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 \(\text{N}_2\text{O}\) and leaching of nitrate from the soil increased. Our aim was to 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 \((\text{NO}_2+\text{NO}_3)\)-\(\text{N}\) 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), \((\text{NO}_2+\text{NO}_3)\)-\(\text{N}\) 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, \(\text{N}_2\text{O}\) was produced mainly by denitrification and approximately 75\% of the denitrification products was \(\text{N}_2\). In the samples from the humus layer, the concentrations of \((\text{NO}_2+\text{NO}_3)\)-\(\text{N}\), 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 \(\text{N}_2\text{O}\) 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 \(\text{N}_2\text{O}\) from soil mainly originates from nitrification or denitrification. The natural fluxes of \(\text{N}_2\text{O}\) from mineral soil sites in Finland are small (Mar-
nitrogen transformations were studied in labora-
tikainen 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 N₂O (Brumme and Beese, 1992). This may not be due to decrease in total denitrification but rather a decreased ratio of N₂O-to-N₂ in response to increased pH of the forest soil (e.g. Nägeli 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 N₂O 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 N₂O production and whether the main product of denitrification was N₂ or N₂O. 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 Hameenlinna (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 Norwegian spruce (Picea abies L.). The site is described in more detail in Lindroos et al. (1998).

Surface water was supplied from a nearby 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 area was divided into five plots (~625 m²), 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 October 1997–18 May 1998) was 1669 m³ m⁻² (plot 5), during continuous infiltration in the summertime (18 May–29 October 1998) 1452 m³ m⁻² (plot 2) and during periodical infiltration in the summertime (8 June–20 July and 17 August–18 September 1998) (plot 3) 607 m³ m⁻². The amounts of irrigation during the previous 2 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 diameter, 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).

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 d using two replicate samples, as described by Smolander et al. (1995). To calculate net ammonification and nitrification, NH₄-N and (NO₂ + NO₃)-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 CaCO₃. 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$_2$H$_2$ at a partial pressure of 2.5 Pa. This concentration of acetylene is reported to be a specific inhibitor of autotrophic nitrification (Klemmedsson et al., 1988). During the 40-day incubation, the samples were aerated and C$_2$H$_2$ 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 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$_2$CO$_3$ or H$_2$SO$_4$. 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 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$_2$O (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$_2$O (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$_2$O production and denitrification enzyme activity (DEA)

A high partial pressure of C$_2$H$_2$ (1–10 kPa) blocks the enzyme nitrous oxide reductase, so that all gaseous denitrification products remain as N$_2$O (Klemmedsson et al., 1977). The contribution of autotrophic nitrification to total N$_2$O production can be estimated under C$_2$H$_2$ at partial pressures of 2.5–5 Pa, because low partial pressures of C$_2$H$_2$ inhibit ammonium oxidase, thus blocking autotrophic nitrification, but have only a small effect on denitrification (Klemmedsson et al., 1988). However, substantial inhibition of the enzyme nitrous oxide reductase can also occur under this low partial pressure of C$_2$H$_2$ 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$_2$H$_2$ that would inhibit nitrification without affecting nitrous oxide reductase. The measurements were made by the procedure described below, except that the partial pressures of C$_2$H$_2$ used were 0, 1, 2, 4, 6, 8, 10, 20, 50 or 100 Pa. The production of N$_2$O-N was the same or slightly smaller in all C$_2$H$_2$ treatments below 8 Pa than with no C$_2$H$_2$, but production increased from 10 Pa with increasing partial pressure of C$_2$H$_2$ (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$_2$H$_2$ for the assay.

N$_2$O production was studied in laboratory incubations at constant temperature (14°C) and moisture

Table 1

<table>
<thead>
<tr>
<th>Plot</th>
<th>Soil layer</th>
<th>pH(H$_2$O)</th>
<th>Organic matter (mg cm$^{-3}$ soil)</th>
<th>Total organic C (mg cm$^{-3}$ soil)</th>
<th>C-to-N ratio</th>
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<tbody>
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<td>81</td>
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<td>74</td>
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<td>26</td>
<td>24</td>
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<tr>
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<td>23</td>
</tr>
<tr>
<td>5</td>
<td>humus</td>
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<td>74</td>
<td>43</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>mineral soil</td>
<td>6.5</td>
<td>60</td>
<td>23</td>
<td>20</td>
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Treatment mean (S.E.M. in parentheses)

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<tr>
<th>Plot</th>
<th>Soil layer</th>
<th>pH(H$_2$O)</th>
<th>Organic matter (mg cm$^{-3}$ soil)</th>
<th>Total organic C (mg cm$^{-3}$ soil)</th>
<th>C-to-N ratio</th>
</tr>
</thead>
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<td>1, 4</td>
<td>humus</td>
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<td>77 (4.0)</td>
<td>43 (0.5)</td>
<td>26 (1.0)</td>
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<tr>
<td></td>
<td>mineral soil</td>
<td>4.9 (0.0)</td>
<td>69 (1.0)</td>
<td>29 (0.5)</td>
<td>23 (0.5)</td>
</tr>
<tr>
<td>2, 3, 5</td>
<td>humus</td>
<td>6.6 (0.0)</td>
<td>76 (1.7)</td>
<td>43 (0.9)</td>
<td>24 (1.5)</td>
</tr>
<tr>
<td></td>
<td>mineral soil</td>
<td>6.5 (0.0)</td>
<td>63 (2.1)</td>
<td>26 (2.0)</td>
<td>22 (1.2)</td>
</tr>
</tbody>
</table>

* Treatment symbols: 1, 4=control; 2, 3, 5=infiltration.
(100% of the WHC) using three replicate soil samples (4 g d.m. humus, 6 g d.m. mineral soil) with either no 
\( \text{C}_2\text{H}_2 \) or with \( \text{C}_2\text{H}_2 \) at partial pressures of 2.5 Pa or 10 
\( \text{kPa} \). \( \text{N}_2\text{O} \) produced was measured after 1 and 2 d in-
cubations using a gas chromatograph (Hewlett Pack-
ard 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 Ar:CH\(_4\) (95:5) as the make-up gas. The tem-
peratures of the detector, injector and column were 
300, 100 and 30°C, respectively. Results given are pro-
duction rates of \( \text{N}_2\text{O}-\text{N} \) between 1 and 2 days. The 
solubility of \( \text{N}_2\text{O} \) 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 \( \text{NO}_3\)-\text{N} concentration of 50 \( \mu \text{g ml}^{-1} \) 
soil water (found to be optimal in preliminary exper-
iments) and a glucose concentration of 15 mg ml\(^{-1} \) 
soil water (shown to be optimal in a study by Priha 
and Smolander, 1999). The water content of the soils 
was adjusted so that the soils were waterlogged. The 
in the bottles was replaced with \( \text{N}_2 \) and \( \text{C}_2\text{H}_2 \) 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 rpm) 
in the dark at 22°C and \( \text{N}_2\text{O} \) 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 \( \text{N}_2\text{O}-\text{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; however, the differences between plots 
were similar when the results were calculated on or-
ganic matter basis.

### 3. Results

#### 3.1. Nitrogen transformations in incubation experiments

\( \text{NH}_4\)-\text{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). (\( \text{NO}_2+\text{NO}_3 \))-\text{N} 
concentrations were negligible in the control soils, while all the soil 
from the infiltration plots contained (\( \text{NO}_2+\text{NO}_3 \))-\text{N}. 
In the mineral soil (\( \text{NO}_2+\text{NO}_3 \))-\text{N} concentrations 
were significantly higher than in the humus.

Net nitrification was determined in incubation exper-
iments. 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.

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 differ-

<table>
<thead>
<tr>
<th>Plot</th>
<th>Soil layer</th>
<th>Initial (( \mu \text{g cm}^{-2} ))</th>
<th>Net formation (( \mu \text{g cm}^{-2} 40 \text{d}^{-1} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( \text{NH}_4)-\text{N} )</td>
<td>( \text{NO}_2+\text{NO}_3)-\text{N} )</td>
</tr>
<tr>
<td>1</td>
<td>humus</td>
<td>10.1</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>mineral soil</td>
<td>2.1</td>
<td>0.1</td>
</tr>
<tr>
<td>2</td>
<td>humus</td>
<td>11.8</td>
<td>11.0</td>
</tr>
<tr>
<td></td>
<td>mineral soil</td>
<td>7.9</td>
<td>33.2</td>
</tr>
<tr>
<td>3</td>
<td>humus</td>
<td>12.2</td>
<td>7.4</td>
</tr>
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<td></td>
<td>mineral soil</td>
<td>11.0</td>
<td>22.9</td>
</tr>
<tr>
<td>4</td>
<td>humus</td>
<td>5.3</td>
<td>0.0</td>
</tr>
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<td></td>
<td>mineral soil</td>
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<td>humus</td>
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<td>11.2</td>
</tr>
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<td></td>
<td>mineral soil</td>
<td>32.6</td>
<td>25.9</td>
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</table>

<table>
<thead>
<tr>
<th>Treatment mean (S.E.M. in parentheses)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, humus</td>
</tr>
<tr>
<td>mineral soil</td>
</tr>
<tr>
<td>2, 3, 5 humus</td>
</tr>
<tr>
<td>mineral soil</td>
</tr>
</tbody>
</table>

\( ^a \) Treatment symbols: 1, 4=control; 2, 3, 5=infiltration.
ences 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 CaCO₃, 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 led to a significant increase in the net formation of mineral N (Fig. 1). Moreover, there was strong net nitrification in both CaCO₃-treated soils.

Humus samples collected from the infiltration plots were regularly treated with 2.5 Pa C₂H₂ during the incubation. C₂H₂ was found to completely inhibit nitrification in all soils (results not shown).

### 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 (NO₂⁺NO₃⁻)-N were small during the 2-week incubation (Fig. 2a and b). When soil suspensions made from the control plot 4 were incubated for two additional weeks, the amount of (NO₂⁺NO₃⁻)-N produced at pH 6.7 was almost 200 µg cm⁻³ but there was negligible production at lower pH values (Fig. 2b).

(NO₂⁺NO₃⁻)-N was produced at much higher concentrations in the soils from the infiltration plots as compared with the control soil suspensions (Fig. 2c and d). (NO₂⁺NO₃⁻)-N production was highest at pH 6.7, whilst there was negligible production at pH 5.3 or under.

![Fig. 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 CaCO₃. The results are the means of three laboratory replicates made of composite samples (±S.E.M.).](image)

### 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).

The numbers of nitrifiers were also determined from the soil suspensions (Fig. 3b). After the 2-week incubation of control soils at pH 6.7, the numbers of NH₄⁺-oxidizers had increased about 600-fold and NO₂⁻-oxidizers about 10-fold. In the suspensions of infiltration soils kept at pH 4.7, the numbers of both NH₄⁺ and NO₂⁻-oxidizers had decreased by about 40 times at the end of the incubation. Only the changes in the numbers of NH₄⁺-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 (Fig. 3a and b).

### 3.4. N₂O production

The production of N₂O-N in soil samples from control plot 1 was negligible (results not shown). With infiltration plots, the N₂O-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 C₂H₂) (Fig. 4a). In the presence of 10 kPa C₂H₂ the production was significantly higher than in the presence of either 2.5 Pa or no C₂H₂; under these treatments the production was similar (not significantly different) (Fig. 4a and b). The difference between the production of N₂O-N in samples under 10 kPa C₂H₂ and with no C₂H₂ varied between soils from different infiltration plots and between the two samplings. On average, humus soils not exposed to C₂H₂ produced 27% of the amount under 10 kPa and the mineral soils produced 18% of the amount under 10 kPa.

### 3.5. Denitrification enzyme activity

Denitrification enzyme activity was measured as N₂O accumulation in the presence of 10 kPa C₂H₂ over 5 h (Fig. 5). N₂O production was significantly greater both in the humus and mineral soil layers with infiltration than in control plots. N₂O production in the humus layer of infiltration plots was significantly higher than in the mineral soil.

### 4. Discussion

Nitrification in ammonium-enriched soil suspensions showed a strong and consistent response to pH (Fig. 2a–d), as had been shown 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 (Fig. 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 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$_2$H$_2$. 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 (Fig. 3a and 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 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 had been 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
\((\text{NO}_2^{+}\text{NO}_3^-)\text{-N}\). The numbers of nitrifiers of the infiltration soils were approximately 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 is thus unable to develop.

The concentrations of \((\text{NO}_2^{+}\text{NO}_3^-)\text{-N}\) 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 \(\text{NO}_3^-\text{-N}\) 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 \((\text{NO}_2^{+}\text{NO}_3^-)\text{-N}\) 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 \((\text{NO}_2^{+}\text{NO}_3^-)\text{-N}\) 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 (Fig. 4a and 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 N₂O 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 N₂O production between the layers in our study is probably also explained by the better availability of organic C in the humus layer than in the mineral soil (Table 1).

Selective use of C₂H₂ was used to differentiate between the N₂O production of nitrification and denitrification. In laboratory experiments with soil samples adjusted to WHC 100%, N₂O production as a by-product of nitrification was negligible since there was no difference between samples treated with 2.5 Pa C₂H₂ or no C₂H₂ (Fig. 4a and b). Thus, it seems that at least during infiltration when the soils are saturated with water, N₂O originates mainly from denitrification. It has been shown also in many other studies that high soil moisture content favours N₂O 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 N₂O but denitrification was more important in autumn.

Because nitrification did not contribute to N₂O production in our experiment, the difference between N₂O accumulation at 10 kPa C₂H₂ and with no C₂H₂ can be considered equivalent to the potential release of N₂ from samples. As only about 25% of the denitrification products was N₂O, N₂ can be considered the dominant product of denitrification in the soils during infiltration. Low pH is known to increase the N₂O-to-N₂ ratio (Focht and Verstraete, 1977), and N₂O 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 N₂ than N₂O 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 N₂O. However, denitrification can be considered as a
positive phenomenon at this study site; it reduces the amount of nitrate in soil mostly as N₂, 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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