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NITRIFICATION AND DENITRIFICATION AS SOURCES OF NITRIC OXIDE AND NITROUS OXIDE IN A SANDY LOAM SOIL

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Summary—Emissions of nitric oxide (NO) and nitrous oxide (N_2O) from a freely drained sandy loam, fertilized with $(NH_4)_2SO_4$ or KNO_3 (100 kg N ha^{-1}) with or without the addition of the nitrification inhibitor dicyandiamide (DCD), were measured. The addition of N fertilizers increased emissions of NO and N_2O. For plots fertilized with $(NH_4)_2SO_4$, NO emissions increased from 2.4 to 46.9 ng NO-N m^{-2} s^{-1} (2.1–40.5 g NO-N ha^{-1} day^{-1}), in the first 7 days after fertilizer application. Nitrous oxide emission rates were considerably lower, ranging from 0.95 to 7.4 ng N_2O-N m^{-2} s^{-1} (0.82–6.4 g N_2O-N ha^{-1} day^{-1}).

Nitrification rather than denitrification was the source of the NO emitted from the soil; additions of DCD inhibited the emissions by at least 92%. Nitrous oxide, on the other hand, was a product of both nitrification and denitrification. When soils were dry, N_2O was produced predominantly by nitrification and DCD reduced emissions by at least 40%. In contrast, in wet conditions denitrification was the main source of N_2O and emissions were not inhibited by DCD.

Nitric oxide emissions correlated significantly with soil temperature (30 mm depth), the air temperature inside the chamber, soil available NH_4^+, and were significantly reduced by watering the soil. Apparent activation energies, calculated from the temperature response in the NO emission rates, ranged from 30 to 71 kJ mol^{-1}. It was concluded from the close links between air temperature in the chamber and the NO emission rates that the NO was produced very close to the soil surface.

During nitrification the rate of depletion of NH_4^+-N emitted as NO-N was 5.5×10^{-5} s^{-1}. It was estimated that for cultivated fields 0.15–0.75% of the applied NH_4^+ fertilizer is released as NO.

INTRODUCTION

Estimates of global NO and N_2O suggest that soils contribute over 50% of the total global N_2O emission and between 8 and 32% of the total NO emission (Bouwman, 1990). The emissions of these gases are of concern because they are involved in important chemical processes in the troposphere and the stratosphere (Crutzen, 1983). Nitric oxide is involved in reactions that lead to a net O_3 production in the troposphere. Following oxidation to nitric acid, it also contributes to acidic deposition in Europe and North America (Logan, 1983; Review Group on Acid Rain, 1990). Nitrous oxide absorbs infrared radiation, and accounts for *ca* 5% of the total greenhouse effect (Bouwman, 1990). There are no chemical sinks for N_2O in the troposphere, therefore it has a long tropospheric lifetime of *ca* 150 yr. The destruction of N_2O in the stratosphere may lead to a net stratospheric O_3 depletion (Crutzen, 1981).

In soil, NO and N_2O are primarily produced biologically, by nitrification and denitrification. Nitric oxide is a direct intermediate of both processes. During nitrification NO is the product of the oxidation of hydroxylamine, and is the precursor of NO_2^- (Haynes, 1986). Denitrifiers both produce NO (reduction of NO_2^-) and consume NO (McKenney *et al.*, 1982) and are thought to contribute very little to the NO emission from soil. Nitrification is therefore believed to be the main source of NO (Anderson and Levine, 1986). Nitrous oxide in soil is produced in the presence of small concentrations of O_2 by nitrifying and denitrifying bacteria. Nitrifiers reduce NO_2^- to N_2O in zones of low O_2 potential in an otherwise aerobic soil (Yoshida and Alexander, 1970). Nitrous oxide is also an intermediate product of denitrification (Payne, 1981), which requires anaerobic conditions, for example, anaerobic microsites in an otherwise aerobic environment (Parkin, 1987). These microsites are often localized areas of intense respirative activity where O_2 demand exceeds supply (Smith, 1980). The proportion of N_2O, relative to that of N_2, produced during denitrification increases as the general O_2 concentration in the soil increases (Firestone and Tiedje, 1979).

The magnitude of the NO and N_2O emission from a soil is dependent on the soil available NH_4^+ and NO_3^- concentration, as well as on other factors such as soil temperature, soil water content, soil aeration (Sahrawat and Keeney, 1986; Galbally, 1989; Shepherd *et al.*, 1991). Fertilized soils are a substantial contributor to the total sources of NO and N_2O

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emissions to the atmosphere (Bouwman, 1990), and are the only biogenic source of atmospheric NO_x and N_2O that can be readily manipulated (e.g. by altering the chemical form of N fertilizer and the timing, method and rate of application). An improved understanding of the relative contributions of nitrification and denitrification to the biogenic emissions of NO and N_2O would therefore help to identify the main mechanisms regulating the emission of these gases. We have measured NO and N_2O emissions from soil to which NH_4^+- and NO_3^--based N fertilizers with or without the addition of the nitrification inhibitor dicyandiamide (DCD) have been applied.

MATERIALS AND METHODS

A greenhouse compartment (25 m^2), containing a freely drained brown earth of the Darvel series (Ragg and Futty, 1967), [81.4% sand, 9.6% silt, 9% clay; organic matter content, 2.8%; pH($CaCl_2$) 5.7] was sown with ryegrass (*Lolium perenne*) in November 1990. Invasion of chickweed (*Stellaria media*) was extensive and eventually covered 40% of the compartment. Once established, the ryegrass–chickweed mixture was cut regularly to a length of 50 mm. Grass cuttings were removed.

The temperature of the compartment was kept between 4 and 25°C. Daylength was extended to 16 h using 400 W high-pressure sodium lamps. The compartment was watered with tapwater, using a hose and attached showerhead. Three manual tensiometers (Burt, 1978) were installed at 200 mm soil depth, to monitor the soil moisture tension. The compartment was divided into six plots of *ca* 2.5 m^2, by sinking rigid plastic strips (150 mm) into the soil to a depth of 70 mm. This was to stop any lateral movement of water in the upper soil layer between plots. At the centre of each plot a galvanized steel frame (0.95 m^2, 150 mm high) was inserted into the soil to a depth of 90 mm. To allow recovery from the soil disturbance, the first fertilizer applications were made a week after inserting the frames, on 14 January 1991. Duplicate plots were fertilized with $(NH_4)_2SO_4$, KNO_3 or KNO_3+10% DCD (Dicyandiamide, Dalgety Agriculture Ltd) at a rate equivalent to 100 kg N ha^{-1}. The appropriate amount of fertilizer for a 1 m^2 area was dissolved in 2 litres of tapwater and was applied using a watering can. Duplicate plots were positioned at diagonally opposite corners of the compartment. Fertilizer application was repeated on 18 and 19 March 1991, when the soil available NH_4^+ and NO_3^- concentrations had returned to base concentrations on all plots. On this occasion the treatments were $(NH_4)_2SO_4$, $(NH_4)_2SO_4$ + 10% DCD and KNO_3.

Fluxes of NO and N_2O were measured by the flow-through (dynamic) chamber method and the closed (static) chamber method, respectively, as described in detail by Skiba *et al.* (1992). For NO flux measurements, a perspex chamber (0.57 m^3) was attached to a galvanized steel frame. Charcoal-filtered (O_3 free) air was pushed through the chamber at a flow rate of 0.079 m^3 min^{-1}. The air inside the chamber was stirred by a fan. Adequate mixing was provided by a tubular baffle made from a thin aluminium sheet. The inlet and outlet air was sampled at 3 min intervals for *ca* 1.5–2 h, using a solenoid valve under the control of a data logger (Campbell Scientific, Model 21X), and was analysed for NO using a chemiluminescence instrument (Thermo Environmental Instruments, Model 42) and for O_3 using a dual channel u.v. photometric ozone analyser (Analysis Automation, Model 427). The O_3 concentration in the chamber did not exceed 2 nl l^{-1}. Reactions between O_3 and NO in the chamber were therefore negligible. The NO flux was calculated from the difference in NO concentration between the air inlet and outlet and the flow rate of air through the chamber. For N_2O flux measurements, a shallow perspex chamber (0.22 m^3) was attached to the frame. The chamber was sealed and air samples (1 ml) in triplicate were taken, typically, 0, 45 and 90 min after sealing the chamber. Samples were analysed for N_2O by electron capture gas chromatography (Philips, Model PU4500).

The temperature of the soil enclosed by chamber (at 30 mm depth) and the air temperature inside the chamber were measured using thermocouples. When NO and N_2O fluxes were measured, a soil sample (two 100 mm cores, 25 mm dia, combined) was taken from each plot. More intensive sampling would have been desirable, but would have caused too much disturbance of the small plots. Soil samples were analysed for available NH_4^+ and NO_3^- (KCl extracts), moisture content (water loss by oven drying) and pH ($CaCl_2$) (Skiba *et al.*, 1992).

RESULTS

The soil and air temperatures inside the flow-through chamber were significantly higher after the second fertilizer application in March, than after the first fertilizer application in January [$\Delta T_{soil} = 3.6$°C and $\Delta T_{air} = 6.4$°C (Table 1)]. After the second fertilizer application watering of the plots was reduced in order to create a more aerobic soil air environment.

Table 1. Soil moisture content, soil temperature (30 mm depth) and air temperature in the flow-through chamber after the first fertilizer application (16 January–6 March 1991) and after the second fertilizer application (18 March–2 April 1991)

	First fertilizer application	Second fertilizer application
Soil moisture content (% dry wt)	18.3 ± 0.2*	17.4 ± 0.2*
Soil temperature (°C)	13.9 ± 0.6†	17.5 ± 1.6‡
Air temperature in flow-through chamber (°C)	15.3 ± 3.1†	21.5 ± 5.3‡

*n = 12.
†n = 18.
‡n = 26.

Differences in the soil moisture tension at 200 mm depth and in the moisture content of soil cores taken from the top 100 mm were significant, but very small ($\Delta_{moisture} = 0.9\%$). Watering of the plots after the second fertilizer application was reduced so that the top few cm of the soil dried out to a greater extent than indicated by the average moisture content of the top 100 mm (Table 1).

Figure 1 shows typical measurements of the NO and N_2O emission rates and soil and flow-through chamber air temperatures from plots that received the different fertilizer treatments. Flow-through and closed chambers were left on a plot for 60–90 min and then were moved to a new plot. In the first 2 weeks after the application of $(NH_4)_2SO_4$ and KNO_3, the rates of NO and N_2O emission from the soil were relatively large [Fig. 2(a), (b)]. When soil available NH_4^+ and NO_3^- concentrations were reduced to background levels (51 and 64 days after fertilizer application) [Fig. 2(c), (d)], the emission rates were very small, < 1.5 ng N_2O-N $m^{-2} s^{-1}$, 64 days after fertilizer application) or not detectable (51 days after fertilizer application). Nitric oxide was either absorbed or emitted at very low rates, ranging from -0.043 to 0.08 ng NO-N $m^{-2} s^{-1}$.

NO flux

Rates of NO emission were significantly greater from plots fertilized with $(NH_4)_2SO_4$ than with KNO_3 ($P < 0.001$, 0.01) [Fig. 2(a)] with an exception on the second day after the first fertilizer application, when both fertilizer treatments gave rise to similar NO emission rates.

The nitrification inhibitor DCD successfully reduced NO emissions. Averaged over the $(NH_4)_2SO_4$ and KNO_3 treatments, the reduction was >92% [Fig. 2(a)]. The NO fluxes from plots that received DCD and KNO_3 were as low throughout the period of the experiment as the NO fluxes measured 51 and 64 days after fertilizer applications without DCD.

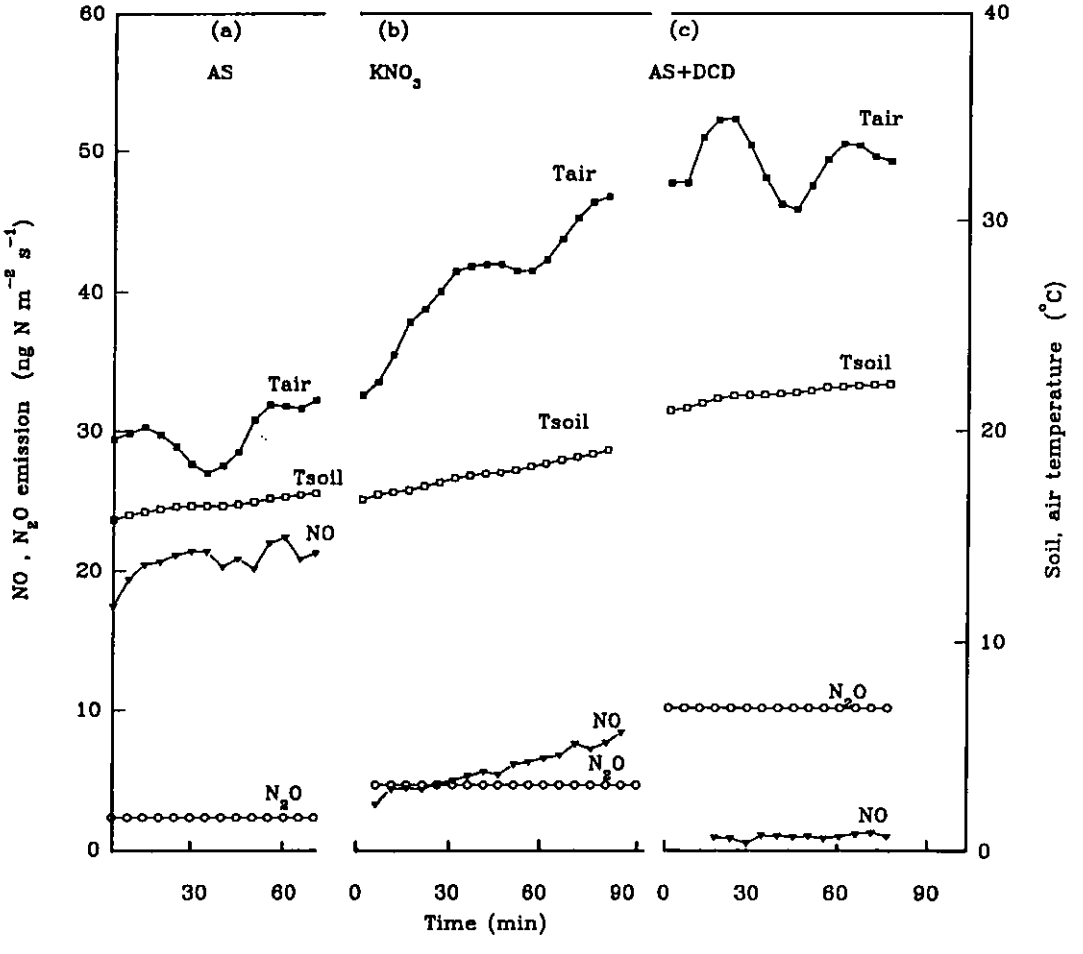

Fig. 1. Measured NO fluxes (▼), N_2O emissions (○), soil temperature at 30 mm depth (□) and flow-through chamber air temperature (■), 21 March 1991, 2 days after second fertilizer application. Measurements were made on a plot fertilized with $(NH_4)_2SO_4$ (AS) (a), KNO_3 (b) and $(NH_4)_2SO_4$ + DCD (10%) (AS + DCD). (c) NO fluxes, soil and air temperature averaged over 6 min intervals; N_2O fluxes: single determination over whole period on each plot—constant rate assumed.

For plots that received $(NH_4)_2SO_4$ and DCD, NO emissions were larger than background emissions for days 2, 7 and 8 after fertilizer application [Fig. 2(a)]; however, even then DCD reduced the NO emission by 95.4, 98.6 and 99.2%, respectively, compared with the treatment with $(NH_4)_2SO_4$ alone.

The NO emission was temperature-dependent. On occasions when, during the 1.5–2 h measuring period, the temperature in the glasshouse compartment changed considerably, significant relationships between NO emission and soil temperature (at 30 mm depth) and air temperature inside the flow-through

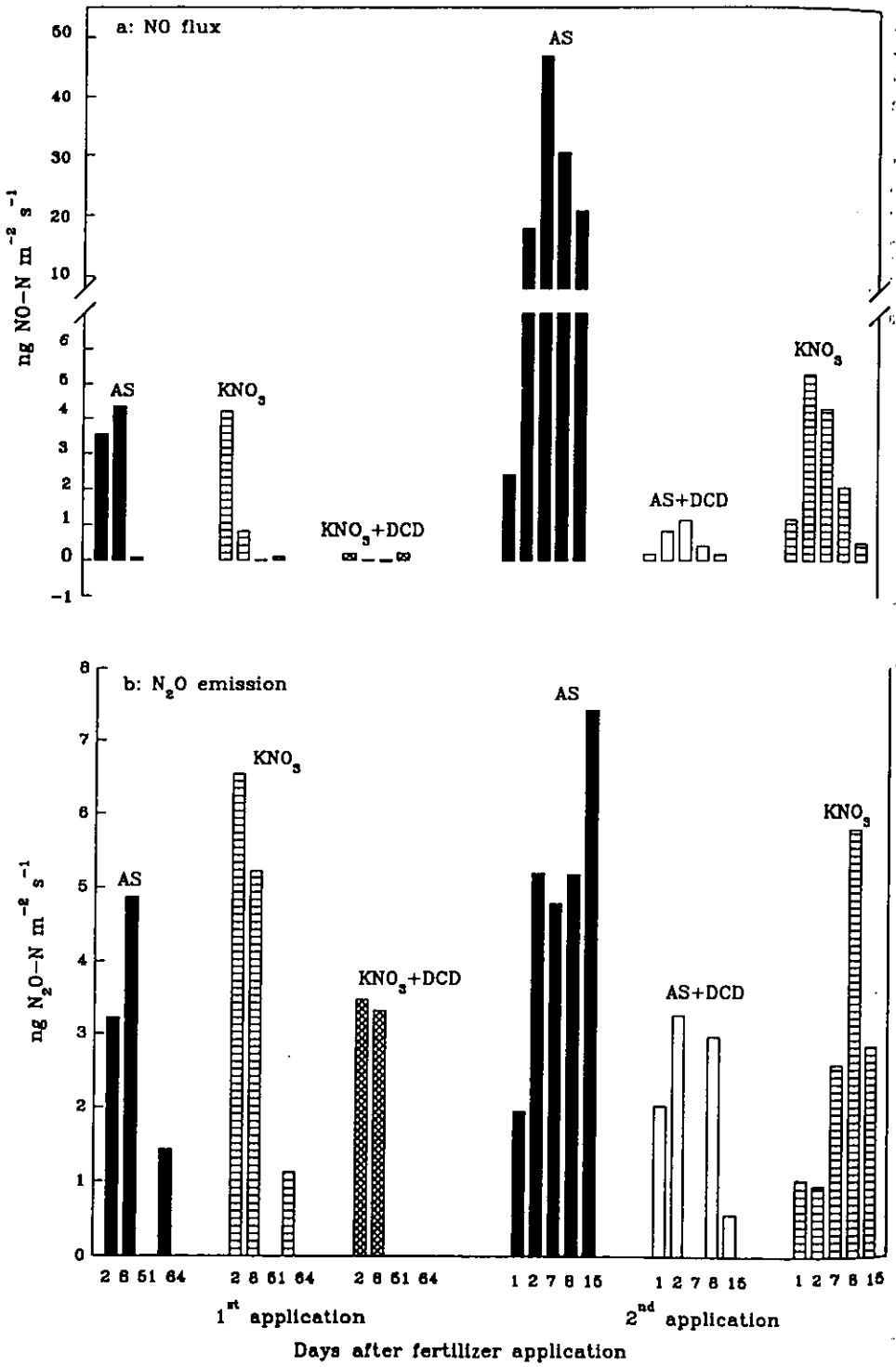

Fig. 2 (a and b)

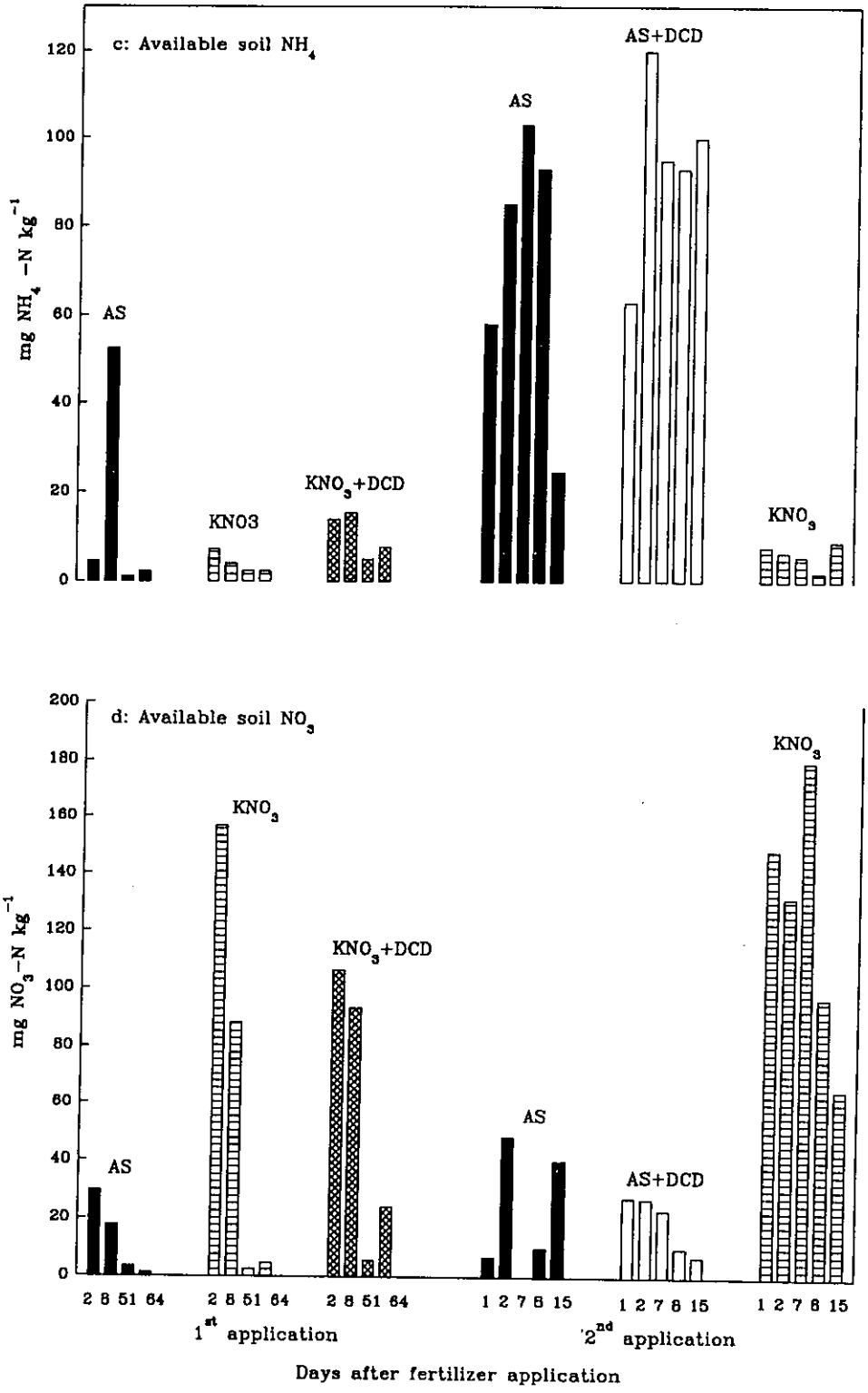

Fig. 2 (c and d)

Fig. 2. The effect of fertilizer type on the (a) NO flux (median of 30 min averages, $n = 2$–11), (b) N_2O emission (median of individual air samples, $n = 1$–6), (c) available soil NH_4^+ and (d) available soil NO_3^- (for both $n = 2$). Duplicate plots were fertilized with $(NH_4)_2SO_4$ (AS) (■), KNO_3 (▤), KNO_3 + DCD (■), or $(NH_4)_2SO_4$ + DCD (AS + DCD) (□).

Table 2. Relationship between NO emission and soil temperature (T_s) at 30 mm depth and flow-through air chamber temperature (T_c). The apparent activation energy (E_a) for NO production was calculated from the Arrhenius equation: ln NO emission = $A - E_a/RT_{s,c}$

Fertilizer applied	Date of flux measurement (1991)	Days after fertilizer application	Apparent activation energy* (kJ mol^{-1})
E_a calculated from the plot: ln NO emission vs soil temperature			
$(NH_4)_2SO_4$			
	19 March	1	213
	20 March	1	292
	21 March	2	101
	22 March	2	98
After watering	2 April	15	764
KNO_3			
	21 March	2	249
After watering	2 April	15	641
E_a calculated from the plot: ln NO emission vs flow-through chamber air temperature			
$(NH_4)_2SO_4$			
	19 March	1	30
	22 March	2	36
	2 April	15	49
KNO_3			
	16 January	1	55
	21 March	2	71
After watering	2 April	15	348

*Correlation coefficients were significant at $P < 0.01$ or $P < 0.001$. Degrees of freedom ranged from 7 to 20.

chamber were observed (Fig. 1). The apparent activation energies (E_a) were calculated from plots of the logarithm of the NO emission rate against the inverse absolute soil temperature and flow-through chamber air temperature (Table 2). Activation energies calculated for changes in NO emission with the soil temperature at 30 mm were unrealistically large, whereas those calculated for changes with the air temperature inside the flow-through chamber resulted in more sensible values consistent with the published data (Johansson and Granat, 1984) (Table 2).

For $(NH_4)_2SO_4$-fertilized plots the E_a value decreased by at least 50% between the first and second day after fertilizer application. When the plots were watered, the E_a values increased 2- to 3-fold.

Nitric oxide emissions were significantly larger after the second fertilizer application in March, when soil temperatures were higher, than after the first application [$P < 0.001$ for $(NH_4)_2SO_4$-fertilized plots, and $P < 0.01$ for KNO_3-fertilized plots, 8 days after fertilizer application] [Fig. 2(a)]. Temperature may, however, not have been the only controlling factor. The reduced soil moisture content after the second fertilizer application would also have contributed to an increase in NO emission.

Watering increased the soil moisture content in the top 100 mm by 2.4% of the dry weight of the soil and significantly reduced the NO emission from $(NH_4)_2SO_4$-fertilized plots but not from plots fertilized with KNO_3 and $(NH_4)_2SO_4$ + DCD (Table 3). For the latter two fertilizer treatments any watering effect was overshadowed by large deviations from the mean.

N_2O emission

After the first fertilizer application, N_2O emissions appeared to be larger from KNO_3-fertilized plots than from those fertilized with $(NH_4)_2SO_4$ or KNO_3 + DCD [Fig. 2(b)]. After the second fertilizer application, however, $(NH_4)_2SO_4$-fertilized plots emitted more N_2O than the other plots, but because of the large variability in the N_2O flux measurements, differences were only significant on one occasion. Eight days after the first and second applications, N_2O emissions from KNO_3-fertilized plots were significantly larger than from plots that were treated with DCD ($P < 0.01$).

Watering the plots significantly increased the N_2O emissions from plots fertilized with KNO_3 and $(NH_4)_2SO_4$ + DCD > 2-fold (Table 3). Nitrous oxide emissions from the $(NH_4)_2SO_4$-fertilized plots before watering were 2.6 times larger than from KNO_3-fertilized plots, but did not differ after watering.

Table 3. Effect of watering on the NO and N_2O fluxes from 1 m^2 plots fertilized with $(NH_4)_2SO_4$, KNO_3 or $(NH_4)_2SO_4$ + DCD, 15 days after second fertilizer application

Fertilizer type	Soil moisture[1] (% dry wt)	NO flux[2] (ng N m^{-2} s^{-1}) mean sg	N_2O flux[3] (ng N m^{-2} s^{-1}) mean sg
$(NH_4)_2SO_4$			
Before watering	18.0	20.7 (0.03)	7.41 (0.04)
		***	NS[4]
After watering	20.4	3.7 (0.11)	6.09 (0.07)
KNO_3			
Before watering	18.0	0.50 (0.13)	2.82 (0.03)
		NS	***
After watering	20.4	0.41 (0.33)	6.54 (0.03)
$(NH_4)_2SO_4$ + DCD			
Before watering	18.0	0.17 (0.24)	0.56 (0.07)
		NS	*
After watering	20.4	0.11 (0.22)	1.55 (0.17)

[1]Mean of triplicates; geometric mean and geometric standard deviation (sg) of the flux measured at 6 min intervals, n = 7–11; geometric mean of 3–6 air samples taken from the closed chamber after sealing it; [4]Student's t-test: NS = difference not significant; *$P < 0.05$; ***$P < 0.001$.

Emission rates for $(NH_4)_2SO_4$-fertilized plots were not affected by soil moisture changes.

$NO:N_2O$ emission ratio

Differences in the $NO:N_2O$ ratio were primarily controlled by the magnitude of the NO flux. Ratios >1 were observed for plots fertilized with $(NH_4)_2SO_4$, with the exception of 2 April (15 days after the second fertilizer application) (Table 4). Then, watering decreased the $NO:N_2O$ ratio to 0.61, by reducing the rate of NO emission. For KNO_3-fertilized plots the $NO:N_2O$ ratio was smaller than that for $(NH_4)_2SO_4$-fertilized plots, except on the second day after the second fertilizer application. The $NO:N_2O$ ratio for KNO_3-fertilized plots was only above unity 1, 2 and 7 days after fertilizer application. For plots that were treated with DCD the $NO:N_2O$ ratio was always <1, and was always smaller than from comparable plots that were not treated with DCD (Table 4). The $NO:N_2O$ ratios were larger after the second fertilizer application, when the soil was warmer and drier, than after the first application. Watering decreased the $NO:N_2O$ emission ratio for all treatments. For $(NH_4)_2SO_4$-fertilized plots a decrease in NO emission was responsible. For plots fertilized with KNO_3 and $(NH_4)_2SO_4$ + DCD the decrease in the ratio was caused by a significant increase in the N_2O emission (Table 3).

NO emission and soil available NH_4^+

A significant correlation between NO emission and soil available NH_4^+ ($r^2 = 0.69$, d.f. = 10) was observed for both fertilizer treatments. When these data were combined with data from earlier field experiments, in which NO fluxes were measured on a clay loam soil sown with *Lolium perenne*, and sandy loam soils sown with winter wheat (Skiba *et al.*, 1992), a significant linear relationship was obtained ($P < 0.05$, with $r^2 = 0.49$ and d.f. = 16) (Fig. 3). The slope provides an estimate of the rate of depletion of NH_4^+-N in the soil by emission as NO-N, of $5.5 \times 10^{-5}\,s^{-1}$.

Table 4. $NO:N_2O$ emission ratios calculated from mean NO and N_2O emissions (ng N $m^{-2}\,s^{-1}$) from replicate plots treated with different N fertilizers

Days after first and second fertilizer application*	$NO:N_2O$		
	$(NH_4)_2SO_4$	KNO_3	KNO_3 + DCD
First application (14 January 91)			
2–3	1.1	0.64	0.05
8–9	1.0	0.16	†
51–52	•	•†	•†
64	0.11	0.11	0.11
Second application (17 March 91)			
			$(NH_4)_2SO_4$ + DCD
1	1.24	1.13	0.09
2	2.16	5.58	0.23
7	9.83	1.67	•
8	5.92	0.36	0.14
15 (before watering)	2.76	0.18	0.03
15 (after watering)	0.61	0.06	0.07

*$N_2O = 0$.
†NO = 0 or negative.

DISCUSSION

The use of NH_4^+ and NO_3^- fertilizer with or without the nitrification inhibitor DCD enabled nitrification and denitrification as sources of fertilizer-derived NO and N_2O emissions to be distinguished. However, DCD did not entirely prevent nitrification; some NO and N_2O associated with nitrification was produced. This seems likely to have been due to the uneven distribution of the inhibitor, resulting in some nitrification of NH_4^+ derived from soil organic matter in microsites not penetrated by the DCD. Additionally, DCD is specific for *Nitrosomonas* (Amberger, 1981) and does not inhibit heterotrophic nitrification (Haynes, 1986). The inhibitory effect of DCD persists on average for 1–3 months, depending on the temperature, water and organic matter content of the soil (Amberger, 1989). Within our study periods the decomposition of DCD therefore was unlikely to have been a problem. On plots that received $(NH_4)_2SO_4$ + DCD, the soil available NH_4^+ concentration did not change significantly in the first 15 days after fertilizer application. Denitrification was the most likely source for N_2O emitted from plots that were fertilized with KNO_3 + DCD or $(NH_4)_2SO_4$ + DCD. For the latter plots the total N_2O emission estimated for the first 8 days after fertilizer application was 1.42 mg $N_2O\,m^{-2}$ and accounted for only 0.03% of the soil available NO_3^- content.

NO flux

Nitrification is generally considered the main source of NO emission from soil (Slemr and Seiler, 1984; Anderson and Levine, 1987). This was also the conclusion reached in this study. Nitric oxide emissions were largest from plots fertilized with $(NH_4)_2SO_4$ (46.9 ng NO-N $m^{-2}\,s^{-1}$, 7 days after the second fertilizer application), and correlated significantly with increasing soil available NH_4^+. The NO emission estimated for the first 8 days after the first fertilizer application, when the soils were wetter and colder, accounted for 0.03% of the N fertilizer applied as $(NH_4)_2SO_4$, but for 0.17% of the N fertilizer applied on the second occasion, when the soils were drier and warmer (Table 1). DCD effectively reduced NO emissions from $(NH_4)_2SO_4$-fertilized plots by at least 92%.

Denitrification was shown to produce NO in laboratory studies (Johansson and Galbally, 1984; McKenney *et al.*, 1982) and in the field, in a savanna climate region in Venezuela (Sanhueza *et al.*, 1990). In our study, however, denitrification was not a significant source of NO, as shown by comparing the plots fertilized with KNO_3 and KNO_3 + DCD. DCD inhibited the NO emission by 96%. This suggests that even on KNO_3-fertilized plots nitrification was

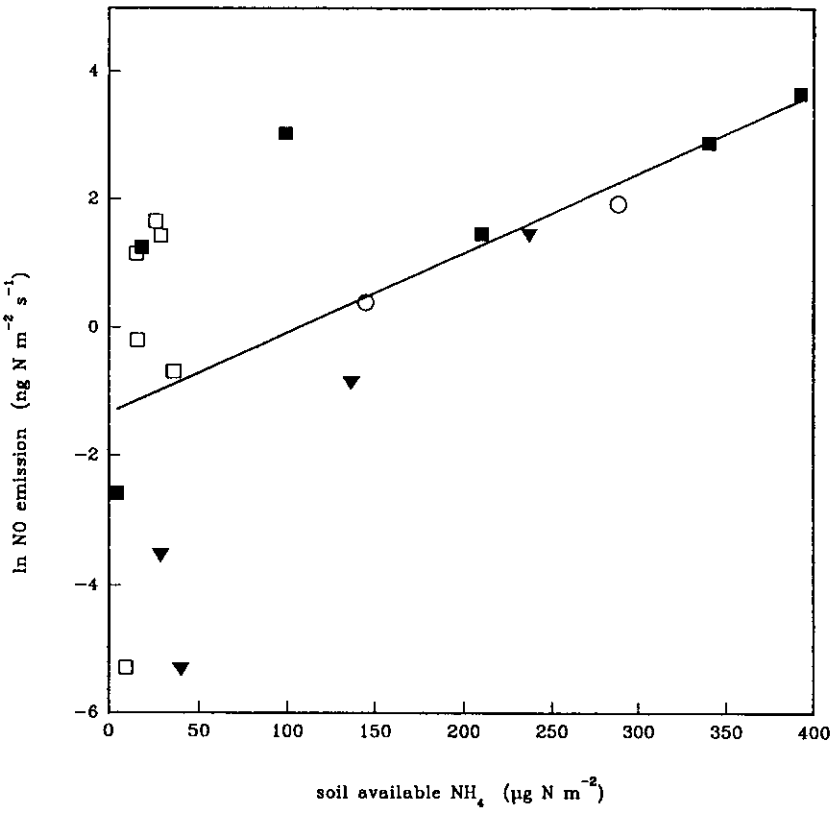

Fig. 3. Relationship between NO emission and soil available NH_4^+. A compilation of data from several experiments: plots of a sandy loam soil in the glasshouse compartments sown with *L. perenne* and fertilized with $(NH_4)_2SO_4$ (■) or KNO_3 (□), 1991; sandy loam soils sown with winterwheat (○), 1990; clay loam soil sown with *L. perenne* (▼), 1990. The solid line was fitted by linear regression.

responsible for most of the NO emitted. The most likely N source of nitrification in these plots was mineralization of organic N. Sorensen (1982) showed that the addition of N fertilizer increased this mineralization, and in our work a 3-fold increase in soil available NH_4^+ was observed immediately after the application of KNO_3. The resulting NH_4^+ concentration was sufficient to support NO emission. In the first 8 days after each fertilizer application, NO emissions were very similar and accounted for 0.14% of the total soil available NH_4^+ present.

Nitric oxide emissions were temperature-dependent. The apparent activation energies (E_a) of NO emissions may provide some indication of the vertical position in the soil profile of NO production, as suggested by Conrad and Seiler (1985) for N_2O emission from soil. The range of E_a-values calculated for changes in NO emission with soil temperature at 30 mm depth (98–292 kJ mol^{-1}) was much larger than reported elsewhere. Activation energies for NO emission usually range between 40 and 100 kJ mol^{-1} (Slemr and Seiler, 1984; Skiba *et al.*, 1992). On the other hand, E_a-values calculated for changes in NO emission with air temperature inside the flow-through chamber were slightly smaller (29.5–70.9 kJ mol^{-1}) than reported elsewhere. As the air inside the flow-through chamber was well stirred, its temperature reflects the temperature at the soil surface more closely than does the soil temperature measured at 30 mm depth. Nitric oxide production therefore was produced much closer to the soil surface than at the depth of 30 mm.

After the second fertilizer application the E_a value decreased significantly within 1 day of fertilizer application, presumably caused by the time it took for the fertilizer to dissolve and to migrate from the soil surface into the top few mm of the soil. The addition of water increased the E_a values *ca* 3-fold, presumably by reducing diffusion rates within the soil and by depressing aerobic processes, such as nitrification. This suggests that, in the soil, E_a-values for a particular reaction depend as much on the physical and chemical conditions of the soil as on the enzyme itself and must not be regarded in the conventional sense as activation energies.

The decrease in the NO emission rate from plots fertilized with $(NH_4)_2SO_4$ after watering can be explained by the same processes that increased the E_a

values, as discussed above. A similar decrease in NO emission was reported by Shepherd *et al.* (1991).

N_2O emissions

Maximum N_2O emission rates (7.4 ng N_2O-N $m^{-2} s^{-1}$, measured 15 days after the second fertilizer application) were considerably smaller than maximum NO emission rates (46.9 ng NO-N $ms^{-2} s^{-1}$, measured 7 days after the second fertilizer application). Comparison with published data on N_2O emissions for a variety of fertilized crops and grasslands also shows that the N_2O emissions measured by us were considerably smaller, and comparable with those from non-fertilized soils (Bouwman, 1990). The soil in the glasshouse compartment was a well-aerated sandy loam and therefore unlikely under normal conditions to develop a large number of anaerobic microsites necessary for N_2O production by denitrification. Application of DCD inhibited N_2O emissions by 40% in the first week after fertilizer application, suggesting that N_2O was emitted at almost equal rates during nitrification and denitrification. Differences in N_2O emission rates between the different fertilizer treatments, however, were not statistically significant. The overall trend, however, implies that the more frequent watering of the plots after the first fertilizer application favoured denitrification (Haynes and Sherlock, 1986) while the infrequent watering after the second fertilizer application favoured N_2O production by nitrification. These trends were confirmed by the observations in Table 3.

The $(NH_4)_2SO_4$-fertilized plots, in which nitrification could be expected to be initially (before watering) the dominant process, produced significantly more N_2O than KNO_3-fertilized plots (by a factor of 2.6), where denitrification would be the major source. Nitrification has been recognized as an important source of N_2O in aerated soils (Davidson *et al.*, 1986). Parton *et al.* (1988) suggested that nitrification is dominant over denitrification until soils become very wet and waterlogged. For comparison, Conrad *et al.* (1983) reported a 4.5-fold increase in N_2O emissions from grass–clover mixtures fertilized with $(NH_4)_2SO_4$, compared with those fertilized with KNO_3. In our work, watering did not alter the N_2O emission rates from $(NH_4)_2SO_4$-fertilized plots, but increased those from KNO_3-fertilized plots, suggesting that, for the latter, the balance between N_2O production from nitrification and denitrification had shifted.

Pure culture studies by Anderson and Levine (1986) showed that for nitrifiers the molar ratio of NO:N_2O produced was usually greater than unity and for denitrifiers was much less than unity. In the more heterogenous environment of the soil, nitrification and denitrification may occur simultaneously and may complicate the interpretation of the molar NO:N_2O ratios observed. Our results, however, suggest that the NO:N_2O emission ratios obtained from soil experiments do provide a valuable indicator of the relative importance of nitrification and denitrification as sources for NO and N_2O.

Conditions that favoured nitrification, e.g. fertilization with NH_4^+ and a lower soil moisture content, were associated with larger molar ratios of NO:N_2O, than conditions that favoured denitrification, such as fertilization with NO_3^-, inhibition of nitrification and increasing the soil moisture content. The steady decrease in the molar NO:N_2O ratio for plots fertilized with KNO_3 in March could be explained by the downward movement of fertilizer with repeated water application, into an environment where anaerobic microsites, necessary for denitrification, are more likely to be present.

Our work has shown that the gaseous loss of NO from well-aerated soils was considerably larger than the loss of N_2O, that NO was a product of nitrification rather than denitrification, and that the rate of NO emission was linearly dependent on the soil available NH_4^+ concentration. A compilation of all NO emission rates measured to date by us for agricultural soils showed that, independent of the physical and chemical soil environment, the rate of depletion of NH_4^+-N by emission as NO-N was $5.5 \times 10^{-5} s^{-1}$. These data may be used to estimate fertilizer N loss as NO to the atmosphere from the whole country. This estimation was based on the assumption that of the 1.5×10^6 tonnes of N applied annually (FMA, 1989) 70% is applied as NH_4^+ or as NH_4^+-forming fertilizers, and is nitrified at a rate of 0.6–2.9 μg N $m^{-2} s^{-1}$ (Verstraete, 1981). This leads to a loss of 0.15–0.75% of the applied NH_4^+ as NO (2.25×10^3 to 1.13×10^4 tonnes per year). For comparison, Bolle *et al.* (1986) estimated that 0.5–2% of the N fertilizer applied was lost as N_2O. Unlike the situation for N_2O, soils are not the major emitter of NO into the atmosphere (Bouwman, 1990), and the NO emissions calculated above correspond to only 0.4–1.9% of the total U.K. NO_x emissions (DoE, 1990). The results, however, showed that for agricultural areas NO loss can be substantial and may be the major source of atmospheric NO on a local scale.

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