

Denitrification in drained and undrained arable clay soil

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SUMMARY

Emissions of nitrous oxide (N_2O) and nitrogen gas (N_2) from denitrification were measured using the acetylene inhibition method on drained and undrained clay soil during November 1980–June 1981. Drainage limited denitrification to about 65% of losses from undrained soil. Emissions from the undrained soil were in the range 1 to 12 g N ha⁻¹ h⁻¹ while those from the drained soil ranged from 0.5 to 6 g N ha⁻¹ h⁻¹ giving estimated total losses ($\text{N}_2\text{O} + \text{N}_2$) of 14 and 9 kg N ha⁻¹.

Drainage also changed the fraction of nitrous oxide in the total denitrification product. During December, emissions from the drained soil (1.8 ± 0.6 g N ha⁻¹ h⁻¹) were composed entirely of nitrous oxide, but losses from the undrained soil (2.7 ± 1.1 g N ha⁻¹ h⁻¹) were almost entirely in the form of nitrogen gas (the fraction of N_2O in the total loss was 0.02). In February denitrification declined in colder conditions and the emission of nitrous oxide from drained soil declined relative to nitrogen gas so that the fraction of N_2O was 0.03 on both drainage treatments. The delayed onset of N_2O reduction in the drained soil was related to oxygen and nitrate concentrations. Fertilizer applications in the spring gave rise to maximum rates of emission ($5\text{--}12$ g N ha⁻¹ h⁻¹) with the balance shifting towards nitrous oxide production, so that the fraction of N_2O was 0.2–0.8 in April and May.

INTRODUCTION

Total denitrification losses from field soils can be measured by three methods. The first method currently available is to use the isotope ¹⁵N to label nitrogen additions to the soil and measure ¹⁵N-labelled gases emitted after denitrification (Rolston & Broadbent, 1977; Colbourn *et al.*, 1984b). One disadvantage of this method is that it does not measure denitrification of the native soil nitrogen but only the denitrification of the added nitrogen. The second method is to deduce denitrification losses from the balance of a nitrogen budget having accounted for crop uptake, soil residues and leaching losses. Once again this needs ¹⁵N and so has the same disadvantage as the first method and it also has the possibility of greater errors accumulated during the budgeting procedure (Dowdell & Webster, 1984).

The third method, the one used in this study, uses acetylene to inhibit the bacterial reduction of nitrous oxide (N_2O) to nitrogen (N_2) (Ryden *et al.*, 1979b). Nitrogen that would have been reduced to nitrogen gas remains as nitrous oxide which can be easily measured by gas chromatography (Hall & Dowdell, 1981). This effect of acetylene on denitrifying bacteria has been demonstrated in laboratory studies (Ryden *et al.*, 1979a) and the method has been used successfully in an irrigated horticultural loam (typic Halploxeroll) (Ryden *et al.*, 1979b), a pasture, clay-loam brown earth (Ochraqualf), (Ryden, 1983) and an arable stagnogley clay soil (typic Haplaquept), (Colbourn *et al.*, 1984a). The advantage of the acetylene inhibition method is that it measures the denitrification of all nitrate nitrogen irrespective of its source and so we adopted the acetylene inhibition method for this study.

We now report the results of the use of the acetylene inhibition method in the field to measure the total denitrification losses from an arable clay soil and to investigate the effect of drainage on

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denitrification. Denitrification in field soils depends on a supply of nitrate and results from the development of anaerobic soil conditions. Smith & Dowdell (1974) found oxygen concentrations in a permeable clay soil were related to the proximity of drains and the height of the water-table. Oxygen inhibits denitrification (Firestone *et al.*, 1979) and so a relationship between drainage and denitrification might be expected. In one published study denitrification was indicated in some poorly drained subsoils in North Carolina whereas higher redox potentials and larger amounts of free nitrate nitrogen were associated with better soil drainage (Gambrell *et al.*, 1975).

EXPERIMENTAL METHODS

Experimental site and treatments

The soil was a Denchworth series clay (typic Haplaquept) developed on Oxford clay (Jarvis, 1973); some physical and chemical details are given in Table 1. Measurements were made on two plots that were part of a larger field experiment on the Brimstone Farm, Faringdon, Oxfordshire which was to investigate the interactions of different methods of tillage and drainage in terms of drain performance, soil physical conditions, crop yield and nutrient losses (Cannell *et al.*, 1984). One of the plots was drained by mole drains spaced 2 m apart at 60 cm depth, which had been drawn in 1978. Both plots were direct-drilled with winter wheat on 1 October 1980. The recent cropping history, which was identical for the two plots, was winter barley in 1977–78 followed by two crops of winter wheat in 1978–80.

No nitrogen fertilizer was applied in the seed-bed. A top dressing of ammonium nitrate of 53 kg N ha⁻¹ was given on 1 April 1981. The drained soil received ammonium nitrate at 96 kg N ha⁻¹ on 21 April 1981. In order to facilitate a related study, the undrained soil was given calcium nitrate at 100 kg N ha⁻¹ on 5 May 1981 instead of the second top-dressing of ammonium nitrate (Colbourn *et al.*, 1984b).

Table 1. Physical and chemical characteristics of the Denchworth series soil at the Brimstone Farm experimental site. Samples for NO₃⁻ and NH₄⁺ determinations collected 10 November 1980.

Depth (cm)	Bulk density (kg dm ⁻³)	Clay* (%)	Total N (g kg ⁻¹)
0–10	0.9	44	3.5
10–20	1.0	44	2.6
20–40	1.3	55	1.3
40–60	1.3	56	1.1
60–100	—	47	0.7

Depth (cm)	Undrained soil				Drained soil			
	pH	Organic C (g kg ⁻¹)	NO ₃ -N (mg kg ⁻¹)	NH ₄ -N (mg kg ⁻¹)	pH	Organic C (g kg ⁻¹)	NO ₃ -N (mg kg ⁻¹)	NH ₄ -N (mg kg ⁻¹)
0–5	6.4	37	—	—	5.7	40	—	—
0–10	6.5	32	2.7	1.3	6.8	38	6.0	1.2
10–20	6.9	18	2.5	1.1	7.2	17	9.1	1.6
20–30	7.6	10	1.2	0.7	7.5	11	3.3	0.7
30–40	7.8	9	0.5	0.5	7.7	9	1.7	0.5
40–50	7.7	8	0.5	0.5	8.0	8	0.8	0.5

*From Jarvis (1973)

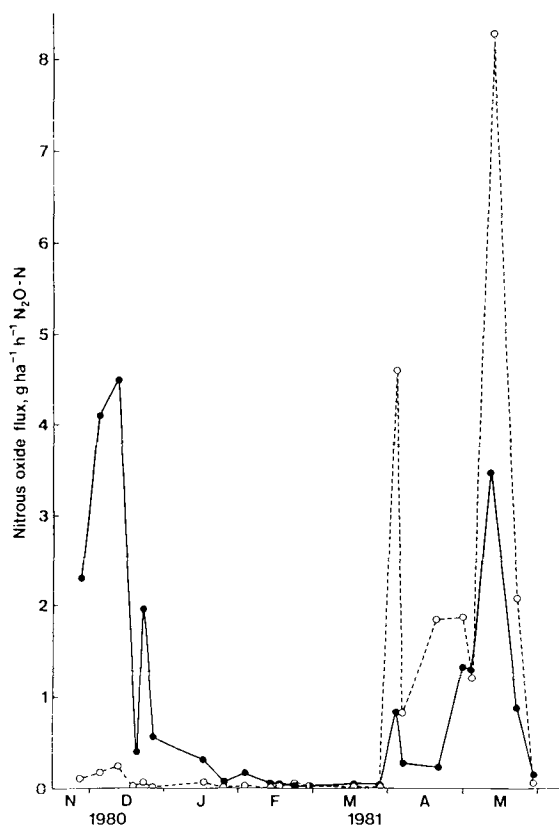


Fig. 1. Nitrous oxide flux from the undrained (---) and from the drained (—) soil in the absence of acetylene, November 1980–July 1981. Fertilizer was applied as ammonium nitrate on 1 April (53 kg N ha^{-1}) to both soils and on 21 April (96 kg N ha^{-1}) to the drained soil. Calcium nitrate was applied on 5 May (100 kg N ha^{-1}) to the undrained soil.

N₂O measurements and C₂H₂ treatments

The nitrous oxide flux from the soil surface was measured using permanently installed flux chambers of 30 cm diameter and 5 l capacity (Burford *et al.*, 1981). On each plot there were two blocks of six flux chambers 4 m apart. One block of six was reserved for the acetylene treatment, the other for measurements of nitrous oxide in the absence of acetylene. Although this experimental design has inherent weaknesses these were accepted because of the overriding requirement to keep the acetylene treated areas physically separate from the untreated areas. It was important to avoid even the remotest possibility of acetylene affecting the measurements of the nitrous oxide flux in the absence of acetylene.

On one occasion in December, February, April and two in May acetylene was introduced into the soil via a gas distribution tube 40 cm underneath the flux chambers (Colbourn *et al.*, 1984a). In April, acetylene was also allowed to enter the soil from the surface via the flux chambers because it was expected that most denitrification would be near the soil surface at this time, as this measurement was made a few days after the application of fertilizer. Also it was anticipated that the diffusion of acetylene into the drying, cracking soil would be unrestricted (Colbourn *et al.*, 1984b). Acetylene was introduced into the closed flux chamber to give a volumetric concentration of 0.1% in the headspace for 1 h. The flux chamber was then opened for at least 30 min for equilibrium concentrations of nitrogenous gases to be re-established before reclosing and measuring the flux of nitrous oxide.

The soil atmosphere, or soil water in saturated soil, was sampled at depths of 5, 10, 15 and 30 cm beneath the flux chambers and at 7.5, 15, 30, 45, 60 and 90 cm beneath other parts of the plot. The sampling devices have been described by Colbourn *et al.* (1984a).

Nitrous oxide and oxygen were measured in gas samples by gas chromatography (Hall & Dowdell, 1981). On occasion when the profile was saturated, water was extracted from the gas sampling probes and the equilibrium concentrations of nitrous oxide in the gas phase in contact with the water were determined chromatographically using a headspace analysis technique (Hall, 1978, 1980). The equilibrium concentration of oxygen in the gas phase in contact with a water sample was determined by injecting a small sample of water into a gas chromatographic column packed with Poropak T which retained the water, leaving oxygen for analysis by an electron capture detector (Hall, 1978). Acetylene was measured with a gas chromatograph fitted with a Poropak Q column and thermal conductivity detector. Nitrate in water was measured colorimetrically (Brewer & Riley, 1965). Meteorological data were collected using an automatic weather station (Didcot Instruments Co., Didcot, Oxon, U.K.).

RESULTS AND DISCUSSION

Nitrous oxide emission was detected throughout the experiment from November 1980 to June 1981 (Fig. 1).

The ranges of loss rates measured were 0.5–12 g N ha⁻¹ h⁻¹ for denitrification and 0.5–5.5 g N ha⁻¹ h⁻¹ for N₂O in the absence of acetylene (Table 2). These were similar to but somewhat lower than those measured by other workers which have been in the range 8–80 g N ha⁻¹ h⁻¹ for a variety of soils at temperatures of 8–20°C or, without acetylene, 0.4–16 g N ha⁻¹ h⁻¹ (Rolston & Broadbent, 1977; Ryden *et al.*, 1979; Ryden, 1983; Webster & Dowdell, 1982). Our observations were made after smaller fertilizer-N applications and at lower soil temperatures.

One effect of drainage in this clay soil was to limit total production of nitrogenous gases. The nitrous oxide flux in the presence of acetylene was always less from the drained soil (Table 2). The total denitrification losses for the period November 1980–June 1981 were estimated as 14 kg N ha⁻¹ and 9 kg N ha⁻¹ from the undrained and drained soils respectively, with standard errors estimated as ±2 and 1 kg N ha⁻¹ respectively.

Drainage also shifted the balance in the composition of the denitrification gases towards nitrous oxide (N₂O), in the autumn and early winter. Nitrous oxide flux from the undrained soil without acetylene was negligible in December but increased greatly after acetylene treatment (Table 2), whereas the introduction of acetylene did not change the already large nitrous oxide flux from drained soil.

Table 2. The concentration of nitrate in soil solution, and the mean rates of nitrous oxide emission from the soil surface in the presence (A) and absence (B) of acetylene. A-B is an estimate of N₂ production

	Undrained				Drained			
	Nitrate (mg N l ⁻¹)	N ₂ O emission data (g N ha ⁻¹ h ⁻¹)			Nitrate (mg N l ⁻¹)	N ₂ O emission data (g N ha ⁻¹ h ⁻¹)		
		A	B	A-B		A	B	A-B
1980 December	6	2.7	0.1	2.6	12	1.7	2.0	-0.3
1981 February	1	2.3	0.1	2.2	5	1.3	0.1	1.2
1981 April	0.1	1.2	0.9	0.3	0.3	0.5	0.3	0.2
1981 May	—	9.6	4.2	5.4	—	5.2	2.0	3.2

Nitrate concentrations were measured near the top of the saturated layer
Coefficient of variation = 130 ± 80%

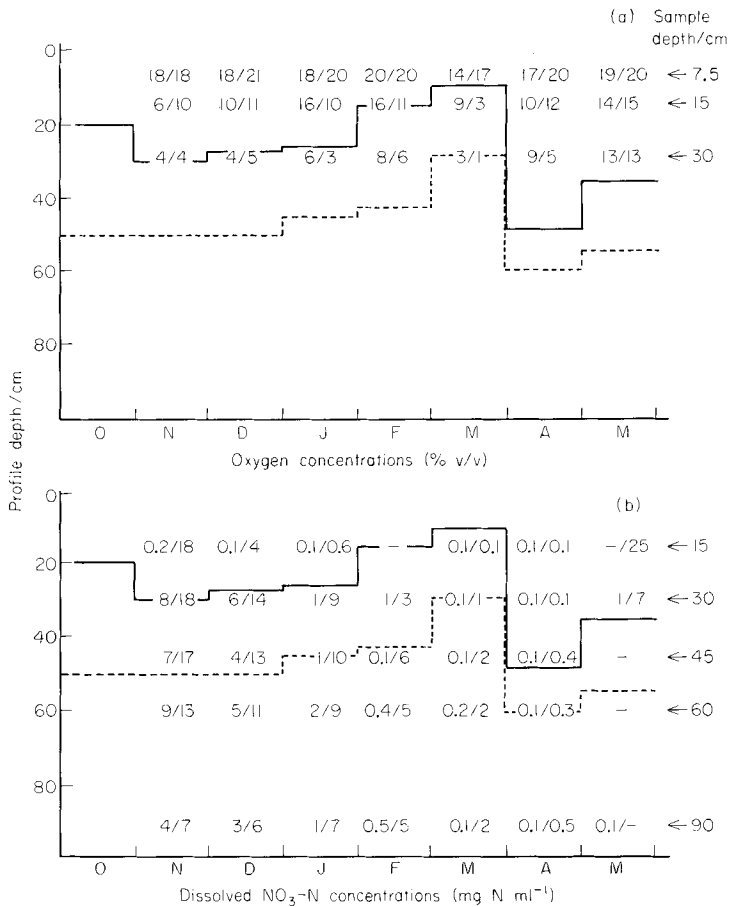


Fig. 2. Variations of concentrations of (a) oxygen, and (b) dissolved nitrate at different depths between October 1980 and May 1981 in Denchworth soil profiles. Mean monthly water tables: — undrained; --- drained. The first of each pair of measurements (e.g. 4/7) refers to an undrained profile, and the second to a drained profile.

Nitrogen gas was the dominant product in winter and nitrous oxide became a greater part of the product after the application of fertilizer nitrogen in the spring (Table 2). There were no significant differences between the drained and the undrained soils after December in the ratio of the two gases produced by denitrification.

A number of factors may have combined to prevent reduction of N₂O to N₂ in the drained soil before the end of December one of which was certainly oxygen (Fig. 2a) since oxygen is a powerful inhibitor of denitrification (Firestone *et al.*, 1979). At 30 cm depth in November the oxygen concentrations were small in both the drained and undrained soils, but during the same month the oxygen concentration was greater in the drained soil at 15 cm. This would have delayed the synthesis of nitrous oxide reductase enzyme and the onset of the reduction of N₂O to N₂ (Smith & Tiedje, 1979). Firestone *et al.* (1979) found that addition of oxygen to give a concentration of 5% by volume slowed down denitrification rate to 0.01% of the anoxic rate but at the same time the N₂O:N₂ ratio of the product changed from 0.5 to 1.2 in favour of N₂O after the 20 min incubation.

Nitrate would also have inhibited N₂O reduction (Blackmer & Bremner, 1978) in the drained soil because nitrate concentration was relatively high (13–18 mg N l⁻¹) at the top of the saturated zone during November (Fig. 2b).

Table 3. The occurrence of dissolved nitrite (mg N l^{-1}) in the undrained (unD) and drained (D) profiles of the Denchworth clay soil November 1980–February 1981

Soil depth (cm)	November		December		January		February	
	unD	D	unD	D	unD	D	unD	D
15	—	—	—	—	—	—	0.1	—
30	0.1	—	0.4	0.4	—	0.2	0.2	—
45	0.1	—	0.2	0.7	—	—	—	—
60	—	0.1	0.2	—	—	—	—	—
90	0.6	—	—	—	—	—	—	—

— no nitrite-N detected; samples tested during the first 2 weeks of February were all negative

Table 4. Monthly mean nitrous oxide concentrations measured in the soil atmosphere, or estimated from water samples, in the absence of acetylene, November 1980–May 1981

Sampling depth (cm)	N_2O concentrations ($\mu\text{l l}^{-1}$)						
	November	December	January	February	March	April	May
<i>Undrained soil</i>							
7.5	2	1	1	0.7	1	4	20
15	5	3	1	2	1	20	400
30	5	4	6	6	0.6	10	800
<i>Drained soil</i>							
7.5	70	3	1	0.5	0.7	1	10
15	900	200	6	9	0.4	30	500
30	400	90	2	1	0.9	9	200

Coefficient of variation = $130 \pm 70\%$

Nitrite (NO_2^-) appeared in the soil water at 30 cm depth during November (Table 3) suggesting that denitrifiers were actively reducing nitrate between 15 cm and 30 cm depth in the undrained soil. The nitrate concentration declined sharply towards the soil surface, while oxygen concentration remained small between 30 cm and 15 cm (Fig. 2). The depth of this denitrification coincides with the water-table but it is also at the bottom of the organic rich topsoil (Jarvis, 1973). The change to the B horizon, reflected in the organic carbon content (Table 1), restricts roots mainly to the ped faces of the strongly prismatic soil structure hence reducing the volume of organically enriched soil in which denitrifiers may flourish.

It seems that, in the drained soil, denitrification occurred in a wide band between 15 cm and 45 cm depth; that is between the saturated zone and the biologically active topsoil (Table 4). The largest nitrous oxide concentrations were measured at a depth of 15 cm (Table 4) and this was matched by the decline in nitrate at 15 cm between November and December (Fig. 2b), but nitrite was found somewhat deeper in the soil profile at 30–45 cm in December (Table 3). Thus the zone of denitrification was at different depths in the two soils because of the difference in depth to the water-table: 50 ± 15 cm in the drained soil, 20 ± 10 cm in the undrained soil (Fig. 2).

Most nitrous oxide was released in autumn and spring. This seasonal pattern of nitrous oxide emission was reflected by the concentration of nitrous oxide in the soil profile (Table 4). Concentrations were greatest (up to $900 \mu\text{l l}^{-1}$) in the drained soil during November and December, and in both profiles during May after the application of fertilizers containing nitrate. The measurements from acetylene treated soil (Table 2) demonstrated that the very low rates of nitrous oxide emission between December and March (Fig. 1), were the result of almost complete reduction of nitrate to nitrogen gas (N_2). Similarly low values of nitrous oxide emission from a Denchworth clay soil after December were reported in an earlier study (Burford *et al.*, 1981) and can also be attributed to reduction of nitrate to nitrogen gas. Denitrification was observed during this period (Table 2) despite low concentrations of nitrate in the soil solution suggesting that nitrogen losses were derived primarily from nitrogen mineralized and nitrified from soil organic matter.

The losses may have been limited by low rainfall (Fig. 3). Between October–December rainfall was 68% of the long-term average for the site; for January and February it was only 46%; but the March rainfall was nearly three times the average. Oxygen concentrations in the soil were relatively high until March (Fig. 2).

In spring, following the application of fertilizer, denitrification increased (Table 2, Fig. 1). Colbourn *et al.* (1984b), using ^{15}N in the undrained soil, measured a rate of $24 \text{ g N ha}^{-1} \text{ h}^{-1}$, equivalent to 4 kg N ha^{-1} for 1 week. This followed heavy and persistent rain during May (Fig. 3).

Estimates of total denitrification losses were made graphically for both drainage treatments by a procedure (shown for the undrained soil: Fig. 4) that allowed the calculation of unbiased means and standard errors. Three sets of horizontal lines were drawn to represent the precision of the field measurements. One set was drawn through the mean nitrous oxide flux rates measured after acetylene treatment on the five occasions specified in Table 2 (line A, Fig. 4). Allowance was made for

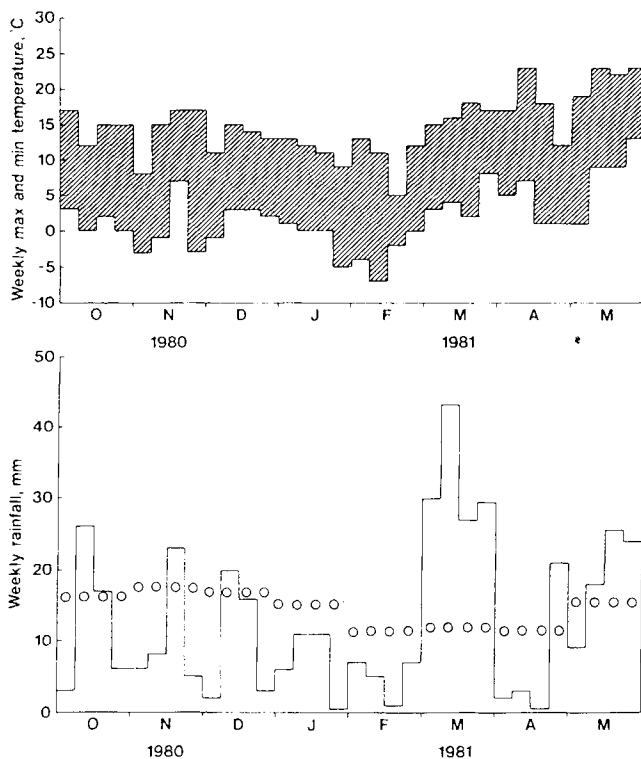


Fig. 3. Air temperature (above) and rainfall (below) at the experimental site, October 1980–May 1981. Open circles show long-term average rainfall at the site.

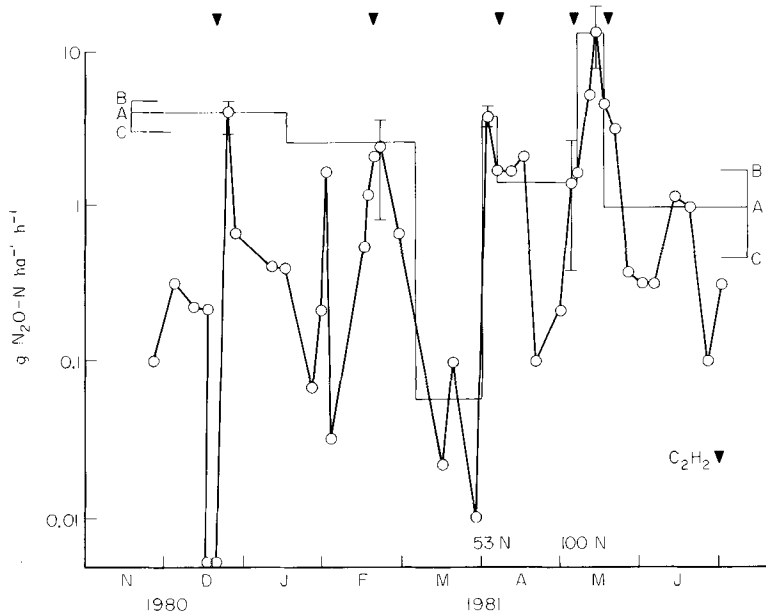


Fig. 4. Nitrous oxide flux from the undrained soil treated with acetylene on five occasions (C_2H_2), November 1980–July 1981. The lines A–C were used in the estimation of the nitrogen losses via denitrification as described in the text. 53 N and 100 N indicate applications of nitrogen fertilizer at 53 kg N ha^{-1} and 100 kg N ha^{-1} . Error bars indicate the standard errors on the mean.

periods of low flux in March and June. A second set was drawn through the mean plus one standard error (line B) and a third set of lines through the mean minus one standard error (line C). When the measured rate of nitrous oxide flux changed significantly, as for example between December 1980 and February 1981, a step was made to the new rate and the horizontal line continued at the new mean flux rate. The area below the line is an estimate of total denitrification loss. The mean of these estimates was taken to be the best estimate of total denitrification loss and was reported above.

The nitrous oxide flux often varied greatly within the area (3 m^2) occupied by six flux chambers. For example, on 5 December 1980, the range of oxide fluxes measured in the six control chambers on the drained soil was $1.1\text{--}7.2 \text{ g N ha}^{-1} \text{ h}^{-1}$, (mean $\pm \text{SE} = 4.1 \pm 0.8 \text{ g N ha}^{-1} \text{ h}^{-1}$). This was typical of our experience on these arable clay soils. Another cause of uncertainty was the realization that the acetylene changed the behaviour of the soil. The nitrous oxide flux from acetylene treated soil continued to exceed that from the untreated soil long after the acetylene could no longer be detected. This may be because the general metabolic activity in the soil was increased by the acetylene metabolism by certain soil bacteria (Germon, 1980) or metabolism of the acetone contaminating the acetylene supply (Gross *et al.*, 1982). Acetylene was used only at 2-monthly intervals to decrease any long-term bias that may have been caused by any such effect, and in April the acetylene treated and untreated soils were interchanged.

CONCLUSIONS

Drainage of the Denchworth series clay soil has limited denitrification especially during the autumn because oxygen could diffuse more deeply into the soil with a lower water-table. The oxygen limited denitrification and inhibited the reduction of N_2O to N_2 gas. The balance of the two gases in the final product was changed, most effectively in November and December 1980. There was a negligible amount of reduction of N_2O to N_2 in the drained soil at that time. Denitrification took place between 10 cm and 50 cm depth.

The higher nitrate concentrations in the drained soil did not increase denitrification loss. In the

undrained soil the rise of the water-table towards the topsoil in the autumn promoted rapid denitrification at the base of the organic rich top soil. The application of inorganic fertilizers and the rainfall in April and May 1981 promoted denitrification irrespective of the drainage status of the soil. Over the period from November 1980 to May 1981 the presence of adequate drainage in this clay soil resulted in a 30–40% decrease in the amount of inorganic nitrogen lost by denitrification.

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