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CONTRIBUTION OF NITRIFICATION AND DENITRIFICATION TO THE NO AND N_2O EMISSIONS OF AN ACID FOREST SOIL, A RIVER SEDIMENT AND A FERTILIZED GRASSLAND SOIL

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Summary—Most studies determining the contribution of nitrification and denitrification to NO and N_2O emissions from soils have been performed in agricultural systems, often with homogenized soil samples. More information about the nitrifier and denitrifier contribution in non-agricultural systems may increase the accuracy of global NO and N_2O emission estimates. We assessed the contributions of nitrification and denitrification to NO and N_2O emissions from three different ecosystems: an acid forest soil; a river sediment in the intertidal zone; and a fertilized peat grassland, using intact soil cores. Samples were taken in the spring of 1993 and the autumn of 1994. Intact soil cores (5 cm deep) were incubated at field temperature in the laboratory and the accumulation of NO and N_2O during 24 h was measured. The nitrification and denitrification contribution was determined by specific inhibition of nitrification. The highest mean N_2O production was in the same range for all sites. Nitrification dominated N_2O production in spring at all sites. In contrast, denitrification was the main source of N_2O in the acid forest soil and grassland soil in the autumn. However, the tight coupling of nitrification and denitrification in the river sediment could have resulted in an over-estimation of the contribution of nitrification to N_2O and NO production. A large part of denitrified N in the acid forest soil was emitted as N_2O, whereas in the river sediment, except for the autumn, the denitrification N_2O-to-N_2 ratio was low, which coincided with a low nitrate content. Nitrification was the dominant NO source in spring at all sites. In autumn, high contributions of both nitrification and denitrification were observed.

INTRODUCTION

Nitrous oxide (N_2O) contributes to the greenhouse effect and is also involved in ozone depletion of the stratosphere (Crutzen, 1981). Most atmospheric N_2O has been emitted from soils and sediments (Duxbury, 1994). Nitric oxide (NO) contributes to photochemical air pollution and is mainly produced by fossil fuel combustion (Melillo *et al.*, 1989). The contribution of soils to global NO_x emissions is estimated at 16%, although the reliability of this estimate is highly uncertain (Bouwman, 1990).

Nitrifying bacteria (chemolithotrophic ammonium oxidizers) and denitrifying bacteria appear to be the main biological sources of N_2O and NO in most natural systems (Firestone and Davidson, 1989), although other microorganisms, such as nitrate respirers, methanotrophs, fungi and heterotrophic nitrifiers, are capable of producing (traces of) N_2O and or NO, at least under laboratory conditions (Smith and Zimmerman, 1981; Krämer *et al.*, 1990; Shoun *et al.*, 1992; Anderson *et al.*, 1993). Chemodenitrification, the chemical reduction of nitrite to NO, appears to be a potential source of NO in acid soils and is stimulated by the presence of organic matter (Blackmer and Cerrato, 1986).

The contribution of nitrification and denitrification to emissions of NO and N_2O have been performed mainly in agricultural systems, which allows for the use of (^{15}N) fertilizers to elucidate the respective role of nitrifiers and denitrifiers. In natural systems, however, addition of fertilizers interferes with the nitrogen allocation in the soil or sediment, making assessments of *in situ* nitrification and denitrification contribution less reliable. The few experiments that have dealt with natural systems without the use of fertilizer were performed with sieved or wetted soil or with flow-through incubation systems (Martikainen, 1985; Davidson *et al.*, 1986; Robertson and Tiedje, 1987; Remde and Conrad,

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1991a; Davidson *et al.*, 1993; Martikainen and De Boer, 1993). Such incubation techniques disturb the oxygen gradients present in the soil and sediment samples. As oxygen is an important factor regarding nitrification and denitrification, as well as the relative production of NO and N_2O (Firestone and Davidson, 1989), disturbance of the oxygen gradient in a soil or sediment sample affects the validity of the assessment of the *in situ* contribution of nitrification and denitrification to N_2O and NO emissions.

Moisture content and mineral N content are also important factors which affect nitrification and denitrification activity (Firestone and Davidson, 1989). In the course of the growing season, decreasing soil moisture and mineral N content through plant uptake could slow down nitrification and denitrification activity in unfertilized soils. Groffman and Tiedje (1989) showed a strong reduction in denitrification activity in temperate forest soils during the course of the summer, with peaks of activity due to rainfall or to the presence of pockets of decaying organic matter. In Danish forest-, grassland- and agro-ecosystems, N_2O emissions were generally maximal in the spring and autumn (Ambus and Christensen, 1995).

We have assessed the contribution of nitrification and denitrification to the NO and N_2O emissions from three different ecosystems, an acid forest soil, a tidal river sediment and a fertilized grassland soil. We took the samples in the spring and autumn, the time of year with the highest expected nitrification and denitrification activity in unfertilized soils in temperate regions. Intact soil cores were used to avoid disturbance of oxygen gradients and other factors which might influence natural NO and N_2O emission.

MATERIALS AND METHODS

Sampling sites

Samples were taken at three sites, an oak–beech forest in Winterswijk, the Netherlands (52°00′N 6°40′E), a tidal river sediment in Burcht, Belgium (51°12′N 4°21′E) and a grassland in Zegveld, the Netherlands (52°10′N 5°00′E). The oak–beech forest in Winterswijk was located on poorly-drained acidic loamy sand, covered by a litter and fermentation layer of 2 cm and is described in detail by Tietema and Verstraten (1992). The sediment banks in Burcht were situated within the tidal freshwater zone of the Scheldt river. A bullrush vegetation covered the sample site. The sediment consisted of slightly alkaline silty clay with a black to grey colour, indicating anoxia. Spots of oxidized iron in the root zone suggested that oxygen diffused through the bullrush into the sediment. This tidal river system has been described in detail by Middelburg *et al.* (1995). The sample site in Zegveld was located on an experimental farm on peat soil and was part of a fertilizer experiment. The peat soil was drained by ditches and had an average ground water level of approximately −55 cm. The soil was covered by a perennial rye-grass sward, which received about 325 kg N ha^{-1} y^{-1} in the form of multiple dressings of calcium ammonium nitrate. Samples from Zegveld were always taken at least 25 d after the last application of fertilizer and as long as 70 d after the last application in the autumn of 1994. The grass was mown throughout the season with an absence of grazing. Further information on the site and its management has been reported by Velthof and Oenema (1995a). Selected properties of the upper 5 cm of the soils and sediment at the time of sampling are shown in Table 1. The Winterswijk, Burcht and Zegveld samples are abbreviated as W,

Table 1. Soil characteristics at the time of sampling of the top 5 cm of an oak–beech forest soil in Winterswijk, a tidal river sediment in Burcht and a fertilized grassland peat soil in Zegveld. The Winterswijk, Burcht and Zegveld samples are abbreviated as W, B and Z, respectively and numbered in order of sampling, spring samples are indicated with s, autumn samples with a

Sample	Date	Temperature (°C)	Moisture (w/w)	Organic matter (w/w)	pH-H_2O	NH_4^+	NO_3^-
						(μmol g^{-1})	
Ws1	12.3.1993	7	0.97	0.26	3.85	2.04 (0.52)	1.01 (0.33)
Ws2	29.4.1993	12	0.71	0.18	3.62	1.74 (0.37)	1.44 (0.29)
Ws3	11.6.1993	15	0.87	0.26	3.53	1.61 (0.44)	1.50 (0.42)
Wa4	17.10.1994	10	1.03	0.43	3.66	0.55 (0.24)	0.27 (0.17)
Wa5	31.10.1994	12	1.64	0.55	3.82	0.57 (0.20)	0.04 (0.03)
Bs1	26.3.1993	8	1.17	0.08	7.33	0.32 (0.20)	0.25 (0.23)
Bs2	7.5.1993	11	0.79	0.09	7.47	0.21 (0.09)	0.14 (0.09)
Bs3	18.6.1993	15	0.87	0.09	7.40	0.35 (0.22)	0.03 (0.03)
Ba4	4.10.1994	12	1.12	0.11	6.98	0.09 (0.03)	0.003 (0.004)
Zs1	16.4.1993	9	1.76	0.75	5.02	1.79 (0.56)	1.77 (1.72)
Zs2	24.5.1993	15	1.03	0.75	5.03	0.83 (0.15)	1.90 (0.70)
Zs3	25.6.1993	15	1.10	0.74	5.05	1.53 (0.44)	4.50 (2.49)
Za4	10.10.1994	11	1.95	0.75	5.25	1.81 (0.93)	1.46 (0.99)

Moisture content, organic matter content and mineral N content are expressed on dry wt basis.
n = 6, except for temperature, (n = 1); standard deviation of mineral N content in parentheses.

B and Z, respectively, and labelled with respect to sampling date, spring samples are indicated with s, autumn samples with a.

Experimental set-up

The sites were sampled three times in the spring of 1993 and once in the autumn of 1994. The site in Winterswijk was sampled once more in the autumn of 1994 during a rainy period. The term "sample" here refers to all the cores taken on one day at one site. Per sample, six sets of three intact soil or sediment cores (5 cm deep, 4.75 cm dia) were taken randomly within the sites. Polyethylene caps were used to close the top and bottom of the stainless steel core jacket after sampling, creating a headspace volume of approximately 100 ml. The upper cap was fitted with a butyl rubber septum to facilitate gas sampling. Care was taken to prevent disturbance of oxygen gradients in the cores during and after sampling. Additional material was sampled for the analysis of soil or sediment characteristics. A grass-plot sampler (upper 5 cm of the soil or sediment) was used in the spring of 1993, whereas three additional cores per set were taken in the autumn of 1994 for soil or sediment analysis. Soil temperature (at 3 cm below surface) was determined at one spot within the site. The cores were transported at ambient temperature to the laboratory and stored without the top lids in an incubator at soil temperature. The samples for soil or sediment characteristics were transported and stored at 4°C and were analysed within a week.

The following treatments were applied prior to incubation, using one core per set resulting in six cores per treatment per sample: (1) control (no additions); (2) inhibition of nitrification—the remaining N_2O and NO accumulation in these cores was used to estimate the contribution of denitrification; and (3) inhibition of denitrifier N_2O reduction—the accumulation of N_2O during incubation was used to estimate the denitrification activity.

The difference in average N_2O production rate per sample between the control core and the nitrification inhibition core was used to determine the contribution of nitrification to the N_2O production rate of that sample. The high NO consumption rate, which is generally observed in soils and sediments, leads to equilibrium concentrations of NO in the headspace in static core incubations. The relative decrease of the average NO equilibrium concentration per sample after inhibition of nitrification was used to estimate the relative contribution of nitrification and denitrification to the NO production of that sample. It is assumed that nitrification and denitrification are the principal NO and N_2O sources.

Nitrification was inhibited (treatment 2) using the short exposure to acetylene method (Kester *et al.*, 1996) adapted for intact cores. With this method (partial) inhibition of N_2O reduction by denitrification does not occur if all acetylene is removed after the exposure period. Acetylene diffuses rapidly even through wet peat soil (Ineson *et al.*, 1991), which makes the short exposure method suitable for short cores as well. However, evaporation of all the acetylene out of the core after exposure takes more time. Acetylene (>99.6% purity, acetone free; HoekLoos, Dieren, the Netherlands) was injected in the headspace (without pressurization) to expose the core to 10 kPa acetylene for 1 h. Afterwards, the top lid of the core was removed and the acetylene was allowed to evaporate during the next day and night. Whenever a nitrification-inhibition core contained traces of acetylene (more than approximately 1 Pa in the headspace) at the first day of incubation, the core was discarded for N_2O production rate measurement in order to avoid interference by partial inhibition of denitrifier N_2O reduction. Due to slow evaporation of acetylene out of the cores, denitrification N_2O production could not be measured in nine of the 78 cores throughout the course of the study. N_2O reduction by denitrification (treatment 3) was inhibited by establishing 10 kPa acetylene partial pressure (without pressurization) in the headspace of denitrification activity cores at the start of the incubation. This treatment inhibits the nitrification N_2O production as well.

All cores were closed and sealed airtight with silicone grease after the evaporation period of the nitrification-inhibition cores. The denitrification activity cores were injected with acetylene up to 10 kPa partial pressure. Subsequently, all cores were stored in the incubator at soil temperature. NO and N_2O concentrations in the headspace were measured within a few hours after the onset of the incubation and once a day thereafter. Incubations were initially carried out for 4 d, but this period was shortened to 1 d later in the study. Following the last sampling of the headspace, the mineral N content was determined. The concentrations of oxygen and acetylene in the headspace were measured daily throughout the incubation period. The N_2O concentrations in the headspace after a few hours of incubation were often not above the background level. The accumulation of NO and N_2O in the headspace after 24 h incubation was used to calculate the NO equilibrium concentration and the N_2O production of a core.

Analytical procedures

N_2O, acetylene and oxygen in the headspace were measured with a g.c. (Carlo Erba GC 6000, Milan, Italy) equipped with an ECD (N_2O below 100 $\mu l\ l^{-1}$) and a HWD (N_2O above 100 $\mu l\ l^{-1}$, oxygen and acetylene). Gasses were separated on a Hayesep Q column (N_2O and acetylene) and a Molsieve 5Å column (oxygen), both operated at 80°C with helium as carrier gas. A soda lime pre-

column was used to absorb carbon dioxide. To calibrate the ECD 48 μl N_2O l^{-1} in nitrogen standard gas (HoekLoos, Dieren, the Netherlands) was used diluted N_2O (>99.7% purity) in air was used to calibrate the HWD. Air was used to calibrate the HWD for oxygen, and diluted acetylene (>99.6% purity) in air for acetylene.

NO was detected with a NO_x analyser (Model 42S, Thermo Environmental Instruments Inc, Franklin, MA, U.S.A.) adapted with a sample mixing unit for small volume samples (Kester *et al.*, 1994). Chemically-produced NO was used to make standards (modification of the method of Goretski *et al.*, 1990). NO was generated by quantitative reduction of nitrite in anoxic vials containing 12 mM potassium iodide in 0.87 M acetic acid. The concentration of NO in the headspace of the vials was calculated using the Bunsen absorption coefficient (Tiedje, 1982).

To determine the soil or sediment characteristics, the samples and cores were crumbled and mixed and leaves, twigs, grass and large roots were removed. Moisture content was determined by drying overnight at 105°C, organic matter content by loss-on-ignition (4 h 550°C). The pH-H_2O was measured in a 1:5 (w/v) soil or sediment–water slurry after 2-h shaking. Concentrations of ammonium, nitrite and nitrate in 2 M KCl extracts of soils and sediment (1:5 w/v, 2-h shaking) were determined with a Technion Traacs 800 autoanalyser (Technion Instruments Corp, Tarrytown, NY, U.S.A.). The nitrite content was always negligible.

Statistical procedures

Arithmetic means were used to calculate mean N_2O production and mean NO equilibrium concentration per sample and treatment. The N_2O production per sample and treatment generally showed log-normal distribution patterns (Wilk–Shapiro normality test, $P < 0.05$), the NO equilibrium concentrations per sample and treatment showed no distinct distribution pattern. The large differences in variance, even after log-normal transformation, prohibited the use of ANOVA techniques to compare the means. The significance of the nitrification contribution to the N_2O production and the NO equilibrium concentration per sample was evaluated by comparing the means of the control cores with the nitrification-inhibited cores using the two sample *t*-test for unequal variance with log-transformed data ($P < 0.05$) and the non-parametric rank sum test ($P < 0.05$), respectively. Differences between samples per treatment were also tested with the two sample *t*-test for unequal variance with log-transformed data ($P < 0.05$) for N_2O production and the rank sum test ($P < 0.05$) for NO concentration. Bonferroni's correction was applied when necessary.

The mean difference in ammonium and nitrate contents between the nitrification-inhibited cores and the control cores per set at the end of the incubation was used to calculate the nitrification ammonium consumption and nitrification nitrate production per sample. Significance of these differences were tested with the paired non-parametric Wilcoxon signed rank test ($P < 0.05$). The difference between nitrification ammonium consumption and nitrification nitrate production per sample was also evaluated with the Wilcoxon signed rank test ($P < 0.05$).

All statistical procedures were performed with the Statistix 4.0 software package (Analytical Software, St Paul's, MN, U.S.A.).

RESULTS

N_2O production

N_2O production during 24 h incubation showed high coefficients of variation, generally above 60%. Mean total N_2O production (control cores) varied considerably between the samples, with the highest (Zs2) and lowest (Wa5) values differing by more than 100-fold (Fig. 1). The highest total N_2O production per site was not significantly different between sites (Ws2, Bs3 and Zs2), although the highest total N_2O production of Zegveld was five times higher than the highest total N_2O production of Winterswijk.

The highest denitrification N_2O production rates at each site (Ws2, Bs3 and Za4) were of similar magnitude. N_2O production by nitrification contributed significantly (>75%) to the total production rate in spring, except for Bs2 and Zs3 (Table 2). In the autumn, however, there was no statistically significant contribution of nitrification. The highest denitrification activities were found in the Burcht

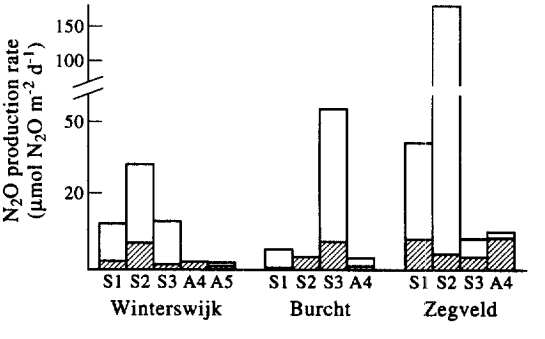

Fig. 1. The means of total and denitrification N_2O production of intact cores of Winterswijk soil, Burcht sediment and Zegveld soil during 24 h incubation. The length of the complete bar shows the total N_2O production, the hatched part shows the denitrification N_2O production. The denitrification N_2O production was measured after inhibition of nitrification. The coefficients of variation ranged from 39 to 225% (mean at 96%) and 19 to 200% (mean at 95%) for total and denitrification N_2O production, respectively. The number of replicates was six except for the denitrification N_2O production of Ws1 ($n = 3$), Ws3 ($n = 4$), Bs1 ($n = 3$) and Bs2 ($n = 5$).

Table 2. The relative contribution of nitrification to the N_2O production and NO equilibrium concentration. The relative contribution was calculated as the ratio (mean control − mean nitrification inhibition)/(mean control)

Sample	N_2O (%)	NO
Ws1	83*	87*
Ws2	77*	91*
Ws3	91*	71*
Wa4	0	30
Wa5	43	83*
Bs1	95*	nd
Bs2	0	nd
Bs3	83*	81*
Ba4	74	0
Zs1	77*	53
Zs2	97*	86*
Zs3	64	46*
Za4	13	0

Relative nitrification contributions calculated from significant decreases of the N_2O production or NO equilibrium concentration after inhibition of nitrification are marked with an asterisk (N_2O, two sample t-test with log-transformed data, one-tailed $P < 0.05$; NO, Wilcoxon rank sum test, $P < 0.05$; $n = 6$). The number of replicates differed for the denitrification N_2O production of Ws1 ($n = 3$), Ws3 ($n = 4$), Bs1 ($n = 3$) and Bs2 ($n = 5$). nd, means not determined. For abbreviations see Table 1.

cores (Fig. 2), but there was no significant difference between the highest denitrification activities of each site (Ws3, Bs3 and Za4).

NO equilibrium concentration

The NO equilibrium concentrations in Burcht were low compared with the concentrations found in Zegveld and Winterswijk (Fig. 3). The highest NO equilibrium concentrations of Winterswijk (Ws1) and Zegveld (Zs2) differed significantly from the highest concentration found in Burcht (Bs3). In Winterswijk, nitrification was the main contributor to the NO production (Table 2), except for Wa4. In Zegveld, nitrification was the dominant NO source in the spring, but denitrification seemed to be the sole provider of NO in the autumn. The low equilibrium concentrations in the early spring in Burcht made it impossible to determine nitrification and denitrification contributions, but later nitrification

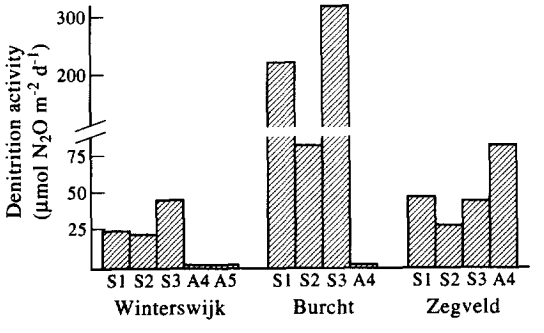

Fig. 2. The mean denitrification activity of intact cores of Winterswijk soil, Burcht sediment and Zegveld soil during 24 h incubation. The production of N_2O after inhibition of N_2O reduction was used to determine the denitrification activity. The coefficients of variation ranged from 27 to 189% (mean at 111%) ($n = 6$).

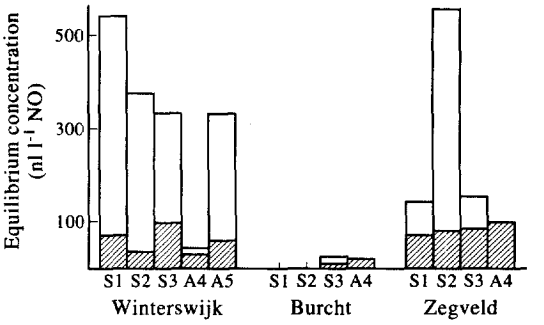

Fig. 3. The mean NO equilibrium concentration in control and nitrification inhibition cores of Winterswijk soil, Burcht sediment and Zegveld soil after 24 h incubation. The length of the complete bar shows the mean NO equilibrium concentration of the control cores, the hatched part shows the NO equilibrium concentration after inhibition of nitrification. The coefficients of variation ranged from 20 to 179% (mean at 73%) and 22 to 122% (mean at 66%) for the concentrations in control and nitrification inhibition cores, respectively ($n = 6$).

was the main contributor, whereas denitrification was the main source of NO in the autumn.

Nitrification ammonium consumption and nitrate production

The nitrification ammonium consumption and nitrate production was estimated by comparing respectively the ammonium and nitrate content at the end of the incubation in the control cores with the corresponding nitrification-inhibited cores (Table 3). The paired comparison of the cores resulted in very large CVs, up to 651%. Significant increases of ammonium content at the end of the incubations caused by inhibition of nitrification were found in all Burcht samples and in Zs1. Significantly lower nitrate contents in nitrification-inhibition cores appeared in Ws1, Ws2, Bs1, Bs2, Zs1 and Zs2. Although the nitrification ammonium consumption and nitrate production differed considerably within the samples, differences were only significant in Burcht, where ammonium consumption exceeded nitrate production in every sample.

The relative production of N_2O during denitrification

The production of N_2O represented between 0.1% (Bs1) and 100% (Wa4) of denitrification products as assessed by denitrification activity (Table 4). For Zegveld, the relative production of N_2O of denitrification was between 5 and 25%. The spring samples of Winterswijk had about the same relative production as the Zegveld samples, but in the autumn N_2O became the major end-product of denitrification. In Burcht, the relative production of N_2O during denitrification was low in the spring, but high in the autumn.

Table 3. Ammonium consumption and nitrate production by nitrification during the incubation. The difference in ammonium and nitrate contents between the control and nitrification-inhibited cores at the end of the incubation period is used to calculate the nitrifier ammonium consumption and nitrate production

Sample	NH_4^+ consumption	NO_3^- production
	(nmol g^{-1} d^{-1})	
Ws1	13 (266%)†	48 (63%)*
Ws2	14 (651%)	41 (122%)*
Ws3	121 (127%)	53 (161%)
Wa4	20 (516%)	52 (114%)
Wa5	54 (427%)	7 (134%)
Bs1	157 (142%)*	19 (80%)*
Bs2	87 (67%)*	16 (151%)*
Bs3	183 (104%)*	13 (150%)
Ba4	13 (105%)*	0
Zs1	406 (151%)*	54 (79%)*
Zs2	211 (147%)	86 (143%)*
Zs3	169 (110%)	130 (97%)
Za4	151 (249%)	144 (553%)

*Significant differences between control and nitrification-inhibited cores are marked with an asterisk (Wilcoxon signed rank test, $P < 0.05$, $n = 6$). For abbreviations see Table 1.
†Coefficients of variation are shown in parentheses.

DISCUSSION

The moisture content of the soil or sediment cores tended to decrease during treatment between 0 and 8% of the initial moisture content (data not shown). This decrease may have enhanced the relative nitrifier contribution; however, soils are also subject to similar drying in the field.

High spatial variability of trace gas fluxes has often been reported (e.g. Ambus and Christensen, 1995; Velthof and Oenema, 1995b). Spatial variability exhibited by denitrification is among the highest reported for soil processes (Parkin, 1990) and also nitrification activity may be subject to high spatial variability (De Boer and Kester, 1996). Due to the high coefficients of variation found in our study, only a few significant differences between sites could be detected despite the large differences in mean values.

Table 4. The relative production of N_2O during denitrification in soil cores. The relative N_2O production by denitrification is calculated as the ratio (mean denitrification N_2O production)/(mean denitrification activity)

Sample	N_2O(%)
Ws1	10
Ws2	36
Ws3	3
Wa4	100
Wa5	81
Bs1	0.1
Bs2	5
Bs3	3
Ba4	70
Zs1	21
Zs2	17
Zs3	8
Za4	13

For abbreviations see Table 1.

N_2O production

Per source, the highest mean N_2O productions of the sites were always within the same range. Nitrification dominated the N_2O production in spring, whereas in autumn denitrification was the main source of N_2O in Winterswijk and Zegveld. Although the relative contribution of nitrification was high in the autumn in Burcht, the absolute production was not significant. The low mineral nitrogen concentration found in the Burcht sediment in autumn has probably been the cause of low rates of both nitrifier and denitrifier N_2O production in the sediment. Denitrification and nitrification activity also decreased in the sediment. The differences in nitrifier contribution to the N_2O production between spring and autumn in the terrestrial soils may have been associated with the higher moisture content in autumn or the low ammonium content in case of the acid forest soil. However, there was no significant decrease in nitrification activity compared with the spring samples from these soils, but that might be due to the poor accuracy of the nitrification activity assessment. The low rate of denitrifier N_2O production and denitrification activity in the acid forest soil in autumn, despite the elevated moisture content, is probably related to the decreased nitrate contents.

However, statistical analysis of the above described relationships (results not shown) was hampered by the large CVs and revealed only a relationship between denitrifier N_2O production and nitrate in Burcht and denitrification activity and nitrate in Winterswijk. Furthermore, differences in scale probably obscure the possibility of finding sound relationships (Robertson, 1994), as regulation of nitrifier and denitrifier N_2O production takes place at micro-scale level, whereas pH and moisture, ammonium, nitrite and nitrate content are average values of a bulk sample.

In agricultural soils, nitrification is generally the main N_2O source under oxic conditions, and the contribution of denitrification increases with increasing moisture content and eventually becomes the main source (Klemedtsson *et al.*, 1988; Tortoso and Hutchinson, 1990; Davidson, 1992; Skiba *et al.*, 1993). Incubations with disturbed samples of non-agricultural systems also showed an important nitrification contribution under oxic conditions (Martikainen, 1985; Martikainen and De Boer, 1993), and only dominant denitrification N_2O production after a prolonged wet period (Davidson *et al.*, 1993). Although we did not find this relationship to be statistically significant in our study, the higher moisture content in the autumn in Winterswijk and Zegveld coincided with the more important role of denitrification in the N_2O production at that time.

NO equilibrium

The NO equilibrium concentrations in the headspaces of the sediment cores were lower than in the terrestrial cores. NO produced in the sediment may have encountered more difficulties in reaching the headspace. The diffusional constraints were probably larger in the wet sediment and nitrate-limited denitrifiers may have consumed a large part of the soil-produced NO before reaching the headspace.

The NO equilibrium concentration can be described by a NO production and consumption model (Remde *et al.*, 1989; Remde and Conrad, 1991b) in which the NO production rate is independent and the NO consumption rate is dependent on the NO concentration. Up to 1 μl NO l^{-1}, consumption of NO can generally be described as being a first-order process dependent on the concentration (Remde *et al.*, 1989; Schuster and Conrad, 1992). The NO concentrations in the headspaces of the cores in this study were always, except for two individual cores, below 1 μl l^{-1} and, therefore, within the first order range. Inhibition of nitrification results in a lower NO production rate and hence in a lower concentration of NO in the headspace at which consumption equals production. Due to the first-order dependency of the NO consumption, the relative decrease of the equilibrium concentration equals the relative decrease of the production rate. This mechanism allowed us to assess the relative contribution of nitrification and denitrification to the NO production of the soil or sediment without knowing the production rate in the core.

Nitrification dominated NO production in the Winterswijk soil, in the Zegveld soil in the spring and in the only sample above detection limit from the Burcht sediment in the spring. Contribution of nitrification to the NO production followed the same pattern as contribution to the N_2O production, except for the Wa5 sample. As yet, the reason for this discrepancy is not understood.

The acidic Winterswijk soil has potential for chemodenitrification according to the criteria of Blackmer and Cerrato (1986), but nitrite was never detected in the samples. However, chemodenitrification in nitrite-poor soils may still occur in nitrite containing acidic microsites around ammonium-oxidizing cells (Firestone and Davidson, 1989). The strong decrease in the NO equilibrium concentration after nitrification inhibition shows that the production of NO in Winterswijk is associated with nitrification, either by direct production or via chemodenitrification.

Nitrification was the dominant NO source whenever high equilibrium concentrations were observed. Conrad (1990) suggests that denitrification plays an important role in NO consumption, and according to Hutchinson *et al.* (1993), the amount of NO escaping from the soil strongly depends on the diffusional constraints in the soil. Hence, a high moisture content contributes to the NO consumption by increasing the denitrifying activity and hampering the escape of NO to the atmosphere. Therefore, the best conditions for the escape of NO are associated with good conditions for nitrification and not for denitrification. However, this theory is only partially supported by the observed moisture contents in our study. It does not explain the origin of the non-nitrifier NO as denitrifiers are, according to this theory, expected to consume denitrifier NO as well as nitrifier NO under wet conditions. However, the relatively constant NO equilibrium concentration in the nitrification-inhibition cores of the terrestrial soils compared with the denitrification N_2O production rate and denitrifier activity, suggest that other sources than denitrification may have been involved in NO production in these cores. Substantial fungal biomass can be found in soils with low pH and high organic matter content. Shoun *et al.* (1992) reported that NO production ability is widely distributed among soil fungi and hence fungi may have been the principal source of NO in nitrification-inhibition cores. Further study with these soils may reveal the existence and identity of other sources.

The dominance of nitrification in the NO emission of undisturbed soils has been reported (Skiba *et al.*, 1993, Vermoesen *et al.*, 1996). However, significant contribution of denitrification to the NO production is sometimes found in soil samples (Remde and Conrad, 1991a; Remde *et al.*, 1993). Remde *et al.* (1993) stated that this denitrifier NO production originated from the upper centimetres of the soil, which shows that whenever denitrification is an important NO source the diffusion pathway must be very short.

N_2O production vs N_2O emission

Total N_2O production in Winterswijk in the spring, extrapolated to annual production, were two to three times greater than the annual N_2O emission reported for acidic deciduous forest soils (Bouwman *et al.*, 1993), but below this range in the autumn. Tietema *et al.* (1991) estimated an annual N_2O emission in the Winterswijk forest for 1987 of 20 kg N_2O-N ha^{-1} y^{-1}, based on weekly measurements with closed chambers. This is about ten times higher than our production rates measured in the spring. However, Tietema *et al.* (1991) reported that three peak emissions accounted for 63% of the annual flux and they observed extended periods with fluxes similar to the total N_2O productions found in our study.

In Burcht, N_2O emissions were measured with closed chambers during the same periods as the samples were taken. In spring the on-site emissions were 1.3–6.6 times higher than the observed total N_2O productions in the cores and followed the same trend, but in autumn emission was 70 times

higher than the production in the cores (F.W.J.A. Van der Nat, pers. commun.). The accumulation of N_2O in the headspace of the cores during 24 h may have resulted in denitrifier N_2O consumption, especially in the nitrate-depleted autumn sample. The on-site measurements were conducted over a shorter time interval and with a larger headspace resulting in much lower concentrations of N_2O and, consequently, less N_2O consumption during the measurements. Assuming that the N_2O concentration is within the range permitting first-order dependency of consumption rate, consumption of N_2O interferes with the determination of N_2O production rates, but not with the estimation of the relative nitrifier and denitrifier contribution.

The total N_2O production rates during spring in Zegveld were in close agreement with on-site N_2O emission rates measured with closed chambers at the same time of the year (Velthof *et al.*, 1996) except for the second sample. However, one core was responsible for the high production in Zs2, without this outlier agreement was restored. There were no data for on-site N_2O emission from Zegveld soil for autumn 1994.

Peak emissions of NO and N_2O

Several authors have reported peak emissions of NO and N_2O after wetting of soil (Davidson, 1992; Davidson *et al.*, 1993; Hutchinson *et al.*, 1993). We collected an extra sample from Winterswijk on the third day of a rainy period in autumn (Wa5). No clear difference in N_2O production rate was found when compared with the dryer Wa4 sample. The relatively low nitrate content in Wa5 suggests that enhancement of denitrification or leaching of nitrate had occurred prior to sampling. During incubation, denitrifier N_2O production was probably limited by nitrate instead of moisture content. The NO equilibrium concentration, however, was strongly elevated after the rainy period in spite of the expected enhanced NO consumption of denitrifiers. The increased NO production resulted from release by nitrifiers, which were probably most active very close to the soil surface considering the high moisture content.

Maximum N_2O fluxes are generally found during spring and autumn (Goodroad and Keeney, 1984; Groffman and Tiedje, 1989; Schmidt *et al.*, 1988; Ambus and Christensen, 1995). Tietema *et al.* (1991) observed the highest fluxes of 1987 in Winterswijk in spring and early summer. In Zegveld, however, the highest N_2O emissions were reported in summer (Velthof *et al.*, 1996). These short periods of high emissions are particularly important for the assessment of the annual nitrification and denitrification contributions, but were not encountered in our sampling programme. Hence, more samples are needed particularly during periods of high emission rates. Nevertheless, the association between nitrification activity and conditions that favour escape of NO from soils suggests that nitrification is the major contributor to the annual NO emission of soils and sediments.

Inhibition of nitrification

The high spatial variability combined with the short incubation period hampered the assessment of nitrification activity. The ammonium accumulation due to inhibition of nitrification in Burcht was significantly higher than the coinciding nitrate depletion for all samples. This suggests that denitrification rapidly depleted the nitrate pool and became inhibited by a lack of nitrate. Hence, the tight coupling of nitrification and denitrification in the Burcht sediment could have resulted in an overestimation of the contribution of nitrification to the NO and N_2O production. The assessment of the denitrification activity in the Burcht sediment may have been influenced by depletion of nitrate as well. In sediments a tight coupling between nitrification and denitrification is often observed (Yoshinari, 1990), which makes nitrification directly involved in the NO and N_2O flux.

The relative production of N_2O during denitrification

A large part of denitrified N in Winterswijk was emitted as N_2O. Low pH tends to inhibit N_2O reduction by denitrifiers (Sahrawat and Keeney, 1986), and N_2O is often found to be the predominant end-product of denitrification in acid soils (Parkin *et al.*, 1985).

In Burcht, the denitrification N_2O-to-N_2 ratio was low, except for the autumn. As mentioned earlier, denitrification in the Burcht sediment is expected to be limited by electron acceptors, which have to be used as efficiently as possible. The high N_2O-to-N_2 ratio in Burcht in autumn is a result of the low denitrification activity even though the high ratio was not expected at the observed low nitrate content.

Conclusions

Per source, the highest mean N_2O production rate for the different sites was always within the same range. Nitrification dominated the NO and N_2O production in spring at all sites. In the autumn denitrification was the main source of N_2O, but the contribution to NO production varied. The tight coupling of nitrification and denitrification in the Burcht sediment could have resulted in an over-estimation of the contribution of nitrification to the NO and N_2O production. Assessment of the denitrification activity in Burcht may also have been hampered by nitrate depletion as well. A large proportion of denitrified N in the acidic Winterswijk soil was emitted as N_2O, whereas in the Burcht sediment, which had a low nitrate con-

tent, the N_2O-to-N_2 ratio was low except for in the autumn.

Nitrification was the dominant NO source whenever high equilibrium concentrations were observed in the cores. The conditions in soil allowing NO to escape are probably associated with nitrification. A high frequency year-round sampling programme with undisturbed cores may reveal more information about contribution of nitrification and denitrification during peak emissions.

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