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Fluxes of nitrous oxide from boreal peatlands as affected by peatland type, water table level and nitrification capacity

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Abstract. Peat soils with high nitrogen content are potential sources of nitrous oxide (N_2O). Fluxes of nitrous oxide were measured *in situ* on nine virgin and ten drained peatlands of different hydrology and nutrient status. Numbers of nitrifying bacteria were estimated in different layers of the peat profiles with a most-probable-number technique. Nitrification potentials were determined in soil slurries of pH 4 and 6 from the profiles of six peat soils. Many virgin peatlands showed low N_2O uptake. Lowering of the water table generally increased the average fluxes of N_2O from the soils, although more in minerotrophic (nutrient rich) than in ombrotrophic (nutrient poor) sites. Ammonium oxidizing bacteria were found on only two sites but nitrite oxidizers were detected in almost all peat profiles. More nitrite oxidizers were found in drained than in virgin peat profiles. Nitrification was enhanced after lowering of the water table in minerotrophic peat but not in ombrotrophic peat. The N_2O fluxes correlated positively with the numbers of nitrite oxidizers, nitrification potential, N, P and Ca content and pH of the soil and negatively with the level of water table (expressed as negative values) and K content of the soil.

Introduction

N_2O is an important component in the atmospheric chemistry both as a greenhouse gas and in the destruction of stratospheric ozone (Cicerone 1987). N_2O is produced in soils by the processes of nitrification and denitrification. Nitrification is an aerobic microbial process which is limited by low oxygen content (Goreau 1980) and low pH (Focht & Verstraete 1977; Ivarson 1977; Roswall & Granhall 1980). In water-logged peat ecosystems the oxygen content as well as pH usually are low. However, nitrifying bacteria adapted to the low pH have been found in acid forest soils (De Boer et al. 1991; De Boer et al. 1992; Martikainen & De Boer 1993; Martikainen et al. 1993a) as well as in a drained peatland (Lång et al. 1994). Denitrification occurs anaerobically when nitrate is available (Focht & Verstraete 1977).

Virgin peat soils have the capacity to denitrify in laboratory experiments (Müller et al. 1980). However, denitrification in peat is often limited by the lack of nitrate (Verhoeven 1986). Drainage has been found to enhance the emissions of N_2O from some peat soils (Goodroad & Keeney 1984; Freeman et al. 1993; Martikainen et al. 1993b) possibly because of increased nitrification activity in the uppermost aerobic peat profile. An increase in nitrification rate has been observed after water table draw-down in some wetlands (Zimenko & Misnik 1970; Neill 1995). Increased nitrification in the surface peat may lead to nitrate leaching to the anaerobic layer and subsequent enhancement of denitrification. The role of nitrification in the production of N_2O in peat profiles is not well known. We examined the relationships between the N_2O fluxes, water table level, occurrence of nitrifiers, nitrifying activity and chemical characteristics of the peat.

Materials and methods

Characteristics of the experimental sites

The experimental sites were located in central Finland (61°47′ N, 24°18′ E) and in eastern Finland (62°46′ N, 30°58′ E). The sites represented several types of peat soils according to their nutrient status and management (Table 1). Some of the virgin peatlands had drained counterparts which originally (30–50 years ago) were similar. The depth of water table was measured from a groundwater well next to each chamber every time when the gas samples were collected. Average depth of the water table during the flux measurement period varied from −4 to −28 cm on the virgin sites and from −13 to −73 cm on the drained sites. Soil pH measured in soil-water suspension (1:2 vol/vol) varied from 3.8 to 5.3. The content of P, K, and Ca in the peats (0–20 cm from the surface) was analysed with an ICP analyser (ARL 358) after HNO_3-H_2SO_4-$HClO_4$ digestion at 200 °C (Allen 1974). Total N was determined with a Leco CHN–600 analyser. Nitrogen content of the soils varied from 170 to 4200 $\mu g\ cm^{-3}$.

Enumeration of nitrifying bacteria

The peat samples for the enumeration of chemolithotrophic ammonium and nitrite oxidizing bacteria were collected in September 1990. A soil profile of 30 cm was divided into layers shown in Table 2 and homogenized manually. The occurrence of bacteria oxidizing ammonium and nitrite was studied in 2–6 layers of the soil profiles with the most probable number (MPN) method using microtiter plates (Rowe et al. 1977). For the dilutions a peat suspension

Table 1. The experimental peatlands and their water level and chemical characteristics in the upper 20 cm.

Site	Classification[1]	WT (cm)		N (μg cm^{-3})		P (μg cm^{-3})		K (μg cm^{-3})		Ca (μg cm^{-3})		pH	
		V	D	V	D	V	D	V	D	V	D	V	D
VIRGIN AND FORESTED SITES													
Minerotrophic													
1	Tall-sedge fen	−4	−31	1300	2300	56	130	30	34	ND	ND	5.0	4.6
2	Tall-sedge pine fen	−21	−38	890	2300	48	83	35	26	2900	6300	4.4	4.0
3	Herb-rich sedge pine-birch fen	–	−50	–	1500	–	48	–	30	–	12400	–	4.5
4	Lagg fen	−5	–	260	–	13	–	20	–	630	–	4.5	–
Ombrotrophic													
5	Dwarf-shrub pine bog	−28	−36	380	380	33	31	23	25	1000	980	3.8	3.8
6	Cottongrass pine bog with *Sphagnum fuscum* hummocks	−18	−24	170	230	13	23	17	26	280	480	4.3	4.1
7	Cottongrass pine bog	−11	−13	530	580	29	38	32	30	1100	1200	3.8	3.8
8	*Sp. fuscum* pine bog	−16	−20	ND	ND	ND	28	ND	9.1	ND	2200	4.3	4.3
9	Low-sedge bog	−12	−27	210	380	16	35	21	32	430	730	4.2	4.0
10	Ridge-hollow pine bog	−13	–	110	–	6.7	–	9.5	–	270	–	4.1	–
OTHER SITES													
11	Cultivated field, originally flark fen	–	−63	–	4200	–	ND	–	ND	–	ND	–	5.3
12	Peat mining area, originally a tall-sedge fen	–	−73	–	ND	–	ND	–	ND	–	ND	–	4.6

WT = average water table depth from surface in 1991 and 1992
V = a virgin subsite, D = a drained subsite
[1] Finnish peatland classification (Ruuhijärvi 1983)
ND = not determined
– = site was not available

containing 10 g of moist peat in 100 ml of distilled water was homogenized with OCI Instruments Omni Mixer. The dilutions began with 150 μl of the suspension in 150 μl of the growth medium, and 12 two-fold dilutions were carried out on microtiter plates. The media for ammonium and nitrite oxidizers were as previously used for acid forest soils in Finland (Martikainen 1985). An incubation period of 11 weeks was used. The growth of ammonium oxidizers was tested by determining the presence of nitrite or nitrate in the medium with diphenylamine (Rowe et al. 1977). The presence of nitrite in the medium for nitrite oxidizers was tested with Griess-Ilosvay reagent (Alexander & Clark 1965). The results were calculated using the table of Rowe et al. (1977) and corrected to correspond cm^3 of soil.

Determination of nitrification potential

Nitrification potentials were determined in the samples from sites 1, 2, 3, 6, 7 and 9. The peat cores (100 cm) for determination of nitrification potential were sampled in August 1992. The cores were cut to sections ±5 cm around five depths: at mean summer water table (MSWT), at MSWT–10 cm, at MSWT–20 cm, at MSWT–30 cm and at MSWT–40 cm. At sites with MSWT below 15 cm from the peat surface the first sampling depths were at MSWT+10 cm and at MSWT+20 cm if needed. The samples were stored unhomogenized at 4 °C until they were homogenized manually a few days before the beginning of the experiment. One peat core on each site was taken from close to the chamber where the highest emissions of N_2O had been observed in 1991–1992 and one core close to the chamber with the lowest emissions of N_2O. Three replicates from both cores were used in the determination of nitrification potential.

Nitrification potentials at pH 4 and 6 were determined by incubating soil samples equivalent to 1 or 2 g dry soil in 100 ml of a mineral medium (Lång et al. 1994). The samples were shaken in 600-ml flasks closed with aluminium foil on a rotary shaker (100 rpm) at 19–21 °C. The pH was adjusted every or every other day with 0.1 M Na_2CO_3 or 1 mM HCl. Samples for nitrate analysis (3 ml) were taken after one, two and three week incubation. The suspension was centrifuged (10 min, 15000 g) and the supernatant was stored at −20 °C. Nitrate and nitrite were determined with a Dionex ion chromatograph and the production rates were calculated using linear regression (Martikainen & De Boer 1993).

N_2O fluxes

In the autumn 1990, aluminium collars (60 × 60 cm) were inserted 30 cm deep in the soil. Every site was equipped with 2–3 collars and boardwalks to minimize the disturbance caused by walking. Depending on the site, the fluxes

Table 2. Mean N_2O fluxes on the sites in 1991 and in 1992 and distribution of ammonium and nitrite oxidizers in the peat profiles.

Site[1]	Mean N_2O flux 1991 ($\mu g\ m^{-2}\ d^{-1}$) V	D	Mean N_2O flux 1992 ($\mu g\ m^{-2}\ d^{-1}$) V	D	Layer (cm)	NH_4^+ oxidizers MPN cm^{-3} V	D	NO_2^- oxidizers MPN cm^{-3} V	D
VIRGIN AND FORESTED SITES									
Minerotrophic									
1	2.3	900	23	260	0–10	0	0	440	$2.8*10^5$
	±16[2]	±270	±12	±91	10–20	0	0	640	4000
					20–30	0	0	165	2100
2	14	33	130	320	0–10	0	0	45	200
	±19	±18	±47	±137	10–20	0	0	36	2500
					20–30	0	0	50	990
3	–	960	–	680	0–6	–	0	–	40
		±140		±160	6–12		0		13
					12–18		0		95
					18–24		0		320
					24–30		0		800
4	–26	–	200	–	0–6	0	–	0	–
	±38				6–12	0		0	
Ombrotrophic									
5	5.6	11	24	74	0–10	0	0	12	72
	±13	±13	±14	±45	10–20	0	0	13	33
					20–30	0	0	30	74
6	−30	−5.3	22	18	0–10	0	0	0	0
	±15	±12	±12	±6.0	10–20	0	0	0	0.6
					20–30	0	0	1.7	150
7	−9.6	22	19	48	0–10	0	0	1.4	20
	±8.5	±35	±12	±30	10–20	0	0	24	10
					20–30	0	0	44	100
8	−1.2	50	ND	ND	plant	0	1200	0	32000
	±25	±25			cover				
					0–5	0	0	ND	1500
					5–10	16	0	550	30
					10–15	ND	0	ND	80
					15–20	ND	0	ND	5.2
					20–25	ND	0	ND	15
9	−15	13	−3.4	13	0–10	0	0	3.6	28
	±8.6	±8.8	±9.4	±16	10–20	0	0	0	76
					20–30	0	0	9	40
10	−0.6	–	2.7	–	0–6	0	–	0	–
	±18		±14						

Table 2. Continued.

Site[1]	Mean N_2O flux 1991 ($\mu g\ m^{-2}\ d^{-1}$) V	D	Mean N_2O flux 1992 ($\mu g\ m^{-2}\ d^{-1}$) V	D	Layer (cm)	NH_4^+ oxidizers MPN cm^{-3} V	D	NO_2^- oxidizers MPN cm^{-3} V	D
OTHER SITES									
11	–	5000	–	7900	0–6	–	$4.7*10^6$	–	$7.4*10^6$
					6–12		$2.1*10^6$		$8.9*10^6$
					12–18		$2.2*10^5$		$8.8*10^6$
					18–24		$2.1*10^5$		$7.2*10^6$
					24–30		$1.7*10^4$		$1.2*10^6$
12	–	67 ±6.0	–	ND	0–5	–	0	–	12

V = a virgin subsite, D = a drained subsite
[1] see site classification in Table 1
[2] result ± standard error
ND = not determined
– = site was not available

were measured 6–15 times from the end of April until the middle of December in 1991. In 1992 the fluxes were measured 1–6 times on different sites. A 60-liter aluminium chamber was used for the sampling. The gas tightness of the chamber was ensured by inserting the chamber on a groove filled with water on the upper end of the collar. Four samples (40 ml) during half an hour were taken in plastic syringes closed with a three-way stopcock (Nykänen et al. 1995a). The samples were analysed within 24 hours after sampling with a gas chromatograph equipped with an electron-capture detector (Martikainen & De Boer 1993). The N_2O fluxes were calculated using linear regression (Nykänen et al. 1995a).

The effect of acetylene (C_2H_2) on N_2O fluxes was studied *in situ* on the drained subsite of site 1 (3 measurement collars in 1992) and on site 3 (2 collars in 1991). C_2H_2 is known to inhibit chemolithotrophic ammonium oxidation and to block the reduction of N_2O to N_2 in denitrification (Hynes & Knowles 1982; Davidson et al. 1986). Inhibition of these processes by C_2H_2 can be used to relate the observed N_2O fluxes to the production processes. Fluxes were measured without C_2H_2 and with 10 % C_2H_2 added into the gas phase of the chamber. Penetration of C_2H_2 to the soil was checked by analysing C_2H_2 in gas samples taken from the soil.

Statistical analysis

All statistical tests were done using SPSS for Windows statistical package. The differences between different groups of sites with respect to bacterial counts or N_2O fluxes were analysed with T-test. Pearson correlation coefficients were determined between the average N_2O fluxes from each chamber in 1991 and 1992, numbers of nitrifying bacteria, nitrification potential in samples taken close to the chambers, water table, pH and N, P, K, and Ca content of the soil. To normalize the distributions log-transformed values of variables related to microbial activity were used in the T-test and correlation analysis. In the principal components analysis pH, N, P, K and Ca contents, average water tables and N_2O fluxes in 1991 and 1992, numbers of nitrite oxidizers and nitrification potentials at pH 4 and 6 were used as variables. Water table levels were expressed as negative values, cm from soil surface.

Results

Nitrifying bacteria

Ammonium oxidizers could be detected only in the peat profiles of two sites, an ombrogenous pine bog and a cultivated field (Table 2). Nitrite oxidizers were observed on all drained sites and on most virgin sites with counts ranging from 0 to $8.9*10^6$ per cm^3 soil. Numbers of nitrite oxidizers were generally lower in virgin peatlands than in their drained counterparts $p = 0.004$). The highest numbers of nitrite oxidizers as well as ammonium oxidizers were found on the site drained for grass cultivation. Numbers of nitrite oxidizers correlated positively with the average N_2O fluxes, water table in 1991, N, P, K and Ca content and pH of the soil (Table 3).

Nitrification potentials

The potential nitrification rates were highest in the minerotrophic drained sites 1 and 3 (Table 4). At the drained site 1 there was vertical variation in the dependency of nitrification with pH so that in the surface layer the nitrification rate was higher at pH 6 in contrast to the two next layers where the activity was higher at pH 4. On the other sites no vertical differences in pH-dependency could be observed. Samples from the virgin site 1 nitrified only at pH 6 and at site 3 all layers had higher nitrification potentials at pH 6. At site 2 some nitrification occurred in the layer 15–25 cm in the drained part but not in the virgin part. Very little nitrification was observed in the ombrotrophic sites except in some samples after a two-week incubation. In

Table 3. Pearson correlation coefficients between biological, physical and chemical characteristics of the peatlands.

	N_2O 1991	N_2O 1992	NO_2 ox	Nit pH4	Nit pH6	WT 1991	WT 1992	N	P	K	Ca	pH
N_2O 1991[1]												
N_2O 1992[1]	0.83 ***											
NO_2 ox[2]	0.85 ***	0.81 ***										
Nit pH4[3]	0.57 **	0.51 *	0.55									
Nit pH6[3]	0.55 *	0.41	0.50	0.74 ***								
WT 1991[4]	−0.58 ***	−0.69 ***	−0.39 *	−0.56 *	−0.46 *							
WT 1992[4]	−0.37 **	−0.51 ***	−0.19	−0.57 **	−0.37	−0.79 ***						
N[5]	0.79 ***	0.79 ***	0.92 ***	0.66 *	0.65 **	−0.64 ***	−0.43 **					
P[5]	0.54 ***	0.53 **	0.78 ***	0.63 **	0.62 **	−0.17	−0.40 **	0.92 ***				
K[5]	−0.24 **	−0.23	−0.49 **	−0.006	−0.03	0.13	−0.08	0.49 **	0.55 ***			
Ca[5]	0.78 ***	0.85 ***	0.77 ***	0.62 *	0.65 **	−0.65 ***	−0.50 **	0.80 ***	0.66 ***	0.31		
pH[6]	0.63 ***	0.50 **	0.57 **	0.19	0.17	−0.32	0.08	0.65 ***	0.31	0.28	0.34	

Asterisks denote two-tailed significances (* $p<0.05$; ** $p<0.01$; *** $p<0.001$)
[1] Fluxes of N_2O *in situ* in 1991 or 1992
[2] Most probable numbers of nitrite oxidizers in the three upper layers of the soil
[3] Nitrification potentials at pH 4 or 6 in the three upper layers of the soil in one-week incubation
[4] Water table depth from surface in 1991 or 1992
[5] Total N, P, K and Ca content in the 0–20 cm layer of the peat
[6] pH measured in soil-water suspension

many samples the nitrification rate increased after the first week. Nitrate loss occurred occasionally. The highest nitrification rates were observed in samples taken near the chambers with the highest N_2O emissions *in situ*. There were positive correlations between nitrification potentials and the N_2O fluxes in 1991 and in the case of nitrification potentials at pH 4 also with the

fluxes in 1992. Also water table levels and N, P and Ca content of the soils correlated with the nitrification potentials (Table 3).

N_2O fluxes

On the virgin sites the fluxes ranged from −30 to 200 μg N_2O m^{-2} d^{-1} in 1991 (Table 2). Many ombrotrophic virgin sites showed net consumption of N_2O. Fluxes on the drained sites were higher than on the virgin sites (p = 0.001) ranging from −5 to 7900 μg m^{-2} d^{-1}. In 1992 the fluxes were generally higher than in 1991 (p = 0.000). Higher fluxes (on both virgin and drained sites) were found on the minerotrophic than on the ombrotrophic sites (p = 0.003). N_2O fluxes were highest from the cultivated field. The N_2O fluxes increased with lowering average water table (Table 3). Soil N, P, Ca, pH and numbers of nitrite oxidizers correlated positively with the N_2O fluxes.

In the principal components analysis (PCA) the first two factors explained 79% of the variance (Figure 1a). With the third factor included to the analysis, 92% of the variance was explained (Figure 1b). The plots of factor 1 versus factor 2 and factor 1 versus factor 3 suggest that content of N, P and Ca and numbers of nitrite oxidizers were closely associated to the N_2O fluxes. According to the PCA nitrification potential at pH 4 was more closely associated to N_2O fluxes than nitrification potential at pH 6 (Figure 1b). The PCA also showed the negative interaction between water level and N_2O fluxes.

The effect of acetylene on N_2O fluxes on two sites is seen in Table 5. In three chambers the emission of N_2O was lowered, in one chamber increased and in one chamber there was no change in the emission with acetylene.

Discussion

Ammonium oxidizers were detected in these peat soils with the MPN-method only in the cultivated peat soil and in the surface layer of site 8, a *Sphagnum fuscum* pine bog. However, nitrite oxidizers were detected in most soils. There are observations that in some soils nitrite oxidizers determined with the MPN techniques can be more abundant than ammonium oxidizers (Belser 1977; Martikainen 1985; Aarnio & Martikainen 1995). It is possible that nitrite oxidizers survive better than ammonium oxidizers in the acid and partly anaerobic peat soil. Acidophilic nitrite-oxidizing bacteria have been found in acid forest soil (Hankinson & Schmidt 1988) but acidophilic ammonium oxidizers have not been isolated. In addition to being aerobic chemolithotrophs nitrite oxidizers are able to grow heterotrophically and anaerobically on nitrate and organic substrates (Freitag et al. 1987; Bock et al. 1988; Woldendorp & Laanbroek 1989; Laanbroek & Schotman 1991). Belser (1977) suggested

Table 4. Nitrification potentials at pH 4 and 6 during a three-week incubation in samples from virgin and drained peatlands of different trophy.

Site[1]	Layer (cm)	NO_3^--N (ng cm^{-3} h^{-1}) 1. Week pH 4	1. Week pH 6	2. Week pH 4	2. Week pH 6	3. Week pH 4	3. Week pH 6
VIRGIN AND FORESTED SITES							
Minerotrophic							
1 virgin	2–12	0	2.2±7.6[2]	0	1.8±0.6	0	0.9±0.3
	12–22	0	0.4±0.2	0	−0.1±0.2	0	0.01±0.01
	22–32	0	0	0	0	0	0
drained	0–5	0.5±0.5	5.4±3.8	2.7±1.2	78±64	ND	ND
	5–15	16±1.7	2.8±1.8	35±6.6	−0.1±0.5	ND	ND
	15–25	11±1.6	−0.3±0.8	31±2.9	−0.4±0.3	ND	ND
	35–45	0	0	0	0	0.4±0.2	0
	55–65	0	0	0	0.3±0.3	0	−0.4±0.4
2 virgin	0–10	0	0	0	0	0	0
	10–20	0	0	0	0	0	0
drained	0–5	−2.3±2.3	0	0	0	0	0
	5–15	0	0	0	0	0	0
	15–25	0.6±1.6	−3.0±0.8	8.6±0.8	2.8±1.0	6.0±0.5	2.7±1.5
3 drained	0–5	4.5±0.0	49±0.0	ND	ND	ND	ND
	5–15	2.7±0.0	26±0.1	ND	ND	ND	ND
	15–25	5.7±0.0	23±0.3	ND	ND	ND	ND
Ombrotrophic							
6 virgin	0–10	0	0	8.4±8.4	17±16	−3.7±3.7	−0.6±1.0
	10–20	0	0	0	0	0	0.2±0.2
drained	0–5	−0.1±0.1	0	0.4±0.4	0	−0.4±0.4	0
	5–15	−0.1±0.1	0	0	−0.6±0.6	0	−0.5±0.4
	15–25	0	0	0	0	0	0
7 virgin	2–12	0	0	0	0	0	0
	12–22	0	0	0	0	0	0
drained	0–5	0	0	1.2±1.2	0	0	0
	5–15	0	0	0	0	0	0
	15–25	0	0	0	0	0	0
9 virgin	0–10	0	0	0	0	0	0
	10–20	0	0	0	0	0	0
drained	0–5	0	0	0	0	0	0
	5–15	0	0	0	0	0	0
	15–25	0	0	0	0	0	0

[1] see site classification in Table 1
[2] result ± standard error
ND = not determined

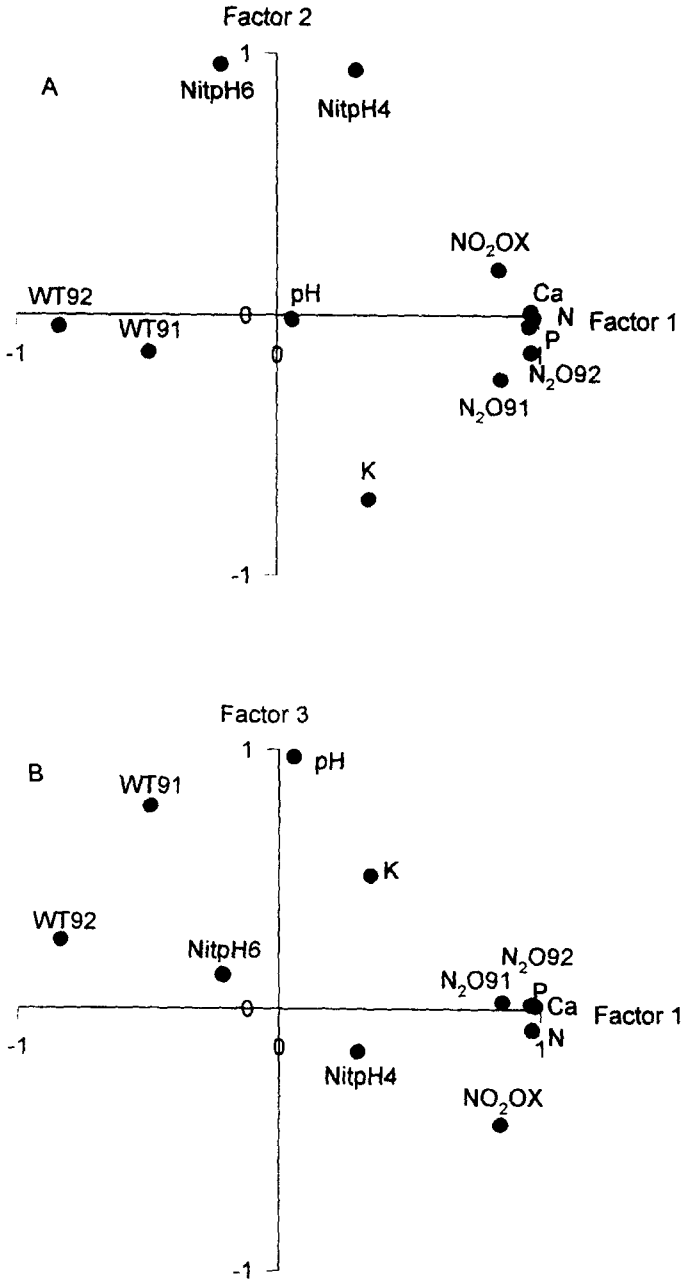

Figure 1. The plots of factors 1 and 2 (a) and factors 1 and 3 (b) in principal components analysis. N_2O flux in 1991 and 1992, nitrification potential at pH 4 (NitpH4) and 6 (NitpH6), numbers of nitrite oxidizers (NO_2OX), water table (WT; negative values as cm from soil surface), pH and content of N, P, K and Ca in the soil were used as variables.

that nitrite oxidizers may grow in higher numbers compared to ammonium oxidizers by means of the nitrite that is reduced from nitrate in anaerobic microsites and diffusing to the aerobic soil space. It is also possible that all strains of ammonium oxidizers adapted to the acid peat soil are not able to grow in the neutral medium used (De Boer et al. 1989). However, a neutral medium is currently the best alternative because there are findings that an acidic medium does not support the growth even of strains isolated from acid soils (Hankinson & Schmidt 1984; Martikainen & Nurmiaho-Lassila 1985). Recently De Boer et al. (1995) succeeded to grow a strain of *Nitrosospira* together with *Nitrobacter* at pH 4 only by immobilizing them in alginate beads or by exposing them to pH-fluctuations which promote formation of cell aggregates.

Nitrification activity is thought to be very low in peat soils (Martin & Holding 1978; Rosswall & Granhall 1980; Verhoeven 1986; Rangeley & Knowles 1988). In the present study most nitrification occurred in samples from the three most minerotrophic drained sites, sites 1–3. On the ombrotrophic sites very little nitrification was observed in the samples both from the virgin and the drained subplots. In the surface layer of drained site 1 nitrification potential was higher at pH 6 than at pH 4 but deeper in the soil the activity was higher at pH 4. In this soil there seemed to be different types of nitrifying bacteria with respect to their pH requirements. In an earlier experiment we found that acetylene did not always inhibit nitrification in the samples from site 1 but the other sites were not included in the study (Lång et al. 1994). Heterotrophic nitrifying microbes are not usually inhibited by acetylene (Hynes & Knowles 1982). Thus, at site 1 there may be heterotrophic nitrifiers in addition to chemolithotrophic nitrifiers. At site 3 the nitrification rates were higher at pH 6 in all layers which, in turn, gives no evidence of vertical differences in nitrifying bacteria. Interestingly, the layers nitrifying most on site 1 were all located above the average water table level and when all sites were considered there was a weak correlation between the nitrification rates and water level (Table 3). Nitrate loss was observed in some slurries. The nitrification rates sometimes changed from positive to negative within one week. Despite constant shaking, the slurries with the poorly decayed heterogeneous peat material may not be totally aerobic and nitrate reduction in anaerobic microsites may consume the nitrate formed (Parkin 1987; Freitag et al. 1987; Bock et al. 1988). Nitrifying activity was quite unevenly distributed in these peat soils. Often only one of the six replicates showed nitrate production. The samples from the vicinity of chambers producing N_2O at higher rates generally had higher nitrification rates than the samples taken near the chambers with low N_2O production rate.

Lowering the water table can enhance nitrification and N_2O fluxes on peatlands (Zimenko & Misnik 1970; Goodroad & Keeney 1984; Freeman et al. 1993; Martikainen et al. 1993b). In spite of the great spatial and temporal variation, there were statistically significant differences in N_2O fluxes between the virgin and drained sites in our study. However, the fluxes had increased more on minerotrophic than on ombrotrophic sites after drainage. In 1992 the N_2O fluxes were generally higher than in 1991 which results from the drier summer and lower water table in 1992. Little is known about N_2O fluxes from peatlands. In Hudson Bay lowland N_2O fluxes from different peatlands ranged from 36 to 120 μg N_2O m^{-2} h^{-1} (Schiller & Hastie 1994). In Wisconsin marshes fluxes of N_2O were 0.7 and 65 μg N m^{-2} h^{-1} from undrained and drained marshes, respectively (Goodroad & Keeney 1984). Fluxes of 6.3 μg N_2O-N m^{-2} h^{-1} were measured in Gulf Coast fresh water marshes (DeLaune et al. 1990). Our results are consistent with these values although they mostly represent quite different types of wetlands.

The highest N_2O fluxes were observed on the site drained for cultivation of grass (site 11) which also had the greatest numbers of nitrifying bacteria. This site had the highest nitrogen content and pH and it was the only site that was yearly fertilized and ploughed. Cultivated organic soils are important sources of N_2O (Li et al. 1994). Ploughing and fertilization together with lowered water table ensure the availability of oxygen and nutrients for the microbes. In contrast, the peat mining area is highly manipulated by peeling off part of the surface peat every year. On the peat mining area the emissions of N_2O as well as the numbers of nitrifiers were low which probably results from transferring away some of the active nitrifiers with the surface peat every summer. However, there are some peat mining areas producing more N_2O than the areas in the present study (Nykänen et al. 1995b).

Fluxes of N_2O correlated with the level of water table, nitrification potential and numbers of nitrite oxidizers. However, we can not conclude from this that the observed N_2O was mainly produced in nitrification. The nitrate produced in the aerobic surface of the peat may leach downwards and N_2O can be formed in denitrification in the deeper layers. The conditions in anaerobic, acid peat are favourable for N_2O production because low pH is known to increase the N_2O to N_2 ratio in denitrification (Focht & Verstraete 1977). The nitrate leached into the anaerobic peat profile can be efficiently denitrified because plenty of substrates, e.g. organic acids from fermentation processes are available. Availability of carbon is known to limit denitrification activity (Bijay-Singh et al. 1988; Drury et al. 1991). In organic soils the availability of organic carbon for denitrifiers is generally high (Li et al. 1994).

The experiment with acetylene (Table 5) revealed the high spatial variation in the processes producing N_2O. The decrease in N_2O fluxes in the presence

of acetylene showed that on different patches on the two sites 0–97% of the N_2O could have been produced in nitrification. However, there also was a patch where the N_2O fluxes rose after the addition of acetylene suggesting that denitrification played a more important role in this case. In the case when acetylene had no effect on N_2O fluxes it could have been that nitrification had no importance in the N_2O production or an increase in the N_2O accumulation in denitrification compensated the N_2O production from nitrification blocked by C_2H_2. By the technique applied the exact differentiation of nitrification and denitrification in N_2O production is impossible. One way to predict the process responsible for N_2O production is to measure water-filled pore space (WFPS) of the soil (Davidson 1993; Weier et al. 1993). According to Davidson's (1993) study denitrification was the main source of N_2O at $> 60\%$ WFPS and the optimum for N_2O production in nitrification at 50% WFPS. There are some measurements of the WFPS from the drained site 3. There the WFPS has been 50–75% in the 0–5 cm layer and 75–95% in the 5–15 cm layer. This data suggests that the moisture conditions in the layer 0–5 cm allow both nitrification and denitrification to take place whereas denitrification is dominating in the layer 5–15 cm. The experiment with acetylene in this soil gave evidence of N_2O production in both processes (Table 5). It must also be taken into account that other processes than chemolithotrophic nitrification and denitrification may be involved in N_2O production. Methanotrophic bacteria are known to oxidize ammonium and to produce N_2O (Yoshinari 1985; Bender & Conrad 1994). Methanotrophs may have significance in N_2O production in drained peat soils. For example the N_2O-producing forested site 3 in the present study is known to oxidize methane at a high rate (Crill et al. 1994). Unfortunately, acetylene can not be used to distinguish between N_2O production from nitrifiers and methane oxidizers because both processes are inhibited by acetylene.

The principal components analysis showed the significance of drainage for N_2O evolution. In Figure 1 it can be seen that lowered water table was related to high fluxes of N_2O and high counts of nitrite oxidizers together with high amounts of N, P and Ca but the amount of K was not related to these variables. The N, P and Ca content per soil volume has been observed to rise after drainage in Finnish peat soils whereas the amount of K rather decreases after drainage (Kaunisto & Paavilainen 1988). Among our sites there was not a clear trend in the amount of K after drainage, and consequently there were poor correlations between K and the other variables.

This study shows the complex pattern of environmental factors regulating N_2O fluxes from peat soils. Many virgin ombrotrophic peatlands act as a weak sink of N_2O. Lowering of water table and cultivation practises increase the microbial activities related to the N_2O emissions. Especially draining

Table 5. Effect of acetylene on N_2O fluxes on drained subsite of site 1 and on site 3.

Site	Chamber	N_2O ($\mu g\ m^{-2}\ d^{-1}$)		Ratio A/B
		Without acetylene (A)	With 10 % acetylene (B)	
1	1	200	60	3.3
	2	500	2290	0.2
	3	350	280	1.3
3	1	140	140	1.0
	2	260	9	29

Fluxes were first measured without acetylene and then a new measurement was made with 10% acetylene in the gas phase.

of nutrient rich peatlands enhances N_2O fluxes (Martikainen et al. 1993b). According to some climate models the summers would be drier and warmer at high latitudes in the future (Manabe & Wetherald 1986; Mitchell 1989). As a result, the northern peatlands would be drier and their N_2O emissions would increase (Martikainen et al. 1993b). Thus, not only changes in land use but also climate change could affect the microbial processes and N_2O fluxes from the northern peatlands.

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References

Aarnio T & Martikainen PJ (1995) Mineralization of C and N and nitrification in scots pine forest soil treated with nitrogen fertilizers containing different proportions of urea and its slow-releasing derivative, ureaformaldehyde. Plant and Soil 27: 1325–1331

Alexander M & Clark FE (1965) Nitrifying bacteria. In: Black CA et al. (Eds) Methods of Soil Analysis, Part 2. American Society of Agronomy, Madison, Wisconsin

Allen SE (1974) Chemical Analysis of Ecological Materials. Blackwell Scientific Publications, Oxford

Belser LW (1977) Nitrate reduction to nitrite, a possible source of nitrite for growth of nitrite-oxidizing bacteria. Appl. Env. Microbiol. 34: 403–410

Bender M & Conrad R (1994) Microbial oxidation of methane, ammonium and carbon monoxide, and turnover of nitrous oxide and nitric oxide in soils. Biogeochem. 27: 97–112

Bijay-Singh, Ryden JC & Whitehead DC (1988) Some relationships between denitrification potential and fractions of organic carbon in air-dried and field-moist soils. Soil Biol. Biochem. 20: 737–741

Bock E, Wilderer PA & Freitag A (1988) Growth of *Nitrobacter* in the absence of dissolved oxygen. Wat. Res. 22: 245–250

Cicerone RJ (1987) Changes in stratospheric ozone. Science 237: 35–42

Crill PM, Martikainen PJ, Nykänen H. & Silvola J. (1994) Temperature and N fertilization effects on methane oxidation in a drained peatland soil. Soil Biol. Biochem. 26: 1331–1339

Davidson EA, Swank WT & Perry TO (1986) Distinquishing between nitrification and denitrification as sources of gaseous nitrogen production in soil. Appl. Environ. Microbiol. 52: 1280–1286

Davidson EA (1993) Soil water content and the ratio of nitrous oxide to nitric oxide emitted from soil. In: Oremland RS (Ed) Biogeochemistry of Global Change, Radiatively Active Trace Gases (pp 369–384). Chapman & Hall, New York

De Boer W, Klein Gunnewiek PJA, Troelstra SR & Laanbroek HJ (1989) Two types of chemolithotrophic nitrification in acid heathland humus. Plant and soil 119: 229–235

De Boer W, Klein Gunnewiek PJA, Veenhuis M, Bock E & Laanbroek HJ (1991) Nitrification at low pH by aggregated chemolithotrophic bacteria. Appl. Env. Microbiol. 57: 3600–3604

De Boer W, Tietema A, Klein Gunnewiek PJA & Laanbroek HJ (1992) The chemolithotrophic ammonium-oxidizing community in a nitrogen saturated acid forest soil in relation to pH-dependent nitrifying activity. Soil Biol. Biochem. 24: 229–234

De Boer W, Klein Gunnewiek PA & Laanbroek HJ (1995) Ammonium oxidation at low pH by a chemolithotrophic bacterium belonging to the genus *Nitrosospira*. Soil Biol. Biochem. 27: 127–132

DeLaune RD, Patrick WH, Lindau CW and Smith CJ (1990) Nitrous oxide and methane emissions from Gulf Coast wetlands. In: Bouwman AF (Ed) Soils and the Greenhouse Effect (pp 497–502). John Wiley & Sons Ltd., England

Drury CF, McKenney DJ & Findlay WI (1991) Relationships between denitrification, microbial biomass and indigenous soil properties. Soil Biol. Biochem. 23: 751–755

Focht DD & Verstraete W (1977) Biochemical ecology of nitrification and denitrification. In: Alexander M (Ed) Advances in Microbial Ecology, Vol 1 (pp 135–214). Plenum Press, New York

Freeman C, Lock MA & Reynolds B (1993) Fluxes of CO_2, CH_4 and N_2O from a Welsh peatland following simulation of water table draw-down: Potential feedback to climatic change. Biogeochem. 19: 51–60

Freitag A, Rudert M & Bock E (1987) Growth of *Nitrobacter* by dissimilatoric nitrate reduction. FEMS Microbiol. Letters 48: 105–109

Goodroad LL & Keeney DR (1984) Nitrous oxide emission from forest, marsh and prairie ecosystems. J. Environ. Qual. 13: 448–452

Goreau TJ, Kaplan WA, Wofsy SC, McElroy MB, Valois FW & Watson SW (1980) Production of NO_2^- and N_2O by nitrifying bacteria at reduced concentrations of oxygen. Appl. Env. Microbiol. 40: 526–532

Hankinson TR & Schmidt EL (1984) Examination of acid forest soil for ammonia- and nitrite-oxidizing autotrophic bacteria. Can. J. Microbiol. 30: 1125–1132

Hankinson TR & Schmidt EL (1988) An acidophilic and a neutrophilic *Nitrobacter* strain isolated from the numerically predominant nitrite-oxidizing population of an acid forest soil. Appl. Env. Microbiol. 54: 1536–1540

Hynes RK & Knowles R (1982) Effect of acetylene on autotrophic and heterotrophic nitrification. Can. J. Microbiol. 28: 334–340

Ivarson KC (1977) Changes in decomposition rate, microbial population and carbohydrate content of an acid peat bog after liming and reclamation. Can. J. Soil Sci. 57: 129–137

Kaunisto S & Paavilainen E (1988) Nutrient stores in old drainage areas and growth of stands. Communicationes Instituti Forestalis Fenniae 145: 1–39

Laanbroek HJ & Schotman JMT (1991) Effect of nitrite concentration and pH on most probable number enumerations of non-growing *Nitrobacter* spp. FEMS Microbiol. Ecol. 85: 269–278

Li C, Frolking SE, Harrish RC & Terry RE (1994) Modeling nitrous oxide emissions from agriculture: a Florida case study. Chemosphere 28: 1410–1415

Lång K, Lehtonen M & Martikainen PJ (1994) Nitrification potentials at different pH values in peat samples from various layers of a drained mire. Geomicrobiol. J. 11: 141–147

Manabe S & Wetherald RT (1986) Reduction in summer soil wetness induced by an increase in atmospheric carbon dioxide. Science 232: 626–628

Martikainen PJ (1985) Numbers of nitrifiers and nitrification in fertilized forest soil. Soil. Biol. Biochem. 17: 245–248

Martikainen PJ & De Boer W (1993) Nitrous oxide production and nitrification in acidic soil from a Dutch coniferous forest. Soil Biol. Biochem. 25: 343–347

Martikainen PJ, Lehtonen M, Lång K, De Boer W & Ferm A (1993a) Nitrification and nitrous oxide production potentials in aerobic soil samples from the soil profile of a Finnish coniferous site receiving high ammonium deposition. FEMS Microbiol. Ecol. 13: 113–122

Martikainen PJ & Nurmiaho-Lassila E-L (1985) *Nitrosospira*, an important ammonium-oxidizing bacterium in fertilized coniferous soil. Can. J. Microbiol. 31: 190–197

Martikainen PJ, Nykänen H, Crill P & Silvola J (1993b) Effect of a lowered water table on nitrous oxide fluxes from northern peatlands, Nature 366: 51–53

Martin NJ & Holding AJ (1978) Nutrient availability and other factors limiting microbial activity in the blanket peat. In: Heal OW & Perkins DF (Eds) Production Ecology of British Moors and Montane Grasslands (pp 113–135). Springer-Verlag, Heidelberg, Germany

Mitchell JFB (1989) The "greenhouse" effect and climate change. Rev. Geophys. 27: 115–139

Müller MM, Sundman V & Skujins J (1980) Denitrification in low pH spodosols and peats determined with the acetylene inhibition method. Appl. Environ. Microbiol. 40: 235–239

Neill C (1995) Seasonal flooding, nitrogen mineralization and nitrogen utilization in a prairie marsh. Biogeochem. 30: 171–189

Nykänen H, Alm J, Lång K, Silvola J & Martikainen PJ (1995a) Emissions of CH_4, N_2O and CO_2 from a virgin fen and a fen drained for grassland in Finland. Journal of Biogeography 22: 351–357

Nykänen H, Silvola J, Alm J & Martikainen PJ (1995b) Fluxes of greenhouse gases CH_4, CO_2 and N_2O from some peat harvesting areas. In: Estonian Ministry of Environment, Environment Information Centre (Ed) Peat Industry and Environment, proceedings, Pärnu, Estonia, Sep 12–15, 1995 (pp 41–43). Tallinna Raamatutrükikoda, Estonia

Parkin T (1987) Soil microsites as a source of denitrification variability. Soil Sci. Soc. Am. J. 51: 1194–1199

Rangeley A and Knowles R (1988) Nitrogen transformations in a Scottish peat soil under laboratory conditions. Soil Biol. Biochem. 20: 385–391

Rosswall T & Granhall U (1980) Nitrogen cycling in a subarctic ombrotrophic mire. Ecol.Bull 30: 209–234

Rowe R, Todd R & Waide J (1977) Microtechnique for most-probable- number analysis. Appl. Env. Microbiol. 33: 675–680

Ruuhijärvi R (1983) The Finnish mire types and their distribution. In: Gore AJP (Ed) Ecosystems of the World. 4B. Mires: Swamp, Bog, Fen and Moor (pp 47–67). Elsevier, Amsterdam

Schiller CL & Hastie DR (1994) The exchange of nitrous oxide within the Hudson Bay lowland. J. Geophys. Res. 99: 1573–1588

Verhoeven JTA (1986) Nutrient dynamics in minerotrophic peat mires. Aquatic Botany 25 117–137

Weier KL, Doran JW, Power JF & Walters DT (1993) Denitrification and the dinitrogen/nitrous oxide ratio as affected by soil water, available carbon, and nitrate. Soil Sci. Soc. Am. J. 57: 66–72

Woldendorp JW & Laanbroek HJ (1989) Activity of nitrifiers in relation to nitrogen nutrition of plants in natural ecosystems. Plant and Soil 115: 217–228

Zimenko TG & Misnik AG (1970) Effect of ground-water level on ammonification and nitrification in peat bog soils. Mikrobiologija 39: 440–449

Yoshinari T (1985) Nitrite and nitrous oxide production by *Methylosinus trichosporium*. Can. J. Microbiol. 31: 139–144