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Potential denitrification rates in acid soils under pine forest

C. KROEZE*, H. G. VAN FAASSEN & P. C. DE RUITER

Institute for Soil Fertility, P.O. Box 30003, NL 9750 RA Haren, Netherlands

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Abstract

Potential rates of N_2O production and N_2O reduction associated with denitrification were measured for samples from two Dutch soils under pine forest, with pH(H_2O) 3.6 and 3.8. Undisturbed cores of the surface 15 cm litter and Ah layer were incubated for several weeks in closed pots, of which the gas phase was examined by gas chromatography for O_2, CO_2, N_2O and C_2H_2. Under anoxic conditions created by soil respiration, average N_2O-N production rates in samples from these soils ranged from 9 to 15 $\mu g\ cm^{-2}\ day^{-1}$ and average N_2O-N reduction rates from 3 to 6 $\mu g\ cm^{-2}\ day^{-1}$. Rates of N_2O production and N_2O reduction were measured at two initial nitrate concentrations: N_2O production rates were almost the same, but N_2O reduction seemed to be delayed by the presence of nitrate. The results indicate that anoxic denitrification peaks might reduce about 1 kg nitrate-N per ha per day in the upper layer of these soils to N_2O and/or N_2.

Keywords: acid soil, pine forest, denitrification, nitrous oxide

Introduction

Natural vegetations suffer from acidifying air pollution in industrial regions, caused by emissions of SO_2, NO_x and NH_3 (Ulrich & Pankrath, 1983). Typical for the Netherlands is an intensive atmospheric ammonium deposition of up to 200 kg per ha per year (van Breemen et al., 1983). In soils, nitrification of the deposited ammonium leads to excess amounts of nitrate, resulting in acidification, an unbalanced nutrient supply for vegetation and high levels of nitrate in groundwater. Denitrification, being a deacidifying and nitrate-removing process, counteracts such undesired effects (Fig. 1).

However, N_2O is an unwanted product: it is one of the so-called greenhouse gases in the atmosphere and causes damage to the stratospheric ozone layer. This detrimental side-effect is absent when N_2O is reduced to N_2. Whether or not N_2O is reduced to N_2 depends on: soil pH, nitrate (Blackmer & Bremner, 1978) and

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* Present address: Department of Environmental Science, University of Amsterdam

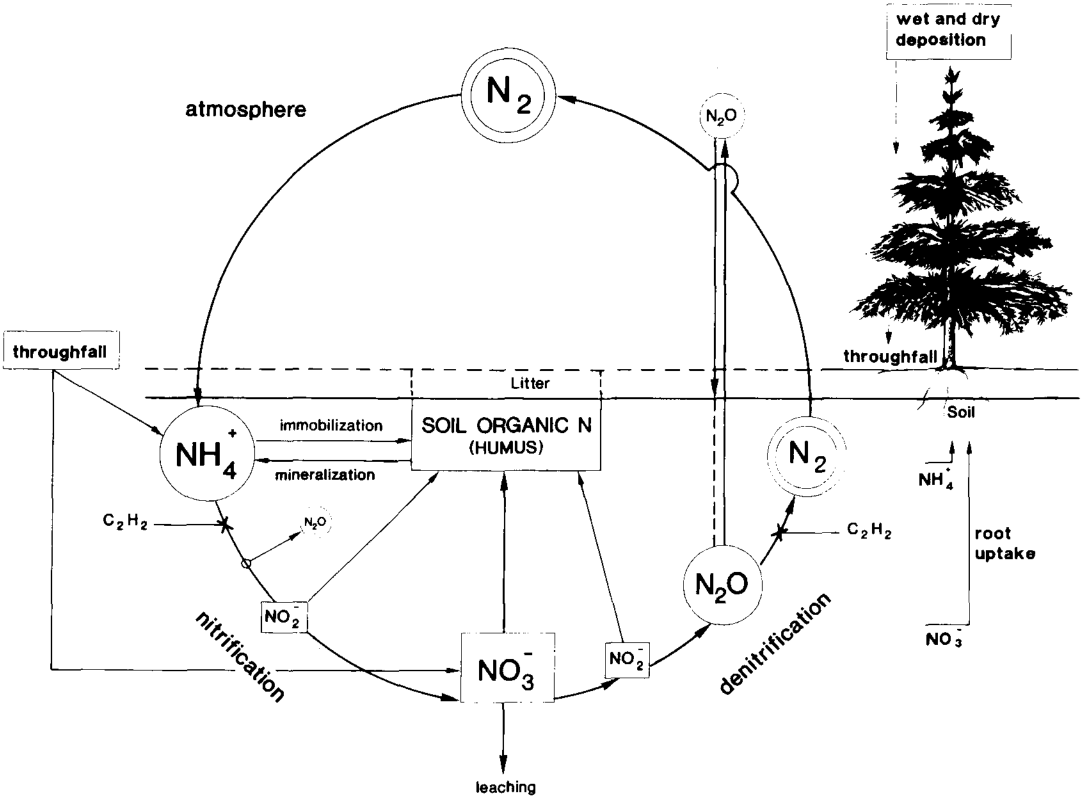

Fig. 1. Scheme showing the role of denitrification in the nitrogen cycle of a forest ecosystem; indicated is the inhibition of N_2O reduction and of NH_4^+ oxidation by C_2H_2.

oxygen concentrations (Firestone, 1982), temperature (Chalamet, 1985) and moisture content.

Most studies on denitrification involve agricultural soils and seldom acid forest soils (Knowles, 1982; Sahrawat & Keeney, 1986; Kuenen & Robertson, 1988). Some factors are known to influence denitrification in forest soils, such as the 'season' and the supply of readily decomposable organic matter (Rashid & Schaefer, 1987, 1988) and moisture content (Binstock, 1984). Parkin et al. (1985) showed that denitrifying microbial populations can adapt to acid environments. So, the objective of this study was to examine whether denitrification can be quantitatively important in Dutch forest soils with low pH, and if so, can any N_2O produced be further reduced to N_2?

To predict the rates of denitrification in forest soils, we have to know how frequently the conditions necessary for denitrification are met, and when they do, at what rates production and reduction of N_2O occur. This study focussed on the latter aspect, the quantification of rates.

Table 1. Relevant characteristics of location and soil surface layers.

	Location, vegetation	
	Olterterp, *Picea sitchensis*	Roden, *Picea abies*
Soil type	gleyic podzol	gleyic podzol
pH(H_2O)	3.6	3.8
pH(KCl)	2.8	2.7
Organic matter (%)		
(0-3 cm Lh layer)	86	74
(4-15 cm Ah layer)	13	17
Bulk density (g cm^{-3})		
(0-15 cm Lh/Ah layer)	0.40+/−0.04	0.39+/−0.05
Nitrate-N (μg cm^{-2})		
(0-5 cm Lh/Ah layer)	10	5
Ammonium-N (μg cm^{-2})		
(0-5 cm Lh/Ah layer)	120	150

Actual field rates of denitrification are difficult to measure, because of the strong peak character (Tietema & Verstraten, 1989). Wet conditions conducive to denitrification strongly limit field measurements of gas exchange, either of N_2O out of the soil or of C_2H_2 into the soil. Therefore we investigated potential rates of N_2O production and N_2O reduction of structurally intact cores from two pine forest soils, with pH(H_2O) of 3.6 and 3.8. We also studied the effect of initial soil nitrate content on these rates, by adding ammonium nitrate solutions to the cores.

Materials and methods

Soil sampling

Soil cores were taken from two locations in the Netherlands, 'Olterterp' and 'Roden'. Some characteristics of these soils are summarized in Table 1. In Olterterp the presence of boulder clay with a low hydraulic conductivity causes a high wetness of the upper layers during a large part of the year.

Undisturbed cores were taken in spring from the 0-15 cm Lh (litter) and Ah layers and incubated in closed plastic pots of about 1.5 l at 20 °C. We sampled on the first rainy day after a dry period of several weeks. The cores were 10 cm in diameter.

The dry matter contents of the cores were determined after drying at 105 °C, the organic matter contents by combustion at 600 °C. The ammonium and nitrate contents of the cores were determined in 1 N KCl extracts (1:2.5) by standard autoanalyser methods; if nitrite was present it was included in the nitrate determined. pH was measured both in 1 M KCl extracts and in 1:2.5 soil:water suspensions.

Gas chromatography

O_2 (including 1 % argon), CO_2, N_2O and C_2H_2 were measured with a Packard 430 gas chromatograph, equipped with a Porapak Q column (2 m × 3.2 mm o.d.; 100-

120 mesh) and a 0.5 nm molecular sieve 13 column (0.6 m × 3.2 mm o.d.; 80-100 mesh) in series. The oven was adjusted to 50 °C, the injector to 80 °C and the thermal conductivity detector to 180 °C. The carrier gas was helium at a flow rate of 15 ml min^{-1}. After O_2 and N_2 had passed both columns and the detector, CO_2, N_2O and C_2H_2 coming from the first column were led directly to the detector by means of a switching system. Changes in N_2 could not be measured against the background of about 80 vol % N_2.

Results from measurements made at daily intervals were converted from vol % to $\mu g\ cm^{-2}$, with a correction for N_2O dissolved in soil water. The limit of detection for N_2O was 0.01 vol % (10 Pa partial pressure).

Experiment 1

In this experiment we estimated rates of production and reduction of N_2O, the former by adding C_2H_2 to the pots to inhibit N_2O reduction. The experimental treatments consisted of four (2 × 2) combinations of factorial levels: location (Olterterp or Roden) and C_2H_2 (supplied or not supplied). Each combination was replicated three times.

Twenty ml C_2H_2 per pot was supplied when bulk oxygen concentrations became less than 6 vol %; thus, until then, nitrification was not affected by C_2H_2. The added C_2H_2 resulted in a gas phase concentration $\geq$ 1 vol %, which in previous experiments was found to be sufficient to inhibit the reduction of N_2O to N_2. Nitrification is known to be already inhibited by C_2H_2 concentrations as low as 0.1 vol %.

Experiment 2

In Experiment 2 the effect of initial nitrate concentration on the rates of N_2O production and N_2O reduction was studied. The soil cores of Experiment 1 were used again, because preliminary experiments had shown that it was safe to assume that all nitrate in these cores had been reduced during Experiment 1. To make sure that no acetylene was left in the cores, they were exposed to the air for a few days. Experimental treatments of Experiment 2 also consisted of four (2 × 2) combinations of factorial levels: location (Olterterp or Roden) and initial nitrate-N additions to the soil samples (equivalent to 60.5 or 30.3 $\mu g\ cm^{-2}$). Each combination was replicated three times. These nitrate additions are comparable to amounts deposited in a 2- to 4-weekly period. Nitrate was added as NH_4NO_3 in 100 ml 'throughfall-solution', with a chemical composition reflecting natural deposition on a pine forest soil in the Netherlands, per litre demineralized water containing 52.3 mg K_2SO_4, 7.2 mg $CaCl_2 \cdot 2H_2O$, 10.2 mg $MgCl_2 \cdot 6H_2O$, 5.8 mg NaCl (pH 4.5; adjusted with H_2SO_4). Although the amounts of nitrate given were realistic, the nitrate concentrations were relatively high. This wetting of the soil core surfaces corresponded to about 13 mm of precipitation, and gave a distribution of water and nitrate that was not homogeneous.

In both experiments the rates of N_2O production and reduction were derived from the periods with a linear increase or decrease in N_2O concentrations in the pots. N_2O reduction rates could be estimated by assuming that N_2O reduction only started when nitrate was completely reduced to N_2O.

Results

Experiment 1

A summary of the results is given in Table 2.

The composition of the gas phase in the pots during incubation changed as indicated in Fig. 2. First pO_2 decreased due to respiration, while pCO_2 increased. N_2O production was observed for all cores when pO_2 had decreased to less than 2 kPa, indicating that anoxic zones had developed. Differences in the period needed to use up the O_2 supply in the pots can account for the fact that N_2O production started at different times (Fig. 3). If no C_2H_2 was added, reduction of N_2O (to N_2) eventually happened. When all O_2 had been consumed, CO_2 concentrations still increased, but at a slower rate, related to denitrification.

Average rates of N_2O-N production ranged from 10 to 15 μg cm^{-2} day^{-1}. Analysis of variance showed that the rates of N_2O production in the cores from Olterterp and Roden were not significantly different. Rates of N_2O production were not significantly affected by acetylene addition.

Total amounts of N_2O-N produced in the Roden cores (43-48 μg cm^{-2}) were significantly larger than those in the Olterterp cores (17-27 μg cm^{-2}; $P<0.002$). Total amounts of N_2O produced were not significantly affected by acetylene.

Table 2. Summary of the results of Experiment 1. Average values of denitrification and respiration rates, and total N_2O production during incubation of undisturbed cores at 20 +/− 1 °C; volume fractions +/− s.d.

	Location, addition of C_2H_2			
	Olterterp		Roden	
	−	+	−	+
(a) Rate of N_2O-N production (μg cm^{-2} day^{-1})	14	12	15	10
(b) Total N_2O-N production (μg cm^{-2})	27	17	43	48
(c) Rate of N_2O-N reduction (μg cm^{-2} day^{-1})	3	0	3	0
(d) Rate of O_2 consumption (μg cm^{-2} day^{-1})	780	710	540	600
Rate of N_2O-N production (% of O_2 consumption) [32/14 × (a)/(d) × 100 %]	4.2	3.7	6.5	4.0
Volume fractions of the cores (cm^3/cm^3)				
— solids	0.20 +/− 0.03		0.19 +/− 0.03	
— water-filled porosity	0.43 +/− 0.05		0.30 +/− 0.05	
— gas-filled porosity	0.37 +/− 0.05		0.51 +/− 0.10	

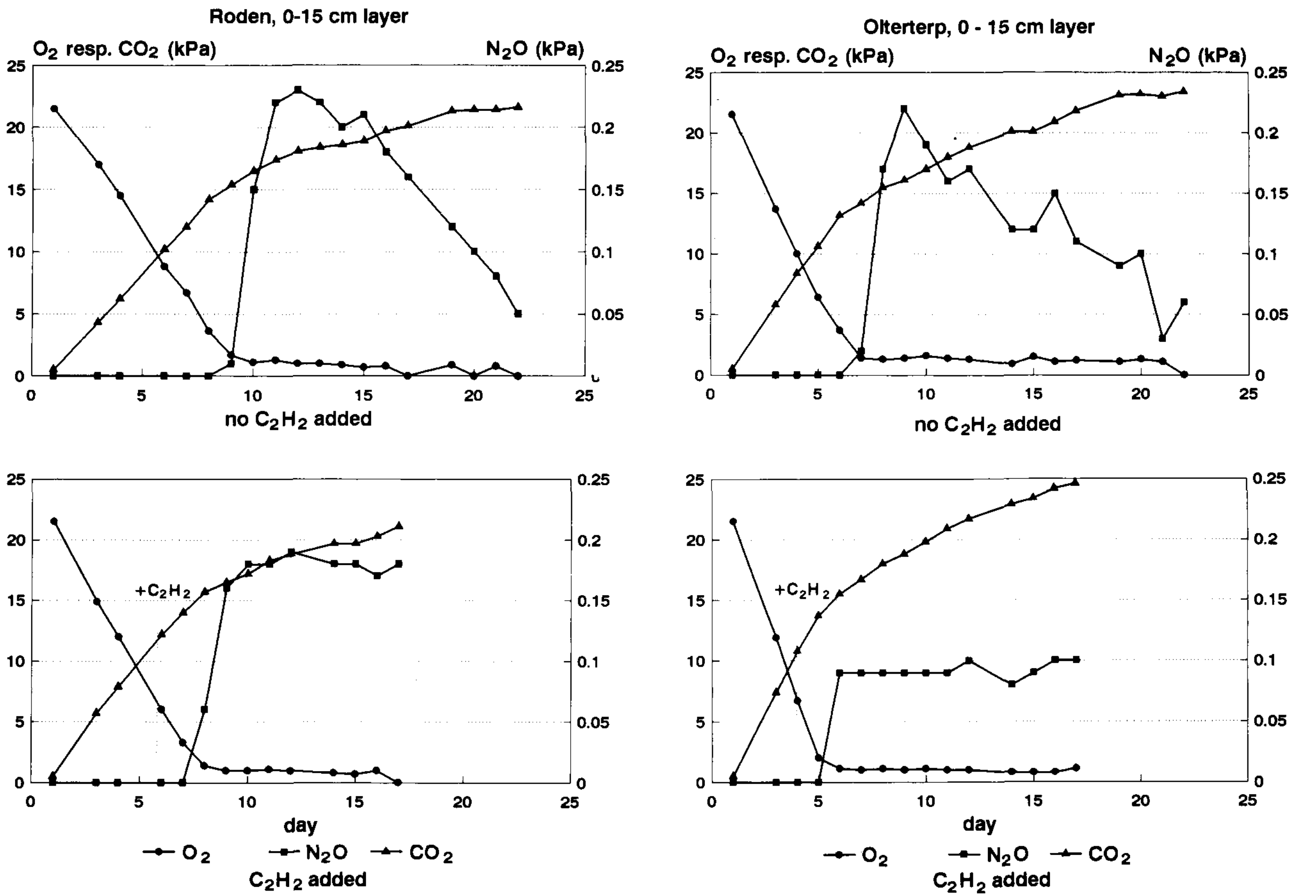

Fig. 2. Typical examples of changes in partial pressures of O_2, CO_2 and N_2O above structurally intact soil cores, incubated in closed pots (Exp. 1), with or without addition of C_2H_2, from Olterterp and Roden. Period: 11 May-1 June.

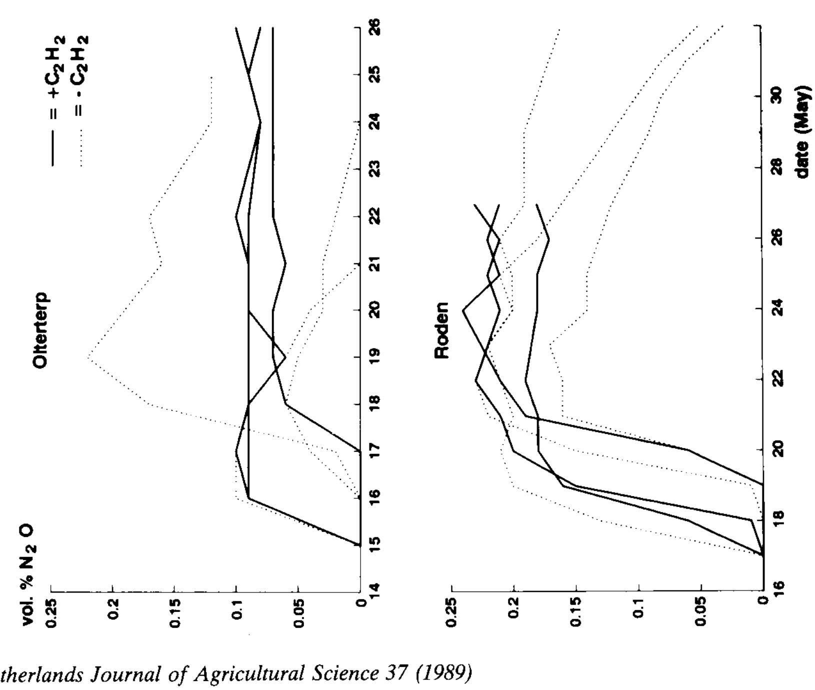

Fig. 3. N_2O concentrations in the gas phase during incubation of undisturbed cores in closed pots (Exp. 1) from Olterterp and Roden.

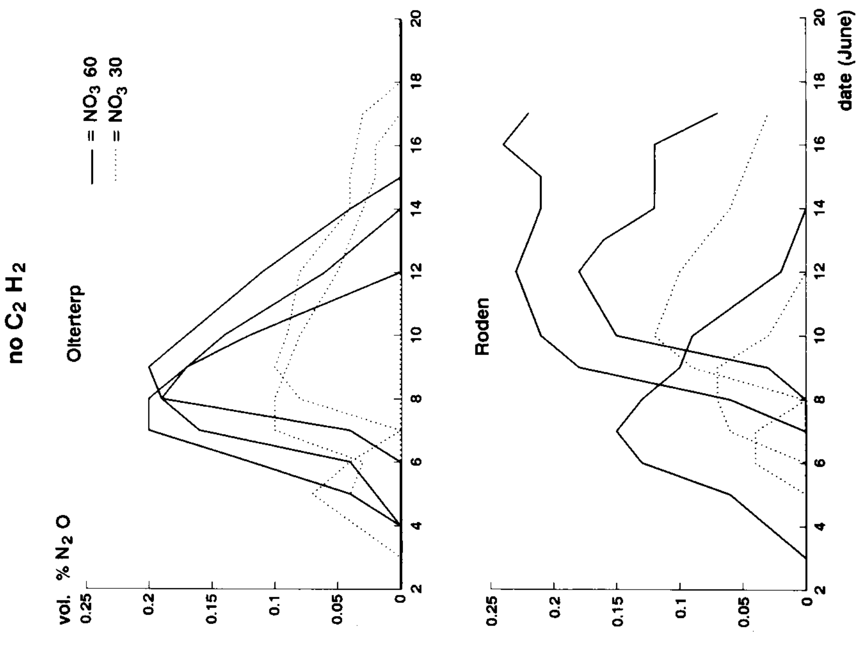

Fig. 4. N_2O concentrations in the gas phase during incubation of undisturbed cores in closed pots (Exp. 2) from Olterterp and Roden. Dashed lines: nitrate-N addition equivalent to 30 μg cm^{-2}; solid lines: nitrate-N addition equivalent to 60 μg cm^{-2}.

The rates of N_2O-N reduction averaged 3 μg cm^{-2} day^{-1}; there was no significant difference between the cores from Roden and Olterterp. The rates of N_2O reduction were about 20 % of the rates of N_2O production, the latter were only 4 to 7 % of the rates of aerobic respiration on an electron-equivalent basis.

Experiment 2

A summary of the results is given in Table 3.

The rates of N_2O-N production measured (9-13 μg cm^{-2} day^{-1}) were similar to those measured in Experiment 1.

Nitrate concentration did not affect the rates of N_2O production significantly (Fig. 4), but the total amount of N_2O produced increased with the amount of nitrate supplied (ANOVA; $P<0.001$). The total amounts of N_2O produced were the same for cores from both locations.

The rates of N_2O-N reduction (3-6 μg cm^{-2} day^{-1}) slightly exceeded those observed in Experiment 1 (0-3 μg cm^{-2} day^{-1}). This might be due to adaptation of the microbial population to the final conditions in Experiment 1, i.e. anoxic and without nitrate, favouring individuals that are able to reduce N_2O to N_2.

Neither the amount of nitrate supplied, nor the location of sampling (Roden or Olterterp) significantly affected the rate of N_2O reduction.

Table 3. Summary of the results of Experiment 2. Average values of denitrification and respiration rates, and total N_2O production during incubation of undisturbed cores at 20 +/− 1 °C; mineral N contents of the cores at the end of the incubation period; volume fractions +/− s.d.

	Location, NO_3-N addition (μg cm^{-2})			
	Olterterp		Roden	
	60.5	30.3	60.5	30.3
(a) Rate of N_2O-N production (μg cm^{-2} day^{-1})	13	9	10	9
(b) Total N_2O-N production (μg cm^{-2})	41	16	40	16
(c) Rate of N_2O-N reduction (μg cm^{-2} day^{-1})	6	3	4	5
(d) Rate of O_2 consumption (μg cm^{-2} day^{-1})	760	610	745	780
Rate of N_2O-N production (% of O_2 consumption) [32/14 × (a)/(d) × 100 %]	3.8	3.2	3.1	2.9
NO_3-N (1-3 cm layer) (μg cm^{-2})	0	0	3	3
NO_3-N (4-15 cm layer) (μg cm^{-2})	0	0	0	4
NH_4-N (1-3 cm layer) (μg cm^{-2})	470	470	380	560
NH_4-N (4-15 cm layer) (μg cm^{-2})	170	175	200	175
Volume fractions of the cores (cm^3/cm^3)				
— solids	0.20 +/− 0.03		0.19 +/− 0.03	
— water-filled porosity	0.52 +/− 0.05		0.39 +/− 0.05	
— gas-filled porosity	0.28 +/− 0.05		0.42 +/− 0.10	

Discussion

The measured potential rates of N_2O-N production were up to 15 μg cm^{-2} day^{-1} (1.5 kg ha^{-1} day^{-1}) and the rates of N_2O-N reduction up to 3 μg cm^{-2} day^{-1} (0.3 kg ha^{-1} day^{-1}). We consider the observed production rates to be relatively high, because within a few days all the nitrate in the soil cores was denitrified. However, these rates were measured under anoxic conditions in the laboratory. Only when such anoxic conditions develop locally in the soil of these forests, e.g. after heavy rainfall, may we assume that on these particular spots denitrification rates will also be high. However, the maximum amount of N_2O-N detected was smaller than the amount added as nitrate-N, indicating that N_2O reduction had started before all nitrate had been reduced to N_2O. A slow escape of N_2O from cores with only few gas-filled pores may be another explanation. Thus, the rate of N_2O production as well as the rate of reduction were perhaps underestimated, as suggested earlier (Letey et al., 1980).

To predict actual denitrification in these forest soils, we must know the frequency and duration of anoxic 'peak events'. When peaks occur frequently, a considerable amount of nitrate might be reduced by denitrification in these soils. As much as 34 kg ha^{-1} NH_4-N and about 10 kg ha^{-1} NO_3-N is deposited annually on these forests (RIVM, 1986). Nitrification of NH_4-N might contribute to the nitrate supply in these soils, but this was not studied. On the other hand nitrate leaching and nitrate uptake by plants may 'compete' with denitrification.

The rates of N_2O production did not depend on the initial nitrate concentration of the soil. This is not in agreement with findings of Robertson et al. (1987), who observed stimulation of denitrification rates by nitrate addition. However, the N_2O production rates they found were relatively low, measured under aerobic conditions and possibly related to nitrification. Furthermore, nitrate supply appeared to regulate denitrification at the site these authors studied.

The total amounts of N_2O produced in Experiment 2 were proportional to the initial nitrate content of the soil. This indicated that the difference between total productions in the cores from Olterterp and Roden in Experiment 1 was caused by differences in initial nitrate content. The difference in initial nitrate content probably also explains the variation in total amounts of N_2O-N measured in similar experiments with soil cores from two national field research sites under Douglas fir forest in Kootwijk (40 μg cm^{-2}) and Speuld (160 μg cm^{-2}). Rates of N_2O production and of N_2O reduction could not be calculated then because of a lower frequency of gas phase sampling (van Faassen, unpublished results).

The present study affirms that N_2O reduction can be delayed by the presence of nitrate. The magnitude of the ratio N_2O/N_2 will therefore depend on the length of the anoxic periods and the amount of nitrate available. N_2O emission into the atmosphere will furthermore depend on the existing soil physical conditions, especially moisture content and gas-filled porosity, which determine the rate of N_2O transport. After rainfall the high water-holding capacity of humus-rich surface layers may result in a low gas-filled porosity and prolonged anoxic conditions, conducive to denitrification.

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