching into the groundwater. The actual risk this poses to groundwater quality depends on the size of the infiltration area in relation to the whole aquifer.

Introduction

Groundwater will be used to an ever-increasing extent by urban water utilities in Finland in the near future. In urban areas groundwater reserves are preferred for a number of reasons. Groundwater usually better fulfils the quality requirements set on drinking water than surface water, which means that groundwater requires less chemical treatment (Hatva, 1996). The organic matter concentrations, for example, are normally relatively high in surface water in Finland compared to natural and artificially recharged groundwater. Water supplied to Finnish communities currently consists of 56 % groundwater, of which 6 % is artificially recharged. By the year 2010 it is expected that 75 % of the domestic water supply will originate from groundwater, of which 15-20 % will be artificially recharged (Hatva, 1996). The development of new forms of artificial recharging, 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 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 basin recharge.

Sprinkling infiltration has been carried out in the Ahvenisto esker in Hämeenlinna, southern Finland, from 1995 to 1998. Changes in soil properties were observed already during the first year (Lindroos et al., 1998; Helmisaari et al., 1998). The pH of the humus layer rose

from about 5 to 6.5 and net nitrification started. Emissions of N_2O from the soil also increased.

Nitrate is a potential source of contamination in groundwater owing to its high mobility. High nitrate concentrations in the groundwater can pose a threat to human health. According to the decision of the Finnish Ministry of Health in 1994, the critical limit for NO_3-N in household water is 6 mg/l. In addition, an increased rate of nitrification may result in increased N_2O emissions (Martikainen, 1996). N_2O, which can be produced in both nitrification and denitrification, is a more efficient greenhouse gas than CO_2, and also participates indirectly in the depletion of stratospheric ozone. In sprinkling infiltration areas, however, gaseous emissions of N can be considered positive phenomenon because they decrease the amount of nitrate likely to be leached from the soil.

Sprinkling infiltration may become an important method for artificial recharge. In addition to Hämeenlinna, other cities in Finland have also started using this method. However, there is no information about the environmental impacts of sprinkling infiltration. Knowledge of the changes in soil processes during and after infiltration is necessary for minimizing the possible negative environmental effects of this method. This study is part of a project being carried out to determine the effects of sprinkling infiltration on forest soil, percolation water and vegetation. In this paper we studied N transformations in the soil, especially nitrification and N losses via leaching and gaseous emissions during the first three years of infiltration. In addition, we assessed the risks of nitrate leaching into the groundwater during sprinkling infiltration and after the cessation of infiltration.

Materials and methods

Site Description. The study was carried out at the experimental sprinkling infiltration site in the Ahvenisto esker area, Hämeenlinna (61°01'N/24°47'E). The annual bulk NH_4-N, NO_3-N and total nitrogen deposition in 1996 and 1997 in this area was approx. 100, 200 and 400 mg m^{-2}, respectively (Lindroos et al., 1999). The esker formation is an important groundwater area. Artificial recharging of the groundwater was started in 1976 using infiltration basins. The quality of the 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 mgl^{-1}.

The sprinkling infiltration was carried out on a relatively steep slope. The forest site was of the fertile *Oxalis-Maianthemum* type (for the Finnish site classification, see Cajander, 1949). The soil texture was a spatial mixture with some areas of sandy till and some of gravel. The soil type was carbic podzol, and the humus type moder. The tree stand consists of a mixture of Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* L.). The percolation water zone of the esker was >10 m thick.

Surface water was obtained from a nearby lake and 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. The sprinkling infiltration (625 m^2) area was divided into seven plots and all except plot 6 were used in this study (Fig. 1, Table 1).

Different infiltration methods (i.e. periodical, summertime, wintertime, see Table 1) were used to find the optimal method for minimizing possible harmful effects, such as soil erosion. The dominant tree species on plots 2 and 3 was Scots pine, and on plots 5 and 6 Norway Spruce (Lindroos et al., 1998). For this reason there were two controls with no infiltration, plots 1 and 4, respectively. Each plot was further divided into 2-3 subplots (I, II and III) (Fig. 1). The amount of irrigation water applied to the site was more than 2000 times the annual precipitation of 600-650 mm (Table 1). Because the groundwater produced by sprinkling infiltration was used for the water supply of the city of Hämeenlinna, the application rate was adjusted by the waterworks.
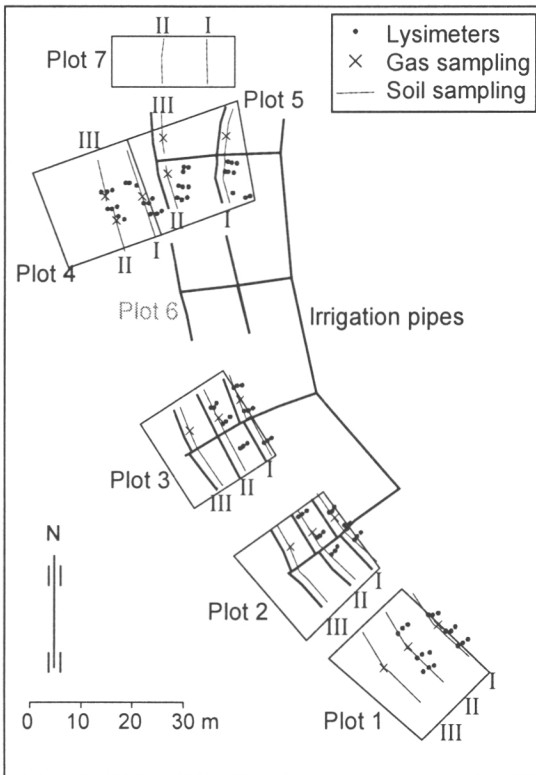
Figure 1. Map of the experimental site. Soil and gas samples were taken at a distance of 2 m from the irrigation pipe.

Table 1. Sample plots and the sprinkling infiltration treatments

Plot	Treatment	Time of irrigation	Irrigation amount m^3 m^{-2}
1	Control for plots 2,3		
2	Continuous infiltration during the summertime	25 June - 30 Oct. 1996	1118
		16 May - 6 Oct. 1997	1258
		18 May - 29 Oct. 1998	1452
3	Periodical infiltration during the summertime	12 June - 12 July, 12 Aug. - 12 Sept. 1996	492
		9 June - 21 July, 18 Aug. - 15 Sept. 1997	634
		8 June - 20 July, 17 Aug. - 18 Sept. 1998	607
4	Control for plots 5,7		
5	Continuous infiltration during the wintertime	14 Dec. 1995 - 3 June 1996	1778
		30 Oct. 1996 - 16 May 1997	1726
		30 Oct. 1997 - 18 May 1998	1669
7	Cessation of infiltration after the 1st year	14 Dec.1995 - 30 Oct. 1996	3234

Soil Sampling. Samples were taken from the humus layer (thickness 5-10 cm) of plots 1-5 two-to-three times during the growing season in 1996-1998. Plot 7 was sampled once or twice during the growing season in 1997 and 1998. 20 to 30 samples (core dia 25 mm) were taken systematically from each subplot (I, II and III, see Fig. 1), and bulked to give three composite samples per plot. In the laboratory green plant material was removed, and the samples were sieved (2.8 mm) and stored in the dark at 4° C for less than 2 weeks before analysis.

The spatial variation in pH was determined on the wintertime infiltration plot. The samples were taken from the humus layer at 2-m intervals; the samples were not sieved.

Determination of soil pH. Soil pH was measured in a water suspension (3:5, v:v, soil:H$_2$O).

Nitrogen transformations in the incubation experiments. Nitrogen transformations were studied using aerobic incubation experiments performed in the laboratory at constant temperature (14° C) and moisture (60% of the water-holding capacity) for 40 days using two replicate samples, as described by Smolander et al. (1995). Net nitrification and formation of mineral N were calculated by subtracting the initial NH_4-N and (NO_2+NO_3)-N concentrations from the final (post-incubation) concentrations.

Measurement of N$_2$O fluxes in the field. Fluxes of N$_2$O from the soil were measured by a static chamber method as described by Nieminen (1998). The measurements were made on each subplot (Fig. 1). Gas samples were taken with 50 ml polypropylene syringes equipped with a three-way stopcock 3, 15 and 30 minutes after the chambers had been installed in the soil. The samples were analyzed within 24 hours of sampling by gas chromatography

(Shimadzu GC-14B) equipped with electron capture and thermal conductivity detectors. N_2O flux was calculated from the linear increase or decrease in the gas concentration (Martikainen et al., 1995; Nieminen, 1998).

Water samples. Samples were taken of infiltration water, percolation water and groundwater. Percolation water was collected below the humus layer using plate lysimeters (6 replications per plot), and by means of suction-cup lysimeters (6 replications per plot) at depths of 40 and 100 cm below the ground surface (Fig. 1). Samples were taken daily at the beginning of the study, but weekly during 1997 and 1998. Groundwater was sampled from a pipe (depth 13.5 m) drilled down to the groundwater in the infiltration area. The NH_4-N, (NO_2+NO_3)-N and total N concentrations were determined by flow injection analysis (FIA). Organic N was calculated as the difference between total N and mineral N.

Statistical analyses. The means of the results from the infiltration plots were compared with those of the control plots by "ANOVA for repeated measures" analysis, in order to determine the overall effect of infiltration on soil nitrogen dynamics (Tabachnick and Fidell, 1989). The different sampling dates were used as the repeated measures. The values from the first sampling were not included because infiltration was not applied on plots 2 and 3 at that time. The data were log-transformed when necessary. Differences between the means were considered statistically significant when $p < 0.05$.

Results and discussion

Soil pH. The pH of the samples from the humus layer of the control plots was about 5 (Fig. 2). Soil pH on the infiltration plots rose to about 6.5 after the start of infiltration (Fig. 2) due to the relatively high pH of the infiltration water (> 7, Lindroos et al., 1998). These samples were collected two meters down slope from the irrigation pipe. There was high variation in soil pH within the plot (Fig. 3). The infiltration water was sprinkled only about one meter up and down slope from the pipe, and the pH of the soil decreased as the distance from the pipe increased (Fig. 3).
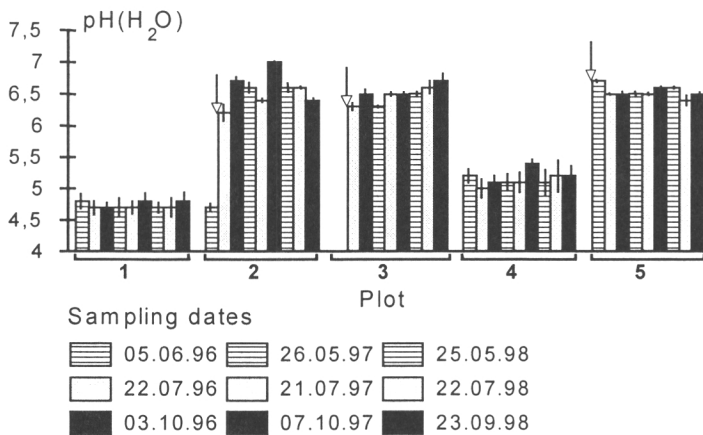

Figure 2. The pH of the samples from the humus layer of plots 1-5 (see Table 1). The results are the means of three subplots (see Fig. 1) (± SEM). Arrows show the initiation of infiltration.

Distance (m)

	2	4	6	8	10	12	14	16	Average
2	6.0	5.4	5.9	5.1	5.8	5.8	6.2	5.9	5.8
4	6.7	6.5	6.6	6.5	6.5	6.5	6.5	6.5	6.5
6	6.6	6.7	6.2	6.7	6.6	6.4	6.4	5.7	6.4
8	6.6	6.7	6.5	6.5	5.9	6.5	6.4	5.0	6.3
10	5.8	-	6.5	6.0	5.5	6.6	4.5	5.5	5.7
12	-	-	5.3	5.3	5.9	5.8	5.8	-	5.6
14	5.9	6.2	5.6	6.3	6.0	6.4	6.0	5.8	6.0
16	6.6	6.5	6.3	6.5	6.3	6.4	6.6	6.5	6.5
18	5.2	4.9	4.9	5.3	5.2	5.8	5.6	5.3	5.3

Irrigation pipe

Figure 3. Spatial variation in the pH of the humus layer on plot 5.

Nitrogen transformations. NH_4-N concentrations in the samples from the humus layer of the infiltrated plots tended to be higher than those of the control plots ($p < 0.06$) (Fig. 4a). (NO_2+NO_3)-N was present only in the soils from the infiltration plots after infiltration had started (Fig. 4b). This is due to the net production of (NO_2+NO_3)-N (Fig. 4c). Net formation of mineral N was significantly higher in the infiltration plots (Fig. 4d). Net nitrification is usually negligible in undisturbed coniferous soils in Finland (e.g. Smolander et al., 1995; Martikainen, 1996; Smolander et al., 1998). The effects of infiltration on net nitrification and formation of mineral N were similar to the effects reported for liming and/or nitrogen fertilization (Smolander et al., 1995) or clear-cutting (Smolander et al., 1998).

The increased NH_4-N concentrations in the infiltrated soils were due to the input of NH_4-N in the large amounts of infiltration water (Table 2) and increased ammonification (Fig. 4d). Measurements of net N mineralization in the laboratory provide an estimate of the pools of mineralizable N present at the time of sampling (Smolander et al., 1995). N mineralization in the infiltrated soils was probably stimulated by the enhanced moisture conditions, especially the wetting/drying cycles (Tietema et al., 1992; van Miegroet and Johnson, 1993) and by the high pH; adjusting the pH of the samples from the control plots to 6.7 with $CaCO_3$ increased N mineralization (Paavolainen et al., 1999). The increased availability of substrate ammonium and moisture, and the wetting/drying cycles probably stimulated nitrification (Tietema et al., 1992; van Miegroet and Johnson, 1993). However, the nitrifiers in the Ahvenisto esker were found to be acid-sensitive, and the increase in soil pH was therefore considered to be the main reason for the initiation of nitrification (Fig. 4c) (Paavolainen et al., 1999).
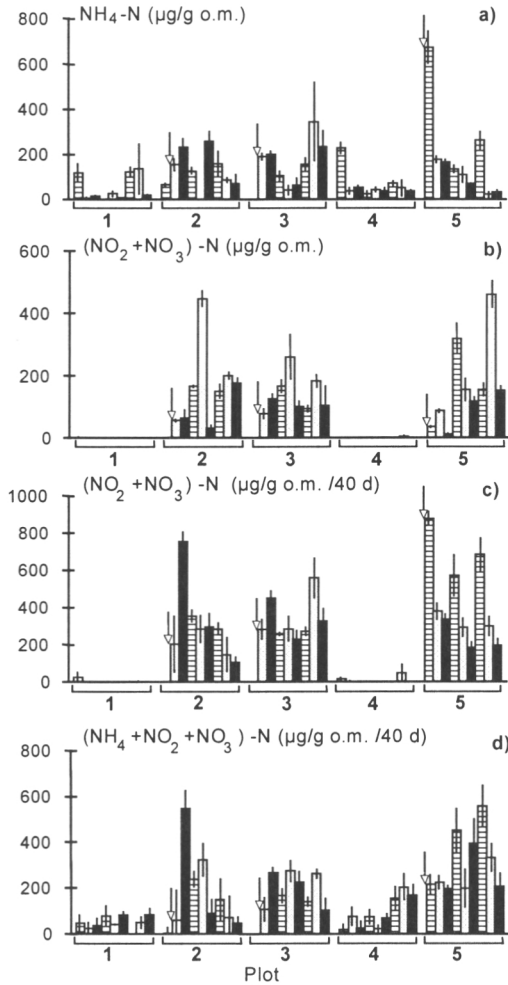
Figure 4. Initial mineral N concentrations (a, b), and the net nitrification and formation of mineral N during a 40-d incubation in the laboratory (c, d) in the samples from the humus layer of plots 1-5 (see Table 1). The results are the means of three subplots (see Fig. 1) (± SEM). Arrows show the initiation of infiltration. For sampling dates see legend in Fig. 2.

Table 2. Mean concentrations of mineral and organic N (mg/l) in infiltration (n = 57) and groundwater (n = 76) during 1996-1998. SEM is indicated in parentheses

	NH_4-N	(NO_2+NO_3)-N	Organic N
Infiltration water	0.01 (0.00)	0.15 (0.02)	0.38 (0.02)
Groundwater	0.00 (0.00)	0.22 (0.01)	0.21 (0.01)

Infiltration was stopped on plot 7 in autumn 1996, and the recovery of the soil processes after the cessation of infiltration was studied. The (NO_2+NO_3)-N concentration in samples from the humus layer did not differ greatly between the samplings, but the net production of (NO_2+NO_3)-N in the laboratory declined with time (Table 3). This could be due to the high

seasonal and year-to-year variation in nitrogen transformations (4a-d), or to the fact that the activity or numbers of the nitrifiers had in fact decreased due to the cessation of infiltration. In spite of intensive nitrification, the pH of the humus layer on plot 7 had not decreased with time (Helmisaari et al., 1999). It would thus appear that the humus layer 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.

Table 3. Nitrogen transformations in the samples from the humus layer of plot 7 (see Table 1). The results are the means of three subplots (see Fig. 1). SEM is indicated in parentheses

Sampling date	Initial (μg / g o.m.)		Net formation (μg / g o.m. / 40 d)	
	NH_4-N	(NO_2+NO_3)-N	(NO_2+NO_3)-N	$(NH_4+NO_2+NO_3)$-N
21.07.1997	45.2 (18.0)	61.0 (6.0)	251.8 (1.6)	220.1 (17.0)
07.10.1997	104.7 (10.6)	70.2 (4.3)	173.7 (23.2)	138.4 (17.2)
23.09.1998	24.5 (0.5)	44.7 (8.0)	77.3 (1.9)	162.7 (17.7)

N_2O fluxes. The fluxes of N_2O were significantly higher in the infiltration plots than in the control plots (Fig. 5). On the plots given continuous or periodical infiltration during the summer, the N_2O emissions increased after the start of infiltration. The main reasons for the increased production of N_2O in the infiltration plots are probably the increased availability of nitrate, and increased soil moisture and pH, all of which are known to increase the production of N_2O (reviewed by Martikainen, 1996). The emissions were generally lower in the plot given wintertime infiltration than in the summertime infiltration plots. This is probably due to differences in the soil moisture; the plots receiving summertime infiltration had a higher soil moisture content during the summer than the wintertime infiltration plot.
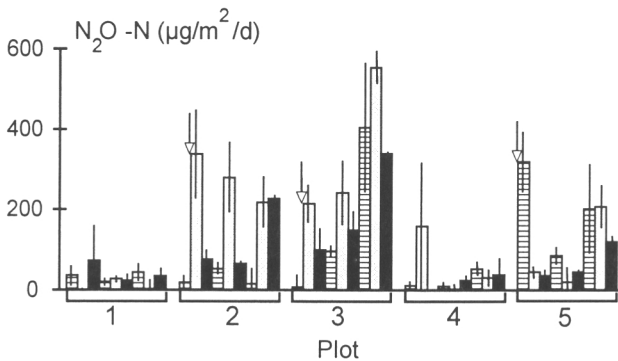

Figure 5. N_2O fluxes measured in the field from plots 1-5 (see Table 1). The results are the means of three subplots (see Fig. 1) (± SEM). Arrows show the initiation of infiltration. For sampling dates see legend in Fig. 2.

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 about 5 times higher than from a poorly drained Norway spruce forest in Sweden (Klemedtsson et al., 1997) but about 10 times lower than that from a forested peatland in Finland (Regina et al., 1998). In an earlier study, Paavolainen et al. (1999) showed that the N_2O is primarily derived from denitrification, and only about 25% of the denitrification products was N_2O. Therefore, 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}.

Nitrogen in percolation and groundwater. Plate lysimeters collect percolation water filtering down through the soil profile, while suction-cup lysimeters primarily sample the water bound on and between the soil particles, as well as percolation water. Therefore, the results obtained using different types of lysimeter on the infiltration plots during breaks in infiltration and on the control plots represent different fractions of soil solution, and thus direct comparison is not fully valid. On the infiltration plots during infiltration, however, large amounts of water move down through the soil profile and the water collected by suction-cup lysimeters was therefore considered to represent percolation water.

The NH_4-N concentration in the percolation water was very low. On the infiltration plots during breaks in infiltration and on the control plots, the NH_4-N concentration at different depths was generally 0.2 - 0.5 mg/l. On the infiltration plots during infiltration the NH_4-N concentration was about 0.02 mg/l.

The (NO_2+NO_3)-N concentration in the percolation water at different depths was appreciable only on the infiltration plots during breaks in infiltration (Fig. 6). Nitrate leaching from undisturbed Finnish forest soils is usually negligible (Soveri and Ahlberg, 1990; Smolander et al., 1995). The forest management practices discussed above, which increase net nitrification, also increase the nitrate concentrations in percolation water (Smolander et al., 1995). In the Ahvenisto esker, nitrate production was initiated by infiltration (Fig. 4c), and it is therefore not surprising that nitrate, being very mobile, is leached from the uppermost soil layers during breaks in infiltration. During infiltration the nitrate produced by the infiltration soils is diluted by the large amounts of infiltration water. Therefore the risk of nitrate leaching seems to be at it highest during breaks in infiltration.

A rough N budget was calculated for the continuous summertime infiltration plot (plot 2) during infiltration. The amount of mineral N added with infiltration water during one summer was similar to the amount collected in the percolation water at a depth of 100 cm; approx. 20 g m^{-2} for NH_4-N and 200 g m^{-2} for (NO_2+NO_3)-N. The amount of N_2O-N produced during one summer, 0.02 g m^{-2}, was very small compared to the amount of nitrate added with the infiltration water. Thus, during infiltration N_2O production seems to have only a very small effect on the nitrate concentrations in the percolation water.
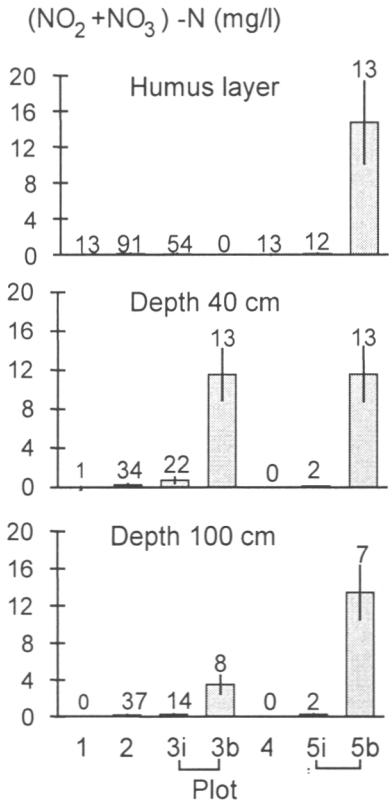
(NO$_2$ +NO$_3$) -N (mg/l)

Figure 6. The (NO$_2$+NO$_3$)-N concentration in percolation water below the humus layer and at a depth of 40 cm and 100 cm on plots 1-5 (see Table 1) during 1996-1998 (mean ± SEM) (i = infiltration, b = break in infiltration). The number of samples is shown above the columns.

Organic N was determined in the percolation water below the humus layer. On the control plots a considerable portion of the N in the percolation water was in an organic form, which is consistent with the results of Smolander et al. (1995) (Fig. 7). During infiltration, the organic N concentration in percolation water was lower on the infiltration plots than on the control plots, whereas during breaks in infiltration the organic N concentration was at the same level on the infiltration and control plots. This suggests that the concentration of organic N leached from the infiltration plots during natural recharge is not higher than that from the control plots.

N-org (mg/l)
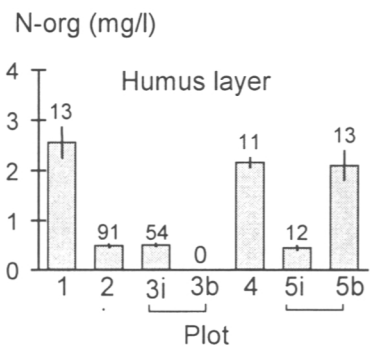
Figure 7. The organic N concentration in percolationwater below the humus layer on plots 1-5 (see Table 1) during 1996-1998 (mean ± SEM) (i = infiltration, b = break in infiltration). The number of samples is shown above the columns.

Infiltration continued throughout the year on some of the plots and thus the concentrations in groundwater represent conditions during infiltration. According to the Hämeenlinna Waterworks, the groundwater used in this study is almost completely derived from this infiltration. The organic N concentration in the groundwater was about half that in the infiltration water, implying that the esker has the capacity to retain organic N (Table 2). The mineral N concentrations were very low and close to the average values for groundwater in Finland (Table 2) (Lahermo et al., 1990; Soveri and Ahlberg, 1990). As stated above, the high (NO_2+NO_3)-N concentration produced during breaks in infiltration is 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 as long as infiltration is continued in the irrigation area. However, this conclusion is based on a three-year experiment, and the long-term effects of infiltration are not yet known.

Soil sampling and the N_2O measurements were made at a distance of 2 m from the irrigation pipe, where the lysimeters were also located (Fig. 1). As the pH of the soil was at it highest at these points (Fig. 3), the results presented in this paper probably represent the maximum effect of infiltration.

Conclusions

As a result of sprinkling infiltration the pH of the humus layer increased from about 5 to 6.7, and caused the initiation of net nitrification in the humus layer. The results of this three-year study show that as long as infiltration continues in the infiltration area, the nitrate produced is diluted by the large amounts of infiltration water and, therefore, the leaching of nitrate does not pose a threat to groundwater quality. N_2O production may have also contributed to the low nitrate concentrations in the groundwater, although during infiltration N_2O production seems to have only a very small effect on the N losses via leaching. However, 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 for 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. Even though it appears that there is no immediate threat to groundwater quality, we must emphazise that the nitrogen cycle in the esker has been drastically changed, and the long-term effects of this are not yet known.

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Paper III

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III

NITRIFICATION AND DENITRIFICATION IN SOIL FROM A CLEAR-CUT NORWAY SPRUCE (*PICEA ABIES*) STAND

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Summary—Laboratory measurements of nitrification and denitrification were made on samples of the humus layer from a Norway spruce (*Picea abies* L.) experimental site that was clear-cut 2 y before this study. For 30 y before the clear-cutting, the stand had been repeatedly fertilized with nitrogen and/or limed. In addition to the nitrogen-fertilized and/or limed experimental plots, there was also a clear-cut control plot and a forested reference plot that was not clear-cut. Aerobic incubation experiments were used to study net production of $(NO_2 + NO_3)$-N, and an aerobic soil-suspension technique was used to study the nature and pH-dependency of nitrification. The numbers of nitrifiers were determined by a MPN method. Nitrification, which was active only in clear-cut plots, was acid-sensitive (no production at pH 4) and autotrophic, as it was inhibited by 2.5 Pa of acetylene. When the amount of NH_4-N was not limiting, as in soil suspension, the pH controlled nitrification: in a pH gradient from 4.2 to 6.2, higher pH values resulted in higher production of $(NO_2 + NO_3)$-N. The soils from the clear-cut plots had an abundant community of nitrifiers, whereas in the forested reference soil, less than 10 NH_4-oxidizers cm^{-3} of soil were observed. Denitrification occurred in soils from all the clear-cut plots, and the main product was N_2. Denitrification started in the forested reference soil only after the addition of NO_3-N in the laboratory, and the main product was N_2O. In the soils from the clear-cut plots, denitrification was limited first by a lack of NO_3-N and second by pH. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Nitrification is considered mainly a harmful process because excess nitrate may be denitrified or leached, leading to loss of nitrogen from the ecosystem and to groundwater pollution. Nitrous oxide (N_2O), one of the end products of denitrification, which acts both as a greenhouse gas and contributes to the destruction of stratospheric ozone, is also produced in nitrification as a by-product of ammonium oxidation.

Hardly any nitrification seems to occur in Finnish acid coniferous soils, unless nitrogen is added via atmospheric input or fertilization and or soil acidity is alleviated by liming (Aarnio and Martikainen, 1992; Martikainen *et al.*, 1993; Priha and Smolander, 1995; Smolander *et al.*, 1995). Clear-cutting can also initiate nitrification in forest soil by increasing both the availability of ammonium and soil pH (Smolander *et al.*, 1998).

Acidity has often been implicated as the factor inhibiting nitrification in coniferous forest soils (Tietema *et al.*, 1992). During the last few years, however, intensive autotrophic nitrification has been observed in acid soils receiving high rates of ammonium deposition (De Boer *et al.*, 1989; Martikainen *et al.*, 1993). Consequently, nitrifiers

were classified as being acid-sensitive or acid-tolerant by using nitrate production in suspensions at pH 6 and 4 as criteria (De Boer *et al.*, 1990). De Boer *et al.* (1990) 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).

We studied the importance of substrate availability (NH_4- or NO_3-N) and pH on nitrification and denitrification in a long-term Norway spruce fertilization experiment, where the risks for nitrification were maximized: a previous long-term increased N input and a previous long-term pH increase (by liming) followed by clear-cutting. Before clear-cutting, nitrification and denitrification occurred only in the plot that had been both limed and fertilized with nitrogen (Priha and Smolander, 1995; Smolander *et al.*, 1998). After clear-cutting nitrification started in all the clear-cut plots (Smolander *et al.*, 1998). Our aim was to investigate further the factors affecting nitrification and denitrification in the soil in the third summer after clear-cutting.

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Table 1. Some characteristics of the experimental soils

Sampling	Plot	Dry matter (%)	Fresh bulk density (g cm^{-3})	Organic matter (% of d.m.)	pH (H$_2$O)
2 August	0(for)	53.9 (0.65)	0.23	62.2 (0.12)	4.1
	0	65.1 (0.07)	0.28	48.0 (0.10)	5.0
	Ca	51.9 (0.16)	0.27	62.6 (0.56)	5.2
	N	66.7 (0.40)	0.31	44.8 (1.25)	4.5
	CaN	60.6 (0.18)	0.32	48.2 (0.24)	5.8
12 September	0(for)	50.8 (0.25)	0.27	57.7 (0.29)	3.8
	0	61.0 (1.89)	0.41	30.1 (2.02)	4.6
	Ca	45.8 (0.18)	0.38	46.6 (0.19)	5.5
	N	62.6 (0.06)	0.46	27.8 (0.72)	4.3
	CaN	59.2 (0.24)	0.51	25.2 (0.13)	6.1

Standard deviation (s.d.) of 2 replicates in parentheses (s.d. in fresh bulk density and pH measurements less than 0.01 and 0.1, respectively).
Plot symbols: 0(for) = forested control, 0 = control, Ca = limed, N = N-fertilized, CaN = limed and N-fertilized; d.m. = dry matter.

MATERIALS AND METHODS

Study site

The study site was a 60 y old Norway spruce (*Picea abies* L.) stand growing on mineral soil in Kerimäki, in the south-eastern part of Finland (61°51′N/29°22′E). The site was fertile, the forest site type, according to the Finnish classification, is *Oxalis–Myrtillus* (OMT) (Cajander, 1949). Soil type was podzol, and the humus type was mor.

The stand was a subject of factorial fertilization experiments established 37 y ago by the Finnish Forest Research Institute. The experimental plots were 15 m × 30 m. The treatments were limed (Ca), nitrogen-fertilized (N), limed and nitrogen-fertilized (CaN), and control (0). In the Ca treatment, finely ground limestone was applied twice, 37 and 15 y before our study, totalling 6000 kg ha^{-1}. In the N treatment, the plot had received nitrogen fertilization seven times, first as ammonium sulphate, then as urea and later as ammonium nitrate with dolomite, totalling 860 kg N ha^{-1}. The last application was 9 y before our study. Fertilization treatments have been described in more detail by Smolander *et al.* (1994).

The stand was clear-cut in January 1993, 2 y before our study. Logging residue was evenly distributed on the surface of each clear-cut plot. There were two controls in the same study site: the control plot (0) mentioned above, which was subjected to clear-cutting, and a forested reference plot (0(for)), which was not clear-cut.

Soil sampling

Soil was sampled on 2 August and 12 September 1995. Twenty samples (core dia, 5 cm) were taken from the humus layer (FH, thickness 5–7 cm) of each plot systematically, and combined to give one composite sample per plot. Green plant material was removed, and the samples were sieved (mesh size, 2.8 mm). Sieved samples were stored in plastic bags at 4°C until further use. Some characteristics of the soil samples are shown in Table 1.

Nitrogen transformations in incubation experiments

Nitrogen transformations were studied in aerobic incubation experiments in the laboratory at constant temperature (14°C) and moisture (60% of the water-holding capacity (WHC)) for 40 d using three replicate samples, as described by Smolander *et al.* (1995). To calculate net ammonification and nitrification, initial NH$_4$-N and (NO$_2$ + NO$_3$)-N concentrations were subtracted from final (post-incubation) NH$_4$-N and (NO$_2$ + NO$_3$)-N concentrations. Net formation of mineral N was estimated as the sum of net ammonification and net nitrification.

Measurement of nitrification in soil suspensions

Nitrification potential was studied using the soil suspension technique described by De Boer *et al.* (1992). Aerobic incubations of the soils were done in 600 ml glass bottles with continuous shaking on a rotary shaker (150 rev min^{-1}) in the dark at 22°C. The suspensions were made of 7.5 g dry matter (d.m.) soil in 300 ml of mineral solution containing NH$_4$-N (see De Boer *et al.*, 1992). The bottles were sealed with rubber septa. To ensure the availability of substrate, 1 ml of a (NH$_4$)$_2$SO$_4$ solution (100 g (NH$_4$)$_2$SO$_4$ l^{-1}) was added every second day. The bottles were opened daily to aerate the samples and to adjust the pH with 0.1–1.0 M Na$_2$CO$_3$ or 0.1–1.0 M H$_2$SO$_4$. The nature of nitrification, autotrophic or heterotrophic, was examined by determining the effect of low partial pressures of acetylene (2.5 Pa) on the production of nitrate. This concentration of acetylene is believed to be a specific inhibitor of autotrophic nitrification (Hynes and Knowles, 1982). For quantitative analyses of (NO$_2$ + NO$_3$)-N, samples (50 ml) of the suspensions were filtered (S&S 589^3), and (NO$_2$ + NO$_3$)-N was measured using a flow-injection analyzer (FIA 5012, Tecator).

The sensitivity of the nitrifying microorganisms towards acidity was determined by comparing the production of (NO$_2$ + NO$_3$)-N in suspensions maintained at pH 4 or 6 using four replicate suspensions at both pH values. Net production of (NO$_2$ + NO$_3$)-
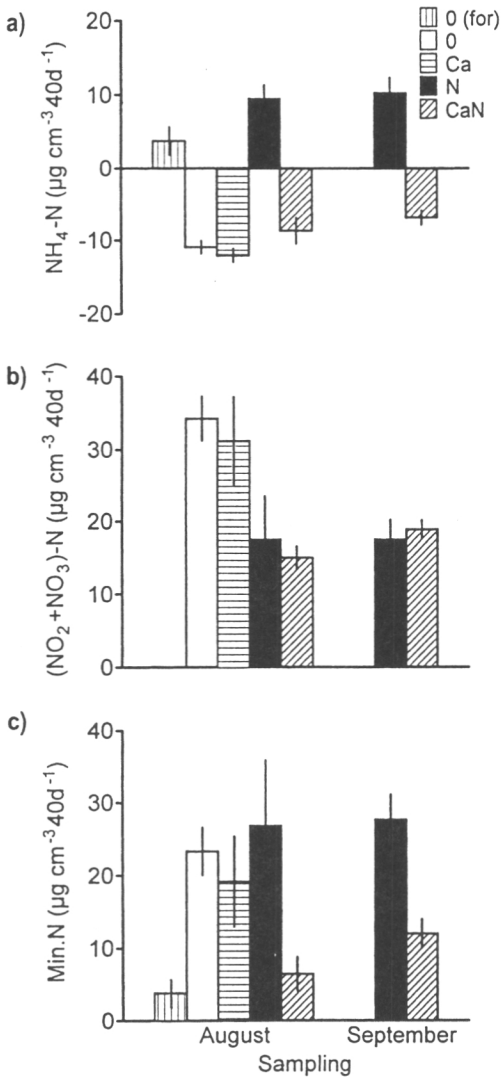
Fig. 1. Nitrogen transformations in incubation experiments from August (all plots) and September (only N and CaN plots) samplings during a 6-week incubation at constant temperature (14°C) and moisture (60% WHC) in the laboratory. (a) Net ammonification, (b) net nitrification and (c) net formation of mineral N (NH_4-N + ($NO_2 + NO_3$)-N). Results are means of three replicate samples (±standard deviation).

N was determined during a 3-week incubation. To study the nature of nitrification, acetylene was added to two replicate suspensions after 2 weeks of incubation.

The response of nitrification to a pH gradient (pH 4.4, 5.2, 5.6 and 6.2) was studied in a 2-week incubation using two replicate samples at each pH value. Another 2-week incubation in a pH gradient of 4.0, 4.5, 5.0, 5.5 and 6.0 was done to determine the nature of nitrification at different pH values. There were four replicates at each pH value, and acetylene was added at the beginning of the experiment to two replicate samples.

Enumeration of autotrophic nitrifiers

The most probable number (MPN) method described by Martikainen (1985) was used to determine the numbers of autotrophic NH_4- and NO_2-oxidizers in the soils. The modified media of Bhuiya and Walker (1977) were used (Martikainen, 1985). In the NH_4-medium and in the NO_2-medium the respective concentrations of $(NH_4)_2SO_4$ and $NaNO_2$ were 0.1 g l^{-1}. The MPN tubes were incubated for 10 weeks at 20–23°C in the dark. The presence of NO_2 and NO_3 was checked by a drop test, as described by Aarnio *et al.* (1996).

Measurement of N_2O production

N_2O production was studied in laboratory incubations at constant temperature (14°C) and moisture (100% of the WHC) for 9 d using three replicate soil samples (4 g d.m.) with either no acetylene or with acetylene at a partial pressure of 10 kPa. This high partial pressure of acetylene blocks nitrous oxide reductase, so that all gaseous denitrification products remain as N_2O (Klemedtsson *et al.*, 1977). The samples were aerated and acetylene added every second day. After 5 d, $NaNO_3$ was added (40 μg NO_3-N g^{-1} d.m.). The production of N_2O was measured daily (except there were no measurements on d 3, so on d 4 the results are reported as the production rate of N_2O during 48 h) by gas chromatography (Hewlett Packard, 6890) using an electron capture detector and a Megapore GS-Q (Scientific) column, 30 m in length. The carrier gas (He) flow rate was 10 ml min^{-1}. The temperatures of the detector, injector and column were 300, 100 and 30°C, respectively. The solubility of N_2O in water was taken into account in the calculations (Moraghan and Buresh, 1977). NH_4- and ($NO_2 + NO_3$)-N concentrations in soil were also measured (FIA; see above) at the beginning, half way through (before adding substrate) and at the end of the experiment.

RESULTS AND DISCUSSION

Ammonification and nitrification

In laboratory incubations, more NH_4-N was produced than consumed in the soils from the N and 0(for) plots, whereas in the soils from the 0, Ca and CaN plots, the opposite occurred (Fig. 1(a)), indicating that at least in the soils from the N and 0(for) plots the amount of NH_4-N was not limiting nitrification. Net production of ($NO_2 + NO_3$)-N was detected only in the soils from the clear-cut plots (Fig. 1(b)). The pH of the soils did not control the production of ($NO_2 + NO_3$)-N (Fig. 1(b), Table 1). The relatively low net production of ($NO_2 + NO_3$)-N in soil from the CaN plot, having the highest pH, was probably the result of a low rate of nitrogen mineralization; i.e. NH_4-N was probably either
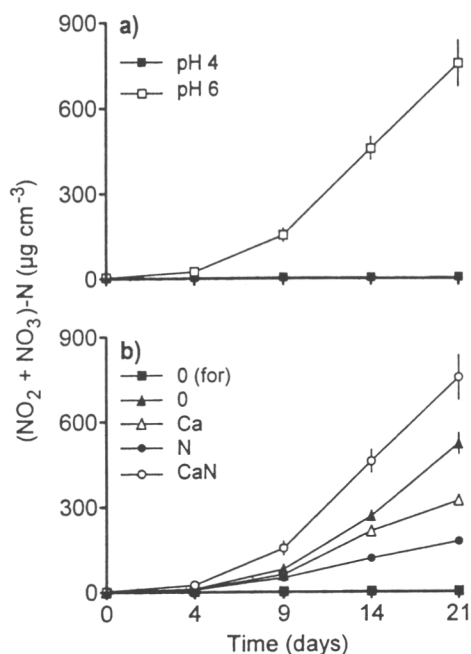
Fig. 2. Accumulation of $(NO_2 + NO_3)$-N in soil suspensions maintained at pH 4 and 6 during a 3-week incubation. (a) Nitrification potential in the soil from the CaN plot at pH 4 and 6 and (b) nitrification potential in soil from all plots at pH 6. Results are means of two replicate soil suspensions (±standard deviation). Soil samples were collected in August.
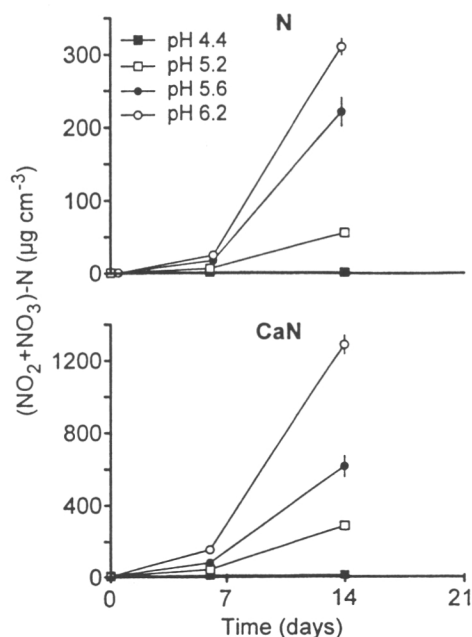

Fig. 3. Effect of pH on the nitrification potential in soil suspensions during a 2-week incubation. (a) N plot and (b) CaN plot. Results are means of two replicate soil suspensions (±standard deviation). Soil samples were collected in September.

produced too slowly for nitrification or mineral N (either NH_4- or NO_3-N) was immobilized. Smolander et al. (1998) noticed, at the same study site, a negative correlation between pH and net formation of mineral N at this high pH range. Denitrification could be another possibility, but it was shown that at these moisture conditions (60% of the WHC), it was of minor importance.

Net nitrification was negligible at pH 4 in suspensions of soil from all plots in a 3-week incubation experiment (results at pH 4 shown only for the CaN plot; Fig. 2(a)). At pH 6, there was nitrification in soil from all the clear-cut plots (Fig. 2(b)). In the soil from the 0(for) plot, net nitrification was not detected even at pH 6. In Finland, there are some coniferous soils that show acid-tolerant nitrification (nitrate production at pH 4). These sites are close to mink farms and, hence, receive high depositions of NH_4 (Martikainen et al., 1993). Although nitrogen input to the soils in the N and CaN plots had been high, acid-tolerant nitrifiers (patterns III and IV), in the sense that De Boer et al. (1990) described the term, did not exist in these soils, instead nitrification was acid-sensitive (nitrate production at pH 6 but not at 4) in soil from all the clear-cut plots (pattern II).

The response of nitrification to a pH gradient from 4.4 to 6.2 was studied in the soils from N and CaN plots because classification of nitrifiers into only two groups (i.e. nitrification in pH 4 or 6) on the basis of a response to pH, while convenient, is artificial. Both soils showed a strict response to the pH gradient: higher pH values resulted in higher production of $(NO_2 + NO_3)$-N (Fig. 3), as also observed in a study by Stams et al. (1990), indicating that when NH_4-N was not limiting, pH controlled nitrification. Stams et al. (1990) observed that this increased production was caused by an increase in the size of the nitrifier community rather than by increased activity of the existing community.

Acetylene, when added in the beginning of the experiment, entirely inhibited nitrification at each pH (Table 2), indicating that nitrification was autotrophic regardless of the pH at which the soil suspension had been maintained. However, when acetylene was added in the middle of an experiment to an already actively nitrifying suspension, it did

Table 2. Production of $(NO_2 + NO_3)$-N in suspensions of soil from N plot during a 2-week incubation

pH	$(NO_2 + NO_3)$-N $\mu g\,cm^{-3}$	
	no C_2H_2	2.5 Pa C_2H_2
4.0	0	0
4.5	2	0
5.0	67	1
5.5	178	1
6.0	278	1

Mean $(NO_2 + NO_3)$-N production $(\mu g\,cm^{-3})$ of two replicate samples (coefficient of variation less than 10%).
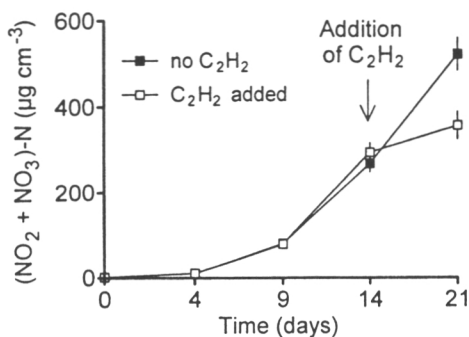
Fig. 4. Effect of low concentration of acetylene (2.5 Pa) on nitrification potential in the soil from the 0 plot. Results are means of two replicate soil suspensions (±standard deviation). Soil samples were collected in August.
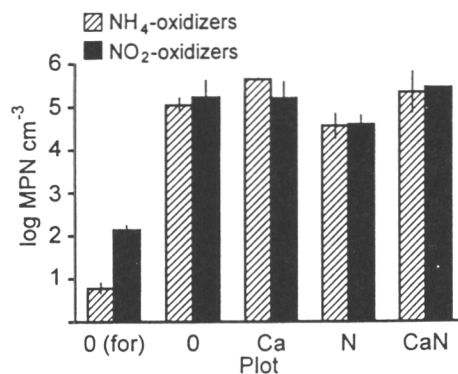

Fig. 5. Numbers of NH_4- and NO_2-oxidizers (MPN) in the soils sampled in August. Standard deviation is shown by bars.

not completely inhibit nitrification (results of 0 plot in Fig. 4). The percentage of inhibition in the different plots was: 0, 75(±8.7)%; Ca, 60(±2.1)%; N, 70(±7.2)%; CaN, 58(±10.6)% (standard deviation in parentheses). Nitrification was observed to be autotrophic also before clear-cutting (Smolander et al., 1995). Killham (1987) found nitrification to be mainly heterotrophic in samples of acid forest soil, but in many recent laboratory studies, autotrophic has dominated heterotrophic nitrification in acid forest soil (e.g. Stams et al., 1990; Martikainen et al., 1993; Persson and Wirén, 1995).

In soil suspension, the production of $(NO_2 + NO_3)$-N was negligible at pH 4.5 or under (Fig. 3, Table 2). However, in the incubation experiment, soil from the N plot nitrified at an initial pH of 4.3 and 4.5 (Table 1, Fig. 1(b)). In the soil suspension technique with continuous shaking, formation of aggregates was not possible, and hence, the actual pH was the measured pH. When the soil incubation method was used, there might have been microsites in the soil with a pH different from the measured mean pH, indicating that nitrification occurred in microsites of pH higher than the surrounding soil. Such microsites may be the result of local intensive ammonification. De Boer et al. (1988) noticed that liberation of NH_4 by ammonifying organisms induces a pH favourable for acid-sensitive NH_4-oxidizing bacteria.

Numbers of autotrophic nitrifiers

Only the soils of the clear-cut plots had an abundant community of nitrifiers, whereas in the soil from the 0(for)plot, less than 10 NH_4-oxidizers cm^{-3} of soil were observed (Fig. 5). The small nitrifier community in soil from the 0(for) plot probably explains why the production of $(NO_2 + NO_3)$-N was negligible in this soil both in soil incubation (Fig. 1(b)) and in soil suspension (Fig. 2(b)) experiments. Before clear-cutting, net nitrification occurred only in the soil from the CaN plot (Smolander et al., 1995, 1998). In the summer following clear-cutting, nitrification started in all the soils from the clear-cut plots but not in the soil from the 0(for) plot (Smolander et al., 1998), indicating that clear-cutting helped to build up increased nitrifier communities. The reason for this is probably increased pH and net formation of mineral N followed by clear-cutting (Table 1, Fig. 1(c), Smolander et al., 1998). One reason could also be allelopathic inhibitors, such as terpenes, which can completely inhibit nitrification (White, 1986). The production of terpenes was high on the 0(for) plot, but on the clear-cut plots terpenes were not detected (Veikko Kitunen, unpublished results).

Of the clear-cut plots, lowest MPN counts were detected in the soil from the N plot (Fig. 5), which is probably explained by the lower pH of this soil compared to the other clear-cut plots (Table 1). This explains why the soil from the N plot had the

Table 3. NH_4- and $(NO_2 + NO_3)$-N concentrations ($\mu g\ cm^{-3}$) in samples without C_2H_2 and in samples under a 10 kPa partial pressure of C_2H_2 during denitrification incubation

Plot	0 d		5 d				9 d			
			no C_2H_2		10 kPa C_2H_2		no C_2H_2		10 kPa C_2H_2	
	NH_4	$NO_2 + NO_3$	NH_4	$NO_2 + NO_3$	NH_4	$NO_2 + NO_3$	NH_4	$NO_2 + NO_3$	NH_4	$NO_2 + NO_3$
0(for)	9.57	0.0	5.9	0.0	5.9	0.0	5.4	0.0	6.2	0.0
0	9.59	2.3	9.2	0.1	10.3	0.0	8.5	0.0	12.7	0.0
Ca	12.46	3.3	15.2	0.0	17.5	0.0	15.7	0.0	19.1	0.0
N	11.37	0.7	12.0	0.0	11.7	0.0	10.8	0.2	13.7	0.0
CaN	10.57	8.9	9.5	4.0	16.1	0.0	3.1	2.8	18.1	0.0

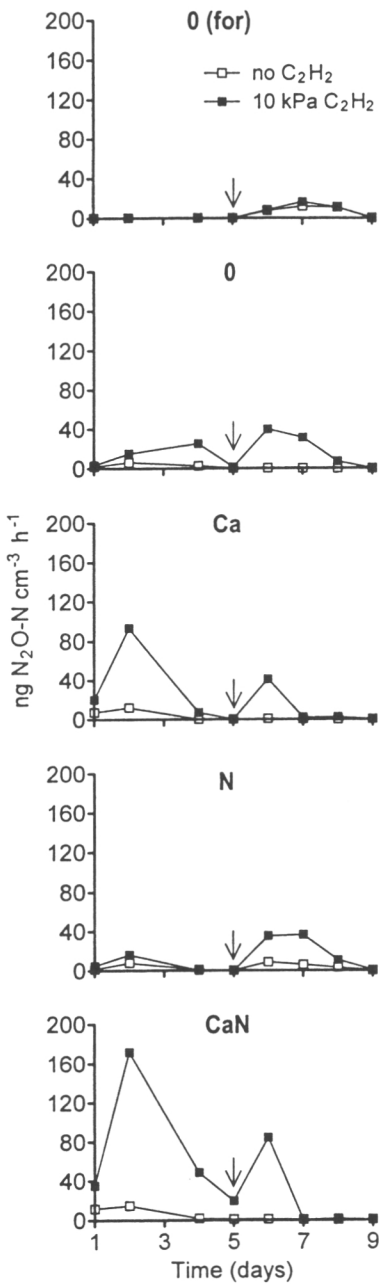Coefficient of variation in three replicate samples less than 10%.

Fig. 6. Production rate of N_2O in the soils during a 9-day incubation with no acetylene and with acetylene at a partial pressure of 10 kPa. Addition of NO_3-N (40 μg g^{-1} dry matter) shown by arrows. Coefficient of variation in three replicate samples was less than 10%. Soil samples were collected in September.

lowest nitrification potential of the clear-cut plots (Fig. 2(b), Fig. 3).

N_2O production

N$_2$O production was studied in a 9 d laboratory incubation. Without the addition of NO$_3$-N, N$_2$O was produced in the soils from the clear-cut plots

only (Fig. 6). Before clear-cutting, denitrification occurred only in the soil from the CaN plot (Priha and Smolander, 1995). This indicates that clear-cutting, which made ($NO_2 + NO_3$)-N available in the soils from the O, Ca and N plots (Smolander et al., 1998), also made denitrification possible. These results are in accordance with field measurements done at this study site, where the enhancing effect of clear-cutting on N$_2$O production was also seen (Martikainen, unpublished results).

Of the clear-cut plots, the rate of denitrification was highest in soil from the limed plots, which also had the highest initial ($NO_2 + NO_3$)-N concentrations (Fig. 6, Table 3). For example, the initial amount of ($NO_2 + NO_3$)-N in the soil from the CaN plot was 8.9 μg cm^{-3} soil of which was denitrified about 7.7 μg N cm^{-3} soil (cumulative production of N$_2$O-N during the first 5 d), whereas in the soil from the N plot only 0.5 μg N cm^{-3} soil was denitrified the initial amount of ($NO_2 + NO_3$)-N being 0.7 μg cm^{-3} soil (Fig. 6, Table 3). After a few days, when the ambient ($NO_2 + NO_3$)-N was exhausted, production of N$_2$O ceased, and recommenced only after addition of NO$_3$-N on d 5 (Fig. 6, Table 3). Of added NO$_3$-N only about 20% was denitrified (for example in the soil from the CaN plot: 12.1 μg NO$_3$-N cm^{-3} soil was added and only 2.2 μg N cm^{-3} soil was denitrified), indicating that most of the added NO$_3$-N was immobilized. Before and after the addition of NO$_3$-N, the soil with the highest pH value (CaN plot) had the highest rate of denitrification (Fig. 6). This indicates that compared with the other soils, the CaN plot had a more effective denitrifying population or more available carbon. It seems that the production of N$_2$O was limited first by a lack of NO$_3$-N and secondly by pH, as also suggested in a study done by Federer and Klemedtsson (1988).

The addition of NO$_3$-N in the laboratory made denitrification possible also in the soil from the 0(for) plot (Fig. 6), even though the production of N$_2$O was very low. In contrast to the soils from the clear-cut plots, equal amounts of N$_2$O were produced in the soil from the 0(for) plot without acetylene as under 10 kPa acetylene. These results indicate that the main product of denitrification in the acid forest soil would be N$_2$O, a harmful greenhouse gas, whereas the main product of denitrification in the clear-cut forest soils was N$_2$. The relative importance of this result in field conditions is, however, difficult to estimate based on short-term laboratory incubations.

In the soil from the CaN plot, NH$_4$-N was consumed only when nitrification was not inhibited (no addition of acetylene), indicating that the soil nitrified during the 9 d incubation (Table 3). However, between d 5 and 9, when the consumption of NH$_4$-N was intense, N$_2$O production in samples lacking acetylene was not detected, suggesting that, in this

experiment, N_2O production by autotrophic nitrifiers was of minor importance.

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Paper IV

IV

Inhibition of nitrification in forest soil by monoterpenes

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Abstract

Nitrate production was detected in untreated soil of a Norway spruce (*Picea abies* L.) stand only after clear-cutting the stand. The aim of this study was to determine whether allelochemical inhibition of nitrification by monoterpenes played any role in inhibiting nitrification in the stand. Therefore, soils from a clear-cut plot and from a forest plot were studied. In the field, monoterpenes (mostly α- and β-pinenes), measured by soil microair diffusive samplers, were intensively produced in the forest plot, but not in the clear-cut plot. In the laboratory, soil samples taken from the forest plot produced only small amounts of monoterpenes, indicating that monoterpenes were mainly produced by the roots and not to great extent by the soil microbial population. The effect of a mixture of monoterpenes (seven major monoterpenes detected in the field) on net nitrification, net N mineralization and denitrification activities of soil from the clear cut plot, and on carbon mineralization of soils from both the forest and clear-cut plots, was studied in the laboratory. In both aerobic incubation experiments and in soil suspensions with excess NH_4-N, nitrification was inhibited by exposure to the vapours of monoterpenes at similar concentrations at which they had been detected in forest plot. This indicates direct inhibition of nitrification by monoterpenes. Exposure to monoterpenes did not affect denitrification. However, it increased respiration activity of both soils. This could also indicate indirect inhibition of nitrification by monoterpenes, due to immobilization of mineral N. Thus it seems that monoterpenes could play a role in inhibiting nitrification in the forest soil.

Introduction

Nitrification was detected in untreated soil of a Norway spruce (*Picea abies* L.) stand only after clear-cutting the stand (Smolander et al., 1998). In further studies Paavolainen and Smolander (1998) found an abundant population of autotrophic nitrifiers only in the soil from the clear-cut area. Also in other studies on undisturbed Finnish coniferous soils, most of the available nitrogen present is usually in the form of ammonium, with little or no nitrate present (Martikainen, 1984; Priha and Smolander, 1995; Smolander et al., 1995). Rice (1984) reviewed experiments concerning depressed nitrification in climax ecosystems, and reported experimental evidence from grasslands and forests for allelochemical inhibition of nitrification. This successional process appears to have adaptive

value, since ammonium ions are less readily leached from the soil than nitrate ions.

It is usually thought that if allelopathy is an inhibitory mechanism of nitrification in soils, then it is likely that the allelopathic agents are various types of polyphenolic compounds including tannins, flavonoids and a variety of other polyphenols (Lodhi and Killingbeck, 1980). In addition, also volatile organics (e.g. terpenes) produced by ponderosa pine were found to inhibit net nitrogen mineralization and nitrification (White, 1986). Monoterpenes could do this by inhibiting nitrification, reducing net N mineralization, enhancing immobilization of NO_3–N relative to NH_4–N, and/or stimulating overall net immobilization of N by providing carbon-rich material (White, 1991). Bremner and McCarty (1988,1996) criticized this hypothesis, and showed that addition of monoterpenes resulted only in N immobilization with no inhibition of ammonium oxidation.

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The aim of this study was to find out whether monoterpenes control nitrification in a Norway spruce stand, and what are the mechanisms envolved.

Materials and methods

Study site

The study site was a 66-year-old Norway spruce (*Picea abies* L.) stand growing on mineral soil in Kerimäki, in the South-Eastern part of Finland (61°51′ N / 29°22′ E). The site was rather fertile, the forest type according to the Finnish classification was *Oxalis-Myrtillus* (OMT) (Cajander, 1949). Soil type was podzol, and the humus type mor.

The stand was a subject of long-term fertilization experiment (Smolander et al., 1998). The stand was clear-cut in January 1993, three years before this study. Logging residue was evenly distributed on the surface of each clear-cut plot. In this study only the untreated (no fertilization) clear-cut plot, and an untreated control plot, which was not clear-cut (forested reference plot) were included. The size of the plots was 15 m × 30 m. The pH(H2O) of the soils from the clear-cut and forested reference plots were 4.9 and 4.2, respectively.

Measurement of VOCs in the field

To collect volatile organic compounds (VOCs) from the soil, microair passive diffusive samplers (3500 Organic Vapour Monitor, 3M) were placed in the forested reference and clear-cut plots in June 11, 1996. A 30-cm-deep hole (dia. 5 cm) was bored into the soil, and the passive diffusive sampler was installed in the hole. The sampler was hanging from an aluminium cap, which covered the hole. Six samplers were evenly distributed on both plots. The ambient air samplers were placed approx. 1.5 m above the surface soil.

After 8 weeks (58 days) all the samplers were removed and the adsorbed VOCs were eluted from the adsorbents using 1.5 mL dichloromethane for 90 min. The solvent was transferred to autosampler vials, and the amount of the compounds were determined by gas chromatography–mass spectrometry (HP 5890A gas chromatograph with 5988A mass spectrometer) operating in total ion monitoring mode (SCAN) using splitless injection and a temperature program starting from 60 °C (0.5 min), and ending to 230 °C at 5 °C/min. The column used was NB-351 (0.2 μm × 0.2 mm × 25 m). Injector temperature was 230 °C and transferline temperature 230 °C. Authentic model compounds were used in the quantitation of the terpenes. Recoveries were tested as recommended by 3 M.

Soil sampling

Twenty samples (core diameter 5 cm) were systematically taken from the humus layer (FH) of the clear-cut (assay soil) and the forested reference (forest soil) plot in June 11, 1996, and combined to give one composite sample per plot. Green plant material was removed and the samples were sieved (mesh size 2.8 mm), and stored in plastic bags at 4 °C for not longer than two weeks before the analyzes. In one experiment in which unsieved forest soil was used, the sample contained also the litter layer and the upper mineral soil horizon (10 cm).

Incubation experiments to study the effect of terpenes on nitrogen mineralization and nitrification

To study nitrogen mineralization in the assay soil three treatments were used: (1) no treatment (control), (2) forest soil treatment, and (3) terpene treatment. Terpene treatment was a mixture of terpenes simulating those collected earlier from the forest plot, and it consisted of α-pinene (38%), β-pinene (38%), limonene (6%), myrcene (6%), camphene (6%), 3-carene (4%) and β-phellandrene (2%).

Mixture of terpenes (3 mL in a petri dish) or fresh unsieved forest soil (60 g f.w. field moisture) were placed in plastic containers (0.75 L), or it was left empty (control). The containers were covered with metal sieves. Assay soil (30 g d.w), with soil moisture adjusted to 60% of the WHC (water holding capacity), was placed in the sieve to avoid contact with the contents of the container except for vapour. There were three replicates of each treatment. The emission of VOCs from the mixture of terpenes was determined by placing, instead of assay soil, a diffusive sampler in the sieve. Containers were covered with foil and incubated in room temperature (22–24 °C) for 6 weeks. Water contents of the assay and forest soil were adjusted weekly. Before and after incubation, NH_4^+-N and (NO_2^- + NO_3^-)-N concentrations of the assay soil were measured as described in Smolander et al. (1995).

Laboratory measurements of the VOCs emitted by the soils

VOCs emitted by the soils were monitored in the incubation experiment described above. A VOC diffusive sampler was placed in the sieve, and below it in the container either assay or unsieved forest soil.

Measurement of nitrification in soil suspensions

Nitrification potential of the assay soil was studied in a 2-week incubation. Aerobic incubations of the soil were done in three replicates, as described earlier by Paavolainen and Smolander (1998), in mineral solution containing ammonium with continuous shaking. The pH of the suspension was kept at 5.0, which was the original pH of the assay soil. The suspensions were made of 7.5 g d.m. soil in 300 mL mineral solution.

The effect of terpenes on nitrification potential was studied by adding mixture of terpenes or α- and/or β-pinene at the beginning of the experiment and after that every third day (one addition 250 μg terpenes mL^{-1} mineral solution). In one of the experiments, the mixture of terpenes was added after a one-week-incubation only. To ensure the availability of the substrate, also ammonium (0.1 g $(NH_4)_2SO_4$ in 1 mL) was added at the same time as monoterpenes.

To study the effect of forest soil on the nitrification potential of the assay soil, soil suspensions were made consisting them both. Assay soil (40 g) and the mineral solution (160 mL) were mixed (Sorval Omni-mixer 17106) for 1 min at half speed. Of this suspension 10 mL was added to a suspension of forest soil (20 g f.w. in 300 mL solution), and kept at pH 5. A suspension of the assay soil (10 mL) in mineral solution (300 mL) served as a control. Ammonium was added every third day as before.

Measurement of denitrification

The effect of monoterpenes on denitrification in assay soil was studied in laboratory incubations. Six replicate soil samples (2 g d.w.) with moisture content adjusted to 100% of the WHC were kept at 14 °C in 125-mL glass bottles, covered with rubber septa, with no acetylene or with acetylene at partial pressure of 10 kPa. Inside the bottles was a small glass bottle (5 mL), which was hanging from the rubber septum. In three replicate samples 0.5 mL mixture of terpenes was added to the small bottle, and in the other three they were left empty. N_2O produced was measured

after 24 and 48 hours by gas chromatography as described by Paavolainen and Smolander (1998). Results are reported as the rate of N_2O-N production between 24 and 48 h. The solubility of N_2O was taken into account in the calculations (Moraghan and Buresh, 1977).

Basal respiration

The effect of terpenes on the activity of heterotrophic bacteria was measured as basal respiration as described by Priha and Smolander (1995). Six replicate soil samples of assay and forest soil (2 g d.w.) with moisture content adjusted to 60% of the WHC were kept at 14 °C for 44–48 hours. In three replicate samples, mixture of terpenes was added (0.5 mL) using the small glass bottles, as in denitrification measurements. The CO_2 production of terpenes was determined in similar bottles but without soil. The CO_2 evolved was measured by gas chromatography (Hewlett Packard 6890) equipped with a thermal conductivity detector and a Megapore GS-Q column, 30 m in length, using He as the carrier gas. The temperatures of the detector, injector, and column were 150°, 120° and 30 °C, respectively. The results given are means of four measurements taken over two weeks. The samples were aerated between measurements.

Statistical analyses

Means of different treatments were compared by student's *t*-test (2 means) or ANOVA (more than 2 means). When using ANOVA, differences between means were determined using Dunnett's test (when different treatments were compared to a control) or Tukey's test (when all treatments were compared). Differences between means were considered statistically significant when $p < 0.05$. The data were log-transformed if necessary.

Table 1. Sum of monoterpenes collected by diffusion sampling

	Field μg cm^{-3} 8 weeks^{-1}		Laboratory μg cm^{-3} 6 weeks^{-1}
	Ambient Air	Soil Microair	Emission from soil
Forest plot	0.4	2060	2.4
Clear-cut plot	0.3	0.1	0.2

Table 2. Monoterpenes collected in the forest plot by diffusive samplers ($n = 6$). Standard deviation in parentheses. There were no significant differences between the means

Monoterpene	$\mu g\ cm^{-3}$	$8\ weeks^{-1}$
3-carene	729	(1782)
alpha-pinene	719	(622)
beta-pinene	409	(583)
myrcene	75	(108)
beta-phellandrene	64	(64)
limonene	45	(82)
camphene	19	(11)

Results

VOCs emitted by the soils

The concentration of monoterpenes in the soil microair was 20 000 times higher in the forest than in the clear-cut plot (Table 1). The concentrations of monoterpenes in the ambient air of both plots were very small. Also in the laboratory, the soil from the forest plot produced more monoterpenes than the soil from the clear-cut plot. The ratios of forest plot: clearcut plot monoterpene production was, however, over thousand times higher in the field measurements than in the laboratory (20 000 in the field and 12 in the laboratory). The amounts of seven major monoterpenes measured in microair of the forest plot are listed in Table 2. The most abundant monoterpene was 3-carene. The amount of 3-carene was, however, over 4000 $\mu g\ cm^{-3}$ only in one sampler and less than 3 $\mu g\ cm^{-3}$ in the other samplers. In these five samplers, where the amount of 3-carene was low, the most abundant monoterpenes detected were α- and β-pinene.

Nitrogen transformations

In the incubation experiments, the assay soil produced 600 $\mu g\ (NO_2^- + NO_3^-)$-N / g d.m. soil in six weeks (Figure 1). Vapours from the forest soil (2 $\mu g\ cm^{-3}$, Table 1) had no significant effect on the net production of NH_4^+-N or $(NO_2^- + NO_3^-)$-N of the assay soil. Exposure to the vapours from the mixture of terpenes (4600 $\mu g\ cm^{-3}$), however, resulted in significantly greater net production of NH_4^+-N and lower net production of $(NO_2^- + NO_3^-)$-N when compared with the assay soil with no exposure (Figure 1). When

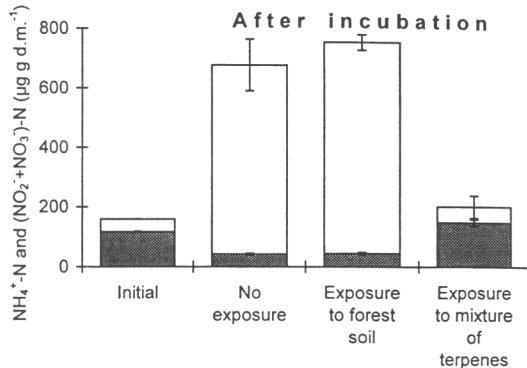*Figure 1.* Concentrations of NH_4^+-N (shaded) and $(NO_2^- + NO_3^-)$-N (white) in the assay soil at the initiation of the incubation and, after a 6-week-incubation in the laboratory (22 °C, 60% of the WHC) incubated with exposure to vapours from forest soil (2 $\mu g\ cm^{-3}$) or from mixture of terpenes (4600 $\mu g\ cm^{-3}$) or no exposure (control). Error bars indicate standard error of 3 replicate samples.
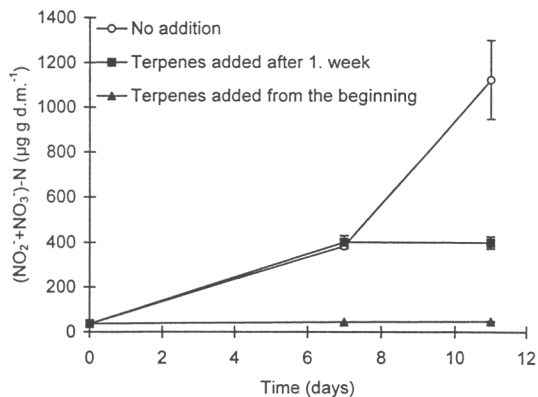

Figure 2. The effect of mixture of monoterpenes on nitrification potential of the assay soil during a 2-week-incubation at pH 5. Addition of monoterpenes (250 $\mu g\ mL^{-1}$ mineral solution) at the beginning or after a one-week-incubation, and after that every third day. Error bars as in Figure 1.

the amounts of mineral N in the soil incubated with the mixture of terpenes were compared to the initial amounts, net production of $(NO_2^- + NO_3^-)$-N was not detected, and the net production of NH_4^+-N was very low. Alltogether, the mixture of terpenes inhibited both net nitrification and net N mineralization.

In soil suspensions, the addition of terpenes inhibited nitrification (Figures 2, 3). The mixture of terpenes totally inhibited net nitrification both when added from the beginning of the experiment or after a one-week-incubation (Figure 2). The addition
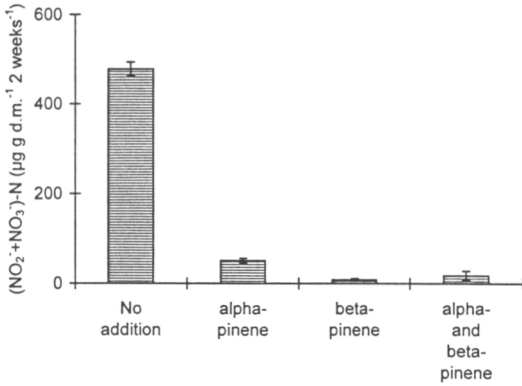
Figure 3. The effect of α- and/or β-pinene on nitrification potential of the assay soil during a 2-week-incubation at pH 5. Addition of pinenes (250 μg mL^{-1} mineral solution) at the beginning of the experiment and after that every third day. Error bars as in Figure 1.
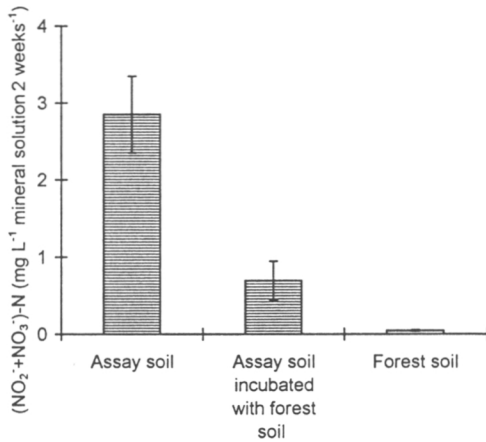

Figure 4. The production of $(NO_2^- + NO_3^-)$-N in soil suspensions of assay soil, assay soil incubated with forest soil and forest soil during a 2-week-incubation at pH 5. Error bars as in Figure 1.
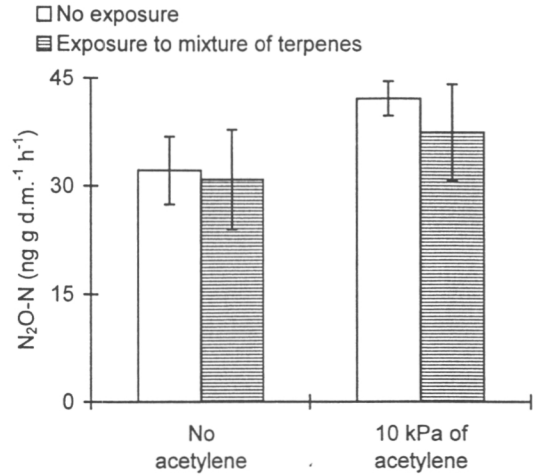

Figure 5. The production of N_2O-N in the assay soil with no C_2H_2 or with C_2H_2 at a partial pressure of 10 kPa incubated with or without exposure to the vapours from the mixture of monoterpenes. Error bars as in Figure 1.
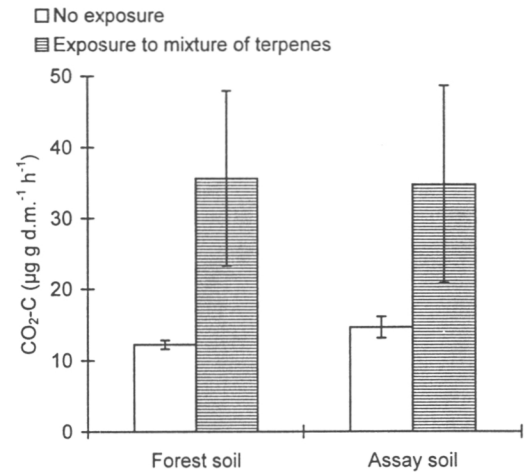
Figure 6. The production of CO_2-C in the forest and assay soils incubated with or without exposure to the vapours from the mixture of monoterpenes. Error bars as in Figure 1.

of terpenes did not, however, result in immobilization of $(NO_2^- + NO_3^-)$-N. Net production of $(NO_2^- + NO_3^-)$-N after a two-week-incubation was significantly lower in soil suspension with β,- or α- and β-pinene than with only α-pinene (Figure 3). The percentage of inhibition by α-pinene alone, β-pinene alone and both together were 90 ($\pm$2)%, 98 ($\pm$1)% and 96 ($\pm$3)%, respectively.

Equal amounts of the assay soil produced significantly less $(NO_2^- + NO_3^-)$-N in forest soil – mineral solution suspension than when suspended in mineral solution only (Figure 4). The contribution of forest soil to the production of $(NO_2^- + NO_3^-)$-N was determined also, but was negligible.

Denitrification

With or without acetylene addition, the mixture of monoterpenes had no significant effect on the production of N_2O in the assay soil (Figure 5).

Basal respiration

The addition of monoterpenes resulted in more than two-fold increase in CO_2 production in the forest and assay soils (Figure 6). The production of CO_2 was not

detected in the absence of soil (results not shown), meaning that autooxidation of monoterpenes did not occur.

Discussion

In the field, the production of monoterpenes was intensive in the forest soil microair, but the quantities were low in ambient air (Table 1). The high amounts of α- and β-pinene measured in the forest soil microair (Table 2) could partly be explained by the injury caused to the roots by the insertion of the diffusive samplers to the soil. Marpeau et al. (1989) observed a large increase of α- and β-pinene in the cortical tissues surrounding an injury, and this effect lasted at least for two months after the injury. In our study 3-carene was produced in high amounts in one location in the forest only. Also Pohjola (1993) observed high variation in monoterpene levels in coniferous trees, and concluded that this indicates strong genetic control (Pohjola, 1993).

In the field the emission of monoterpenes probably is not as constant as the emission from the mixture of monoterpenes in the laboratory. If these values are, however, calculated per week, the emissions in the field and laboratory are of the same magnitude: 260 and 770 $\mu g\,cm^{-3}\,week^{-1}$. In the laboratory, this amount was sufficient to significantly inhibit net N mineralization and nitrification (Figure 1). In a study by White (1991), where α-pinene and limonene were added to a soil from grassland area, net production of nitrate was also inhibited in a 21-day laboratory incubation. Comparison of terpene emissions in the laboratory and in the field is difficult, but considering the high variation in the production of terpenes in the field (Table 2), the concentrations used in the laboratory are most probably also met up in nature.

Addition of monoterpenes inhibited the production of $(NO_2^- + NO_3^-)$-N also in soil suspensions (Figures 2, 3). This inhibition was not due to enhanced immobilization of NO_3^- -N relative to NH_4^+-N, as nitrate was not immobilized during experiment (Figure 2). Monoterpenes were added continuously to ensure the availability of this potential inhibitor, since White (1991) observed that, when pure liquid of monoterpene was added to soil, only 0.4% of it could be extracted from the soil at the end of a 21-day incubation. The total amount added in two weeks was 1000 $\mu g\,cm^{-3}$ soil suspension. This is also in the same order of magnitude as the concentrations in the forest

soil microair in the field (Table 1). In soil suspensions lack of NH_4^+-N did not limit nitrification. In laboratory incubations no net consumption of NH_4^+-N was detected in the terpene treated soil, indicating that the amount of NH_4^+-N was not limiting nitrification either (Figure 1). Therefore our results imply to allelopathic inhibition of nitrification by monoterpenes i.e. terpenes had a direct effect on cell physiology of nitrifiers as also suggested by White (1988, 1991).

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 basal respiration by the vapours from the mixture of terpenes could point to this (Figure 6). In soil suspension the addition of monoterpenes resulted also in enhanced immobilization of ammonium: ammonium was consumed as much in the non-nitrifying samples with added monoterpenes as in the nitrifying control samples (results not shown).

The terpene vapours from the forest soil ($2\,\mu g\,cm^{-3}$, Table 1) did not affect nitrogen mineralization (Figure 1). This indicates that the monoterpene production by the forest soil was not high enough to inhibit nitrification. Thus, if monoterpenes are the inhibitory agents in the forest soil, then the forest soil itself is not the main emitter of terpenes, but the high production of terpenes probably requires the presence of a living root system. This is supported by the VOC measurements, where the ratios of forest plot:clearcut plot terpene production was over thousand times higher in the field than in the laboratory incubations (Table 1). Also Stahl and Parkin (1996) concluded that even though terpenes appear to be the VOCs most abundantly produced by fungi, their usefulness as indicators of fungal activity in soil may be limited by the fact that they are also commonly produced by plant roots (Stahl and Parkin, 1996).

There is no clear consensus of the purpose of hydrocarbon production and emission by vegetation (Benjamin et al., 1996). Terpenes, however, are probably produced by the plants as a defence against plant pathogens (Cheniclet et al., 1988), but may perhaps also indirectly affect plant nutrition. Norway spruce prefers NH_4^+ over NO_3^- as a nitrogen source (Kronzucker et al., 1997). It might be possible that Norway

spruce produces monoterpenes also to influence the rates of nitrification and nitrate leaching, which, in turn, influences the amounts of NH_4^+ and NO_3^- available for uptake. Also earlier findings suggest that the reason why climax communities produce chemicals which inhibit nitrification, is to retain soil nitrogen as in the form of NH_4^+ to enhance the long-term maintenance and stability of an ecosystem by reducing energy requirements and by increasing retention of nitrogen in upper soil horizons by decreasing leaching (Rice and Pancholy, 1972).

In the forest soil less than $10 \, NH_4^+$-oxidizers cm^{-3} soil were detected, whereas in the clear-cut plot the number was over $10^5 \, NH_4^+$-oxidizers cm^{-3} soil (Paavolainen and Smolander, 1998). In a ponderosa pine community, low numbers of nitrifiers were suggested to be a direct result of toxic effects from secondary plant chemicals, like condensed tannins (Lodhi and Killingbeck, 1980). In soil suspension experiments forest soil inhibited the production of $(NO_2^- + NO_3^-)$-N of the assay soil, even though the vapours from the forest soil had no effect on nitrification of the assay soil (Figure 1, 4). This could be explained either by immobilization of $(NO_2^- + NO_3^-)$-N by forest soil, or by inhibition of nitrification by water-soluble, non-volatile inhibitors, like polyphenols, as suggested by Lodhi and Killingbeck (1980). The low number and activity of nitrifiers in the forest soil could be the sum of several factors: production of monoterpenes and polyphenolic compounds by spruce, and as suggested by Smolander et al. (1998) lower pH and rate of nitrogen mineralization as compared to the clear-cut plots.

Denitrification was not affected by addition of monoterpenes (Figure 5). Thus, monoterpenes were either not a useful carbon source for the denitrifiers or the amount of carbon was not limiting denitrification during these short-term laboratory incubations.

In conclusion, monoterpenes were detected in significant amounts in the soil microair of the forest only, and not in the clear-cut area. In the laboratory experiments they inhibited production of $(NO_2^- + NO_3^-)$-N in soils when applied at concentrations at which they had been detected in the field. Direct inhibition of net nitrification by monoterpenes was evident, but the results indicated also indirect inhibition due to enhanced immobilization of NH_4-N. Thus it seems that monoterpenes could partly explain the negligible nitrification observed in the forest soil.

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V

Nitrification and denitrification in forest soil subjected to sprinkling infiltration

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Abstract In Ahvenisto esker, southern Finland, artificial recharging of groundwater has been done by sprinkling infiltration i.e. by sprinkling lake water directly onto forest soil. Due to infiltration, the pH of the humus layer rose from about 5 to 6.5, nitrification was initiated, and the fluxes of N_2O and leaching of nitrate from the soil increased. The aim was to further study nitrogen transformations in different soil layers, and to determine the response of nitrification to pH. Nitrification in ammonium-enriched soil suspensions was pH-dependant in a gradient from 4.7 to 6.7. In the soils subjected to infiltration the production of (NO_2+NO_3)-N was inhibited by decreasing the pH to 5.3 or lower. Low pH also led to decreased numbers of nitrifiers. In the soils not subjected to infiltration (control soils), (NO_2+NO_3)-N production initiated at pH 6.7, and the numbers of nitrifiers increased. In incubation experiments, with no added ammonium, the adjustment of pH to 6.7 also initiated nitrification in the control soils. Thus, increase in soil pH was the main reason for initiation of nitrification at this site. During infiltration, N_2O was produced mainly by denitrification, and approx. 75% of the denitrification products was N_2. In the samples from the humus layer the concentrations of (NO_2+NO_3)-N, the net production of mineral N and net nitrification were in general less whereas denitrification enzyme activity and denitrification potential were higher than in the samples from the mineral soil layer. The mineral soil may therefore contribute substantially to the leaching of nitrate.

Keywords: Nitrification, denitrification, forest soil, groundwater, sprinkling infiltration

1. Introduction

Sprinkling infiltration is a new method for recharging groundwater reserves in which the raw water is sprinkled directly onto the forest soil from a network of pipes. The effects of sprinkling infiltration on forest soil, percolation water and vegetation were studied in the Ahvenisto esker in Hämeenlinna, southern Finland (Helmisaari et al., 1998). In the first year of infiltration pH rose from about 5 to 6.5 in the humus layer and nitrification was initiated. The fluxes of N_2O from the soil also increased (Helmisaari et al., 1998; Lindroos et al., 1998).

Net nitrification is usually negligible in Finnish acid coniferous soils, unless nitrogen is added via atmospheric input or fertilization, or soil acidity is alleviated by liming (Aarnio and Martikainen, 1992; Martikainen et al., 1993; Priha and Smolander, 1995; Smolander et al., 1995). Clear-cutting can also initiate nitrification in forest soil, the main reasons for which are probably the increase in both the availability of ammonium and soil pH (Paavolainen and Smolander, 1998; Smolander et al., 1998) and the reduction of allelopathic inhibitors such as terpenes (Paavolainen et al., 1998).

The flux of N_2O from soil mainly originates from nitrification and/or denitrification. The natural fluxes of N_2O from mineral soil sites in Finland are small (Martikainen et al., 1994), but all factors that enhance the availability of ammonium and nitrate can also favour the production of nitrogen gases (e.g. Priha and Smolander, 1995; Paavolainen and Smolander, 1998). Liming of forest soil has, however, been observed to decrease the emissions of N_2O (Brumme and Beese, 1992). This may not be due to decrease in total denitrification but rather a decreased ratio of N_2O to N_2 in response to increased pH of the forest soil (e.g. Nägele and Conrad, 1990; Willison and Anderson, 1991; Brumme and Beese, 1992).

In the Ahvenisto esker the leaching of nitrate can pose a threat to the quality of groundwater (Helmisaari et al., 1998, Lindroos et al., 1998). Increased production of N_2O in the groundwater recharge area, 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.

Here we studied effects of increased pH on the initiation of nitrification after sprinkling infiltration, and whether nitrification rates could be controlled by regulating soil pH. We also determined the contribution of nitrification and denitrification in N_2O production, and whether the main product of denitrification was N_2 or N_2O. Nitrogen transformations were studied in laboratory experiments both in the humus and mineral soil layers.

2. Materials and methods

2.1. Site description

The study site was the experimental sprinkling infiltration site in the Ahvenisto esker area of Hämeenlinna (61°01'N/24°47'E). According to the Finnish classification of Cajander (1949), the forest site was fertile *Oxalis-Maianthemum* type. The forest stand was a mixture of Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* L.). The site is described in more detail in Lindroos et al. (1998).

Surface water was supplied from a near-by lake and routed through the plots by a network of pipes. Water was sprinkled directly onto the forest floor from the irrigation pipes. The sprinkling infiltration (625 m^2) area was divided into five plots representing two controls (plots 1 and 4), continuous infiltration during the summertime (plot 2), periodical infiltration during the summertime (plot 3) and continuous infiltration during the wintertime (plot 5). The dominant tree species on plots 1, 2 and 3 was Scots pine and on plots 4 and 5 Norway spruce (Lindroos et al., 1998) and for this reason plot 1 served as a control for plots 2 and 3 and plot 4 served as a control for plot 5. Each plot was further divided into three subplots.

Sprinkling infiltration was performed during 1995 – 1998. The amount of irrigation water applied to the site was more than 2000 times the annual precipitation of 600 – 650 mm. The amount of irrigation water applied during the winter period (30 Oct. 1997 – 18 May 1998) was 1669 m^{3}m^{-2} (plot 5), during continuous infiltration in the summertime (18 May – 29 Oct. 1998) 1452 m^{3}m^{-2} (plot 2), and during periodical infiltration in the summertime (8 June – 20 July and 17 Aug. – 18 Sept. 1998) (plot 3) 607 m^{3}m^{-2}. The amounts of irrigation during the previous two years were of the same magnitude (Lindroos et al., 1998).

2.2. Soil Sampling

Soil was sampled on 25 May and 23 September 1998. Twenty samples (core dia, 2.5 cm) were taken from the humus layer (thickness 5-10 cm) and, on 25 May, also from the mineral soil (the uppermost 0-10 cm) of each subplot systematically, and bulked into three samples per plot. The samples were collected from a distance of 2 m from the irrigation pipe in infiltration plots and from corresponding places in the control plots. Green plant material was removed, and soils were sieved (humus 2.8 mm mesh, mineral soil 2 mm mesh) and stored in the dark at 4°C for not longer than 4 weeks before the analyses. Some characteristics of the soils collected in May are shown in Table 1. The soils sampled in September were used only in denitrification measurements. In some of the analyses the samples from the three subplots were studied separately, whereas in some analyses composite samples were used (i.e. the samples from the subplots were combined).

Table 1. Some soil characteristics. The values are means of three subplots

Plot	Soil layer	pH(H_2O)	Organic matter, mg / cm^3 soil	Total organic C, mg / cm^3 soil	C:N
1	Humus	4.7	81	43	25
	Mineral soil	4.9	59	28	22
2	Humus	6.6	74	45	27
	Mineral soil	6.5	62	26	24
3	Humus	6.5	79	42	24
	Mineral soil	6.4	67	30	21
4	Humus	5.1	73	42	27
	Mineral soil	4.8	61	29	23
5	Humus	6.6	74	43	22
	Mineral soil	6.5	60	23	20

Treatment mean (SEM in parentheses)

Plot	Soil layer	pH(H_2O)	Organic matter, mg / cm^3 soil	Total organic C, mg / cm^3 soil	C:N
1, 4	Humus	4.9 (0.2)	77 (4.0)	43 (0.5)	26 (1.0)
	Mineral soil	4.9 (0.0)	60 (1.0)	29 (0.5)	23 (0.5)
2, 3, 5	Humus	6.6 (0.0)	76 (1.7)	43 (0.9)	24 (1.5)
	Mineral soil	6.5 (0.0)	63 (2.1)	26 (2.0)	22 (1.2)

Treatment symbols: 1, 4 = control; 2, 3, 5 = infiltration

2.3. Nitrogen transformations in incubation experiments

Nitrogen transformations were studied in aerobic incubation experiments in the laboratory at constant temperature ($14°C$) and moisture ((60% of the water-holding capacity (WHC)) for 40 days using two replicate samples, as described by Smolander et al. (1995). To calculate net ammonification and nitrification, NH_4-N and (NO_2+NO_3)-N concentrations were subtracted from final (post-incubation) concentrations. Net formation of mineral N was estimated as the sum of net ammonification and nitrification.

To study the effect of pH increase on net nitrification, the pH of six replicate humus samples from control plots was increased up to 6.7 before the incubation with a predetermined amount of $CaCO_3$. At the end of incubation, three replicates were used for pH measurements and the rest for mineral N determinations.

To determine whether nitrification was autotrophic or heterotrophic, three replicate humus samples from the infiltration plots were incubated with C_2H_2 at a partial pressure of 2.5 Pa. This concentration of acetylene is reported to be a specific inhibitor of autotrophic nitrification (Klemedtsson et al., 1988). During the 40-day incubation, the samples were aerated and C_2H_2 was reapplied three times a week.

2.4. Measurement of nitrification in soil suspensions

Response of nitrification to a pH-gradient (4.7, 5.3, 6.0 and 6.7) was investigated in 2-week soil suspension experiments as described earlier by Paavolainen and Smolander (1998). Briefly, two replicates of each (3.75 g d.m.) were incubated with continuous shaking in mineral solution (150 ml) containing ammonium. The pH of the soil suspensions was adjusted daily with either $0.1 - 1.0$ M Na_2CO_3 or H_2SO_4. To ensure the availability of substrate, 1 ml of a $(NH_4)_2SO_4$ solution (100 g $(NH_4)_2SO_4$ l^{-1}) was added after the first sampling. Suspensions of soil from the control plot 4 were further incubated for two additional weeks.

2.5. Enumeration of autotrophic nitrifiers

The most probable number (MPN) method was used to determine the numbers of autotrophic NH_4- and NO_2- oxidizers in the soil samples as described by Paavolainen and Smolander (1998). In addition the numbers of nitrifiers were determined from soil suspensions both after the first day and after the 2-week incubation. The first dilution was done from the soil samples by mixing 20 g of fresh soil with 180 ml of sterilized H_2O (1 min at half speed in a homogenizer, Sorval Omni-Mixer 17106), and from soil suspension by mixing 2 ml of the soil suspension with 18 ml of sterilized H_2O (3 min at full speed in a vortex, Scientific Industries Vortex-genie 2). The MPN tubes were incubated for 10 weeks at $20°C$ in the dark.

2.6. Measurement of N_2O production and denitrification enzyme activity (DEA)

A high partial pressure of C_2H_2 (1-10 kPa) blocks the enzyme nitrous oxide reductase, so that all gaseous denitrification products remain as N_2O (Klemedtsson et al., 1977). The contribution of autotrophic nitrification to total N_2O production can be estimated under C_2H_2

at partial pressures of 2.5-5 Pa, because low partial pressures of C_2H_2 inhibit ammonium oxidase, thus blocking autotrophic nitrification, but have only a small effect on denitrification (Klemedtsson et al., 1988). However, substantial inhibition of the enzyme nitrous oxide reductase can also occur under this low partial pressure of C_2H_2 leading to underestimation of nitrifier nitrous oxide production (Kester et al., 1997).

Preliminary experiments were done with humus and mineral soils to determine the optimal low partial pressure of C_2H_2 that would inhibit nitrification without affecting nitrous oxide reductase. The measurements were done with the protocol described below, except that the partial pressures of C_2H_2 used were 0, 1, 2, 4, 6, 8, 10, 20, 50 or 100 Pa. The production of N_2O-N was the same or slightly smaller in all C_2H_2 treatments below 8 Pa than with no C_2H_2, but production increased from 10 Pa with increasing partial pressure of C_2H_2 (results not shown). These results showed that in these soils significant inhibition of enzyme nitrous oxide reductase occurs at partial pressures of 10 Pa and higher. Therefore we chose 2.5 Pa C_2H_2 for the assay.

N_2O production was studied in laboratory incubations at constant temperature (14°C) and moisture (100% of the WHC) using three replicate soil samples (4 g d.m. humus, 6 g d.m. mineral soil) with either no C_2H_2, or with C_2H_2 at partial pressures of 2.5 Pa or 10 kPa. N_2O produced was measured after 1 and 2 d incubations using a gas chromatograph (Hewlett Packard 6890 series), equipped with an electron capture detector and a Megapore GS-Q column (J&W Scientific), 30 m in length, using He (10 ml min⁻¹) as carrier gas and ArCH₄ (95:5) as the make-up gas. The temperatures of the detector, injector and column were 300, 100 and 30° C respectively. Results given are production rates of N_2O-N between 1 and 2 days. The solubility of N_2O in water was taken into account in the calculations (Moraghan and Buresh, 1977).

Denitrification enzyme activity (Luo et al., 1996) was measured from the soils using the same amounts of soil as above, but adding solutions of KNO_3 and glucose to give a NO_3-N concentration of 50 µg ml⁻¹ soil water (found to be optimal in preliminary experiments) and a glucose concentration of 15 mg ml⁻¹ soil water (shown to be optimal in a previous study by Priha and Smolander, 1999). The water-content of the soils was adjusted so that the soils were waterlogged. The air in the bottles was replaced with N_2, and C_2H_2 was added to give a partial pressure of 10 kPa as described by Priha and Smolander (1999). The samples were incubated for 5 h with continuous shaking (150 rev min⁻¹) in the dark at 22° C, and N_2O produced was measured as described above.

2.7. Statistical analyses

The statistical analyses were performed with ANOVA. Differences between means were considered statistically significant when $p < 0.05$. The results from the nitrogen transformations and N_2O production -measurements and MPN analyses were log-transformed. Even though the infiltration treatments differed, in order to see the general effect of infiltration we compared the means of the results from infiltration plots with those of the control plots. To compare humus and mineral soils, the results are expressed on volume basis.

3. Results

3.1. Nitrogen transformations in incubation experiments

NH_4-N concentrations tended to be higher in the humus and mineral soil layers of the infiltration plots than of the control plots, but the differences were not statistically significant ($p = 0.1$ in both humus and mineral soil) (Table 2). (NO_2+NO_3)-N concentrations were negligible in the control soils, while all the soil from the infiltration plots contained (NO_2+NO_3)-N. In the mineral soil (NO_2+NO_3)-N concentrations were significantly higher than in the humus.

Net nitrification was determined in incubation experiments. Net nitrification was negligible in the control soils (Table 2). Net nitrification was intensive in both soil layers in the infiltration plots, particularly in the mineral soil of the wintertime infiltration plot.

Table 2. Nitrogen transformations during 40-d incubation of the soils in the laboratory. The values are means of three subplots

Plot	Soil layer	Initial, $\mu g / cm^3$		Net formation, $\mu g / cm^3 / 40d$	
		NH_4-N	(NO_2+NO_3)-N	(NO_2+NO_3)-N	$(NH_4+NO_2+NO_3)$-N
1	Humus	10.1	0.0	0.1	-1.7
	Mineral soil	2.1	0.1	0.4	21.0
2	Humus	11.8	11.0	20.8	10.7
	Mineral soil	7.9	33.2	35.5	31.1
3	Humus	12.2	7.4	21.5	11.1
	Mineral soil	11.0	22.9	20.6	16.2
4	Humus	5.3	0.0	0.1	11.2
	Mineral soil	5.5	0.2	0.0	36.1
5	Humus	19.3	11.2	49.4	39.7
	Mineral soil	32.6	25.9	91.7	68.4
Treatment mean (SEM in parentheses)					
1, 4	Humus	7.7 (2.4)	0.0 (0.0)	0.1 (0.0)	4.8 (6.5)
	Mineral soil	3.8 (1.7)	0.2 (0.0)	0.2 (0.2)	28.6 (7.5)
2,3,5	Humus	14.4 (2.4)	9.9 (1.2)	30.6 (9.4)	20.5 (9.6)
	Mineral soil	17.2 (7.8)	27.3 (3.1)	49.3 (21.6)	38.6 (15.5)

Treatment symbols: 1, 4 = control; 2, 3, 5 = infiltration

With the exception of the samples collected from the humus layer of control plot 1, the net formation of mineral N was always positive (Table 2). The differences between the control and infiltration soils or between the different soil layers were not statistically significant. However, in all the soils net formation of mineral N tended to be greatest in the mineral soil layer.

Before the incubation, humus samples from the control plots were treated with $CaCO_3$, raising the pH to 6.7. At the end of the incubation, the pH of these soils was 7.1 (plot 1) and 7.3 (plot 4). The rise in pH lead to a significant increase in the net formation of mineral N (Fig. 1). Moreover, there was strong net nitrification in both $CaCO_3$-treated soils.

Humus samples collected from the infiltration plots were regularly treated with 2.5 Pa C_2H_2 during the incubation. C_2H_2 was found to completely inhibit nitrification in all soils (results not shown).
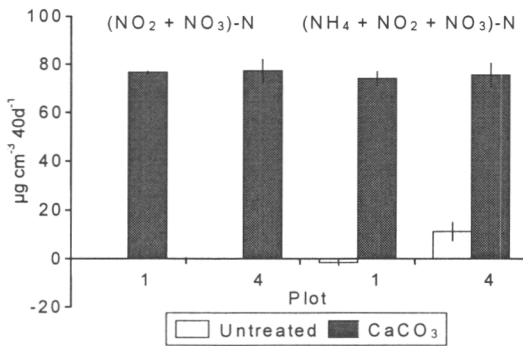

Figure 1. The effect of pH increase on net nitrification and formation of mineral N in 40-d laboratory incubation. The pH of samples from the humus layer of control plots 1 and 4 was increased up to 6.7 with $CaCO_3$. The results are the means of three laboratory replicates made of composite samples ($\pm$ SEM).

3.2. Nitrification potential in soil suspensions

Ammonium-enriched suspensions of soils were incubated at pH values 4.7, 5.3, 6.0 and 6.7. In control soils, nitrification started at pH 6.7, but the quantities of (NO_2+NO_3)-N were small during the two-week incubation (Figs. 2a, b). When soil suspensions made from the control plot 4 were incubated for two additional weeks, the amount of (NO_2+NO_3)-N produced at pH 6.7 was almost 200 $\mu g\ cm^{-3}$ but there was negligible production at lower pH values (Fig. 2b).

(NO_2+NO_3)-N was produced at much higher concentrations in the soils from the infiltration plots as compared with the control soil suspensions (Figs. 2c, d). (NO_2+NO_3)-N production was highest at pH 6.7, whilst negligible production occurred at pH 5.3 or under.
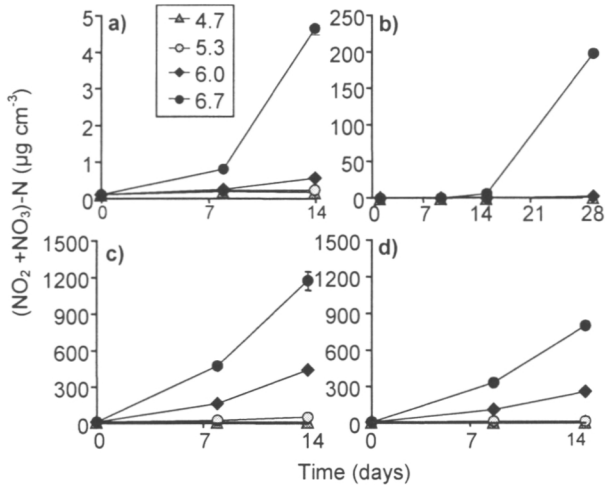
Figure 2. Effect of pH on the nitrification potential in ammonium-enriched soil suspensions during a 2- or 4-week incubation. The results are means of two laboratory replicates (± SEM), made of composite samples from the humus layer of control plots (a) 1 and (b) 4 and of infiltration plots (c) 2 and (d) 5. The infiltration treatments are given in section 2.1.

3.3. Numbers of autotrophic nitrifiers

Numbers of nitrifiers were determined by the MPN method. The soils from the infiltration plots were found to have significantly higher numbers; about 500 times that of the control soils (Fig. 3a).
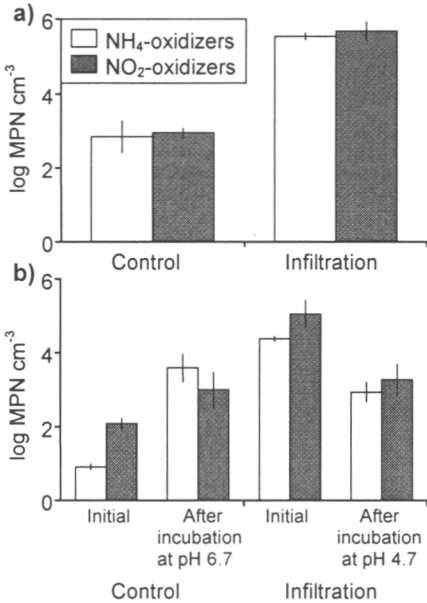

Figure 3. Numbers of NH_4- and NO_2-oxidizers (MPN) in the composite samples from the humus layer. The results are means of control plots 1 and 4 and of infiltration plots 2 and 5 (± SEM). (a) Determination from soil samples and from (b) soil suspension at the beginning and after the 2-week incubation at 6.7 (control soils) or at pH 4.7 (infiltration soils). The infiltration treatments are given in section 2.1.

The numbers of nitrifiers were also determined from the soil suspensions (Fig. 3b). After the two-week incubation of control soils at pH 6.7, the numbers of NH_4- oxidizers had increased about 600-fold and NO_2- oxidizers about 10-fold. In the suspensions of infiltration soils kept at pH 4.7, the numbers of both NH_4- and NO_2- oxidizers had decreased by about 40 times at the end of the incubation. Only the changes in the numbers of NH_4- oxidizers were statistically significant.

The counts of nitrifiers were smaller when the extraction was done from the soil suspensions (at the beginning of the experiment) rather than directly from the corresponding soil (Figs. 3a, b).

3.4. N_2O production

The production of N_2O-N in soil samples from control plot 1 was negligible (results not shown). With infiltration plots the N_2O-N production was significantly less in samples taken from the mineral soil than from the humus layer under all the treatments (2.5 Pa, 10 kPa and no C_2H_2) (Fig. 4a). In the presence of 10 kPa C_2H_2 the production was significantly higher than in the presence of either 2.5 Pa or no C_2H_2; under these treatments the production was on the same level (not significantly different) (Figs. 4a, b). The difference between the production of N_2O-N in samples under 10 kPa C_2H_2 and with no C_2H_2 varied between soils from different infiltration plots and between the two samplings. On average, humus soils not exposed to C_2H_2 produced 27% of the amount under 10 kPa, and the mineral soils produced 18% of the amount under 10 kPa.
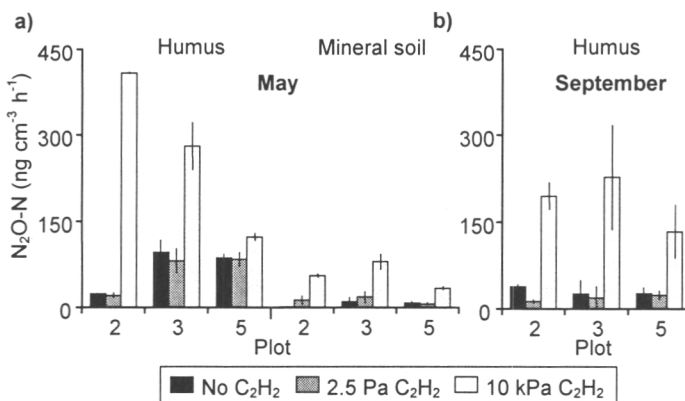

Figure 4. Production of N_2O-N with no C_2H_2 and with C_2H_2 at partial pressure of 2.5 Pa or 10 kPa in the soils from the infiltration plots 2, 3 and 5 in May and September. The results are (a) means of three laboratory replicates made of composite samples (± SEM) and (b) means of the three subplots (± SEM). The infiltration treatments are given in section 2.1.

3.5. Denitrification Enzyme Activity

Denitrification enzyme activity was measured as N_2O accumulation in the presence of 10 kPa C_2H_2 over a period of 5 hours (Fig. 5). N_2O production was significantly greater both in the humus and mineral soil layers with infiltration than in control plots. N_2O production in the humus layer of infiltration plots was significantly higher than in the mineral soil.
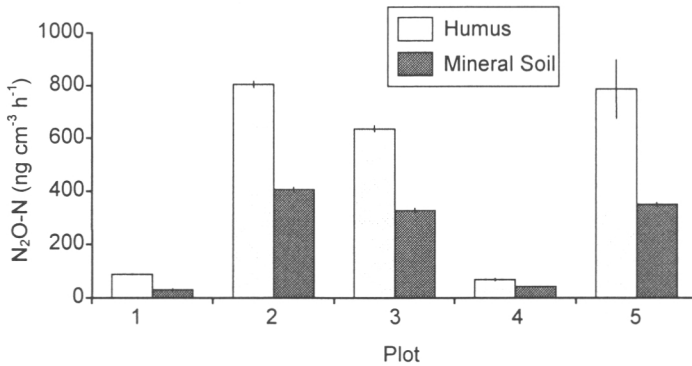

Figure 5. Denitrification enzyme activity (DEA) of the composite soil samples from control (1,4) and infiltration plots (2,3,5). The results are means of three laboratory replicates (± SEM). The infiltration treatments are given in section 2.1.

4. Discussion

Nitrification in ammonium-enriched soil suspensions showed a strong and consistent response to pH (Figs. 2a-d), as was also shown previously with soils collected from a clear-cut Norway spruce stand (Paavolainen and Smolander, 1998). In the control soils with an original pH of about 5 (NO_2+NO_3)-N production was initiated by increasing the pH to 6.7. Conversely, in the soils treated with infiltration with an original pH of 6.7, the production of (NO_2+NO_3)-N could be inhibited by decreasing the pH to 5.3 or less. The effect of pH also became evident in incubation experiments with no added ammonium, where increasing the pH up to 7.0 by $CaCO_3$ initiated nitrification in the control soils (Fig. 1). Thus, the increase in soil pH of the humus layer from about 5 to 6.5, caused by the high pH of the infiltration water (about 7, Lindroos et al., 1998), is likely to be the major reason for the initiation of nitrification in the Ahvenisto esker. If required, nitrification could probably be controlled by decreasing the pH of the infiltration water. The increased NH_4-N concentrations in the infiltration plots also enhanced the activity of the nitrifiers. However, when ammonium was present the soil pH was the factor in determining the rate of (NO_2+NO_3)-N production.

(NO_2+NO_3)-N production in soil suspensions was negligible at pH 4.7 and 5.3 (Figs. 2a-d). The production of (NO_2+NO_3)-N was also negligible at pH 6 in the control soils, while infiltration soils showed activity at this pH. This indicates adaptation of the nitrifiers in infiltration soils to a pH close to 6. Nitrification is reported to be performed by both acid-tolerant and acid-sensitive nitrifiers (De Boer et al., 1990), and according to this

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classification the nitrifying populations found in this study site were acid-sensitive. Nitrification was found to be autotrophic, as infiltration soils failed to nitrify in the presence of 2.5 Pa C_2H_2. Autotrophic and acid-sensitive nitrification was also found in the humus layers of spruce forests in Sweden and Denmark (Persson and Wirén, 1995) and in Finland (Paavolainen and Smolander, 1998).

The numbers of nitrifiers were determined both directly from the soils and from the soil suspensions (Figs. 3a, b). Lower yields were obtained from the soil suspensions than from the soil. The reason for this could be the different treatments; in contrast to the soil samples, the soil suspensions were not homogenised and it is likely that the nitrifying bacteria were not extracted as efficiently.

In soil suspensions, the numbers of NH_4-oxidizers in the control soils were increased by 600-fold after incubation for 2 weeks at pH 6.7 (Fig. 3b). The numbers of NO_2-oxidizers did not increase as much, as also previously shown by Stams et al. (1990). With infiltration soils the numbers of nitrifiers decreased during the incubation at pH 4.7. These results clearly show that when ammonium was present, pH controlled the numbers of nitrifiers and thus also the production of (NO_2+NO_3)-N. The numbers of nitrifiers of the infiltration soils were approx. 500 times higher than those of the control soils (Fig. 3a). Therefore the increase in pH of the infiltration soils (Helmisaari et al., 1998; Lindroos et al., 1998) has probably caused a similar increase in the numbers of nitrifiers in the forest soil as observed in the laboratory. In control soils, the small nitrifying community, although present, is inhibited by the low pH and thus unable to develop.

The concentrations of (NO_2+NO_3)-N in the mineral soil were roughly double those present in the humus layer (Table 2). Persson and Wirén (1995) found that in laboratory incubations of acid forest soils more NO_3-N was sometimes formed in the 0-10 cm mineral soil layer than in the humus layer. Also in our study, net formation of mineral N and net nitrification was equal, or higher in the mineral soil and this partly explained the large (NO_2+NO_3)-N concentrations of the mineral soil. The mineral soil is therefore a suitable habitat for the nitrifiers, and the soil layers underneath the humus layer might substantially contribute to NO_3 leaching. The differences in (NO_2+NO_3)-N concentrations between the layers could also be explained by the increased biomass of grasses on the infiltration plots (Helmisaari et al., 1998). If nitrate is present in the soil, grasses prefer it over ammonium as a nitrogen source (Falkengren-Grerup and Lakkenborg-Kristensen, 1994). The majority of the grass roots will only penetrate the humus layer, leaving nitrate in the mineral soil relatively unexplored.

In denitrification enzyme activity measurements, with only a short incubation time, enzyme activity is dependant on pre-existing denitrifying enzymes, whereas in denitrification potential measurements the longer incubation time allows the synthesis of new enzymes (Luo et al., 1996). In the control soils DEA was less than with the infiltration soils (Fig. 5), and due to the lack of nitrate denitrification potential was negligible. In the infiltration soils the DEA values obtained were about three times higher than those of Priha and Smolander (1999) for pine and spruce forests in Finland. Infiltration increased the moisture content of the soil, promoting microsites with anoxic conditions, increased the soil pH and nitrate concentrations, all of which can lead to enhanced denitrification populations (reviewed by Martikainen, 1996).

Both DEA and denitrification potential were higher in the humus layer than in the mineral soil (Figs. 4a,5). Henrich and Haselwandter (1997) found denitrification to be considerably higher in the humus layer of an acid spruce forest stand than in the mineral soil, and they attributed this to the higher nitrate content of the humus layer. In our study, however, the concentrations were higher in the mineral soil. In terms of substrate availability, denitrification would therefore be expected to occur more freely. In forested peatland in Finland higher production of N_2O in the upper layer (0-5 cm) compared with the 5-10 cm layer was explained by poorer availability of C compounds in the lower layer, and thus decreased activity of heterotrophic denitrifiers (Regina et al., 1998). The difference in N_2O production between the layers is probably also explained in our study by the better availability of organic C in the humus layer than in the mineral soil (Table 1).

Selective use of C_2H_2 was used to differentiate between the N_2O production of nitrification and denitrification. In laboratory experiments with soil samples adjusted to WHC 100%, N_2O production as a by-product of nitrification was negligible since there was no difference between samples treated with 2.5 Pa C_2H_2 or no C_2H_2 (Figs. 4a, b). Thus it seems that at least during infiltration when the soils are saturated with water, N_2O originates mainly from denitrification. It has been shown also in many other studies that high soil moisture content favours N_2O production by denitrification (Inubushi et al., 1996; Bollmann and Conrad, 1998). Also the dominant production process did not vary seasonally, in contrast to the study of Kester et al., (1997), where in spring nitrification was the principal source of N_2O but denitrification was more important in autumn.

Because nitrification did not contribute to N_2O production in our experiment, the difference between N_2O accumulation at 10 kPa C_2H_2 and with no C_2H_2 can be considered equivalent to the potential release of N_2 from samples. As only about 25% of the denitrification products was N_2O, N_2 can be considered the dominant product of denitrification in the soils during infiltration. Low pH is known to increase the N_2O to N_2 ratio (Focht and Verstraete, 1977), and N_2O is considered to be the main product of denitrification in acid forest soils (e.g. Nägele and Conrad, 1990; Kester et al., 1997; Paavolainen and Smolander, 1998). Thus, the conditions in this forest soil with high pH are more favourable for N_2 than N_2O production. One should be cautious, however, in extrapolating results obtained from short-term laboratory incubations to field conditions.

5. Conclusions

In the forest site subjected to sprinkling infiltration, nitrification in the humus layer was shown to be pH dependant. Thus the increase in soil pH was most likely the major reason for the initiation of nitrification after the infiltration started, and if required, nitrification could probably be controlled by decreasing the pH of the infiltration water. During infiltration, denitrification is mainly responsible for the production of N_2O. However, denitrification can be considered as a positive phenomenon at this study site; it reduces the amount of nitrate in soil mostly as N_2, i.e. in a form that is not harmful to the atmosphere. The mineral soil may contribute substantially to the leaching of nitrate, since the net production of mineral N and net nitrification were in general higher and denitrification enzyme activity and denitrification potential lower in the samples from the mineral soil layer than in those from the humus layer.

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