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Nitrogen loss from grassland on peat soils through nitrous oxide production

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Abstract

Nitrous oxide (N_2O) in soils is produced through nitrification and denitrification. The N_2O produced is considered as a nitrogen (N) loss because it will most likely escape from the soil to the atmosphere as N_2O or N_2. Aim of the study was to quantify N_2O production in grassland on peat soils in relation to N input and to determine the relative contribution of nitrification and denitrification to N_2O production. Measurements were carried out on a weekly basis in 2 grasslands on peat soil (Peat I and Peat II) for 2 years (1993 and 1994) using intact soil core incubations. In additional experiments distinction between N_2O from nitrification and denitrification was made by use of the gaseous nitrification inhibitor methyl fluoride (CH_3F).

Nitrous oxide production over the 2 year period was on average 34 kg N ha^{-1} yr^{-1} for mown treatments that received no N fertiliser and 44 kg N ha^{-1} yr^{-1} for mown and N fertilised treatments. Grazing by dairy cattle on Peat I caused additional N_2O production to reach 81 kg N ha^{-1} yr^{-1}. The sub soil (20–40 cm) contributed 25 to 40% of the total N_2O production in the 0–40 cm layer. The N_2O production:denitrification ratio was on average about 1 in the top soil and 2 in the sub soil indicating that N_2O production through nitrification was important. Experiments showed that when ratios were larger than l, nitrification was the major source of N_2O. In conclusion, N_2O production is a significant N loss mechanism in grassland on peat soil with nitrification as an important N_2O producing process.

Introduction

Interest in nitrous oxide (N_2O) production in soils is predominantly based on the contribution of N_2O to the enhanced greenhouse effect (Wang et al., 1976) and the decay of stratospheric ozone (Crutzen, 1970). However, N_2O production through nitrification and denitrification may be a substantial fraction of the total gaseous nitrogen (N) loss from soils and important to the N balance of grasslands. The N_2O produced in the soil will most likely escape to the atmosphere as N_2O or, after further reduction, as N_2. In the atmosphere, N_2O and N_2 are relatively stable gases that are not directly available for plant uptake. Managed grasslands on peat soil have large N losses through denitrification (Guthrie and Duxbury, 1978; Koops et al., 1996) and have large N_2O emissions (Duxbury et al., 1982; Velthof et al. 1994; Velthof and Oenema, 1995). The large losses are related to the relatively wet conditions combined with high mineralisation rates (e.g. Grootjans et al., 1985) which will favour both denitrification and N_2O production through nitrification; additional N fertiliser input may further increase gaseous N losses.

Denitrification is an anaerobic microbiological process in which organic carbon (C) is used as an energy source and nitrate (NO_3^-) is used as an electron acceptor and is reduced to the gaseous N compounds N_2O and dinitrogen (N_2) (Reaction 1). Translated to field situations, denitrification in soils will occur under wet circumstances where oxygen (O_2) is limited and NO_3^- and organic C are available for micro-organisms.

The relative amount of N_2O produced through denitrification can be expressed as $N_2O/(N_2O+N_2)$, i.e. N_2O produced divided by total gaseous N production

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through denitrification. This ratio is influenced by a number of soil variables: increased soil moisture contents will lead to low ratios whereas increased NO_3^- contents of the soil will result in relatively high ratios (Firestone et al., 1979; Letey et al., 1980b; Weier et al., 1993). These studies also showed a decrease of the ratio in time being ascribed to a lag phase in the N_2O reductase production. Nitrous oxide may be subjected to further reduction to N_2 by denitrifiers when it is trapped in the soil water (Bandibas et al. 1994; Højberg et al., 1994). The pH also has an influence on the $N_2O/(N_2O+N_2)$ ratio; in general the ratio decreases with pH exceeding 5 to 6 (Granli and Bøckman, 1994).

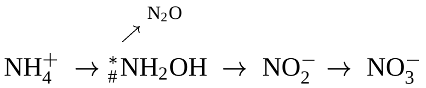
Nitrous oxide can also be formed during nitrification (Reaction 2), i.e. oxidation of ammonium (NH_4^+) (Blackmer et al., 1980; Bremner and Blackmer, 1978; Lipschultz et al., 1981). Both autotrophic (Blackmer et al., 1980) and heterotrophic (Tortoso and Hutchinson, 1990) nitrifying bacteria are capable of N_2O production. The amount of N_2O produced through nitrification increases with decreasing O_2 concentrations (Bremner and Blackmer, 1979; Lipschultz et al., 1981). Goodroad and Keeney (1984) reported that 0.1–0.2% of the nitrified N in silt loam soil was produced as N_2O in relatively dry soil but at larger water contents this figure increased to more than 1%. Nitrous oxide production through nitrification can be an important mechanism for potential N loss. Hendrickson et al. (1980) observed that 35% of the total gaseous N loss from an agricultural watershed was through N_2O production by nitrification.

Nitrite (NO_2^-) is an intermediate in both the nitrification and the denitrification process. Reduction of NO_2^- from nitrification is called nitrifier-denitrification (Castignetti and Hollocher, 1982; Poth and Focht, 1985), and this pathway will also lead to the production of N_2O and N_2 (Reaction 3). Poth and Focht (1985) found that *Nitrosomonas europaea* was capable of denitrification of NO_2^- to N_2O. Castignetti and Hollocher (1982) found that a heterotrophic nitrifier of the *Alcaligenes* species was capable of oxidising hydroxylamine to NO_2^- and was able to synthesise the full complement of denitrifying enzymes. Other micro-organisms that are capable of nitrifier-denitrification also exist (Robertson, 1988).

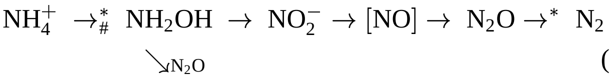
The aim of the present study was to quantify N_2O production in grassland on peat soils in relation to N input and to determine the relative contribution of nitrification and denitrification to N_2O production. Two representative peat soils with different ground water levels and different N management were selected. Nitrous oxide production was measured by incubation of intact soil cores allowing rapid gas exchange between soil and headspace. Production of N_2O was compared to denitrification activity in order to asses the relative contribution of denitrification and nitrification to the total N_2O losses. In a number of experiments N_2O production through denitrification was measured with the nitrification inhibitor methyl fluoride (CH_3F) (Miller et al., 1993).

Reactions:

$$NO_3^- \rightarrow NO_2^- \rightarrow [NO] \rightarrow N_2O \xrightarrow{*} N_2 \qquad (1)$$



$$NH_4^+ \rightarrow {}^{*}_{\#}\overset{\nearrow N_2O}{NH_2OH} \rightarrow NO_2^- \rightarrow NO_3^- \qquad (2)$$



$$NH_4^+ \rightarrow^{*}_{\#} \underset{\searrow N_2O}{NH_2OH} \rightarrow NO_2^- \rightarrow [NO] \rightarrow N_2O \rightarrow^{*} N_2 \qquad (3)$$

(1) Denitrification
(2) Nitrification
(3) Nitrifier-denitrification
* Blockage by acetylene
# Blockage by methyl fluoride

Materials and methods

Site and treatment descriptions

Two sites on different peat soils were selected at the Regional Research Centre “Zegveld”, in the western part of the Netherlands (52° 08’ N 4° 48’ E). These sites are further referred to as Peat I and Peat II. The main differences between Peat I and Peat II were the organic matter content and the ground water level. Peat I had a lower organic matter content in the top 20 cm (39%) than Peat II (44%). Mean ground water level on Peat I was 30 cm below the surface whereas on Peat II it was 45 cm below the surface (Koops et al., 1996). The sites were divided into plots (3 × 15 m) on which several treatments were applied in a randomised block design.

Samples were taken from unfertilised (0 N treatment) plots and from adjacent plots that were fertilised (+ N treatment) with Calcium-Ammonium-Nitrate (CAN). On Peat I a rotational grazing treatment (+ NG treatment) was also included. The stocking rate was adjusted such that the sward could be grazed in one day, this resulted in stocking rates of about 360 cows ha^{-1}. The + NG treatment received as much N fertiliser as the + N treatment and was applied immediately after grazing. The average annual amount of applied N was 435 kg N ha^{-1} yr^{-1} on Peat I and 340 kg N ha^{-1} yr^{-1} on Peat II, split in 6 to 7 dressings of 25 to 90 kg N ha^{-1} per dressing.

Sampling and incubation

Soils were sampled on a weekly basis in 1993 and 1994 using an intact soil core incubation method as introduced by Ryden et al. (1987) with some modifications (Koops et al., 1996). Soil cores were taken at random from the plots, at the + NG treatment fresh dung patches were excluded. However, urine and old dung patches were included in the sampling. Incubation took place in PVC containers (volume 3 L) in which 4 soil cores were placed. Soil cores were taken from the top soil (0–20 cm depth; diam 47 mm) and from the sub soil (20–40 cm depth; diam 35 mm). Incubation took place at temperatures close to the soil temperature at time of sampling, the difference between field and incubation temperature was never more than 2 °C. Headspace sampling for N_2O took place after 4 and 24 h of incubation.

Methyl fluoride and acetylene incubations

To determine the contribution of denitrification to the total N_2O production, parallel incubations were carried out with and without methyl fluoride (CH_3F) or acetylene (C_2H_2). Samples were taken on Peat I from the + NG treatment and on Peat II from the + N treatment in May and June 1994. Methyl fluoride (Scotts Specialty Gases) and C_2H_2 (generated from calcium carbide and water) were added to give 8% (v/v) in the headspace of separate incubation containers. Methyl fluoride is a gaseous nitrification inhibitor (Reaction 2, #) that does not interfere with the $N_2O/(N_2O+N_2)$ ratio from denitrification (Miller et al., 1993). Nitrous oxide produced in the presence of CH_3F thus originates from denitrification. Total denitrification activity was quantified by measuring the N_2O produced after incubation with C_2H_2 (Koops et al., 1996; Yoshinary and Knowles, 1976). Acetylene blocks both N_2O reductase (Reaction 1, *) and NH_4^+ oxidation and thus N_2O production by nitrification (Reaction 2, *). Nitrifier-denitrification derived N_2O production is blocked by both C_2H_2 and CH_3F (Reaction 3, * and #). Hence, N_2O production through nitrifier-denitrification can not be separated from the N_2O production through nitrification.

The sum of nitrification and nitrifier-denitrification derived N_2O production was calculated from N_2O production in containers without addition of specific inhibitors minus N_2O production in the presence of CH_3F. The $N_2O/(N_2O+N_2)$ ratio from denitrification was calculated from N_2O production in the presence of CH_3F divided by the total denitrification as measured in the presence of C_2H_2.

Sample analysis

The N_2O concentrations in the headspace of the incubation containers with and without C_2H_2 were determined with a Brüel and Kjær 1302 multi-gas monitor, a photoacoustic infrared gas analyser. Nitrous oxide concentrations in containers with CH_3F were measured with a gas chromatograph because of the interference of CH_3F on the determination of N_2O by the gas analyser. The gas chromatograph (Unicam 4880) was equipped with Haysep Q (80–100 mesh) columns at 60 °C, N_2 carrier gas at 0.47 mL s^{-1} and a ^{63}Ni electron capture detector at 350 °C. The difference between the analysis methods was < 6% in the concentration range from 5 to 100 ppmv N_2O.

Nitrate and NH_4^+ contents of the soil samples were determined, after drying at 40 °C and subsequent extraction with 0.01 *M* $CaCl_2$, with standard auto-analyser techniques (Houba et al., 1989). Soil water content was determined by drying at 105 °C immediately after the last headspace sampling. The water filled pore space (WFPS) was calculated from the soil water content, bulk density and the total porosity of the soils.

Calculations

The N_2O production rate was calculated from the concentrations of N_2O in the headspace after 4 and 24 h of incubation. The measured concentrations of N_2O were corrected for the amount of N_2O in the air filled pore space and dissolved in the soil water assuming diffusion equilibrium (Tiedje, 1982). Dissolved N_2O, already present in the soil water would have diffused into the headspace in the first 4 h of incubation. How-

Table 1. Average annual N_2O production and denitrification in kg N ha^{-1} yr^{-1}, 0 N = mown and no N fertiliser, + N = mown and N fertiliser application (435 kg N ha^{-1} yr^{-1} on Peat I and 340 kg N ha^{-1} yr^{-1} on Peat II), + NG = grazed and N fertiliser application. Standard deviation in parentheses

	Peat I			Peat II	
	0 N	+ N	+ NG	0 N	+ N
N_2O production					
Top soil (0–20 cm)	24(9)	27(16)	61(8)	21(12)	27(14)
Sub soil (20–40 cm)	12(1)	19(12)	20(9)	11(4)	14(3)
Total (0–40 cm)	36(8)	46(28)	81(18)	32(16)	41(18)
Denitrification					
Top soil (0–20 cm)	23(3)	31(11)	63(42)	17(12)	31(25)
Sub soil (20–40 cm)	4(2)	10(1)	12(5)	6(4)	13(12)
Total (0–40 cm)	27(1)	41(12)	75(47)	23(16)	44(38)

ever, some of the measurements done in 1993 were measured only at 24 h and required a correction (Koops et al., 1996).

Annual N losses through N_2O production were determined by linear integration of the N_2O production rates per year, using the trapezoidal rule. The N_2O production rates were log-normally distributed. Log-transformation of the data was carried out before determination of the Pearson's correlation coefficients between N_2O production rates and WFPS, NO_3^- and NH_4^+. The average coefficient of variation between duplicate samples was 55% ($n = 139$). All statistical calculations were carried out using SPSS/PC (Huizingh, 1993).

Results

The variations in N_2O production rates over a 2 year period are shown in Figures 1 and 2. Rates on the 0 N and + N treatments were similar on both soils. On Peat I, the + NG treatment N_2O production rates reached 2000 g N ha^{-1} day^{-1}, whereas maximum rates on the + N treatment were 500 g N ha^{-1} day^{-1}. Peak production rates often occurred after N fertiliser application and grazing. Average annual N_2O production in the 0–40 cm soil layer was 36, 46 and 81 kg N ha^{-1} yr^{-1} on 0 N, + N and + NG treatments of Peat I, respectively (Table 1). On Peat II the average annual N_2O production was 32 and 41 kg N ha^{-1} yr^{-1} on the 0 N and + N treatments, respectively. Despite the larger N fertiliser input on Peat I than on Peat II and the different ground water level, N_2O production was not significantly different between the soils ($p < 0.05$). Apparent N loss through N_2O production was 2 and 3% of the applied fertiliser N on Peat I and Peat II, respectively.

The sub soil was a significant site of N_2O production; the contribution of the sub soil to the total N_2O production ranged from 25 to 41%. Production of N_2O in the sub soil was significantly ($p < 0.05$) higher on the + N and + NG treatments than on the 0 N treatment. This indicates that the effect of N fertiliser application was not restricted to the top soil. Apparently, part of the fertiliser N was transported to the sub soil and there converted to N_2O.

The N_2O production:denitrification ratio (Figures 3 and 4) gives an insight into the relative importance of nitrification in N_2O production. A ratio larger than 1 implies that N_2O production through nitrification must have occurred, because N_2O production through denitrification cannot exceed total denitrification (i.e. N_2O+N_2). Ratios were highly variable but were on average about 1 in the top soils (Figures 3a and 4a) and about 2 in the sub soils (Figures 3b and 4b). Hence nitrification must have been an important N_2O producing process in these peat soils, especially in the sub soil.

A more precise distinction between N_2O released from nitrification and denitrification was made by using CH_3F as a specific nitrification inhibitor. Table 2 shows the results of incubations with and without CH_3F or C_2H_2 addition. These single independent sample sets were taken from the + NG treatment of Peat I and the + N treatment of Peat II to include large denitrification rates. Nitrous oxide production through denitrification was expected to be of significance at

Table 2. Nitrous oxide production in g N ha^{-1} day^{-1} during incubation with and without CH_3F or C_2H_2 in the headspace. Data represent single independent sample sets. Production of N_2O through nitrification was calculated from the N_2O production without inhibitor minus N_2O production in the presence of CH_3F (i.e. N_2O production by denitrification). The ratio $N_2O/(N_2O+N_2)$ in the denitrification process was calculated from N_2O production in the presence of CH_3F divided by the total denitrification as determined by incubation with C_2H_2

No addition (A)	With C_2H_2 (B)	With CH_3F (C)	N_2O production: denitrification (A:B)	% N_2O from nitrification 100×(A-C):C	$N_2O/(N_2O+N_2)$ of denitrification (C:B)
			Ratios > 1		
32	0	0	–	100	–
47	0	0	–	100	–
171	0	0	–	100	–
144	3	0	48.0	100	0.0
87	10	0	8.7	100	0.0
81	10	0	8.1	100	0.0
140	24	0	5.8	100	0.0
117	44	16	2.7	86	0.4
34	21	0	1.6	100	0.0
24	18	0	1.3	100	0.0
			Ratios < 1		
495	539	0	0.92	100	0.0
23	50	9	0.46	61	0.2
64	140	1	0.46	98	0.0
1166	3177	2181	0.37	–[a]	0.7
535	1984	966	0.27	–[a]	0.5
17	399	0	0.04	100	0.0

[a] Percentage apparently less than zero.

high denitrification activities. Cores for the separate incubations were taken in pairs close to each other to minimise the effects of spatial variation. However, this did not prevent N_2O production through denitrification exceeding total N_2O production in a number of cases. In one case this was probably due to a lower WFPS of the incubation without inhibitor, for the other case no unequivocal explanation is available. Nevertheless, some main trends can be distinguished. For N_2O production:denitrification ratios larger than 1 almost all the N_2O was produced by nitrification. Ratios smaller than 1 were mostly associated with high denitrification activities. Notable N_2O production through denitrification occurred at very high denitrification activities only. The $N_2O/(N_2O+N_2)$ ratio from denitrification was < 0.7 in these samples and in most other instances was zero.

There was a significant correlation between denitrification activity and N_2O production rate in the top soils of both Peat I and Peat II for all treatments (Table 3). In the sub soil the correlation between denitrification activity and N_2O production was much less than in the top soil. This confirms indirectly the relative importance of nitrification for N_2O production in the sub soil. Correlation coefficients between N_2O production rates and WFPS, NO_3^- and NH_4^+ are given in Table 4. In

Table 3. Pearson's correlation coefficients between denitrification activity and N_2O production rates in the top and sub soil. One tailed significance: $^* = 0.01$, $^{**} = 0.001$

Treatment	Top soil	Sub soil
Peat I		
0 N	0.73^{**}	0.16
+ N	0.81^{**}	0.45^{**}
+ NG	0.69^{**}	0.11
Peat II		
0 N	0.58^{**}	0.48^{**}
+ N	0.55^{**}	0.41^{*}

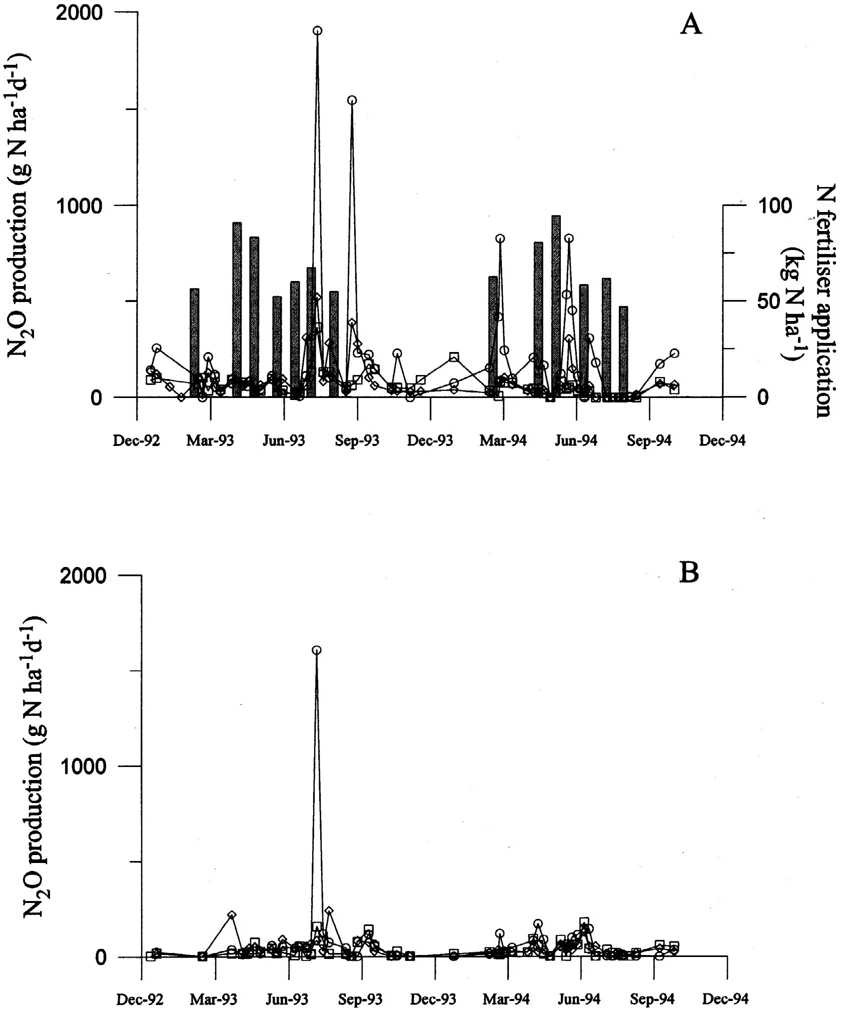


Figure 1. Production rates of N_2O in the top soil (**A**) and sub soil (**B**) of Peat I in g N ha^{-1} day^{-1} over a 2 year period. □ = 0 N treatment; ○ = + N treatment; ◇ = + NG treatment. Bars indicate time and amount of N fertiliser application.

the top soils WFPS was the most significant factor; correlation between N_2O production and NO_3^- content was only significant in the 0 N treatments. In the sub soils there was no significant correlation between WFPS, NO_3^- and NH_4^+ contents and N_2O production, indicating that other factors were more important.

Discussion

Nitrous oxide production

Nitrous oxide produced in soil will eventually reach the atmosphere as N_2O or N_2 and may be seen as an N loss mechanism for grassland. Measurements of N_2O production in peat soils are still scarce. Jarvis et al. (1994), using a similar soil core incubation technique, reported N_2O production rates of 20–200 g N ha^{-1} day^{-1} from peat soil after the application of cattle slurry. In the present study, N_2O production from grazed grassland ranged from 0 to 2000 g N ha^{-1} day^{-1} (Figure 1) and the average annual production of N_2O in the peat soils ranged from 32 to 81 kg N ha^{-1} yr^{-1} (Table 1). Although not wholly comparable, our values are of the same order of magnitude as those measured by Duxbury et al. (1982) who found annual losses of 7–165 kg N ha^{-1} yr^{-1} from organic soils in Florida. Much lower values (0.5–1.5 kg N ha^{-1} yr^{-1})

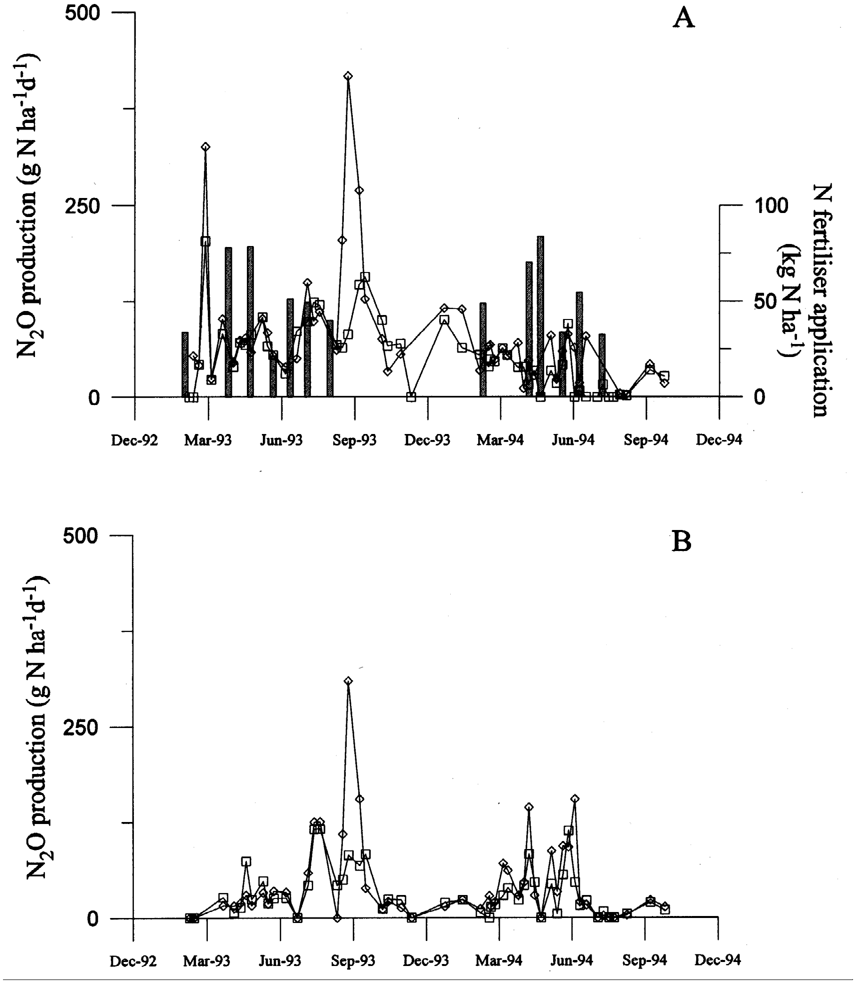


Figure 2. Production rates of N_2O in the top soil (**A**) and sub soil (**B**) of Peat II in g N ha^{-1} day^{-1} over a 2 year period. ☐ = 0 N treatment; ○ = + N treatment. Bars indicate time and amount of N fertiliser application.

were found by Martikainen et al. (1993) on northern peatlands in Finland. The differences found may be explained in part by the difference in climatic conditions. Velthof and Oenema (1995) reported total losses from peat soils in the Netherlands in the range of 2–32 kg N ha^{-1} over a nine-month period from March to November. However, in the latter three studies N_2O emissions were measured with a flux chamber method.

A major question to be answered is which part of the N_2O produced in the soil is actually emitted as N_2O to the atmosphere. Many studies have dealt with the emission of N_2O from the soil surface measured with the use of flux chambers or flux gradient and micro meteorological techniques (Mosier, 1989). The N_2O produced in the soil is not necessarily emitted from the soil as N_2O, but may be further reduced to N_2. The diffusion from the soil is controlled by soil structure and soil moisture content (Bandibas et al., 1994; Højberg et al., 1994). The amount of N_2O that is reduced further to N_2 is reflected by the difference between measured N_2O production in soil cores and N_2O surface fluxes. We were able to compare annual N_2O production with the annual N_2O emissions as determined by Velthof et al. (1994) at the same sites (Table 5). It is clearly shown that production can be 2.5 to 18 times greater than emission. Peat I has a smaller N_2O emission than Peat II whereas N_2O production in the 0–40 cm layer in both soils is comparable. The difference in N_2O emission between the soils is probably related to the lower ground water level in Peat II. Nitrous oxide produced is less retained by the soil water in Peat II and thus less subjected to further reduction to N_2 than in Peat I. The

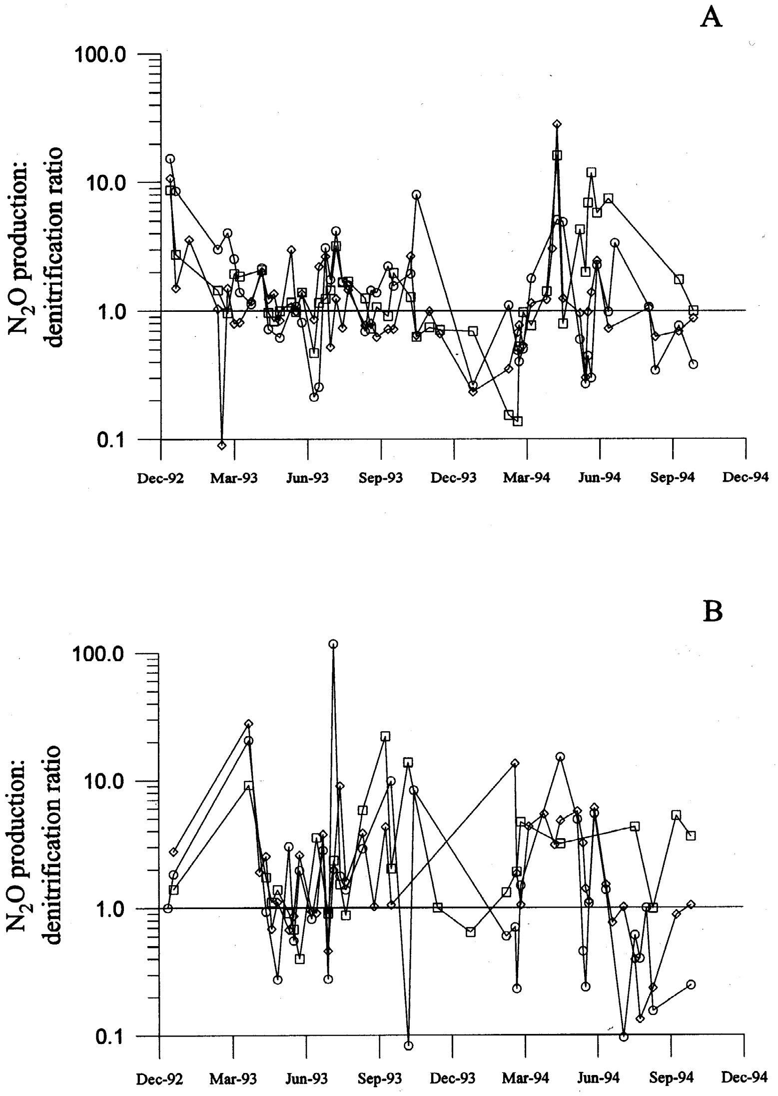


Figure 3. Time course of the N_2O production:denitrification ratios in the top soil (**A**) and sub soil (**B**) of Peat I. ☐ = 0 N treatment; ○ = + N treatment; ◇ = + NG treatment.

data suggest further that the differences decrease with increasing N input. Evidently, N_2O production rates as measured in our study are larger than the N_2O surface fluxes and should be interpreted carefully. They are, however, in combination with denitrification measurements valuable methods for the assessment of gaseous N losses.

Effects of N fertiliser application and grazing

Total N_2O production in + N treatments was 1.3 times larger than in the 0 N treatments on both sites (Table 1). The apparent N fertiliser loss by N_2O production was 2 and 3% of the applied N fertiliser on Peat I and Peat II, respectively. The estimated additional N input by urine and dung on the + NG treatment on Peat I was ca. 200 kg N ha^{-1} yr^{-1} (Velthof and Oenema, 1995). Considering this with the fertiliser input, on the + NG treatment about 7% of the total applied N was lost through N_2O production. This is much larger than the 2 to 3% of N fertiliser lost from the + N treatment. Obviously, grazing had stimulating effects on N_2O production. Grazing increases the NH_4^+ and NO_3^- contents of the soil and this increases the poten-

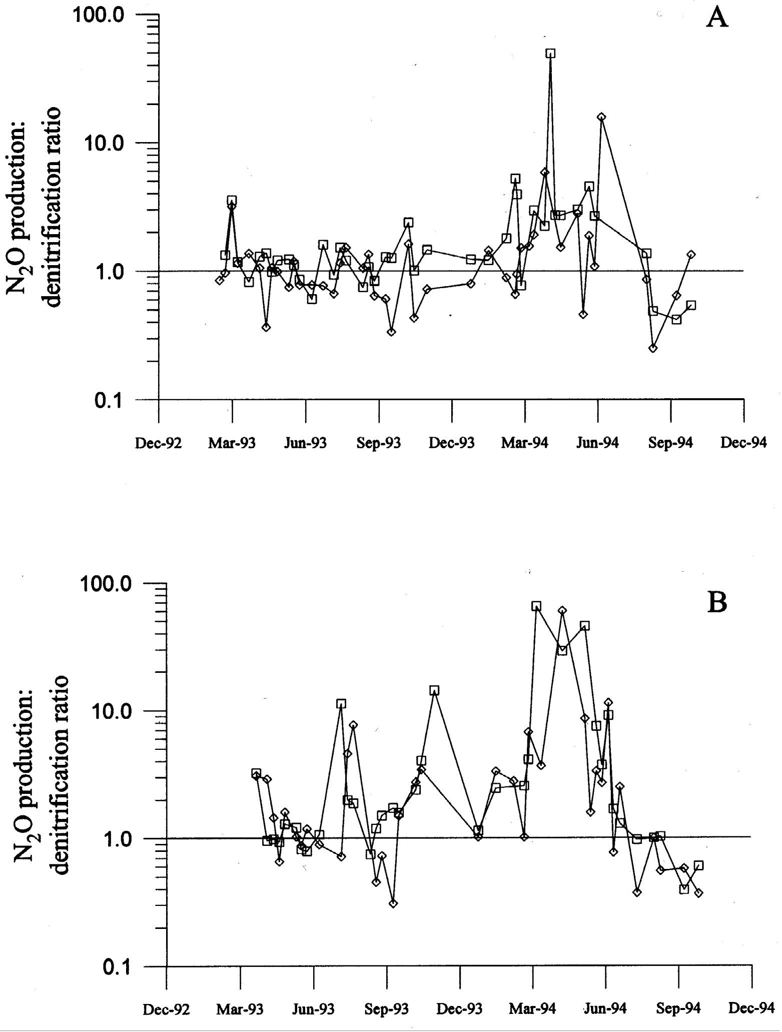


Figure 4. Time course of the N_2O production:denitrification ratios in the top soil (**A**) and sub soil (**B**) of Peat II. ☐ = 0 N treatment; ○ = + N treatment.

tial for nitrification and denitrification, accompanied by increased N_2O production. Another possible stimulating effect could have arisen from input of organic C in urine and dung. Organic C is an important rate determining factor for denitrification and heterotrophic nitrification. Furthermore, grazing may also compact the soil resulting in locally reduced pore space. However, this did not result in a detectable higher average WFPS of the + NG treatment (data not shown).

N_2O production in the sub soil

The contribution of the 20–40 cm soil layer to the total N_2O production in the 0–40 cm layer ranged from 25 to 41% (Table 1) and underlines its importance in this respect. It is possible that N_2O is subjected to further reduction because of the larger moisture content of the sub soil, which may lead to an underestimation of N_2O production (Bandibas et al., 1994; Højberg et al. 1994). However, the diameter of the soil columns provided rapid diffusion to the headspace, so reduction of N_2O in the soil should not have happened to a large extent.

The N_2O production in the sub soil was larger on the + N and + NG treatments than on the 0 N treatment, indicating that part of the applied fertiliser N was converted to N_2O in the sub soil. However, additional N input was not reflected in the correlation coef-

Table 4. Pearson's correlation coefficients between N_2O production rates and WFPS, NO_3^- and NH_4^+ contents of the soil, determined after ln-transformation for the top and sub soil. One tailed significance: * = 0.01, ** = 0.001

Treatment	Soil layer	WFPS	NO_3^-	NH_4^+
Peat I				
0 N	Top soil	0.77^{**}	0.68^{**}	-0.27
	Sub soil	0.13	0.04	0.11
+ N	Top soil	0.74^{**}	-0.06	-0.45^*
	Sub soil	0.35	0.09	-0.01
+ NG	Top soil	0.46^*	0.02	-0.05
	Sub soil	-0.07	-0.12	-0.14
Peat II				
0 N	Top soil	0.59^{**}	0.45^*	-0.23
	Sub soil	0.03	0.10	-0.02
+ N	Top soil	0.54^{**}	0.07	-0.13
	Sub soil	0.19	-0.19	-0.03

Table 5. Nitrous oxide emission as measured by flux chambers and N_2O production as measured by incubation in the 0–40 cm layer at Peat I and Peat II. Annual averages (N_2O emission 1992–1993 from Velthof et al. (1994), N_2O production 1993–1994, this study) in kg N ha^{-1} yr^{-1}

Site	Treatment	N_2O emission	N_2O production
Peat I	0 N	2	36
	+ N	9	45
	+ NG	15	82
Peat II	0 N	9	32
	+ N	18	42

ficients between N_2O production and NO_3^- and NH_4^+ contents of the soil (Table 4). Correlation coefficients were low and not significant, indicating that mineral N was not limiting the N_2O production in the sub soil. Correlation between WFPS and N_2O production was not significant, indicating that other soil variables or conditions were more important for N_2O production, these variables are not yet defined and further research is desirable.

Sources of N_2O

Ratio N_2O production:denitrification

Drained peat soils have large mineralisation rates, resulting in large amounts of NH_4^+ and high nitrification rates (Grootjans et al., 1985). The ground water level of Peat I is maintained at relatively high levels to reduce the subsidence of the soil. On Peat II, the ground water level is kept to about 20 cm below that of Peat I, to increase the bearing capacity in the growing season, especially in early spring. In the winter season, ground water levels may reach the surface on both sites. Clearly, these differences between the sites and the changes during the year contribute to differences in soil environments that are favourable for denitrification and N_2O production through nitrification. The process that is most rate determining can be deduced qualitatively from the ratio between N_2O production and denitrification. This ratio is on average 1 in the top soil of both sites and about 2 in the sub soil (Table 1). Maximum $N_2O/(N_2O+N_2)$ from denitrification is 1, that is when no N_2 is formed and the sole end-product of denitrification is N_2O (Reaction 1). A ratio of N_2O production:denitrification > 1 implies that production of N_2O through nitrification must have occurred. An average ratio of 2 in the sub soil reflects the importance of N_2O production through nitrification which is assumed to be the main N_2O producing process but may also include N_2O production through nitrifier-denitrification activities (Reaction 3). Nitrifier-denitrification is suspected to play an important role in N_2O production because of the changing aeration status of the soils when going from the top to the sub soil. The limited O_2 availability, in combination with the low NO_3^- contents, suggests that under these conditions NO_2^- from nitrification of NH_4^+ is directly converted into N_2O and/or N_2 (Reaction 3) rather than further nitrified to NO_3^- (Reaction 2). In peat soils, and especially when drained, large mineralisation rates accompanied by large nitrification rates may occur. As nitrification is a major source of NO_3^- in peat soils, it is not only important in producing N_2O directly, but also with respect to denitrification.

Nitrification and denitrification

The N_2O production:denitrification ratios < 1 are more difficult to interpret and need additional information from the use of a specific nitrification inhibitor such as CH_3F. Although the samples were taken from treatments on which relatively large denitrification activities were expected, only a few samples showed N_2O production:denitrification ratios > 1 (Table 2). In all samples with ratios > 1, nitrification was the major N_2O producing process (86–100% of the total N_2O production). Ratios < 1 indicated N_2O production through denitrification especially when denitrification rates were very large.

It is shown that the $N_2O/(N_2O+N_2)$ ratio in the denitrification process was < 0.70 (Table 2). The ratio increased with increasing denitrification activity. These results agree with other studies in which the $N_2O/(N_2O+N_2)$ ratio from the denitrification process was determined with N-isotopes. The observed $N_2O/(N_2O+N_2)$ ratio after 10 days of pre-incubation of soil with $^{15}NO_3^-$ did not exceed 0.3 (Letey et al., 1980a). In another study, initial ratios were higher, up to 1.0 (Letey et al., 1980b) but decreased rapidly to < 0.1. Firestone et al. (1979) found a maximum ratio of 0.2 under the most rigorous conditions for denitrification, using $^{13}NO_3^-$. De Klein (1994) found a ratio of 0.4 in peat soil cores that were treated with $^{15}NO_3^-$ and measurements with soil chambers resulted in a higher ratio of 0.6. All these studies were carried out in laboratory experiments or involved the addition of NO_3^- and are therefore difficult to extrapolate directly to field conditions. Nevertheless, increased N_2O production during increased denitrification activity as found in these studies was confirmed in our work. Furthermore, we provided additional evidence that the $N_2O/(N_2O+N_2)$ ratio is small at moderate denitrification rates and that under these conditions most of the measured N_2O production must have arisen from nitrification.

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