Journal of Soil Science, 1984, **35**, 11–22,

Estimation of the total gaseous nitrogen losses from clay soils under laboratory and field conditions

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SUMMARY

Acetylene blockage was evaluated as a method for measuring losses of $N_2O + N_2$ from two Denchworth series clay soils. The denitrification potential in anaerobic, dark incubations at 20°C with nitrate (equivalent to 100 kg N ha^{-1} 0–20 cm depth), maximum water holding capacity, and acetylene (1%), was equivalent to 32 ± 11 and 39 ± 6 kg N ha^{-1} per day for the two 0–20 cm soils and was positively correlated with carbon content ($r = 0.98$). After 4 days N_2O was reduced to N_2 in the presence of C_2H_2.

In April 1980 following irrigation (24 mm) and applications of ammonium nitrate (70 kg N ha^{-1}) and acetylene, the mean nitrous oxide flux from soil under permanent grass was 0.05 ± 0.01 kg N_2O–N ha^{-1} per day for 8 days.

In June 1980, the losses of nitrogen from cultivated soils under winter wheat after irrigation (36 mm) and acetylene treatment were 0.006 ± 0.002 and $0.04–0.07 \pm 0.01$ kg N ha^{-1} per day respectively before and after fertilizer application (70 kg N ha^{-1}). The nitrous oxide flux in the presence of acetylene decreased briefly, indicating that nitrification was rate determining in drying soil.

INTRODUCTION

Recent studies of nitrous oxide emissions from arable (Burford *et al.*, 1981) and permanent grass swards (Webster & Dowdell, 1982) have shown that these gaseous losses (5–8 kgN ha^{-1} per annum) are rarely of agricultural significance. Losses of nitrogen gas from denitrification are thought to be much greater (C.A.S.T., 1976) but only recently have simple techniques become available to estimate them. The techniques depend upon the inhibition of nitrous oxide reductase by acetylene (Yoshinari & Knowles, 1976; Smith *et al.*, 1978) allowing the nitrogen to be emitted in equivalent amounts in the readily measured form, nitrous oxide. Field and laboratory techniques using acetylene to measure denitrification have been described (Ryden *et al.*, 1979*a*, *b*; Veen *et al.*, 1979; Lippold *et al.*, 1981). We now report laboratory incubations using acetylene, comparing the potential for denitrification in soils from two arable sites, both classified as Denchworth series, but developed on different parent clays and following either many years of arable (the Brimstone site) or grassland (the Compton Beauchamp site). We also report field observations on the use of acetylene to estimate the total gaseous losses of N in a clay soil under grass and under winter wheat.

EXPERIMENTAL

Soils

The measurements were made on non-calcareous clay soils (stagnogleys, Denchworth series; Jarvis, 1973) from two sites in Oxfordshire where long-term cultivation experiments were in

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progress comparing the growth of winter wheat after direct-drilling and after conventional tillage (Cannell, 1979; Cannell *et al.*, 1980). The soil at the Compton Beauchamp site has developed on Gault clay and had been in grass for at least 20 years before 1974; part of the site still remains under permanent grass. The soil at the Brimstone site has developed on Oxford clay and was sampled after 4 years of arable cropping that had followed many years in an arable/grass rotation. The 0–20 cm topsoils contain 450–500 g kg^{-1} clay and in their undisturbed state have a strong fine angular blocky structure. The structure becomes prismatic below 30 cm (Jarvis, 1973; Cannell *et al.*, 1980).

Laboratory study on potential rates of denitrification

The soil profiles at both sites were sampled in triplicate by coring (7.5 mm diameter; 60 cm depth) after harvesting winter wheat in August 1980. The cores were sectioned into 0–10, 10–20, 20–40 and 40–60 cm portions and some relevant chemical and physical properties were determined (Table 1).

Table 1. Physical and chemical characteristics of the soils at four depths: pH measured in 1 : 2.5 soil : water slurry, extractable-N in August 1980, organic carbon (g kg^{-1}) and bulk density (D_B, kg dm^{-3})

		mg N kg^{-1}			
Depth (cm)	pH	NO_3	NH_4	Org-C	D_B
Compton Beauchamp					
0–10	6.7	7.2	4.1	37	0.8
10–20	6.7	4.7	4.2	35	0.9
20–40	6.9	1.5	1.8	17	1.1
40–60	6.9	<0.7	<0.8	9	1.2
Brimstone					
0–10	6.6	4.1	2.1	29	0.9
10–20	6.8	1.9	1.6	22	1.0
20–40	7.5	<0.7	<0.8	7	1.3
40–60	7.8	<0.7	<0.8	5	1.3

Fresh samples from each section of the core, equivalent to 25 g oven dry soil, were weighed into six 175 ml glass bottles (total internal volume 190 ml). The bottles were sealed with plastic caps fitted with three-way nylon taps and rubber septa. Two ml of a potassium nitrate solution (0.044 M; 50 mg N kg^{-1} of soil, equivalent to 100 kg N ha^{-1}, 0–20 cm depth) was added with enough water (approximately 3–8 ml) to bring the water content of the soils to their maximum water holding capacity. All six bottles were flushed with helium for 5 min and 2 ml acetylene per bottle (to give a concentration of 1%) was added to three of the bottles. The bottles were shaken thoroughly and incubated at 20°C, normally for 18 h, in the dark. After incubation, samples (5 ml) of the gas in the bottles were collected into glass syringes for immediate analysis. Six hours after sampling, the bottles were flushed again with helium, and acetylene was added as before but no further nitrate was added (Table 2).

Field study of N_2 emission

Three sub-plots (6 m^2) were chosen at the Compton Beauchamp site, one on an area of permanent grass, one on direct-drilled land and the third on land sown after mouldboard ploughing.

Table 2. Details of incubations of replicated 25 g soil samples incubated (a) under helium, (b) with 1% acetylene, (c) with 2 ml 0.04 M KNO_3 showing number of replicates (n) and length of incubation (t, hours)

Soil samples		Incubation						
						n		
Core	Depth (cm)	n	Date	Day	t (h)	*a*	*b*	*c*
CB1,	0–10	6	19/8	1	4	6	3	6
BR1	10–20	6	20	1	18	6	3	0
	20–40	6	21	2	18	6	3	0
	40–60	6	22	3	18	6	3	0
CB2,	0–10	6	27/8	1	18	6	3	6
BR2	10–20	6	28	2	18	6	3	0
	20–40	6	29	3	18	6	3	0
	40–60	6						
CB3,	0–10	6	2/9	1	18	6	3	6
BR3	10–20	6	3	2	18	6	3	0
	20–40	6	4	3	18	6	3	0
	40–60	6	5	4	18	0*	0	0
			6–8	5+	90	0*	0	0

* On these occasions the bottles were not flushed out at the beginning of the incubation.

The rate of nitrous oxide emission was measured using flux chambers, i.e. rings 30 cm deep of 30 cm diameter plastic pipe, inserted into soil to give a 5 litre volume above the soil surface (Burford *et al.*, 1981). Industrial grade acetylene was passed via a distribution-board comprising a valve, a pressure gauge and four flow meters, to sintered glass gas-distribution tubes set in sleeves of 17 mm diameter plastic pipe and inserted obliquely in the soil beneath the centre of the flux chamber. Acetylene was allowed to flow into the soil (line pressure 0.7 bar) for 3–4 h each day around midday, 1 h at 200 ml min^{-1} was followed by 2–3 h at 40 ml min^{-1} to aid distribution through the soil profile and penetration of the finer pore space. The dry soil in June needed faster rates of flow (500 and 100 ml min^{-1}) to maintain the target concentration, based on values stated in the literature (Yoshinari & Knowles, 1976; Ryden *et al.*, 1979*a*) of 0.1–2% in the soil gas space. Acetylene was injected at 10, 20, 40 and 60 cm depth on 14, 15 and 16 April and only at 40 cm on 2, 3, 9, 10 and 23, 24 June.

The acetylene concentration in the soil atmosphere was measured using sampling probes made from lengths of 21 mm diameter plastic pipe, with the ends covered by fine-mesh plastic screens to form chambers approximately 10 ml in volume. These were inserted in the soil to 15 cm and 30 cm depth at 20 cm and 40 cm from the centre of the flux chamber.

Sampling equipment was installed on the sub-plots immediately after sowing of the winter wheat in October 1979. Acetylene was applied to the grass sub-plot in April 1980 and to the cultivated sub-plots in June. On the grassland, the soil beneath one flux chamber was treated with acetylene and another was used as a control, but on the cultivated land two flux chambers were treated and four kept as controls on each sub-plot.

Nitrous oxide and acetylene fluxes were measured using glass syringes to take 5 ml aliquots of the atmosphere within the flux chamber at 0, 2, 4, 6, 8 and 10 min or 0, 5, 10 and 15 min after closing it. The longest time interval was employed when fluxes were small to increase precision. The flux rate was calculated from the rate of increase in nitrous oxide concentration in the chamber (Burford *et al.*, 1981), and a similar calculation was used for the rate of emission of acetylene. Samples (2 ml) of the soil atmosphere obtained from probes were analysed for acetylene concentration.

Ammonium nitrate solution (0.125 M) was applied to the grass sub-plot in two equal applications equivalent to 70 kg N ha^{-1} (35 mg N kg^{-1}, 0–20 cm depth) on 10 and 11 April followed by a third application on 15 April bringing the total to 210 kg N ha^{-1} to ensure that nitrate supply did not become limiting as a result of uptake by the grass sward. The supply of nitrogen made available in the soil (0–20 cm depth) was therefore 53 mg N kg^{-1} ammonium and 53 mg N kg^{-1} nitrate. One acetylene treated flux chamber and one control on each of the winter wheat sub-plots received a similar application (70 kg N ha^{-1}) on 10 June.

Measurements at an automatic weather station on the site gave estimates of rainfall and potential evaporation on the plots. Irrigation was measured and applied by hand (see Figs 2 and 3). Soil temperature ranges were 9–16°C in April and 13–24°C in June (Figs 2 and 3). Rainfall amounted to 78 mm in March but only 11 mm in April (Fig. 2). The irrigations in April added an additional 24 mm. By the beginning of June the soil moisture deficit at the site was close to 70 mm and this was only reduced to 63 mm by the rainfall in the middle of the month (Fig. 3) because of the high potential evaporation. The irrigations in June (36 mm, Fig. 3) reduced the soil moisture deficit further.

Analytical methods

Nitrous oxide was estimated using a gas chromatograph equipped with a backflush system to remove acetylene (Hall & Dowdell, 1981). Acetylene was measured by gas chromatography with a flame ionization detector after separation on Poropak-T. Nitrite, nitrate and

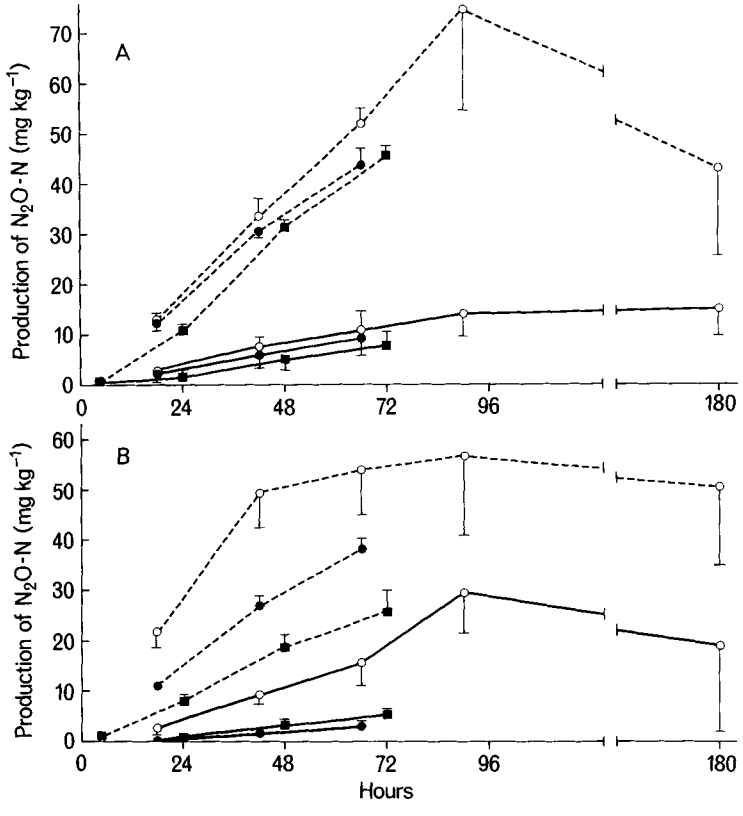

Fig. 1. Denitrification in Denchworth series clay soils: (A) Compton Beauchamp; (B) Brimstone, at two depths 0–10 cm (broken line), 20–40 cm (solid). Means are of triplicate laboratory incubations with standard error bars. Three soil cores from each site were sampled (CB1, BR1, ■; CB2, BR2, ●; CB3, BR3, ○) and subsamples were incubated with added nitrate in the presence of acetylene (see Table 2).

Table 3. Nitrous oxide production from the two soils incubated under helium either with (A) or without acetylene (B) for individual days and the total (∑) for 72 h

		N_2O (mg N kg^{-1})			
		Day			72 h
	Depth (cm)	1	2	3	∑(SE)*
Compton Beauchamp					
(A)†	0–10	15	20	14	49 (4)
	10–20	13	19	16	48 (9)
	20–40	3	5	3	16 (1)
	40–60	1	2	1	4 (1)
(B)†	0–10	13	10	8	31 (9)
	10–20	8	8	7	23 (8)
	20–40	2	3	3	8 (1)
	40–60	1	2	1	4 (1)
Brimstone					
(A)†	0–10	16	18	8	42 (11)
	10–20	15	14	5	34 (13)
	20–40	2	4	4	10 (3)
	40–60	1	2	3	6 (2)
(B)†	0–10	15	15	4	34 (6)
	10–20	11	6	1	18 (4)
	20–40	<1	1	<1	<2 (1)
	40–60	<1	<1	<1	<2 (<1)

* SE = standard error.
† (A–B) = the reduction of N_2O to N_2 in the absence of acetylene.

ammonium were measured colorimetrically on 1 M KCl extracts (10 g soil : 500 ml KCl) using an autoanalyser (Brewer & Riley, 1965; Crooke & Simpson, 1971). Organic carbon was measured colorimetrically on representative subsamples of the soils by the method of Walkley & Black (1934).

RESULTS AND DISCUSSION

Laboratory study

N_2+N_2O production. Both soils had the potential to denitrify rapidly large quantities of added nitrogen (Fig. 1, Table 3).

Potential rates of gaseous nitrogen loss can be estimated from our results. The maximum daily rate of N_2O production from the Compton Beauchamp 0–10 cm topsoil in the absence of acetylene was 13 mg N kg^{-1} (Table 3). Taking the weight of a hectare of soil to the depth of 10 cm as 10^6 kg (bulk density approximately 1 kg dm^{-3}, see Table 1), this rate of N loss as N_2O was equivalent to 13 kg N ha^{-1} per day and the maximum loss from the same soil layer in the presence of 1% acetylene was 20 kg N ha^{-1} per day. Similarly, the 0–10 cm Brimstone soil denitrified at maximum daily rates of 15 kg N ha^{-1} as N_2O and 18 kg N ha^{-1} in total (N_2O + N_2). In total, the maximum potential daily gaseous nitrogen losses from the top 20 cm of these two soils were 39 ± 6 and 32 ± 11 kg N ha^{-1} when the nitrate supply was plentiful.

The mole fraction of nitrous oxide in the denitrification product was smallest (0.2) in Brimstone subsoil 20–60 cm, but was otherwise 0.5 or greater despite the oxygen concentration being consistently less than 1.5% (mean ± standard error, n = 128, 0.5% ± 0.4).

Possibly the maximum rate of N_2 production had not been achieved by the end of the study. Letey *et al.* (1980) found N_2 emission was still increasing after 7 days from a loam incubated under similar conditions. In accordance with the results of other incubation studies (Matsubara & Mori, 1968; Firestone & Tiedje, 1079; Letey *et al.*, 1980), the maximum N_2O release (day 1) preceded the maximum N_2 release (days 2 or 3).

Soil effects. The amount of N lost as N_2 in the presence of acetylene during 3 days from the 0–10 and 10–20 cm Compton Beauchamp soil almost equalled the amount of nitrate added initially (Table 3). The Compton Beauchamp topsoil (0–10 cm) tested beyond 3 days (CB3) continued to denitrify (Fig. 1A) so that the total N_2O–N produced in the presence of acetylene (75 ± 20 mg N kg^{-1}, $n = 3$, equivalent to 75 kg N ha^{-1}, 0–10 cm) exceeded the sum of the NO_3^-–N added initially and the endogenous extractable soil-N reported in Table 1, but the Brimstone soil did not continue denitrifying in the same way (Fig. 1B). The pattern of response was consistent for all three soil cores from Compton Beauchamp (Fig. 1A) but the Brimstone soil cores were more diverse (Fig. 1B). One of the Brimstone soil cores, 0–10 cm depth (BR3), maintained a fast initial rate (1.6 mg N kg^{-1} h^{-1}, equivalent to 38 kg N ha^{-1} per day) for nearly 40 h and subsequently slowed to a second phase (0.2 mg N kg^{-1} h^{-1}, 5 kg N ha^{-1} per day) which was slower than the mean rate of the other two replicate samples (0.45 mg N kg^{-1} h^{-1}, 11 kg N ha^{-1} per day). This variability is inherent in the soil as sampled from the field and may reflect past management practices such as old drainage systems or variations in soil parent material.

The top layers (0–20 cm) of both soils denitrified significantly more of the added nitrogen than the subsoils (20–60 cm). The production of N_2O correlated positively with the carbon content of the soil ($r = 0.98$***) and negatively with soil depth ($r = -0.32$**) (Tables 1 and 3) which can be understood because denitrification depends on the readily oxidizable energy sources present in the soil (Burford & Bremner, 1975) or added to it (Jacobson & Alexander, 1980). Maximum reduction of N_2O to N_2 took place in the 10–20 cm soil (Table 3) suggesting that the population of N_2O reducing bacteria or enzyme activity was potentially greater at that depth whereas NO_3^- and NO_2^- reducers or reductases were potentially more active nearer the soil surface. This distribution of microbial activity may result from larger amounts of nitrate in the surface soil or less available oxygen below 10 cm. A depth factor related to denitrifier populations or enzyme activity has not been reported from soil column studies, perhaps because the experimental conditions have given either nearly all N_2O (Gilliam *et al.*, 1978) or all N_2 (Starr *et al.*, 1974).

Acetylene blocking. The N_2O concentration decreased when the bottles containing samples of Compton Beauchamp soil (CB3, Table 2) were left unflushed for a further 90 h (Fig. 1). The decrease in N_2O concentration after prolonged incubation with acetylene, which suggests that nitrous oxide reductase had become adapted to acetylene and was no longer blocked by it, is consistent with observations made by Yeomans & Beauchamp (1978). Ryden *et al.* (1979*a*) observed that this reduction of N_2O in the presence of acetylene occurred when the nitrate had been exhausted which may suggest an interaction between acetylene and nitrate. Recently Kaspar (1982) reported that acetylene blocking was ineffective in marine sediments with nitrate concentrations of 5 or 10 μM. However, the decrease was not entirely consistent. One replicate of soil CB3 0–10 cm showed no significant change during the 90 h (from 2300 to 2400 μl N_2O l^{-1}). In contrast 98% of the N_2O was lost from the other two replicates, mean: 4500 decreased to 90 μl N_2O l^{-1}). All three replicates of soils CB3 10–20 cm; BR3 0–10 cm; and BR3 10–20 cm gave similar decreases of 95–98% (means: 4600–230; 460–10; 100–5 μl N_2O l^{-1} respectively) but there was little if any decrease with acetylene treated subsoils (20–60 cm). As one would expect, the N_2O concentration of the controls with no acetylene also decreased: for soil CB3 10–20 cm the decrease was 99.9% (3000 ± 860 to 3 ± 1 μl N_2O l^{-1}).

The use of acetylene could cause changes in the biological system. We assumed, in the absence of any firm indication in the literature, that 0.1–2% C_2H_2 was unlikely to cause any major change in the soil microbial populations. Germon (1980) has now reported that 2% acetylene increased the rate of nitrate reduction in soil and more recently Gross *et al.* (1982)

have identified the acetone impurity in commercial acetylene as providing a substrate for denitrifiers. The increase due to commercial acetylene averaged 40% more than nitrate reduction in the absence of acetylene by three soils after 4, 11 or 18 days incubation (Germon, 1980). As the 2% limit was exceeded in our experiments (Fig. 2) this excess acetylene could have modified the soil environment by excluding oxygen or providing a carbon source for soil microbes. However, much of the excess acetylene would have been lost quickly, especially from the drier, ploughed soil. The acetylene contained acetone which supplied the treated soil with carbon potentially available for microbial growth. Its effect in these soils is not known but it has been recently stated that it promoted denitrification in Iowa soils (Gross *et al.*, 1982). Further work is needed. In subsequent work we have moved site between successive measurements.

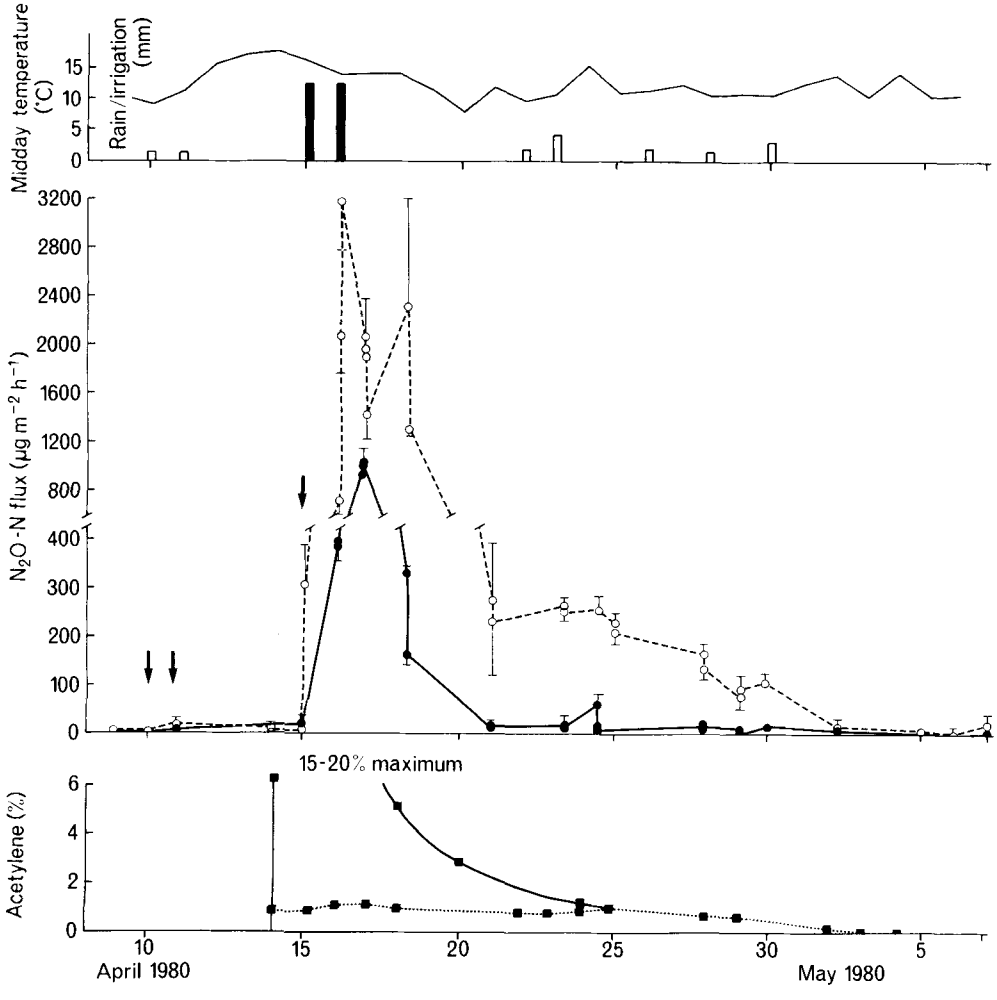

Fig. 2. Effects of ammonium nitrate fertilizer and irrigation on the nitrous oxide flux from a permanent grass sward on acetylene treated (broken line) and control (solid) Denchworth series clay soils, April 1980. Individual flux, calculated from N_2O concentration of air in closed chamber, with standard error bars. Arrows indicate applications of nitrogen fertilizer. Irrigation (solid), rainfall (open) and midday air temperature are shown above. Acetylene concentration in soil air at 15 cm depth in acetylene treated soil, measured (dotted line) and calculated (solid line, see text), shown below.

Field study

N_2+N_2O flux. The measured nitrous oxide flux from the clay soil under grass following treatment with acetylene in April (Fig. 2), gave a total gaseous nitrogen loss of 2.1 kg N ha^{-1} over 21 days, three-quarters of it as N_2, that is 2% of the fertilizer-N. The initial peak (Fig. 2) followed the addition of ammonium nitrate when the nitrate available in the pasture soil was only small (less than 0.1 mg l^{-1}, 7 μM, in soil solution). Webster & Dowdell (1982) measured similar N_2O peaks following fertilizer applications to permanent grass in lysimeters especially with coincident rain or irrigation. After the initial peak caused by fertilizer and irrigation, the nitrous oxide flux from the acetylene treated soil became steady at 210 μg N m^{-2} h^{-1} (0.05 kg N ha^{-1} per day) about 10 times that from the untreated soil and 100 times the flux before the fertilizer was applied. Ryden (1981) measured similar rates on another grassland site in southern England in the same year. Contrary to results of laboratory experiments (Yeomans & Beauchamp, 1978), the inhibitory effect of acetylene seemed to last for 10–12 days (see below). The nitrous oxide flux from treated soil decreased almost simultaneously with the disappearance of acetylene from the soil.

The total gaseous nitrogen losses (N_2O+N_2) in June from the cultivated clay soil were 0.4–0.7 kg N ha^{-1} over 20 days, i.e. up to 1% of the fertilizer-N. The results from both the

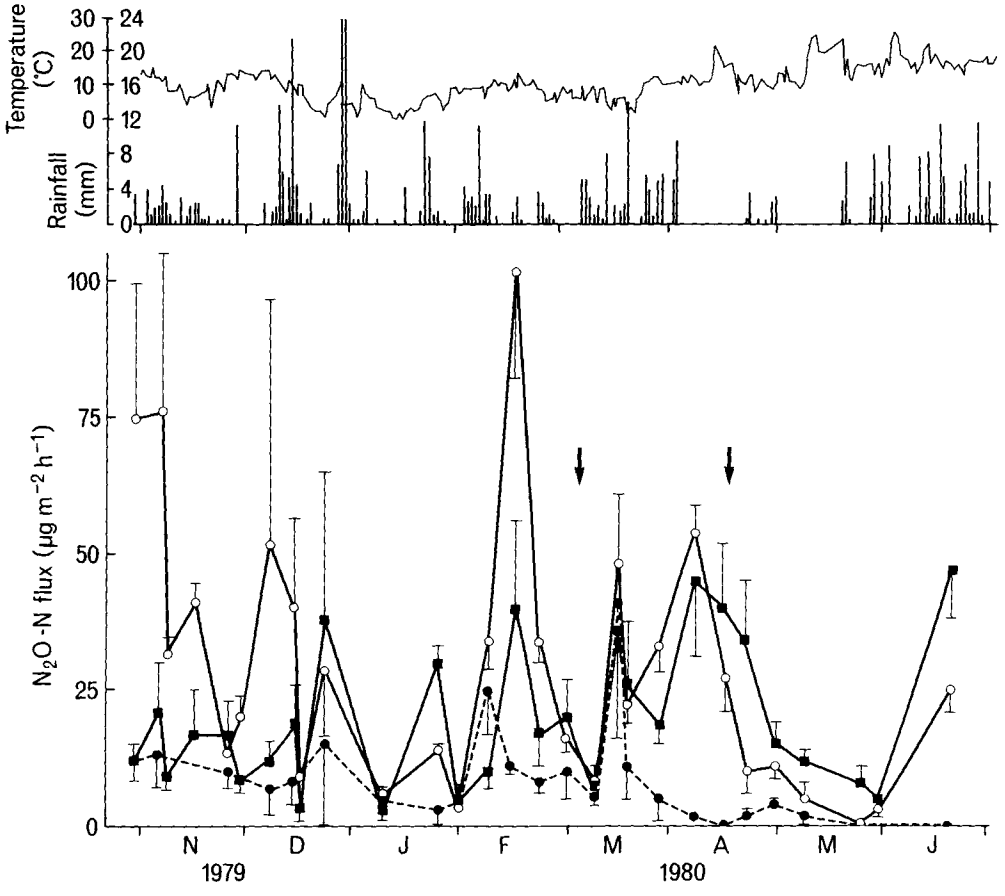

Fig. 3. Nitrous oxide flux from Denchworth series clay soil in the field under three management systems, October 1979 to June 1980. Means of duplicate fluxes from permanent grass (broken line), and winter wheat (solid line) on direct-drilled (○) and ploughed plots (●) with standard error bars. Arrows indicate nitrogen fertilizer applications to the winter wheat. Rainfall and 09.00 hours air temperature shown above.

Table 4. Nitrous oxide emission from direct-drilled (DD) or ploughed (PL) Denchworth series clay soil, June 1980, showing the effects of adding ammonium nitrate fertilizer at 70 kg N ha^{-1} ($+NH_4NO_3$)

			N_2O (kg N ha^{-1})			
			DD		PL	
Date	No. days	C_2H_2	x*	(SE)*	x	(SE)
3–9 June	6	0	0.02	0.003	0.03	0.004
		+	0.03	0.006	0.05	0.009
10–27 June	17	0	0.25	0.07	0.20	0.05
		+	0.6	0.2	0.6	0.2
10–27 June $+NH_4NO_3$	17	0	0.4	0.1	0.5	0.1
		+	0.6	0.2	0.7	0.2

* x = mean, SE = standard error.

direct-drilled and ploughed soils have been averaged because the nitrous oxide fluxes did not differ significantly between them in this experiment (Table 4). Nitrous oxide fluxes had differed between the two cultivations earlier in the growing season, particularly in November 1979 and February 1980 (Fig. 3), and in other seasons (Burford *et al.*, 1981). Rainfall after 9 June (Fig. 4) increased the rate of nitrous oxide emission (Table 4). Fertilizer nitrogen increased N_2O emission from soils not treated with acetylene (Table 4).

The loss of nitrogen as nitrous oxide from the three soil management systems between November 1979 and June 1980 (Fig. 3) was 0.4, 1.3 and 1.1 kg N ha^{-1} for grass, direct-drilled and ploughed respectively. These were low compared to previous years' results (Burford *et al.*, 1981) because there were smaller nitrous oxide losses following fertilizer applications in March and April associated with low temperatures in mid-March and low rainfall in April (Fig. 3).

Acetylene distribution in the field soil. The distribution of acetylene in the soil was not a problem in laboratory incubations but could be in the field especially when the soil is a well-structured clay like the Denchworth series. Ryden *et al.* (1979*b*) evaluated acetylene distribution in a horticultural loam in the Santa Maria Valley in California and theoretically by analogy to heat flow and using a gas diffusion equation of unsaturated conditions. In theory, if an adequate concentration is established in the large pore space, diffusion of the gas to the centre of small soil structures takes only minutes; however, for structures greater than 5 cm radius it would take hours, if not days. We measured acetylene concentrations in the large air filled pore space of the soil in the field. For a more precise evaluation of acetylene distribution a micro-sampling technique would be needed.

In April, the target of 0.1–2% acetylene was attained at 15 cm (Fig. 2). At 30 cm depth the concentration exceeded 1% for 12 days. The concentration declined with time after injection, with distance from the centre of the flux chamber and towards the soil surface. Acetylene fluxes exceeded 1 ml m^{-2} min^{-1} (1.4 l m^{-2} per day) for 12 days.

Initially the method of analysis used for acetylene greatly underestimated concentrations greater than 1%. The probable acetylene concentrations (Figs 2 and 4) have been calculated from more reliable measurements made later on the same soils under similar conditions, using the same rates of addition. The high acetylene concentrations (>10%) present in the soil atmosphere would have made oxygen concentrations proportionately smaller but would have caused only transient anaerobic volumes. For a few days, acetylene would have been about equivalent to oxygen in terms of their respective concentrations in free air space. The acetylene concentration in free air space would have promoted diffusion of acetylene into the

anoxic zones where nitrous oxide was being reduced to nitrogen and therefore increased the effectiveness of the reductase blocking action. A proportion of the acetylene would have dissolved in the soil water and diffused in aqueous phase. However, as acetylene in the air-filled pore space was continuously being lost to the atmosphere this dissolved acetylene diffused to the gas/liquid boundary and came out of solution. It is therefore very significant that the nitrous oxide flux decreased to the same rate as the control coincident with the cessation of a measurable flux of acetylene at the soil surface (Fig. 2) indicative of the acetylene concentration effectively becoming zero in soil solution at this time and ceasing to block nitrous oxide reduction in the anoxic zones.

In June (Fig. 4) the acetylene concentration decreased quickly after the first two injections. Only on 23 and 24 June, after irrigation and rainfall amounting to 70 mm, did the concentration at 15 cm remain near 1%. The acetylene moved quickly in the ploughed soil towards the surface and away from the chamber in early June but on the 23 and 24 June it dispersed less rapidly. The acetylene dispersed more slowly in the direct-drilled soil even when dry; in particular there was noticeably less lateral movement than in the ploughed soil.

Acetylene interaction with soil-N. Nitrification was important in these soils in June. The

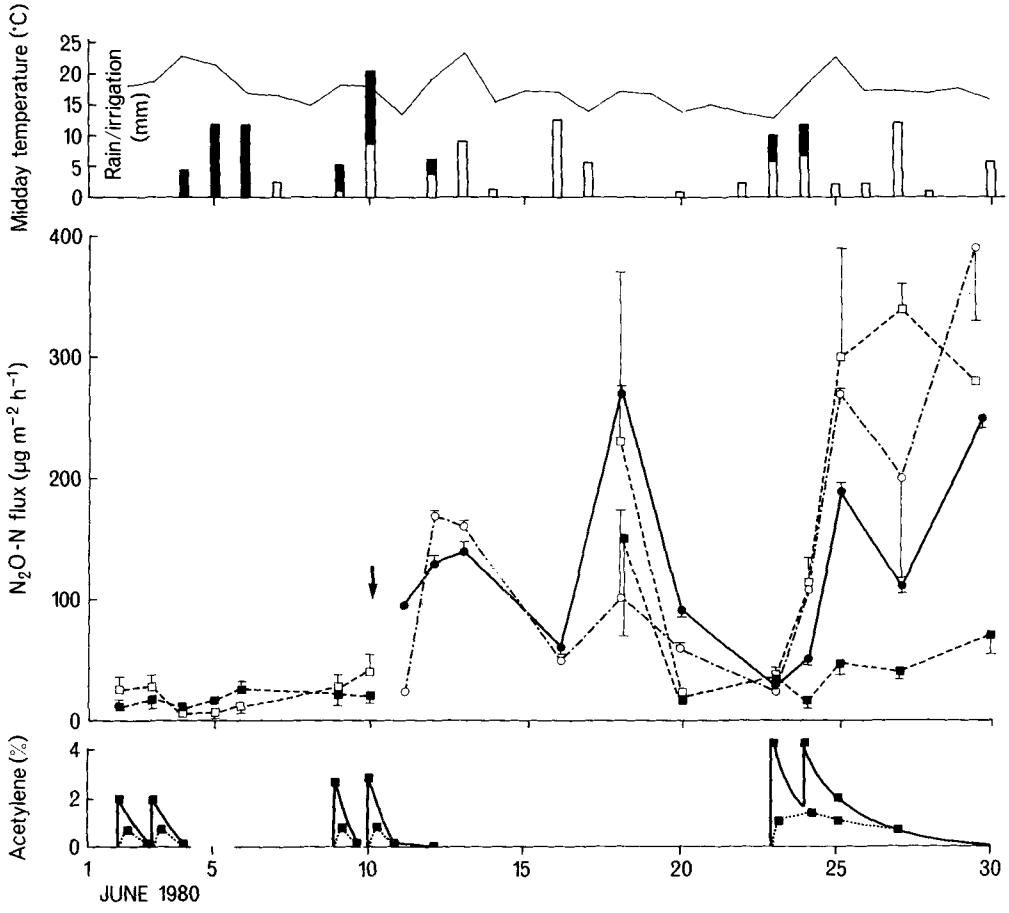

Fig. 4. Nitrous oxide flux from acetylene treated (□, ○) and control (■, ●) cultivated Denchworth series clay soil, June 1980. Arrow indicates application of nitrogen fertilizer to one acetylene treated (○) and one control (●) replicate. Individual flux, calculated from N_2O concentration of air in closed chamber with standard error bars. Irrigation (solid), rainfall (open) and midday air temperature are shown above. Acetylene concentration in soil air at 15 cm depth in acetylene treated soil measured (dotted) and calculated (solid, see text), shown below.

nitrous oxide fluxes from the soils treated with acetylene were 40–290 μg N m^{-2} h^{-1} (0.01–0.07 kg N ha^{-1} per day, Fig. 4) and exceeded those from the untreated soil for the first few days. Subsequently they become smaller. This could be attributed to acetylene blocking nitrification (Hynes & Knowles, 1978) and either cutting off the supply of nitrate to the pool available for denitrification or directly preventing the emission of nitrous oxide from nitrification itself (Bremner & Blackmer, 1978). It cannot be said for certain that nitrous oxide was being evolved from nitrification directly but it remains a possibility. For either reason, nitrification had become the rate determining step for gaseous N losses from the drying soil.

Fertilizer appeared to change the balance of denitrification products. Thus of similar total gaseous losses (25–30 June) the mole fraction of nitrous oxide was 0.60 from soil with added fertilizer but 0.17 from the unfertilized soil (Fig. 4). Nitrate was apparently inhibiting N_2O reduction in the fertilized soil (Blackmer & Bremner, 1978). The rainfall in mid-June considerably enhanced denitrification even in the absence of added fertilizer nitrogen (Table 4). Cycles of mineralization, nitrification and denitrification in rain wetted warm soil could involve relatively large quantities of soil nitrogen during the summer months depending on the pattern of rainfall. In 5 days (25–30 June) 0.36 kg N ha^{-1} were evolved at an average flux of 300 μg N m^{-2} h^{-1} (0.072 kg N ha^{-1} per day, Fig. 4).

Field measurement of denitrification. The acetylene blockage technique needs testing under a wider range of soil conditions. At best, measuring the nitrous oxide flux is once removed from measuring the actual process of denitrification, nitrate reduction. The inhibition of nitrous oxide reduction by acetylene has been seen to be complete in laboratory trials (Smith *et al.*, 1978), but in a wet soil in the field the effect on nitrous oxide flux has been delayed and the apparent response to acetylene prolonged. In the field, the diffusion rate and the diffusion path length for acetylene and nitrous oxide may delay the measured response to acetylene (Jury *et al.*, 1980). Note, for example, the long-term increase in N_2O flux we observed in April compared with the short times in our laboratory study and in the report by Yeomans & Beauchamp (1978). In a drier soil, or when nitrogen has been supplied to the soil surface as fertilizer, the relationship between the denitrification process and the measured nitrous oxide flux is much closer and the response is much more rapid (Jury *et al.*, 1980). Measurement of the nitrous oxide concentrations in the soil profile during treatment with acetylene would facilitate interpretation of the results in any future experiments.

Clearly the total gaseous N losses measured in these field trials fall well short of the potential measured in the laboratory. The conditions in the field, especially oxygen concentrations, limited the losses at the time of study. However, conditions in the field approach those used in the laboratory incubation if heavy rain follows a fertilizer application; gaseous losses would then represent a large fraction of the fertilizer applied. Potentially 30–40 kg N ha^{-1} per day can be denitrified in these clay soils but in the field we measured less than 0.1 kg N ha^{-1} per day.

ACKNOWLEDGEMENTS

We thank our colleagues at Letcombe Laboratory for their assistance. The work was funded by the Ministry of Agriculture, Fisheries and Food. One of us (M.M.I.) acknowledges financial assistance by the International Atomic Energy Agency under their Technical Assistance Programme 1980.

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(Received 5 October 1982)