



Environmental factors controlling NO_3^- leaching, N_2O emissions and numbers of NH_4^+ oxidisers in a coniferous forest soil

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Abstract

Main and interactive effects of temperature, throughfall volume and NH_4^+ deposition on soil solution NO_3^- concentrations, N_2O emissions and numbers of NH_4^+ oxidisers were investigated in a controlled laboratory experiment. Large intact soil cores from a *Picea abies* (L.) Karst. stand were incubated according to an ‘incomplete factorial design’ at 4, 12 or 20°C and watered every 2 weeks with 300, 500 or 700 ml (442, 737 and 1032 mm yr^{-1}) of a natural throughfall solution enriched with 0, 37.5 or 75 kg $\text{NH}_4^+ - \text{N ha}^{-1} \text{ yr}^{-1}$. Watering and sampling were performed every 2 weeks, during a 112 d period. At d 112, a temperature optimum for $\text{NO}_3^- - \text{N}$ concentrations in the leachate, $\text{NO}_3^- - \text{N}$ fluxes and numbers of NH_4^+ oxidisers in the mineral soil layer was determined at ca. 11°C. $\text{NO}_3^- - \text{N}$ concentrations also decreased with throughfall volume, towards a minimum at 590 ml, with temperature however contributing most to modelling $\text{NO}_3^- - \text{N}$ concentrations and the two factors acting independently. The model explained 59% of the variability in the data, and the regression between observed and predicted concentrations was highly significant ($P < 0.0001$, $r^2 = 0.93$). $\text{NO}_3^- - \text{N}$ fluxes increased quadratically with throughfall volume, and throughfall volume and NH_4^+ deposition interacted significantly in determining the numbers of NH_4^+ oxidisers in the mineral soil layer. Numbers of NH_4^+ oxidisers were higher in the humus layer and decreased with increasing temperatures. N_2O fluxes increased quadratically with temperature, and the linear and quadratic effects of throughfall volume (maximum at 500 ml). Results suggest that optimum temperatures for net nitrification may have been overestimated in previous studies by the use of disturbed soils. © 1999 Elsevier Science Ltd. All rights reserved.

1. Introduction

An improved understanding of the controls of nitrification in acid forest soils is necessary to predict the effects of high N availability through atmospheric deposition and increased temperatures due to global warming. Nitrification, the biological conversion of organic or inorganic nitrogen compounds from a reduced to a more oxidised state (Alexander et al., 1960), is a key process in the nitrogen cycle, controlling N losses through the production of the highly mobile nitrate anion. In the past, forest ecosystems were limited by N

availability, with a tight internal N cycle and low N leaching rates. However, large parts of Europe now receive N inputs by throughfall exceeding the capacity of the system to absorb this N and N leaching is frequently observed (Gundersen, 1995). In such ‘N-saturated’ ecosystems, nitrification controls soil solution nitrate losses, associated with soil acidification, cation leaching and aluminium release (Reuss and Johnson, 1986; Gundersen and Rasmussen, 1990; Carnol et al., 1997a), as well as nitrous oxide emissions (Maag and Vinther, 1996). Nutrient imbalances for tree nutrition, aluminium toxicity to roots, an enhancement of the emission of the greenhouse gas N_2O are possible consequences (Nihlgård, 1985). This change in N cycling process of forests has revealed our ignorance about the

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controls of nitrification, especially in acid forest soils (Killham, 1990).

Microbial N transformations in soils are largely dependent on environmental conditions. In particular, temperature, moisture and substrate dependence of nitrifiers have frequently been studied in relation to culture growth characteristics, or nitrate production in soil laboratory incubations. However, it is not clear how these controls operate under natural conditions in acid forest soils. Much work has been performed on the individual relationships between specific environmental factors and nitrifying activities (Emmer and Tietema, 1990; Ellert and Bettany, 1992). However, nitrifier activity is not simply a result of the addition of the effects of individual factors, but from interactions between them (Laudelout et al., 1976; Quinlan, 1980). Studies on the interaction of several environmental factors in the control of nitrification are scarce, and their relative importance remains unclear.

Water acts as a transport and diluting agent, as well as a necessary component for functioning of biological reactions. The influence of water on nitrification in soils is also closely linked to oxygen availability, as autotrophic nitrifiers are obligate aerobes. Water content has been reported to stimulate nitrification up to an optimum, where oxygen diffusion becomes limiting (Schrödter and Tietjen, 1971). The optimum moisture conditions reported for nitrification frequently vary between studies. This could be explained by differing moisture contents leading to different matric potentials depending on soil texture, and thus representing varying availability of water to the microorganisms (Swift et al., 1979). Increased ammonium supply in throughfall may stimulate nitrification (Stams et al., 1991), but responses vary in different soils (Carnol et al., 1997a). Temperature dependence is often described by Q_{10} (temperature coefficient), or Arrhenius functions, assuming a linear response of nitrification to temperature increase. Because these functions do not accommodate for biological optima, quadratic ('bell shaped') response functions have been recommended (Schrödter and Tietjen, 1971; Ellert and Bettany, 1992).

Our objective was to provide an integrated study on the dependence of nitrogen transformations in an acid forest soil on temperature, throughfall volume and NH_4^+ deposition. We chose an experimental design permitting the analysis of linear, quadratic and interactive effects, using minimally disturbed soil columns, incubated for 4 months in the laboratory. We used one soil from the European project CORE (Berg et al., 1997; Carnol et al., 1997a,b), and, for comparative purposes, adopted an identical lysimeter design and sampling procedure.

2. Materials and methods

2.1. Lysimeter sampling and soil description

Soil cores were taken from Grizedale forest (National Grid Ref. SD326915), UK, beneath a Norway Spruce stand (*Picea abies*), planted in 1950. The site was located on an acid brown soil with clay texture, and slight seasonal waterlogging. Mean thickness of the humus and mineral horizons was 3.8 and 10 cm, respectively. Soil $\text{pH}_{\text{H}_2\text{O}}$ was 3.7 in the upper mineral layer, and mean C and N contents were 41.1% and 1.7% in the organic layers, and 32.4% and 2% in the mineral layers (Carnol et al., 1997b). Mean annual air temperature and rainfall were 12.8°C and 1556 mm, respectively (1989–1990), with a total N deposition of 15 kg ha⁻¹ yr⁻¹ (Carnol et al., 1997a). Average soil temperature at 5 cm depth was 7.8°C, with a mean monthly minimum of 3.3°C in January, while the mean monthly maximum of 11.8°C occurred in July (data from September 1992–1993). Plexiglas cylinders (14.4 cm inner dia, 24 cm deep) were fitted onto a metal corer, which was driven into the ground, using a large rubber mallet. After checking that the soil cores were intact, the bases were trimmed so that the length of the soil column was 17.5 cm. A 5 cm depth of acid washed sand was placed at the bottom of each core and a cap was fitted and sealed with neutral silicone adhesive. A ceramic cup (P80, KPM Berlin) was inserted diagonally into the sand and connected to a 2 l glass bottle to which a suction of 65 kPa was applied (Raubuch, 1992). The cores were covered with polythene bags to minimise evaporation but enabling diffusion of gases (Bremner and Douglas, 1971), and incubated at 12°C in the dark for 2.5 months in order to minimise effects of experimental disturbance. During this time, they were watered every 2 weeks with 500 ml filtered throughfall (see below).

Forty-five cores were then randomly assigned to the different treatments, with three replicates per treatment, and incubated for 4 months in the dark. They were watered every 2 weeks from a stock of filtered throughfall, collected at Grizedale forest during previous months (stored at 2°C). $(\text{NH}_4)_2\text{SO}_4$ was added at 0, 37.5 or 75 kg N ha⁻¹ yr⁻¹ in addition to the throughfall (0, 1.44 and 2.88 kg N ha⁻¹ mixed with the throughfall at each watering), and leachates were collected every 2 weeks and analysed for volume, pH, NO_3^- -N and NH_4^+ -N.

2.2. Experimental design

In order to study main and interactive effects of the three factors temperature (TEM), throughfall volume (VOL) and NH_4^+ -N deposition (DEP), we used an 'incomplete factorial' design (Box and Behnken, 1960)

Table 1
Combination of factors used according to the 'incomplete factorial' Box–Behnken experimental design for 3 factors at 3 levels

TEM (°C)	VOL (ml (2 weeks) ⁻¹ core ⁻¹)	DEP (kg N ha ⁻¹ y ⁻¹)
20	500	75
4	500	0
20	500	0
4	500	75
20	700	37.5
4	300	37.5
20	300	37.5
4	700	37.5
12	700	75
12	300	0
12	700	0
12	300	75
12	500	37.5
12	500	37.5
12	500	37.5

consisting of a selected, equilibrated subset of a complete factorial design. In this approach, all levels of all factors are varied in a controlled and equilibrated way, offering numerous advantages for experimental setup and analysis of results (Goupy, 1988). The design consisted of 12 combinations of 3 factors at 3 levels (–1, 0, +1) and required the addition of 3 central points (all factors at level 0). Due to the high heterogeneity of soils, three replicate lysimeters were established for each combination, leading to a total of 45 experimental units. Levels for the 3 chosen factors were within the range observed in the field (temperature: 4, 12, 20°C; throughfall volume: 300, 500, 700 ml 2 weeks⁻¹ core⁻¹) or within ranges observed in Europe in high deposition areas (NH₄⁺–N: 0, 37.5, 75 kg ha⁻¹ yr⁻¹). Combination of the factors according to the experimental design is shown in Table 1. Throughfall volumes of 300, 500 and 700 ml 2 weeks⁻¹ core⁻¹ correspond to 442, 737 and 1032 mm yr⁻¹, respectively. Ambient throughfall NH₄⁺–N deposition (DEP = 0) was 4.9, 8.1 and 11.4 kg ha⁻¹ yr⁻¹ for a throughfall volume of 300, 500 and 700 ml 2 weeks⁻¹ core⁻¹, respectively and, for clarity, notation of additional experimental input has been used.

2.3. Enumeration of NH₄⁺ oxidizers

NH₄⁺ oxidizers were enumerated using a most probable number (MPN) technique. The medium contained (l⁻¹): KH₂PO₄, 100 mg; NaCl, 500 mg; MgSO₄·7H₂O, 40 mg; CaCl₂, 20 mg; FeSO₄·7H₂O, 2.46 mg; Na₂EDTA, 3.31 mg; NaMoO₄·2H₂O, 0.1 mg; MnCl₂, 0.2 mg; ZnSO₄·7H₂O, 0.1 mg; CuSO₄·5H₂O, 2 µg; (NH₄)₂SO₄, 330 mg (De Boer et al., 1988). After autoclaving, the pH was adjusted to 7.3 with sterile NaOH. At the end of the 4 month incubation under differing

conditions (see above), all cores were analysed individually for NH₄⁺ oxidisers in the humus and mineral layers. Humus and mineral layers of each core were separated and homogenised manually. Wet soil, 5 g, was transferred into a blender, and 95 ml of sterile medium added. The sample was blended for 60 s at half speed and 0.5 ml of this suspension was diluted 2-fold in sterile microplates containing 0.5 ml of the MPN medium per well. Medium, 2 ml, was then added to each well, the microplates packed in aluminium foil and incubated at 25°C for 10 weeks. NH₄⁺ oxidation was determined by the diphenylamine spot test (Morgan, 1930), and the MPN of nitrifiers estimated using statistical tables (Rowe et al., 1977).

2.4. N₂O emission study

During N₂O measurement, air-tight Perspex caps were mounted on top of the lysimeters using a wide rubber band, and left for 24 h. The volume of the Perspex chamber was ca. 1000 cm³ which allowed 24 h incubation without developing anaerobic conditions (T. Nielsen, pers. comm.). Gas samples were taken after 24 h incubation from the lysimeter headspace by inserting a syringe through the rubber septum of the cap. After flushing the syringe three times with 1 ml air from the cap, a 4.5 ml sample was injected into a 3 ml vacuum venvoject tube (Terumo Europe N.V., Leuven). N₂O sampling was performed every 2 weeks immediately prior to watering the lysimeters, as tests had shown higher emission after dry periods and a reduction after watering events. Control air samples of the constant temperature rooms were taken at the beginning of each 24 h incubation to determine background air concentrations, and were subtracted from measurements in order to calculate net flux rates.

2.5. Chemical analysis

Leachates were filtered (Whatman GF/C) prior to chemical analysis and pH was measured in an unfiltered subsample. Methods included ion chromatography (NO₃⁻–N), colorimetry (NH₄⁺–N) and gas chromatography (N₂O). N₂O analysis was performed at the Department of General Microbiology, University of Copenhagen. Other chemical analyses were performed by the Chemistry Section, ITE Merlewood, UK.

2.6. Statistical analysis

Main, quadratic, and interactive effects of the three factors were analysed by a least square regression technique using the RSREG procedure in SAS (SAS Institute Inc., 1989). A lack-of-fit test was included to

Table 2

Soil solution NO_3^- -N concentrations (NO_3C , mg l^{-1}), NO_3^- -N fluxes (NO_3F , $\text{kg ha}^{-1} 2 \text{ weeks}^{-1}$), MPN of NH_4^+ oxidisers in the humus (MPNH, MPN g^{-1}) and mineral soil layers (MPNM, MPN g^{-1}), and N_2O -N fluxes (N_2OF , $\text{ng cm}^{-2} \text{ d}^{-1}$) from lysimeters after 4 months incubation under different experimental treatments

TRMT	TEM	VOL	DEP	NO_3C	NO_3F	MPNH	MPNM	N_2OF
a	4	300	37.5	11.8 (3.1)	2.1 (0.6)	299 (348)	79 (70)	5.5 (7.5)
b	4	500	0	12.0 (1.3)	3.4 (0.8)	541 (180)	80 (84)	-2.0 (0.3)
c	4	500	75	10.0 (0.9)	3.3 (0.8)	514 (141)	60 (53)	3.2 (8.1)
d	4	700	37.5	10.1 (1.4)	3.7 (1.0)	478 (202)	15 (26)	37.1 (61.4)
e	20	300	37.5	12.2 (4.8)	2.2 (0.7)	177 (149)	45 (47)	167.4 (138.1)
f	20	500	0	6.2 (3.9)	1.8 (1.1)	224 (148)	0 (0)	502.1 (119.6)
g	20	500	75	8.0 (2.9)	2.5 (0.9)	207 (188)	35 (44)	340.4 (287.7)
h	20	700	37.5	6.9 (5.6)	3.0 (2.3)	62 (54)	80 (118)	121.0 (151.2)
i	12	300	0	19.1 (5.8)	3.3 (0.9)	464 (122)	194 (136)	0.6 (2.5)
j	12	300	75	17.3 (5.9)	2.9 (1.0)	371 (21)	279 (66)	24.7 (45.2)
k	12	700	0	15.2 (3.0)	6.4 (1.4)	351 (39)	126 (73)	42.1 (63.0)
l	12	300	75	16.9 (1.2)	7.0 (0.6)	356 (38)	48 (51)	7.7 (9.5)
m	12	500	37.5	15.1 (2.1)	4.4 (0.7)	369 (230)	88 (75)	67.0 (152.8)

(TEM = temperature, $^{\circ}\text{C}$; VOL = volume, ml; DEP = NH_4^+ -N deposition, $\text{kg ha}^{-1} \text{ yr}^{-1}$). Mean \pm S.D. of 3 replicates (6 replicates for treatment m).

test whether the quadratic model used was adequate (SAS Institute Inc., 1989). Nonsignificant factors were then eliminated and a new regression equation fitted using the REG procedure (SAS Institute Inc., 1989). The resulting regression equations were used to generate response surfaces in the range of the factors variables studied. If more than two factors occurred in the equation, other factors were set to their mean level for response surface generation. Correspondence between measured values (means of three replicates) and model predictions was assessed by linear regression using the REG procedure (SAS Institute Inc., 1989) and visual examination of observed and predicted values. Nitrate fluxes were calculated for individual lysimeters by multiplying soil solution concentration by leachate volume.

3. Results

3.1. NO_3^- concentrations

At the first sampling day, just after conditioning (preincubation), NO_3^- -N concentrations in soil solutions across the treatments were between 6.7 ± 1.7 and $9.0 \pm 0.9 \text{ mg l}^{-1}$. At the end of the 4 months incubation, concentrations ranged from 6.2 ± 3.9 to $19.1 \pm 5.8 \text{ mg l}^{-1}$ (Table 2). After 4 months under constant conditions, concentrations stabilised at 4 and 20°C , but were still increasing at 12°C . The global model for the effects of environmental factors on soil solution NO_3^- concentrations was significant ($P < 0.05$) from d 42–56 onwards (Table 3). Factors with significance $P < 0.2$ at d 112 were selected to construct a reduced model. However, DEP was not

retained, because this factor was not significant in the reduced model, and its exclusion did not reduce the overall r^2 . The quadratic effect of VOL was kept in the model at $P = 0.12$, because it improved the overall fit of the model slightly (from $r^2 = 0.56$ to 0.59). The resulting regression equation of linear (TEM, VOL) and quadratic (TEM^2 , VOL^2) effects of retained factors on NO_3^- -N concentrations (NO_3pred , mg l^{-1}) in lysimeter leachates at d 112 was ($r^2 = 0.59$; adj. $r^2 = 0.55$; $P = 0.0001$; individual P values in brackets)

$$\begin{aligned} \text{NO}_3\text{pred} = & 16.026(0.0146) + 2.273(0.0001)*\text{TEM} \\ & - 0.046(0.0690)*\text{VOL} \\ & - 0.102(0.0001)*\text{TEM}^2 \\ & + 0.0000393(0.1185)*\text{VOL}^2. \end{aligned} \quad (1)$$

Note that the parameter estimates do not express the individual contribution of the factors, because their value depends on the measurement unit. The positive sign of the parameter estimate for TEM indicated a positive relationship between TEM and NO_3^- concentrations. This relationship was not linear and showed a maximum, as indicated by the significant and negative sign of the quadratic term. VOL affected NO_3^- concentrations negatively, with a slight upward curvature. The interactive term between these two factors was not significant, meaning that the form of response of one factor was the same at different levels of the other factor.

The good fit of the model was demonstrated by the relationship between the mean measured and the predicted values (Fig. 1), with an r^2 of 0.93. The intercept

Table 3

Summary statistics for the regression of linear, quadratic and interactive effects of all factors on NO_3^- -N concentrations in lysimeter leachates at all samplings

	Day								
	1	14	28	42	56	70	84	98	112
r^2	0.09	0.129	0.137	0.276	0.406	0.529	0.619	0.643	0.623
Model	0.94	0.82	0.79	0.21	0.02	0.0009	0.0000	0.0000	0.0000
TEM	ns	ns	ns	ns	c	a	a	a	a
DEP	ns	ns	ns	ns	c	ns	ns	ns	c
VOL	ns	ns	ns	c	a	a	b	c	c
TEM*TEM	ns	ns	ns	a	a	a	a	a	a
DEP*TEM	ns	ns	ns	ns	ns	ns	ns	ns	ns
DEP*DEP	ns	ns	ns	ns	ns	ns	ns	ns	ns
VOL*TEM	ns	ns	ns	ns	ns	ns	ns	ns	ns
VOL*DEP	ns	ns	ns	ns	c	c	ns	ns	ns
VOL*VOL	ns	ns	ns	c	a	b	b	ns	c

r^2 : r -square of the model, MODEL: P value for total regression, ns: not significant, (a) $P < 0.05$, (b) $P < 0.1$, (c) $P < 0.2$.

term was not significantly different from 0. The relative influence of TEM and VOL has been illustrated by plotting the response surface of predicted concentrations resulting from the quadratic regression equation (Fig. 2). The temperature effect was most important, with a maximum at 11°C . At the mean VOL level (500 ml), the maximum predicted concentration was $15.4 \text{ mg NO}_3^- \text{N l}^{-1}$. At the same VOL, the predicted concentration was lower at 20°C than at 4°C (7.5 and $10.2 \text{ mg NO}_3^- \text{N l}^{-1}$, respectively). Increasing VOL influenced NO_3^- concentrations negatively, with a minimum at 590 ml. Above 590 ml, con-

centrations increased again, but this increase was relatively low ($0.5 \text{ mg NO}_3^- \text{N l}^{-1}$ at 12°C). The concentration range produced by varying VOL at 12°C

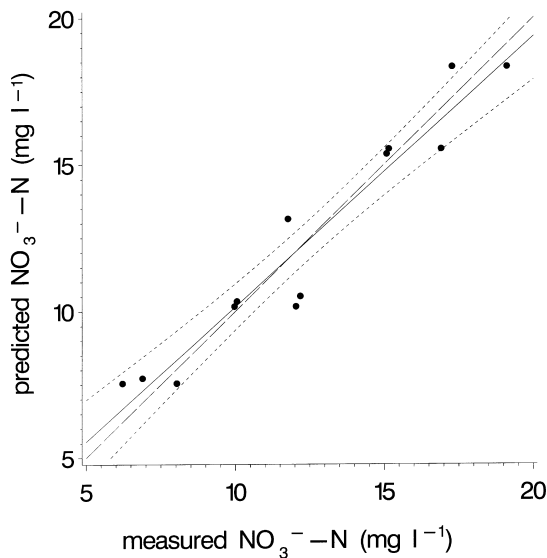


Fig. 1. Linear regression of predicted (parameters from Eq. (1)) against measured (means of three replicates) values of NO_3^- -N concentrations in lysimeter leachates at d 112 ($r^2 = 0.93$, $P = 0.0001$). The regression line for the samples (—), 95% confidence limits (---) and an equivalence line (— · —) are shown.

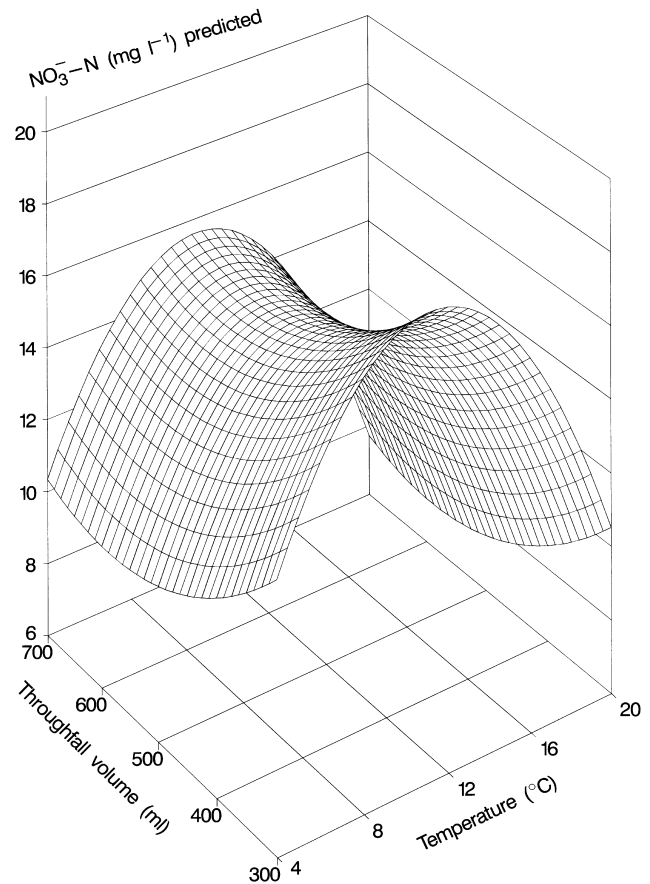


Fig. 2. Response surface of predicted NO_3^- -N concentrations in soil solution depending on temperature and throughfall volume, simulated by using the regression parameters from Eq. (1).

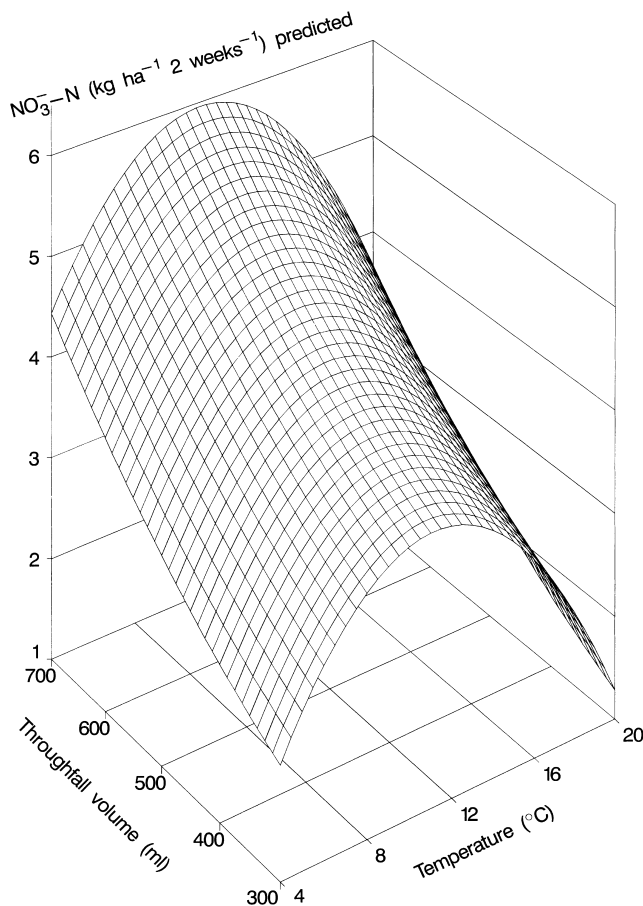


Fig. 3. Response surface of predicted NO_3^- -N fluxes from lysimeters depending on temperature and throughfall volume, simulated by using the regression parameters from Eq. (2).

was 15.04 to 18.33 mg l^{-1} . Varying TEM at VOL 500 simulated concentrations in the range of 7.54 to 15.42 mg l^{-1} .

3.2. NO_3^- -N fluxes

NO_3^- -N fluxes ranged from 1.8 ± 1.1 to 7.0 ± 0.6 kg ha^{-1} 2 weeks $^{-1}$ at incubation day 112 (Table 2). As for soil solution NO_3^- concentration, effects of environmental factors on NO_3^- fluxes were significant ($P < 0.05$) from d 42–56 onwards. After fitting the complete model at d 112, three factor terms were retained for constructing the reduced model. Whilst the individual parameter estimate for VOL was not significant in the complete model, the global test on this factor was highly significant ($P = 0.0003$), and inclusion of the quadratic effect of the VOL into the reduced model was significant, yielding the highest r^2 (0.63). TEM showed a positive effect up to a maximum at 11°C. The regression equation of linear (TEM) and quadratic (TEM^2 , VOL^2) effects of retained factors on NO_3^- -N fluxes (NO_3flux , kg ha^{-1} 2 weeks $^{-1}$) from lysimeter leachates at d 112 was ($r^2 = 0.63$; adj.

$r^2 = 0.60$; $P = 0.0001$; individual P values in brackets)

$$\begin{aligned} \text{NO}_3\text{flux} = & -0.634(0.3763) \\ & + 0.661(0.0001) * \text{TEM} \\ & - 0.0296(0.0001) * \text{TEM}^2 \\ & + 0.00000591(0.0001) * \text{VOL}^2. \end{aligned} \quad (2)$$

Linear regression between predicted and measured values was highly significant ($P = 0.0001$), with an r^2 of 0.86 (adjusted $r^2 = 0.85$). Increasing VOL affected NO_3^- fluxes positively, with a slight upward curvature (Fig. 3). Fluxes at 500 ml VOL, simulated for the different temperatures had a minimum value of 2.23 and a maximum value equivalent to 4.54 kg ha^{-1} 2 weeks $^{-1}$. Doubling the throughfall volume at 12°C from 300 to 600 ml caused a 1.5× increase in NO_3^- flux.

3.3. Most probable numbers of NH_4^+ oxidisers

The most probable numbers (MPN) of NH_4^+ oxidisers g^{-1} dry soil ranged between 0 to 906 in the humus layer, and 0 to 350 in the mineral layer, across individual columns of the different experimental conditions. Means were 62 ± 54 to 541 ± 180 in the organic layers and 0 ± 0 to 279 ± 66 in the mineral layer (Table 2). In the complete model for the humus layer ($r^2 = 0.40$, $P = 0.0237$), only the interaction term of VOL with TEM had a P value below 0.2. However, the global test for TEM was significant ($P = 0.0031$) and the lack-of-fit test was not significant, indicating that a quadratic regression model was adequate to describe the data. In a reduced model, the quadratic effect of TEM was significant, whilst its interaction term with VOL was not significant. The regression equation of the quadratic effect of TEM (TEM^2) on most probable numbers of NH_4^+ oxidisers ($\text{MPN}_{\text{humus}}$) g^{-1} d.w. in the humus layer at d 112 was ($r^2 = 0.31$; adj. $r^2 = 0.29$; $P = 0.0001$; individual P values in brackets)

$$\text{MPN}_{\text{humus}} = 481.362(0.0001) - 0.771(0.0001) * \text{TEM}^2. \quad (3)$$

The model indicated that the MPN of NH_4^+ oxidisers in the humus layer decreased from 469 to 173 when increasing the temperature from 4 to 20°C. Linear regression between predicted and observed values was highly significant ($P = 0.0001$), with an r^2 of 0.75 (adjusted $r^2 = 0.73$).

For the mineral layer, variables with $P < 0.2$ were retained to construct the reduced model, and they were all significant. The regression equation of retained factors on most probable numbers of NH_4^+ oxidisers (MPN_{min}) g^{-1} d.w. in the mineral layer at d 112 was ($r^2 = 0.43$; adj. $r^2 = 0.34$; $P = 0.0012$; individual P

values in brackets)

$$\begin{aligned} \text{MPN}_{\text{min}} = & 260.347(0.0843) \\ & + 27.532(0.0036) * \text{TEM} \\ & - 1.145(0.0545) * \text{VOL} \\ & - 1.1966(0.0020) * \text{TEM}^2 \\ & + 0.0297(0.0296) * \text{DEP}^2 \\ & - 0.00451(0.0356) * \text{VOL} * \text{DEP} \\ & + 0.00112(0.0604) * \text{VOL}^2. \end{aligned} \quad (4)$$

Linear regression between predicted and observed values was highly significant ($P = 0.0001$), with an r^2 of 0.78 (adjusted $r^2 = 0.76$). The temperature effect was characterised by a maximum at 11.5°C, with the response function being independent of the other factors (Fig. 4). At a VOL of 500 ml and DEP of 37.5 kg ha⁻¹ yr⁻¹, maximum MPN was 83 bacteria g⁻¹ d.w. VOL and DEP showed a significant interaction term (Fig. 5). At DEP = 0 and TEM = 12°C, predicted bacterial numbers ranged between 125 and 175 g⁻¹, with a minimum at 512 ml. At highest NH₄⁺–N deposition,

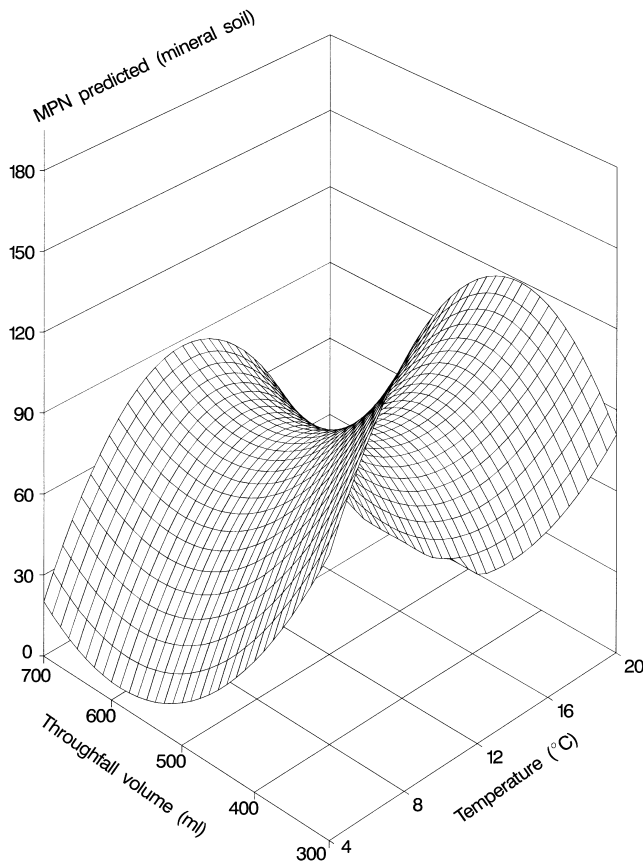


Fig. 4. Response surface of predicted most probable number of NH₄⁺ oxidisers (MPN, g⁻¹) in the mineral layer depending on temperature and throughfall volume, simulated by using the regression parameters from Eq. (4) with the other factors at their mean level.

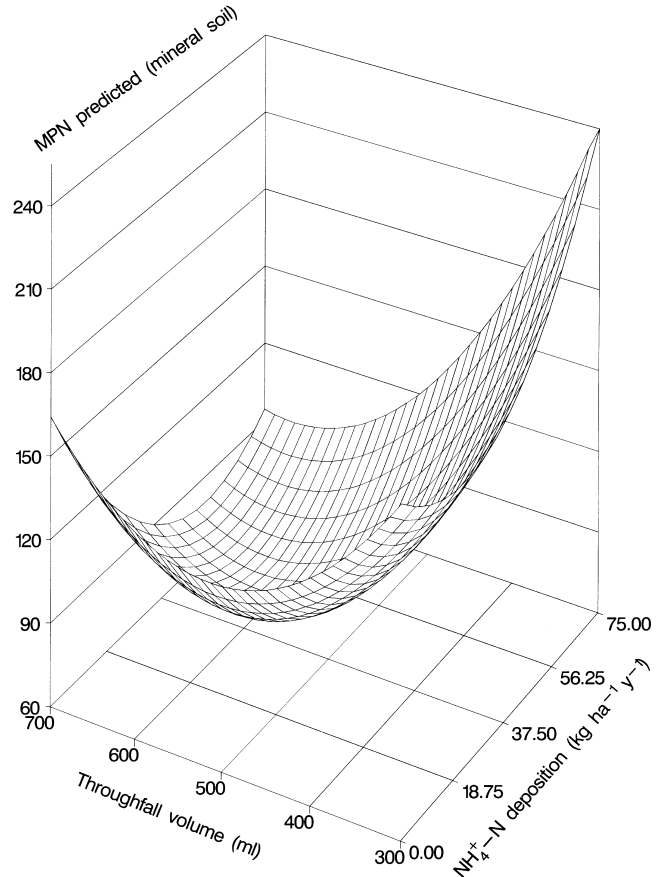


Fig. 5. Response surface of predicted most probable number of NH₄⁺ oxidisers (MPN, g⁻¹) in the mineral layer depending on NH₄⁺ deposition and throughfall volume, simulated by using the regression parameters from Eq. (4) with the other factors at their mean level.

and an increase in VOL from 300 to 700 ml, caused a decrease in bacterial numbers from 241 to 94 g⁻¹. Similarly, an increase in DEP at high VOL caused a decrease in bacterial numbers, but an increase at low volumes (Fig. 5). A linear regression model between measured numbers of NH₄⁺ oxidisers in the mineral soil layer and NO₃⁻–N concentrations in soil solutions at the last sampling date was significant ($P = 0.0001$, $r^2 = 0.41$, adjusted $r^2 = 0.40$). However, such a regression was not significant for the humus layer.

3.4. N₂O–N fluxes

In contrast to the variables analysed previously, the effects of factors on net N₂O–N fluxes were significant from d 14 onwards, with similar r^2 values at all sampling dates. Linear and quadratic effects of VOL and the quadratic effect of TEM were significant from d 14 to d 112 ($P < 0.1$). The linear effect of TEM was only significant until d 56 ($P < 0.2$), and the quadratic effect of DEP until d 98 ($P < 0.2$). However, lack-of-fit statistics were near significance ($P < 0.1$) at all

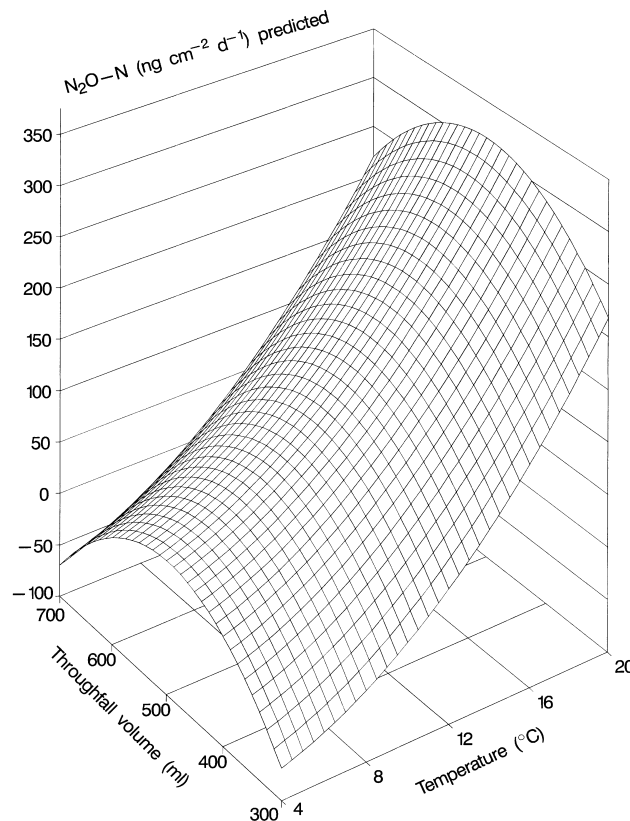


Fig. 6. Response surface of predicted $\text{N}_2\text{O-N}$ fluxes from lysimeters depending on temperature and throughfall volume, simulated by using the regression parameters from Eq. (5).

sampling dates, indicating that a quadratic model might not be the most appropriate, and that a higher term model may be necessary. Data were highly skewed, but residuals of the regression model were normally distributed. Furthermore, as logarithmic transformation did not improve the fit of the model, the raw data were used. A reduced model was constructed with the three factor terms significant at d 112, explaining 47% of the variation in the data. The regression equation linear (VOL) and quadratic (TEM^2 , VOL^2) effects of retained factors on $\text{N}_2\text{O-N}$ fluxes (N_2O , $\text{ng cm}^{-2} \text{d}^{-1}$) from lysimeters at d 112 was ($r^2 = 0.47$; adj. $r^2 = 0.43$; $P = 0.0001$; individual P values in brackets)

$$\begin{aligned} \text{N}_2\text{O} = & -585.935(0.0194) \\ & + 2.395(0.0225)*\text{VOL} \\ & + 0.751(0.0001)*\text{TEM}^2 \\ & - 0.00239(0.0223)*\text{VOL}^2. \end{aligned} \quad (5)$$

Even though a higher order model might produce a better fit, linear regression between mean measured and predicted values was significant ($P = 0.0001$), with an r^2 of 0.76 (adjusted $r^2 = 0.73$). VOL made a significant contribution as a linear and quadratic term, and

the simulation data (Fig. 6) showed a maximum at 500 ml. However, the influence of VOL, yielding predicted fluxes from 25.5 to 121.7 $\text{ng cm}^{-2} \text{d}^{-1}$ at 12°C, was low compared to the effect of TEM. $\text{N}_2\text{O-N}$ fluxes increased quadratically with TEM, with a projected maximum value of 313.9 $\text{ng cm}^{-2} \text{d}^{-1}$ at 20°C, at optimum VOL (500 ml). Mean measured positive $\text{N}_2\text{O-N}$ fluxes ranged from $0.6 \pm 2.5 \text{ ng cm}^{-2} \text{d}^{-1}$ ($0.16 \pm 0.50 \text{ ng g}^{-1}$) to $502.1 \pm 119.6 \text{ ng cm}^{-2} \text{d}^{-1}$ ($169.73 \pm 150.75 \text{ ng g}^{-1}$) (Table 2). In contrast to soil solution NO_3^- concentrations, $\text{N}_2\text{O-N}$ fluxes stabilised rapidly under all experimental conditions. However, variability between replicates was high (coefficient of variation 6–500%).

4. Discussion

Intact soil cores were incubated in the laboratory using an incomplete factorial design to determine the effects of temperature, throughfall volume and increased NH_4^+ supply on soil solution NO_3^- concentration, N_2O emissions and numbers of NH_4^+ oxidisers in an acid coniferous forest soil. Temperature was found to be the most important factor controlling NO_3^- concentrations in soil solution, and, together

with throughfall volume, explained 59% of the variability in the data after 112 d of incubation. The optimum temperature for NO_3^- concentrations and leaching fluxes determined from our study was 11°C which contrasts with the optimum temperature of ca. 30°C for nitrification in laboratory culture conditions (Table 4). Similarly, optima for net nitrification during soil incubation are most frequently reported to be ca. 25–30°C.

We propose two explanations for this major difference between our observations and those in the literature. Firstly, we studied leachate concentrations, whilst most incubation studies report total net potential production rates. Although this may result in some discrepancy we consider that leachate concentrations would be proportional to net production rates for a given rainfall volume and soil texture, which would result in similar temperature optima being found. The second, and more likely explanation, is that the differences in incubation conditions used to determine net nitrification optima were responsible (Table 4). In most studies, soils are dried, sieved, amended or agitated during incubation, which modify soil conditions, particularly for gas diffusion and substrate supply. For example, higher net nitrification and mineralisation rates have been reported for percolated cf. closed systems (Koopmans et al., 1995). Additionally, Quinlan (1980) demonstrated that the optimum temperature for nitrification increased with substrate concentration and it is clear that temperature optima are a product of experimental conditions. These differences may be why optimum temperatures reported for some laboratory studies are higher than those actually occurring in the field, as found by Stark (1996). An optimum at 10°C for NO_3^- leaching was also determined for an acid brown soil, by incubation of intact soil columns, under 200 mm month⁻¹ rainfall (Buldgen, 1982). In our study, the optimum temperature determined corresponded well with mean field temperatures.

The optimum temperature determined in the current study might not directly represent a physiological optimum of the nitrifying organisms, since leachate concentrations are the resultant of mineralisation–nitrification–immobilisation processes, each with their own temperature response. The decrease in NO_3^- leaching above 11°C might, for example, be due to increased microbial N immobilisation, since increasing N immobilisation with temperature has been reported (Nicolardot et al., 1994), and recent evidence also suggests a strong link between NO_3^- leaching and microbial NO_3^- uptake (Stark and Hart, 1997). These authors demonstrated high NO_3^- production rates and microbial NO_3^- uptake in coniferous soils, suggesting that the frequently observed low NO_3^- concentrations in forest soils might not be caused by competition with

heterotrophs for NH_4^+ , as proposed by Riha et al. (1986), but by high NO_3^- immobilisation.

Soil O_2 consumption increases with temperature (Salonius, 1978), and a reduction in nitrification at decreased partial pressures of O_2 has been reported (Alexander, 1961; Laudelout et al., 1976). This may have been an important factor in the current work and is supported by the observations of lower numbers of NH_4^+ oxidisers above 11°C. Additionally, N losses through N_2O emissions were insufficient to explain the decrease in observed NO_3^- -N fluxes, but we did not measure total gaseous N losses and cannot fully exclude increased denitrification as a contributory factor. However, the higher N_2O emissions also indicate an increase in anaerobic microsites.

Water input acts as a transport medium and diluting agent as well as being a necessary prerequisite for growth and survival of the soil biota. Water content also determines oxygen diffusion, which may become limiting for nitrifiers at high water contents. The observed reductions in NO_3^- concentrations with increasing throughfall volume, could be either due to simple dilution or a decrease in the nitrification capacity due to oxygen limitation. Increases in the total nitrate fluxes indicate a stimulation of bacterial growth and activity to increased water supply.

The concentrations of NO_3^- in forest soil solution are important determinants of ionic equilibrium in soil solutions, and are closely linked with acidification (Reuss and Johnson, 1986). Acidification due to NO_3^- leaching was strongly supported by our data, with the temperature-based model for leachate H^+ concentrations following the same pattern as for NO_3^- leaching, with highest H^+ concentrations at ca. 13°C.

Surprisingly, increased $(\text{NH}_4)_2\text{SO}_4$ deposition did not significantly affect NO_3^- concentrations or fluxes during this 4 month laboratory study, unlike results from a parallel field experiment in which the same soil showed increased NO_3^- leaching 4 months after increasing the $(\text{NH}_4)_2\text{SO}_4$ supply in throughfall (Carnol et al., 1997a). The reasons for these differences in behaviour between field and laboratory are not understood and merit further investigation.

No significant interactions of the control of net nitrate leaching were found between the three factors studied. By applying a quadratic model to the NO_3^- content of a soil at different times of the year, Schrödter and Tietjen (1971) also concluded an independent influence of temperature and moisture on nitrification. Other authors, however, found significant interactions between these two factors (Goodroad and Keeney, 1984; Emmer and Tietema, 1990; Maag and Vinther, 1996). Experimental conditions (see above) might be responsible for these apparent differences with our study, yet we emphasise that the current

Table 4
Temperature optima for nitrification reported in the literature

Optimum (°C)	Sample	Method (handling, temperatures, incubation time)	Notes (max. net NO_3^- -N production, characteristics)	Reference
40	0–10 cm, Tippera clay loam, pH 7.0	sieved, mixed with acid-washed sand, 20–60°C, 28 d	0.85 $\mu\text{g g}^{-1} \text{d}^{-1}$	Myers, 1975
35	idem	idem, amended with NH_4^+ , 20–60°C, 28 d	4.6 $\mu\text{g g}^{-1} \text{d}^{-1}$ still measurable at 60°C	Myers, 1975
30	Plano silt loam, pH 4.7–6.7	wet sieved, stored at 0°C, 100 $\mu\text{g N}$ as $(\text{NH}_4)_2\text{SO}_4$, 10–30°C, 0.1–0.3 $\text{m}^3 \text{m}^{-3}$, 5 d	6, 7.6, 11.2 $\mu\text{g g}^{-1} \text{d}^{-1}$ (pH 4.7, 5.1, 6.7, respectively) optimum at highest water content and temperature	Goodroad and Keeney, 1984
30	pure cultures <i>Nitrosomonas</i> sp., <i>Nitrobacter</i> sp.			Bock and Koops, 1992; Koops and Möller, 1992
29–55 (NH_3 oxidation)	mixed culture (Thames Estuary water) and pure cultures of <i>Nitrobacter winogradskyi</i>	application of rate laws reported in literature	optimum temperature increases with log substrate concentration	Quinlan, 1980
10–33 (NO_2^- oxidation)				
25	oak-beech litter and fermentation layer, pH_{KCl} 3.3	dried, sieved, 0–30°C, 4 weeks	ca. 575 $\text{mg N kg}^{-1} \text{month}^{-1}$ quadratic dependence on temperature, mean field temperature: 12.5°C, inhibition at 30°C	Emmer and Tietema, 1990
20	Lyngby sandy loam, $\text{pH}_{\text{CaCl}_2}$ 6.6	dried, sieved, stored at 2°C, field capacity adjusted to 40–100% with NH_4Cl solution, 5–20°C, 11 d	ca. 3.7 $\mu\text{g g}^{-1} \text{d}^{-1}$ (100% field capacity), hydrothermal interaction	Maag and Vinther, 1996
20	Gray Luvisol/Chernozem (previously cropped with barley), pH 6.1–7.2	dried, sieved, amended with 100 $\mu\text{g g}^{-1}$ $(\text{NH}_4)_2\text{SO}_4$, –4–40°C, 9–167 d	3.02–3.72 $\mu\text{g g}^{-1} \text{d}^{-1}$, reduction after max., Michaelis–Menten kinetics	Malhi and McGill, 1982
10	organic layer, acid brown soil, <i>Fagus sylvatica</i> and <i>Picea abies</i> stands pH 3.2–3.8	intact columns, 4–15°C, 30 d; 200 mm month^{-1}	10.75 (beech)–11.17 (spruce) $\text{mg } 100 \text{ g}^{-1}$	Buldgen, 1982

work was designed to mimic transformations occurring in the field.

Numbers of NH_4^+ oxidisers were low, ranging from 0–906 and 0–350 g^{-1} dry soil in the humus and mineral layers, respectively. Similar low population sizes for acid forest soils have been reported previously (Hankinson and Schmidt, 1984; Martikainen, 1985; Pennington and Ellis, 1993). The MPN technique, applied in acid soils, might only recover part of the active nitrifiers, and systematically underestimate bacterial numbers (Belser, 1979). It has been argued that the MPN technique is unsuitable for enumerating acid-tolerant NH_4^+ oxidisers, and lower MPN numbers in the lower soil layers could be due to a vertical gradient of acid-sensitive to acid-tolerant nitrifiers towards the lower soil layers (De Boer et al., 1992). However, it is reassuring that the temperature response of the NH_4^+ oxidisers in the mineral layer was similar to that for NO_3^- concentrations, with a significant correlation between these two measurements.

Despite the high variability in the N_2O emissions of soil cores within the same treatment, significant effects were detected. The model showed lower r^2 values than for NO_3^- data, but predicted and mean measured values corresponded well, with temperature and volume being the most important factors. It is interesting to note that at low temperatures and low through-fall volumes, N_2O fluxes were negative but above 12°C simulated fluxes were positive, increasing up to the highest temperature studied. This result is consistent with the reported effects of temperature on soil N_2O emissions (Goodroad and Keeney, 1984; Sitaula and Bakken, 1993; Maag and Vinther, 1996). Within a global change perspective, our model would predict an increase of $3.1 \text{ kg ha}^{-1} \text{ yr}^{-1}$ $\text{N}_2\text{O-N}$ emissions with a temperature increase of 4°C (optimum throughfall) in this nitrifying clay soil.

In our study, mean $\text{N}_2\text{O-N}$ fluxes across the treatments after 4 months incubation were 6 to $5021 \mu\text{g m}^{-2} \text{ d}^{-1}$ with reported values for coniferous forests being in the ranges of 2 to $880 \mu\text{g m}^{-2} \text{ d}^{-1}$ (Bowden et al., 1990), 100 to $410 \mu\text{g m}^{-2} \text{ d}^{-1}$ (Sitaula et al., 1995) and 240 to $408 \mu\text{g m}^{-2} \text{ d}^{-1}$ (Martikainen and De Boer, 1993). On a yearly basis, fluxes in our study, calculated from data at d 112, would correspond to $0.02\text{--}18.3 \text{ kg N}_2\text{O-N ha}^{-1} \text{ yr}^{-1}$. Losses from untreated forest ecosystems normally range between 0.05 and $15 \text{ kg N}_2\text{O-N ha}^{-1} \text{ yr}^{-1}$ (Dutch and Ineson, 1990).

The use of an incomplete factorial design proved to be a powerful tool in studying the combined effects of three factors on nitrification, combining a manageable number of experimental units with the application of rigorous statistical analysis. This method has numerous advantages over more 'classical' approaches (in which one variable is manipulated at a time) enabling

the detection of interactions between factors, whilst permitting detection of optima and the possibility of modelling the process (Murphy, 1977; Goupy, 1988). In spite of these benefits, planned approaches of this type are rare in soil research yet should be given serious consideration at the experimental design stage.

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References

- Alexander, M., Marshall, K.C., Hirsch, P., 1960. Autotrophy and heterotrophy in nitrification. In: Transactions 7th International Congress of Soil Science, Madison, pp. 86–591.
- Alexander, M., 1961. Introduction to Soil Microbiology. John Wiley, New York.
- Belser, L.W., 1979. Population ecology of nitrifying bacteria. Annual Review of Microbiology 33, 309–333.
- Berg, M.P., Verhoef, H.A., Anderson, J.M., Beese, F., Bolger, T., Coûteaux, M.-M., Ineson, P., McCarthy, F., Palka, L., Raubuch, M., Splatt, P., Willisson, T., 1997. Effects of air pollutant–temperature interactions on mineral-N dynamics and cation leaching in replicate forest soil transplantation experiments. Biogeochemistry 39, 295–326.
- Bock, E., Koops, H.P., 1992. The genus *Nitrobacter* and related genera. In: Balows, A., Trüper, H.G., Dworkin, M., Harder, W., Schleifer, K.-H. (Eds.), The Prokaryotes: A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications, 2nd edn. Springer Verlag, New York, pp. 2302–2309.
- Bowden, R.D., Steudler, P.A., Melillo, J.M., Aber, J.D., 1990. Annual nitrous oxide fluxes from temperate forest soils in the northeastern United States. Journal of Geophysical Research 95, 13997–14005.
- Box, G.E.P., Behnken, D.W., 1960. Some new three level designs for the study of quantitative variables. Technometrics 2, 445–475.
- Bremner, J.M., Douglas, L.A., 1971. Use of plastic film for aeration in soil incubation experiments. Soil Biology & Biochemistry 3, 289–296.
- Buldgen, P., 1982. Features of nutrient leaching from organic soil layer microcosms of beech and spruce forests: effects of temperature and rainfall. Oikos 38, 99–107.
- Carnol, M., Ineson, P., Anderson, J.M., Beese, F., Berg, M.P., Bolger, T., Coûteaux, M.-M., Cudlin, P., Dolan, S., Raubuch, M., Verhoef, H.A., 1997a. The effects of ammonium sulphate deposition and root sinks on soil solution chemistry in coniferous forest soils. Biogeochemistry 38, 255–280.
- Carnol, M., Ineson, P., Dickinson, A.L., 1997b. Soil solution nitrogen and cations influenced by $(\text{NH}_4)_2\text{SO}_4$ deposition in a coniferous forest. Environmental Pollution 97, 1–10.
- De Boer, W., Duyts, H., Laanbroek, H.J., 1988. Autotrophic nitrification in a fertilized acid heath soil. Soil Biology & Biochemistry 20, 845–850.
- De Boer, W., Tietema, A., Klein Gunnewiek, P.J.A., Laanbroek, H.J., 1992. The chemolithotrophic ammonium-oxidizing commu-

- nity in a nitrogen-saturated acid forest soil in relation to pH-dependent nitrifying activity. *Soil Biology & Biochemistry* 24, 229–234.
- Dutch, J., Ineson, P., 1990. Denitrification of an upland forest site. *Forestry* 63, 363–377.
- Ellert, B.H., Bettany, J.R., 1992. Temperature dependence of net nitrogen and sulfur mineralization. *Soil Science Society of America Journal* 56, 1133–1141.
- Emmer, I.M., Tietema, A., 1990. Temperature-dependent nitrogen transformations in acid oak-beech forest litter in The Netherlands. *Plant and Soil* 122, 193–196.
- Goodroad, L.L., Keeney, D.R., 1984. Nitrous oxide production in aerobic soils under varying pH, temperature and water content. *Soil Biology & Biochemistry* 16, 39–43.
- Goupy, J., 1988. *La Méthode des Plans d'Expériences: Optimisation du Choix des Essais and de l'Interprétation des Résultats*, Dunod, Paris.
- Gundersen, P., 1995. Nitrogen deposition and leaching in European forests: preliminary results from a data compilation. *Water, Air and Soil Pollution* 85, 1179–1184.
- Gundersen, P., Rasmussen, L., 1990. Nitrification in forest soils: effects from nitrogen deposition on soil acidification and aluminium release. *Reviews of Environmental Contamination and Toxicology* 113, 1–45.
- Hankinson, T.R., Schmidt, E.L., 1984. Examination of an acid forest soil for ammonia- and nitrite-oxidizing autotrophic bacteria. *Canadian Journal of Microbiology* 30, 1125–1132.
- Killham, K., 1990. Nitrification in coniferous forest soils. *Plant and Soil* 128, 31–44.
- Koopmans, C.J., Lubrecht, W.C., Tietema, A., 1995. Nitrogen transformations in two nitrogen saturated forest ecosystems subjected to an experimental decrease in nitrogen deposition. *Plant and Soil* 175, 205–218.
- Koops, H.P., Möller, U.W., 1992. The lithotrophic ammonia-oxidizing bacteria. In: Balows, A., Trüper, H.G., Dworkin, M., Harder, W., Schleifer, K.-H. (Eds.), *The Prokaryotes: A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications*, 2nd edn. Springer Verlag, New York, pp. 2625–2637.
- Laudelout, H., Lambert, R., Pham, M.L., 1976. Influence du pH et de la pression partielle d'oxygène sur la nitrification. *Annales de Microbiologie* 127A, 367–382.
- Maag, M., Vinther, F.P., 1996. Nitrous oxide emission by nitrification and denitrification in different soil types and at different soil moisture contents and temperatures. *Applied Soil Ecology* 4, 5–14.
- Malhi, S.S., McGill, W.B., 1982. Nitrification in three Alberta soils: effect of temperature, moisture and substrate concentration. *Soil Biology & Biochemistry* 14, 393–399.
- Martikainen, P.J., 1985. Numbers of autotrophic nitrifiers and nitrification in fertilized forest soil. *Soil Biology & Biochemistry* 17, 245–248.
- Martikainen, P.J., De Boer, W., 1993. Nitrous oxide production and nitrification in acid soil from a Dutch coniferous forest. *Soil Biology & Biochemistry* 25, 343–347.
- Morgan, M.F., 1930. A simple spot-plate test for nitrate nitrogen in soil and other extracts. *Science* 71, 343–344.
- Murphy, T.D., 1977. Design and analysis of industrial experiments. *Chemical Engineering* 6, 168–182.
- Myers, R.J.K., 1975. Temperature effects on ammonification and nitrification in a tropical soil. *Soil Biology & Biochemistry* 7, 83–86.
- Nicolardot, B., Fauvet, G., Cheneby, D., 1994. Carbon and nitrogen cycling through soil microbial biomass at various temperatures. *Soil Biology & Biochemistry* 26, 253–261.
- Nihlgård, B., 1985. The ammonium hypotheses: an additional explanation to the forest dieback in Europe. *Ambio* 14, 2–8.
- Pennington, P.I., Ellis, R.C., 1993. Autotrophic and heterotrophic nitrification in acidic forest and native grassland soils. *Soil Biology & Biochemistry* 25, 1399–1408.
- Quinlan, A.V., 1980. The thermal sensitivity of nitrification as a function of the concentration of nitrogen substrate. *Water Research* 14, 1501–1507.
- Raubuch, M., 1992. Wechselwirkungen zwischen mikrobiologischen und chemischen Parametern in Waldböden unter Berücksichtigung des atmosphärischen Eintrags. *Berichte des Forschungszentrums Waldökosysteme, Reihe A, Bd. 89*, Göttingen.
- Reuss, J.O., Johnson, D.W., 1986. Acid deposition and the acidification of soils and waters. In: *Ecological Studies* 59. Springer Verlag, New York.
- Riha, S.J., Campbell, G.S., Wolfe, J., 1986. A model of competition for ammonium among heterotrophs, nitrifiers, and roots. *Soil Science Society of America Journal* 50, 1463–1466.
- Rowe, R., Todd, R., Waide, J., 1977. Microtechnique for Most-Probable-Number Analysis. *Applied and Environmental Microbiology* 33, 675–680.
- Salonius, P.O., 1978. Effects of mixing and various temperature regimes on the respiration of fresh and air-dried coniferous raw humus materials. *Soil Biology & Biochemistry* 10, 479–482.
- SAS Institute Inc., 1989. *SAS/STAT User's Guide*, Version 6, Vols. 1 and 2. SAS Institute Inc., Cary, NC.
- Schrödter, H., Tietjen, C., 1971. Statistische Betrachtungen zur Frage der Abhängigkeit der Nitrifikation von Bodentemperatur und Bodenfeuchtigkeit. *Agricultural Meteorology* 9, 77–91.
- Sitaula, B.K., Bakken, L.R., Abrahamsen, G., 1995. CH₄ uptake by temperate forest soil: effect of N input and soil acidification. *Soil Biology & Biochemistry* 27, 871–880.
- Sitaula, B.K., Bakken, L.R., 1993. Nitrous oxide release from spruce forest soil: relationships with nitrification, methane uptake, temperature, moisture and fertilization. *Soil Biology & Biochemistry* 25, 1415–1421.
- Stams, A.J.M., Boeltink, H.W.G., Lutke-Schipholt, I.J., Beemsterboer, B., Woittiez, J.R.W., Van Breemen, N., 1991. A field study on the fate of ¹⁵N-ammonium to demonstrate nitrification of atmospheric ammonium in an acid forest soil. *Biogeochemistry* 13, 241–255.
- Stark, J.M., 1996. Modeling the temperature response of nitrification. *Biogeochemistry* 35, 433–445.
- Stark, J.M., Hart, S.C., 1997. High rates of nitrification and nitrate turnover in undisturbed coniferous forests. *Nature* 385, 61–64.
- Swift, M.J., Heal, O.W., Anderson, J.M., 1979. *Decomposition in Terrestrial Ecosystems*. Blackwell, Oxford.