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Source: *Biogeochemistry*, Vol. 12, No. 3 (Mar., 1991), pp. 189-205

Published by: [Springer](#)

Stable URL: <http://www.jstor.org/stable/1468776>

Accessed: 18/09/2014 09:02

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Role of nitrification and denitrification for NO metabolism in soil

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Accepted 3 December 1990

Key words: aerobic soil, anaerobic soil, chlorate, ¹⁵N, nitrapyrin, nitrous oxide

Abstract. Release and uptake of NO was measured in a slightly alkaline (pH 7.8) and an acidic (pH 4.7) cambisol. In the alkaline soil under aerobic conditions, NO release was stimulated by ammonium and inhibited by nitrapyrin. Nitrate accumulated simultaneously and was also inhibited by nitrapyrin. ¹⁵NO was released after fertilization with ¹⁵NH₄NO₃ but not with NH₄¹⁵NO₃. The results indicate that in aerobic alkaline cambisol NO was mainly produced during nitrification of ammonium. The results were different under anaerobic conditions and also in the acidic cambisol. There, NO release was stimulated by nitrate and not by ammonium, and was inhibited by chlorate and not by nitrapyrin indicating that NO production was exclusively due to reduction of nitrate. The results were confirmed by ¹⁵NO being released mainly from NH₄¹⁵NO₃ rather than from ¹⁵NH₄NO₃. The observed patterns of NO release were explained by the NO production processes being stimulated by either ammonium or nitrate in the two different soils, whereas the NO consumption processes being only stimulated by nitrate. NO release was larger than N₂O release, but both were small compared to changes in concentrations of soil ammonium or nitrate.

Introduction

Nitric oxide plays an important role in the chemistry of the atmosphere and is believed to result in an increase of tropospheric ozone (Crutzen 1979; Singh 1987). NO is emitted into the atmosphere mainly by anthropogenic processes, such as combustion of fossil fuels and biomass burning. However, soils obviously can also act as a significant source for atmospheric NO. Numerous field measurements have confirmed that NO is produced and emitted from soils (for review see Conrad 1991; Johansson 1989). A few field studies demonstrated that atmospheric NO can also be taken up by soils (Slemr & Seiler 1984; Johansson 1984; Johansson &

Granat 1984). The operation in soil of both microbial production and uptake of NO was corroborated by laboratory experiments which also demonstrated the existence of a NO compensation mixing ratio at which production and consumption of NO just balance each other (Johansson & Galbally 1984; Remde et al. 1989).

Experiments with microbial cultures have shown that NO can be produced by nitrifying as well as by denitrifying bacteria. However, the relative importance of these two groups of microorganisms for NO metabolism in soils is not well understood (Firestone & Davidson 1989; Conrad 1991). Therefore, we studied the effects of incubation conditions on the production of NO in two different soils, to elucidate the importance of either nitrification or denitrification. NO production by nitrification was enhanced by addition of ammonium under aerobic conditions, and was inhibited by treatment of the soil with nitrapyrin. NO production by denitrification, on the other hand, was enhanced by addition of nitrate under anaerobic conditions, and was inhibited by treatment of the soil with chlorate. The origin of the released NO was in addition studied by measuring the conversion of either ^{15}N -labelled ammonium or ^{15}N -labelled nitrate to ^{15}NO . The results show that NO release was dominated by nitrification in one soil, and by denitrification in the other demonstrating that both processes can be of importance for control of NO emission into the atmosphere.

Methods

Two different soils were used, an acidic and an alkaline cambisol, which were sampled from the A_h horizon (about 10 cm deep) of locations in the vicinity of Konstanz as described by Remde et al., 1989. The soil samples were stored in a cold room (8 °C) in fresh state for up to 6 weeks. The soils were homogenized and passed through a screen (2mm mesh) immediately before use. The origin and main characteristics of the soils are given together with the average concentration (\pm SD) of ammonium, nitrite, and nitrate in Table 1.

The soils were either incubated aerobically (air atmosphere) or anaerobically (N_2 atmosphere). The soils were either left untreated (control) or were treated with ammonium or nitrate to stimulate nitrification or denitrification, respectively. In some experiments, the soils were treated with ammonium nitrate which was labelled with ^{15}N in either the ammonium or the nitrate position to trace the origin of the released NO. Nitrapyrin or chlorate was added to inhibit ammonium oxidation or nitrate reduction, respectively. The soil treatments were done by spraying

2–7 ml of either water (control) or solutions of nitrogen compounds or inhibitors onto soil samples (150 g) and mixing them. An aliquot (100 g) was placed in an Erlenmeyer flask (1000 ml) used as incubation vessel. Measurements of NO release were started 1 h afterwards. The rest of the soil was used for chemical analysis. The following concentrations were applied: NH_4Cl or KNO_3 (84 or 840 ppm N or $\mu\text{g N g}^{-1}$ soil), ^{15}N -labelled NH_4NO_3 (3.5 mg N g^{-1} soil), nitrapyrin ($10 \mu\text{g g}^{-1}$ soil), and KClO_3 ($240 \mu\text{g g}^{-1}$ soil). Nitrapyrin (Dow Chemicals, Midlands, MI) was applied 12 h (incubation at 8°C) before the start of the experiment to overcome the lag phase of inhibition of the nitrification activity (Belser & Schmidt 1981). The inhibitory effect of nitrapyrin was assessed over the whole period of the experiment. Chlorate (Fluka, Neu-Ulm, Germany) was applied when NO release had reached maximum rates. Since the effect of chlorate is short term (Karki et al. 1972), its inhibitory effect was assessed over a period of only 1 h.

The effect of nitrogen addition on NO release was measured in three sets of experiments between 1987 and 1988. In the first experiment with samples taken in August 1987, the soils were fertilized with 840 ppm N, in the other two experiments with soil samples taken in August and September 1988, the soils were fertilized with only 84 ppm N. The maximum rates of NO release and the total amounts of NO released during the measurement period of 3–4 days are summarized in Table 2. In the second set of experiments, we also determined the release of N_2O and the change in concentrations of ammonium, nitrite and nitrate in the soil during an incubation period of 66 h. The experiments were done with unfertilized, ammonium-fertilized and nitrate-fertilized soil under aerobic and anaerobic incubation conditions. One replicate was incubated in the presence of nitrapyrin to inhibit nitrification, and another replicate was used to test the influence of the denitrification inhibitor chlorate. The results are shown in Tables 3–5. In the third set of experiments (Table 6) we determined the gross rates of NO production and the rate constants of NO consumption at a time, when the NO release rates in the fertilized samples had reached their maximum.

The soil samples were incubated at 25°C and flushed with a constant flow rate ($600\text{--}2000 \text{ ml min}^{-1}$) of either humidified N_2 or air. NO or NO_2 was not detectable in these gases. Gas flow rates were measured and controlled by mass flow controllers (HiTech, Wagner, Offenbach, Germany). Mixing ratios of NO, NO_2 , and N_2O were measured at the inlet and outlet of the incubation vessel. Release of NO, NO_2 and N_2O was measured using the experimental setup described earlier (Remde et al. 1989). NO was analyzed with a Thermo Electron NO_x analyzer (Hopkinton, MA) based on the chemoluminescence technique. NO_2 was converted to NO by

a molybdenum converter heated to 400 °C and subtracting the original NO signal (Slemr & Seiler 1984). NO₂ was never detected during our experiments. N₂O was analyzed by gas chromatography with an electron capture detector (Perkin Elmer F22, Überlingen, Germany) described by Conrad & Seiler (1980). The experimental set-up allowed detection of NO or N₂O release rates higher than 0.05 ng h⁻¹ g⁻¹ soil. The net release rates (J) were calculated from the flow rate and the difference in mixing ratios at the inlet and outlet, and were integrated over the total incubation time (66 h) to obtain the total amount of N released as nitrogen oxides.

The gross rate of NO production (P), and the pseudo-first-order rate constant of NO consumption (*k*) were determined from measurements of net NO release rates (J) at different NO mixing ratios (*m*) using the model described by Remde et al. (1989). The model assumes that the observed net NO release is the result of simultaneous NO production and NO consumption, the latter being a pseudo-first-order reaction with respect to the NO mixing ratio. The values of J and *m* were related to each other by the following equation

$$J = P - km$$

The compensation mixing ratio (*m*_c) was calculated for zero NO flux (J = 0) by

$$P = km_c$$

The release of ¹⁵N-labelled NO was measured over an incubation period of 66 h after the soil was fertilized with ¹⁵N-labelled NH₄NO₃. ¹⁵N-labelled NH₄NO₃ (10–13% enrichment) was obtained from Stohler/Kor Stable Isotopes, Innerberg, Switzerland. The released ¹⁵N-labelled NO was collected by passing the gas stream at the outlet of the incubation vessel through 10 ml of a solution of 2 M KMnO₄ in 0.5 M H₂SO₄. NO (also NH₃ or NO₂) is oxidized to nitrate in this solution (Smith & Chalk 1979). The formed nitrate was converted to NH₃ and transferred by water vapour distillation into 10 ml 50 mM HCl (Bremner & Keeney 1965). The resulting solution of NH₄Cl was evaporated at 40 °C under vacuum to dryness. The ratio of ¹⁵N/¹⁴N of the resulting crystals was analyzed in a Finnigan MAT 312 mass spectrometer (Borman et al. 1985).

At the end of incubation the soil was collected from the flask, extracted with 1M KCl (1 g per 2.5 ml) on a shaker (100 rpm) at 8 °C for 16 h, and centrifuged. The supernatant was stored in a deep freeze (−40 °C) until colorimetric analysis of ammonium, nitrite, and nitrate (Schlichting & Blume 1966).

Results

The average concentrations (\pm SD) of ammonium, nitrite, and nitrate in the unfertilized soils are shown in Table 1. Fertilization with ammonium or nitrate increased the concentrations of these compounds. The average concentrations (\pm SD) at the beginning of the experiments are shown in the footnotes of Tables 3 and 4. The range of the initial concentrations of the fertilized samples was relatively large. This variability (\pm 10–20%) was probably due to inhomogeneities which were not completely averaged out by sampling aliquots of the soils which had been sprayed and mixed with fertilizer solution. The initial nitrate concentrations generally were lower than expected from the amount of N added (84 ppm). Possibly, some of the nitrate had immediately been decomposed after addition of the fertilizer.

When soil samples were fertilized with nitrogen, the rates of NO and N₂O release increased usually reaching a maximum several hours to one day after addition. Efflux rates then decreased again and reached the rates of the unfertilized control after 2–4 days. Addition of nitrogen apparently stimulated the processes responsible for NO and N₂O production. However, production of NO₂ was never observed.

The results show that the NO release rates of the unfertilized controls were similar, but not identical in the different sets of experiments (Table 2). NO release was generally stimulated by anaerobic incubation conditions, except in the alkaline cambisol fertilized with 840 ppm ammonium-N. Addition of fertilizer also stimulated NO release, but to different extents depending on the soil type. In the alkaline cambisol, NO release was

Table 1. Origin and characteristics of the soils.

Location	Wollmatingen	Mainau forest
Soil type	Alkaline cambisol (loamy clay)	Acidic cambisol (sandy clay loam)
Use	Barley field	Oak forest
pH	7.8	4.7
H ₂ O [%]	34	23
org.C [%]	1.36	6.16
Total N [%]	0.08	0.32
C/N	16.81	19.55
NH ₄ ⁺ [ppm]	13.9 \pm 3.1	9.2 \pm 3.4
NO ₃ ⁻ [ppm]	3.2 \pm 0.5	2.8 \pm 1.4
NO ₂ ⁻ [ppm]	0.3 \pm 0.2	0.4 \pm 0.2

Table 2. Maximum NO release rates and total amounts of NO released in unfertilized and fertilized alkaline and acidic cambisol.

Experiment	Maximum NO release rate [ng NO-N h ⁻¹ g ⁻¹] (total NO released ¹ [ng NO-N g ⁻¹ soil])		
	August 1987 840	August 1988 84	September 1988 84
<i>Alkaline cambisol</i>			
H ₂ O [%]	32.2	22.0	24.0
<i>Aerobic incubation</i>			
no addition	0.67 (20)	0.61 (29)	0.58
+ NH ₄ ⁺	67.9 (970)	5.70 (179)	2.70
+ NO ₃ ⁻	13.0 (130)	0.89 (36)	0.87
<i>Anaerobic incubation</i>			
no addition	24.0 (210)	45.0 (179)	18.3
+ NH ₄ ⁺	4.28 (60)	48.0 (209)	4.70
+ NO ₃ ⁻	486 (12940)	1050 (2840)	858
<i>Acidic cambisol</i>			
H ₂ O [%]	33.3	35.1	37.4
<i>Aerobic incubation</i>			
no addition	3.65 (140)	0.67 (22)	0.60
+ NH ₄ ⁺	3.87 (190)	0.72 (28)	0.56
+ NO ₃ ⁻	63.9 (2220)	4.60 (159)	2.73
<i>Anaerobic incubation</i>			
no addition	7.80 (160)	12.0 (54)	7.43
+ NH ₄ ⁺	4.10 (120)	22.5 (89)	2.36
+ NO ₃ ⁻	155 (4930)	2004 (8310)	195

¹ Determined from integration of individual release rates measured during a total incubation period of 72 h and 66 h in Aug 1987 and Aug 1988, respectively.

stimulated mainly by ammonium under aerobic conditions, but stimulated by nitrate under anaerobic conditions. In the acidic cambisol, on the other hand, NO release was only stimulated by nitrate, both under aerobic and anaerobic conditions. Under aerobic conditions, NO release was consistently higher, when 840 ppm versus 84 ppm of fertilizer-N were applied. However, under anaerobic conditions, increased nitrogen content did not result in stimulated NO release.

The concentration changes of ammonium, nitrite, and nitrate in soil were determined together with the total amounts of NO and N₂O released

(Tables 3 and 4). A net increase or decrease is indicated by a positive or negative value, respectively. The tables include experiments in which the soils were fertilized with ammonium and simultaneously treated with nitrapyrin to inhibit nitrification. The total amounts of NO (and N₂O) released were only small compared to the changes of ammonium or nitrate concentrations, especially under aerobic incubation conditions when NO accounted for only < 2.5% of the net change of soil nitrate. The amount of released NO was generally larger than that of N₂O.

The net changes of soil ammonium and nitrate under the different incubation conditions indicate whether ammonium oxidation (nitrification) or nitrate reduction (denitrification) was occurring. Nitrification occurred only in the alkaline cambisol under aerobic conditions, as evidenced by the accumulation of nitrate. Even nitrite accumulated somewhat in the ammonium-treated soil. Nitrapyrin significantly inhibited the decrease of ammonium and the accumulation of nitrate and nitrite only under these conditions (Table 3). However, the depletion of ammonium apparently

Table 3. Transformation of nitrogen in fertilized and unfertilized alkaline cambisol.

Change during 66 h [$\mu\text{g N g}^{-1}$ soil]	Unfertilized control ¹	NH ₄ Cl ²	Soil treated with NH ₄ Cl ² + nitrapyrin	KNO ₃ ³
<i>Aerobic incubation</i>				
NH ₄ +	-5.7	-31.4	-4.8	-6.1
NO ₂ -	-0.1	+1.6	-0.2	-0.1
NO ₃ -	+4.8	+17.3	-1.4	+1.7
NO	+0.029	+0.179	+0.016	+0.036
N ₂ O	n.d. ⁴	+0.119		n.d.
Sum	-1.0	-12.2	-6.4	-4.4
<i>Anaerobic incubation</i>				
NH ₄ +	-4.0	-11.2	-7.8	-8.6
NO ₂ -	-0.1	-0.3	-0.1	-0.1
NO ₃ -	-2.8	-2.7	-1.2	-36.2
NO	+0.179	+0.209	+0.209	+2.84
N ₂ O	n.d.	n.d.		+1.05
Sum	-6.7	-14.0	-8.9	-41.0

¹ Initial concentrations in the control are given in Table 1.

² Initial concentration of ammonium-N: 83.4 ± 15.2 ppm.

³ Initial concentration of nitrate-N: 59.5 ± 10.9 ppm.

⁴ n.d. = not detectable.

was not balanced by the production of nitrite plus nitrate. This discrepancy can be explained by denitrification of the produced nitrate and nitrite to NO, N₂O and most of all, N₂. However, since ammonium was also depleted under anaerobic conditions, we assume that it was also immobilized by incorporation into microbial biomass.

Except for aerobically incubated alkaline cambisol, nitrate concentrations decreased in all other experiments indicating that denitrification was more active than nitrification. Decrease of nitrate was especially large in the nitrate-fertilized samples, but was not significantly enhanced by anaerobic incubation of the acidic cambisol. The total depletion of inorganic nitrogen compounds was not only due to loss of nitrate by denitrification, but also to loss of ammonium. The loss of ammonium was probably by immobilization, since it was similar under aerobic and anaerobic conditions and in presence and absence of nitrapyrin (Table 4). Immobilization of ammonium was larger in the acidic cambisol, probably because of the higher C/N ratio in this soil.

Table 4. Transformation of nitrogen in fertilized and unfertilized acidic cambisol.

Change during 66 h [$\mu\text{g N g}^{-1}$ soil]	Unfertilized control ¹	NH ₄ Cl ²	Soil treated with NH ₄ Cl ² + nitrapyrin	KNO ₃ ³
<i>Aerobic incubation</i>				
NH ₄ ⁺	-1.7	-48.5	-39.2	-1.9
NO ₂ ⁻	-0.1	-0.2	-0.4	-0.1
NO ₃ ⁻	-1.3	-1.1	-0.9	-36.2
NO	+0.022	+0.028	+0.028	+0.159
N ₂ O	n.d. ⁴	n.d.		n.d.
Sum	-3.1	-49.8	-40.5	-39.0
<i>Anaerobic incubation</i>				
NH ₄ ⁺	-1.0	-69.2	-49.9	-2.9
NO ₂ ⁻	-0.4	-0.1	-0.3	-0.2
NO ₃ ⁻	-1.8	-1.8	-2.8	-23.8
NO	+0.054	+0.089	+0.089	+8.31
N ₂ O	n.d.	n.d.		+2.16
Sum	-3.2	-71.0	-52.9	-16.4

¹ Initial concentrations in the control are given in Table 1.

² Initial concentration of ammonium-N: 105.9 ± 17.3 ppm.

³ Initial concentration of nitrate-N: 47.5 ± 4.7 ppm.

⁴ n.d. = not detectable.

NO was released in all experiments irrespectively of the predominance of nitrification or denitrification activity in soil. However, addition of nitrapyrin inhibited the release of NO only in the alkaline cambisol (Table 5) which also exhibited nitrification activity under aerobic conditions (Table 3). Chlorate, on the other hand, an inhibitor of nitrate reduction, inhibited NO release only slightly under these conditions, but was an efficient inhibitor under anaerobic incubation conditions (Table 5), when denitrification was predominant. Chlorate (but no nitrapyrin) was also an efficient inhibitor in the acidic cambisol (Table 5) which exhibited denitrification activity under both aerobic and anaerobic conditions (Table 4).

The net release (J) of NO is the result of simultaneous NO production and consumption in soil (Remde et al. 1989). The effect of addition of ammonium or nitrate on the rate of NO production (P) and the rate constant (k) of NO consumption is shown in Table 6. Note that gross rates of NO production are necessarily higher than net rates of NO release documented in Table 1. Table 6 includes the NO compensation mixing ratios (m_c) which are defined as the concentration at which production is equal to consumption. The results confirm that NO production was stimulated by anaerobic incubation conditions and was additionally stimulated, if nitrate was added. Under aerobic conditions, NO production was stimulated by ammonium in the alkaline cambisol and by nitrate in the acidic cambisol. The stimulation increased proportional to the amount of ammonium or nitrate added. NO consumption, on the other hand, was only stimulated by nitrate both under aerobic and anaerobic conditions. Stimulation of NO production in general was more pronounced than stimulation

Table 5. Effect of inhibitors on the release of NO under aerobic and anaerobic incubation conditions.

Experiment	Inhibition [%] by			
	Nitrapyrin		Chlorate	
	Aerobic	Anaerobic	Aerobic	Anaerobic
<i>Alkaline cambisol</i>				
no addition	83	0	7	90
+ NH ₄ Cl	91	0	6	93
+ KNO ₃	87	0	12	92
<i>Acidic cambisol</i>				
no addition	0	0	90	93
+ NH ₄ Cl	0	0	90	90
+ KNO ₃	0	0	92	92

Table 6. Effect of fertilization on production rate (P), consumption rate constant (k), and compensation mixing ratio (m_c) of NO in alkaline and acidic cambisol.

Experiment	P [ng N h ⁻¹ g ⁻¹]	k [cm ³ h ⁻¹ g ⁻¹]	m_c [ppbv]
<i>Alkaline cambisol</i>			
<i>Aerobic incubation</i>			
no addition	0.62	39	16
+ NH ₄ Cl (84 ppm)	2.82	33	85
+ NH ₄ Cl (840 ppm)	31.6	36	880
+ KNO ₃ (84 ppm)	0.92	40	23
+ KNO ₃ (840 ppm)	6.5	125	52
<i>Anaerobic incubation</i>			
no addition	27.9	163	182
+ NH ₄ Cl (84 ppm)	5.6	201	27
+ KNO ₃ (84 ppm)	1165	959	1214
<i>Acidic cambisol</i>			
<i>Aerobic incubation</i>			
no addition	0.73	47	16
+ NH ₄ Cl (84 ppm)	0.72	44	16
+ NH ₄ Cl (840 ppm)	1.5	36	41
+ KNO ₃ (84 ppm)	4.89	67	72
+ KNO ₃ (840 ppm)	48.3	102	472
<i>Anaerobic incubation</i>			
no addition	9.0	101	89
+ NH ₄ Cl (84 ppm)	5.0	36	124
+ KNO ₃ (84 ppm)	213	260	819

of NO consumption, so that fertilization or anaerobic incubation conditions usually resulted in increased NO compensation mixing ratios.

The enrichment of ¹⁵N/¹⁴N in NO released from NH₄NO₃-fertilized alkaline and acidic cambisol was measured under aerobic and anaerobic incubation conditions (Table 7). Using unlabelled NH₄NO₃, the released NO generally exhibited the same low enrichment typical for natural nitrogen as in the added fertilizer. Using ¹⁵NH₄NO₃, NO was significantly enriched in ¹⁵N if it was released from the alkaline cambisol incubated under aerobic conditions demonstrating that NO was produced from nitrification of ammonium. Under anaerobic conditions, however, addition of labelled ammonium did not result in a distinctive enrichment with ¹⁵N of the released NO. Instead, the addition of NH₄¹⁵NO₃, resulted in an

enrichment of the released NO demonstrating that NO originated from nitrate reduction under these conditions. In the acidic cambisol, NO became enriched mainly from labelled nitrate rather than from labelled ammonium both under anaerobic and aerobic conditions (Table 7).

Under aerobic conditions, the enrichment of NO with ^{15}N from $\text{NH}_4^{15}\text{NO}_3$ was low. However, it increased with the ^{15}N enrichment of the applied fertilizer indicating that some of the released NO originated from the nitrate moiety (Table 7). However, even under anaerobic incubation conditions, the enrichment of the released NO never reached the value of that of the added nitrate, although nitrate was added in about 1000 fold excess to the concentration in unfertilized soil. Dilution of the label by oxidation of $^{14}\text{NH}_4^+$ to $^{14}\text{NO}_3^-$ can be ruled out from the control with $^{15}\text{NH}_4\text{NO}_3$. The same is true for a possible volatilization of $^{14}\text{NH}_3$ and trappment in the permanganate solution. Hence, activities of reductive NO

Table 7. Release of ^{15}NO from labelled NH_4NO_3 added to alkaline or acidic cambisol.

Experiment	Enrichment with ^{15}N [%] in added NH_4NO_3	released NO
<i>Alkaline cambisol</i>		
<i>Aerobic incubation</i>		
NH_4NO_3	0.33	0.33 ± 0.03
$^{15}\text{NH}_4\text{NO}_3$	9.23	5.33 ± 0.4
$\text{NH}_4^{15}\text{NO}_3$	10.3	0.44 ± 0.15
$\text{NH}_4^{15}\text{NO}_3$	20	0.94
$\text{NH}_4^{15}\text{NO}_3$	30	1.46
<i>Anaerobic incubation</i>		
NH_4NO_3	0.33	0.33 ± 0.03
$^{15}\text{NH}_4\text{NO}_3$	9.23	0.9
$\text{NH}_4^{15}\text{NO}_3$	10.3	2.7 ± 0.3
<i>Acidic cambisol</i>		
<i>Aerobic incubation</i>		
NH_4NO_3	0.33	0.30 ± 0.03
$^{15}\text{NH}_4\text{NO}_3$	9.23	0.94 ± 0.14
$\text{NH}_4^{15}\text{NO}_3$	10.3	2.55 ± 0.1
<i>Anaerobic incubation</i>		
NH_4NO_3	0.33	0.32 ± 0.03
$^{15}\text{NH}_4\text{NO}_3$	9.23	1.04 ± 0.07
$\text{NH}_4^{15}\text{NO}_3$	10.3	4.35 ± 0.3
$\text{NH}_4^{15}\text{NO}_3$	30	8.68 ± 0.09

production must either have discriminated against the heavier isotope (Blackmer & Bremner 1977) or the production of NO partially depended on a pool of nitrogen which did not completely equilibrate with the $^{15}\text{NO}_3^-$ sprayed onto the soil.

Discussion

Our observations demonstrate that nitrification as well as denitrification may be important for NO production in soils and consequently may be responsible for NO emission under field conditions. Assaying two different soils under aerobic conditions, NO production in the alkaline cambisol was stimulated by ammonium, inhibited by nitrapyrin and released ^{15}NO originating from labelled ammonium. In contrast, NO production in the acidic cambisol was stimulated by nitrate, inhibited by chlorate, and released ^{15}NO originating from labelled nitrate. Hence, NO production under aerobic conditions was due to oxidation of ammonium (nitrification) in the alkaline cambisol, and due to reduction of nitrate (denitrification) in the acidic cambisol. In both soils, NO production under anaerobic conditions was due to reduction of nitrate.

Aerobic NO production by ammonium-oxidizing bacteria is not unexpected, as it was observed in pure cultures of autotrophic nitrifiers (Lipschultz et al. 1981; Anderson & Levine 1986; Remde & Conrad 1990) and heterotrophic nitrifiers (Papen et al. 1989). NO production in the alkaline cambisol probably was due to autotrophic nitrifiers, since the activity was sensitive to nitrapyrin, an inhibitor that is relatively specific for the ammonium monooxygenase-containing nitrifiers (reviews by Kuenen & Robertson 1988; Bedard & Knowles 1989). Nitrapyrin also inhibits methanotrophs which also are heterotrophic nitrifiers (Bedard & Knowles 1989). However, two strains of methanotrophs proved to be effective only in production of N_2O and consumption of NO, but not in production of NO (Krämer et al. 1990).

NO production by nitrate-reducing bacteria in aerobic soil is not unexpected. Even aerobic soils contain sufficient anaerobic microniches to sustain anaerobic denitrification (Parkin 1987). Furthermore, many denitrifying bacteria are also active under aerobic conditions (Kuenen & Robertson 1988). The significant stimulation of NO production by anaerobiosis and nitrate addition suggests that anaerobic denitrifiers prevail over aerobic ones and indicates that O_2 and even nitrate are proximate regulators of NO-producing denitrifiers or other nitrate-reducing bacteria. It must be pointed out that our experiments did not strictly distinguish between respiratory denitrification and other modes of nitrate

reduction (compare Tiedje 1988). However, nitrate reduction did neither result in a detectable accumulation of nitrite nor of ammonium, so that nitrate respiration and dissimilatory nitrate reduction to ammonium did not appear to be dominant processes.

Chemical production of NO (Chalk & Smith 1983), e.g. by decomposition of nitrite, apparently was not significant in our experiments, since otherwise metabolic inhibitors should not have been efficient. The unimportance of chemodenitrification is explained by the low concentrations of nitrite in the soils studied. Although we cannot exclude that some of the nitrite produced by nitrifiers or nitrate reducers was chemically decomposed to NO, such a chemical reaction was certainly controlled by the availability of nitrite, i.e. by the nitrite-producing bacteria.

The reason why NO production was dominated by nitrifiers in the alkaline soil and by denitrifiers in the acidic soil is yet unclear, but is probably due to the different microbial communities in the two soils. The alkaline soil apparently contained an active nitrifying population, whereas the acidic soil did not. However, our results cannot be extrapolated to other alkaline or acidic soils. Usually both nitrifying and denitrifying bacteria are sensitive to low pH. Nevertheless, there are several examples where nitrification or denitrification was demonstrated in acidic soils (e.g. Parkin et al. 1985; Martikainen 1985). While the predominance of nitrification or denitrification in the soils studied here describes a relatively simple situation, other soils may exhibit a large range of variable contributions of nitrification versus denitrification for NO release which may furthermore be complicated by the effects of changing environmental parameters. Quantification of the microbial sources for NO thus has common problems with that for N₂O (Firestone & Davidson 1989; Conrad 1991).

Another important question is the partitioning of nitrogen during nitrification or nitrate reduction towards NO production. Unfortunately, our data do not allow us to derive exact rates for nitrification and denitrification. Nitrification rates can either be based on the decrease of ammonium or the increase of nitrate. Both approaches may be biased. Because of the large activity of ammonium immobilization (especially in the acidic cambisol), ammonium oxidation rates probably are better approximated by the accumulation of nitrate, however, may be underestimated due to the loss of nitrate by denitrification. Denitrification rates can be derived from the decrease of nitrate, but only, if corrected for simultaneous nitrification and if immobilization of nitrate is relatively small.

Being aware of these limitations, it may nevertheless be worthwhile to calculate the amounts of NO released relative to the change in the nitrate

pool. As a result, NO production by the nitrification in the aerobically incubated alkaline cambisol accounted for 0.6 to 2.1% of the net accumulation of nitrate. NO production by denitrification in the aerobically incubated acidic cambisol accounted for 0.4 to 2.5% of the net decrease of soil nitrate. Under aerobic conditions, the percentage loss of nitrogen as NO apparently was independent of the pathway by which it was produced. Only under anaerobic conditions, when NO was produced from denitrification of nitrate, did NO production account for 3 to 9% of the net decrease of soil nitrate (and even for 35% in case of the nitrate-fertilized acidic cambisol).

The increased loss of nitrate-N under anaerobic conditions may partially be explained by the relatively larger stimulation of NO production versus NO consumption reactions in the soil resulting in increased NO compensation mixing ratio under anaerobic conditions. This phenomenon has already been described (Remde et al. 1989) and is confirmed here. Interestingly, NO production rates and thus, compensation mixing ratios were substantially lower, if the soils were not amended with nitrogen resulting in lower values than observed in the same soils by us earlier (Remde et al. 1989). This discrepancy is possibly due to seasonal changes in NO production and is presently under investigation. Another interesting feature was the apparent inhibition of NO production by ammonium addition under anaerobic conditions (Tables 2 and 6). The mechanistic basis of this phenomenon is presently not understood and needs further research.

NO production and consumption reactions were differentially affected by the availability of ammonium or nitrate. Thus, NO consumption was only stimulated by nitrate, but not by ammonium, whereas NO production was stimulated by both. The mechanistic basis for this observation is still unclear, but we suggest that in contrast to NO production, NO consumption in the soils studied was due to denitrifiers only. There is sufficient evidence that reductive consumption of NO is common among denitrifiers (e.g., Zafiriou et al. 1989; Remde & Conrad 1991), while NO consumption by autotrophic nitrifying bacteria remains to be demonstrated.

Our measurements show that release of NO was generally larger than release of N₂O. Molar ratios of NO/N₂O were in a range of > 1 to 8. Similar ratios (2 to 14) were observed in laboratory (McKenney et al 1982) and field studies (Slemr & Seiler 1984). Other investigators found that NO release was smaller than N₂O release, e.g. NO/N₂O ratios often being < 0.1 in laboratory (Keeney et al. 1979) and field studies (Johansson & Granat 1984; Anderson & Levine 1987). Hence, NO release cannot be predicted from N₂O release and vice versa. More research is necessary to understand the control of NO production and consumption

in soil and to improve models of NO flux between soil and atmosphere (Galbally & Johansson 1989).

Acknowledgements

We thank Dr K. Knoll (University of Konstanz) and Drs S. Slanina and J. Woittiez (ECN, Petten, The Netherlands) for the mass spectrometric analysis of samples, M. Baumgärtner (University of Konstanz) for helpful discussion, and Dr R. D. Bowden (MBL, Woods Hole) for critically reading the manuscript. We thank the Dow Chemicals Company (Midland, MI, USA) for a gift of nitrapyrin. This work is a contribution to the EUROTRAC-BIATEX project and was financially supported by the Bundesministerium für Forschung und Technologie (07EU719) and the Fonds der Chemischen Industrie.

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