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Denitrification Losses from a Clay Soil Measured by Acetylene Blocking

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ABSTRACT

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A study was made to assess denitrification loss from arable land. An acetylene-blocking technique was used to measure denitrification in a poorly drained, arable, clay soil in Oxfordshire, U.K. The denitrification loss from the soil, in this field experiment, was estimated as greater than 5 kg N ha^{-1} between October and June. The fastest loss of N_2O was in the autumn with emission of N_2O to the atmosphere estimated as 0.5 kg N ha^{-1} between October and March, and 0.2 kg N ha^{-1} after spring fertiliser applications. Acetylene depressed production of N_2O in drier soils in late spring. Soil-atmosphere analysis and platinum electrodes, used to measure redox potentials in the soil, provided estimates of the soil volume involved in the denitrification process. In autumn, denitrification occurred throughout the top 60 cm of the soil, but in late winter it virtually stopped because of lack of nitrate. In a 30-day period after the spring fertiliser applications it was restricted, by advancing aeration in the drying soil and by nitrate distribution, to about 15% of the soil volume. The disruption of denitrification by aerated autumn rain and the dominance of N_2O in spring, both indicate that denitrification occurs in thin anoxic zones on ped surfaces. The diffusion of acetylene in wet, winter soils was slow and may have caused underestimation of denitrification. The decline of denitrification in winter was simply a regression with time but, in spring, it correlated strongly with rainfall. Twenty percent of autumn fertiliser-N may have been lost to the atmosphere.

INTRODUCTION

Over 10% of the fertiliser-N for winter cereals in the U.K. has been applied, commonly, in the autumn (Cooke, 1980). A large part of this autumn N has been lost, either by leaching (Dowdell et al., 1987) or by denitrification. A 10% loss of autumn fertiliser-N represents some 4.5 10^3 tonnes of N contributing to the atmospheric balance of N_2O. The overall objective of this study was to assess this loss.

Acetylene blocking of the second stage of the denitrification process in soils has been used in field studies for several years (Ryden et al., 1979; Colbourn et al., 1984). In this technique, the acetylene stops the biological reduction of the intermediate product, nitrous oxide (N_2O), to nitrogen gas (N_2) (Yoshinari and Knowles, 1976). The distribution of acetylene in uniform loamy soils appears to be adequate (Ryden et al., 1979). However, the use of acetylene blocking in clay soils raises questions as to the effectiveness of the method under these conditions (Colbourn et al., 1984; Colbourn and Harper, 1988). Good measurements of denitrification on clay soils were needed as part of a study of nitrogen losses from arable land (Colbourn, 1985).

The objectives of this study were: (1) to provide estimates of the annual nitrogen loss from the soil owing to denitrification; (2) to measure seasonal patterns of denitrification and to relate them to soil conditions; (3) to quantify N_2O released from the soil and to estimate the annual input to the atmosphere; (4) to assess the practical application of acetylene blocking by taking measurements on a poorly drained, uniform clay soil.

METHODS

Experimental design and practice

The field equipment and operational procedures used by the acetylene-blocking technique were developed from those described by Colbourn et al. (1984). A random-block design was used, having 4 blocks each comprising 8 (2×2 m) plots. Within each plot, half was assigned randomly to be treated with acetylene and half remained untreated. Within each block, the 8 plots were used in sequence to avoid any complications arising from the repeated use of acetylene at one location. Hence, the plots gave information on season, since the plots represented a succession of measurements from autumn through to spring.

Acetylene from an industrial gas cylinder (British Oxygen Company plc) was bubbled through water to trap acetone, and led to each block through 6-mm nylon tubing. Only one plot in each block was actively treated at any one time. Acetylene was distributed in the soil from one sintered-glass gas-distribution tube (porosity 1, Gallenkamp, Loughborough), set into a stem of plastic pipe (Durapipe ABS 3/8 class T), installed vertically at the centre of the treated half of the plot, 20-cm depth. Acetylene was allowed to infuse into the soil at 100 ml min^{-1} for 1 h and for 2 h at 40 ml min^{-1}.

Acetylene was applied first on 27 October 1981. In some conditions acetylene was also applied to the soil surface at a concentration of 25% in a closed chamber 30-cm diameter, 7-cm high, for 1 h. This surface application of acetylene ended half an hour before the measurement of nitrous-oxide flux started, to allow time for equilibration within the soil. The distribution of acetylene was checked at intervals by sampling the soil atmosphere around the gas-distribution tube. The observed distribution was compared with gas diffusion rates in similar soils (Ball et al., 1981). The diffusion coefficient for acetylene in air was taken to be 0.15 $cm^2 s^{-1}$ and 1.1 $10^{-5} cm^2 s^{-1}$ in water.

Sampling and measurement in the field

The flux of nitrous oxide from the soil surface was measured by using a closed chamber, 30-cm diameter and 7-cm high, as described by Colbourn et al. (1984). At the time of sampling the chamber was closed by a metal lid weighted against a rubber seal on the top edge of the chamber. Gas samples were withdrawn from the chamber in 5-ml glass syringes at 2-min intervals: 0, 2, 4, 6 and 8 min. The pistons of the syringe were carefully greased with silicone vacuum grease to prevent gas exchange. The filled syringes were closed by 3-way syringe stopcocks (Nipro, Henlys Medical Supplies, London). Samples were taken daily following acetylene treatment.

Soil samples were collected from 0–10-cm topsoil on 22 September and 7 October 1981 and from 0–30-cm soil on 18, 19 and 25 January 1982. Soil water or soil atmosphere samples were collected routinely once a week at depths 7.5, 15, 30, 45 and 60 cm. The soil atmosphere samplers were sintered bronze cups, similar to those used by Dowdell et al. (1979).

Redox potential and oxygen flux were measured with platinum electrodes at 15, 30 and 45-cm depths and a silver/silver chloride anode contacting the wet soil surface.

Analytical techniques

Gas samples were analysed by gas chromatography as described previously (Hall, 1978; Hall and Dowdell, 1981; Colbourn et al., 1984). Soil samples were extracted with 1 M KCl and analysed colorimetrically by an auto-analyser system as described in earlier work (Colbourn et al., 1984).

Description of study site

The random-block design was established on direct-drilled arable land adjacent to the Brimstone field trial described by Cannell et al. (1984). Some details of the pelo-stagnogley soil are given in Table 1. A former cultivation pan had been broken in 1979 and direct-drilling management was imposed in 1980. Mean annual rainfall has been estimated as 680 mm (Anonymous, 1984). The rainfall distribution during the course of this experiment is shown in Fig. 1. The land had no artificial drainage, all the old drainage systems had been blocked (Harris et al., 1984).

Winter wheat was sown on 8 October 1981 with fertiliser containing 24 kg N ha^{-1}. In the previous spring, 1981, a total of 150 kg N ha^{-1} had been applied

TABLE 1

Characteristics of the Denchworth series soil

Depth (cm)	Clay 2 μm^1 (%)	Organic-C^2 (g kg^{-1})	Total-N^2 (g kg^{-1})	BD^3 (g cm^{-3})	Porosity^3 (%)
0-15	54	28	3.2	1.0	66
15-30	56	10	1.5	1.2	57
30-45	60	7	1.0	1.3	50

[1] From Cannell et al. (1984).
[2] From Colbourn et al. (1984).
[3] From Douglas et al. (1980).
BD = Bulk density.

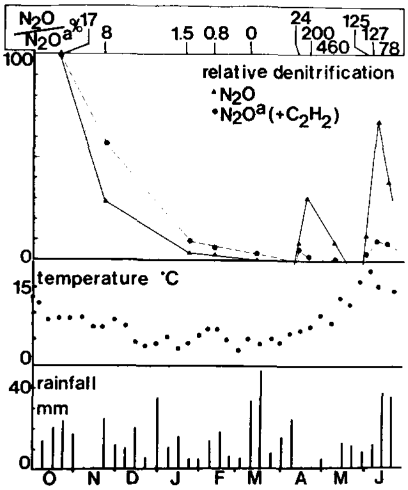

Fig. 1. Rainfall, temperature and denitrification at the field site, October 1981–June 1982: rainfall plotted as the weekly total (mm), weekly mean soil temperatures measured at 15-cm depth, and denitrification plotted relative to the rate in October 1981 (= 100). The rates of N_2O emission and N_2O emission after treating the soil with acetylene are shown. The ratios of the two rates are given as percentages at the top.

in wet conditions to the winter wheat crop which was harvested on 20 August 1981. In the spring of 1982, fertiliser (NH_4NO_3) was applied on 24 March, 14 and 29 April at 35, 57 and 57 kg N ha^{-1}.

RESULTS

Acetylene distribution in the soil

Acetylene diffused outwards from a small acetylene-saturated volume of soil close to the gas-distribution tube representing a total pore volume of 200–500

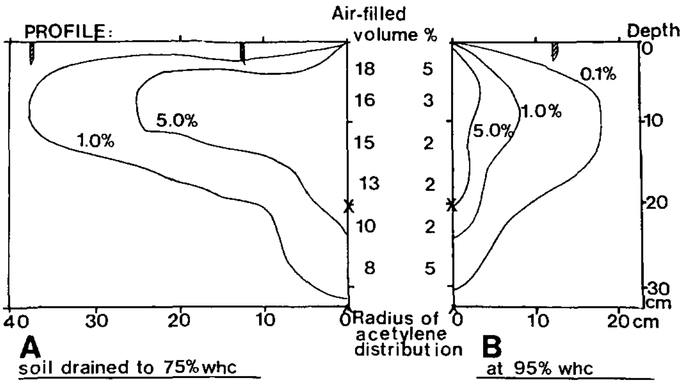

Fig. 2. The distribution of acetylene from a gas distribution tube placed centrally at 20-cm depth (X) in the Denchworth series soil, 24 h after infusion: (A) dispersion in the soil profile at field capacity (75% water-holding capacity), vertical section showing air-filled porosities at 6 depths; (B) dispersion in a wet soil profile (95% water-holding capacity), vertical section showing air-filled porosities at 6 depths.

cm^3 (total porosity 57% at 20-cm depth; Douglas et al., 1980). Diffusion was most rapid in the more open surface soil, giving concentrations greater than 0.1% to 35-cm depth within a 45-cm radius at field-capacity water content. Concentrations greater than 1% persisted near the distribution tube and in the 0–12-cm soil layer for over 24 h (Fig. 2). In wetter soil conditions, dispersion was restricted to a radius of 20 cm at concentrations greater than 0.1% after 24 h.

Diffusion rates, calculated from appropriate diffusion coefficients (Ball, 1981), gave comparable distributions after 24 h.

Oxygen in the soil: aerobic/anaerobic interface

Low oxygen concentrations ($<10\%$) were common in topsoil during the winter months, below 20 cm, from November to April, and persisted deeper in the soil until May (Table 2). Nitrous-oxide concentrations in soil air were unstable during the autumn ($\leqslant 60\ \mu l\ l^{-1}$). In the presence of acetylene, much more N_2O accumulated ($\leqslant 400\ \mu l\ l^{-1}$) which persisted longer (Table 3). Smaller amounts accumulated in late January after the thaw ($\leqslant 30$–$70\ \mu l\ l^{-1}$; $+C_2H_2$) but little thereafter.

Redox potentials at 3 depths showed the return of aeration in spring. Uniformly reduced conditions in March gave way to full aeration by mid-May. Redox potentials characteristic of denitrification were found deeper in the soil as time went on, but were most frequent at 30-cm depth.

An analysis of the redox potentials shows the distribution of the aerated and the potentially denitrifying volumes in the soil profile during the spring of

TABLE 2

Oxygen in soil air during autumn, winter, spring and summer; mean concentrations

Depth (cm)	Oxygen (%)[1]			
	Oct.-Dec.	Jan.-March	March-May	May-June
5-10	18	18	20	21
10-20	16	12	17	20
20-40	10	11	10	18
40-60	5	6	6	14

[1] Average coefficient of variation = 0.6. Typical coefficient of variation at 5-10 cm = 0.2; 10-20 cm = 0.4; 20-40 cm = 0.7; 40-60 cm = 1.2.

TABLE 3

Nitrous oxide in soil air during autumn, winter, spring and summer; mean concentrations

Depth (cm)	Nitrous oxide (μl l^{-1})[1]							
	Oct.-Dec.		Jan.-March		March-May		May-June	
	0	+	0	+	0	+	0	+
5-10	8	450	2	20	1	1	1	1
10-20	12	380	2	24	1	2	1	1
20-40	11	600	1	18	1	1	1	1
40-60	7	660	1	14	1	3	1	1

[1] 0 = no acetylene, + = following acetylene infusion. Average coefficient of variation = 1.3. Typical coefficients of variation at 5-10 cm = 1.0; 10-20 cm = 1.2; 20-40 cm = 1.5; 40-60 cm = 1.7.

1982. The frequencies of observations within specified 100-mV ranges were mapped onto charts for each period. The resulting distribution was contoured and regions were designated as 'aerated' (>400 mV), 'denitrifying' (0–400 mV), or 'reduced' (<0 mV) and the relative soil volumes in each category were estimated (Fig. 3).

Nitrate availability

Nitrate concentrations were unstable in the autumn (3–17 μg N g^{-1} soil, 0–10 cm; 8–23 μg N ml^{-1} soil water) showing cycles of net nitrification and net 'loss' related to rainfall. After a small increase late in January, nitrate declined to a minimum (0.2 μg N ml^{-1}) by 11 March. Fertiliser increased nitrate concentrations to 75 μg N ml^{-1} in April.

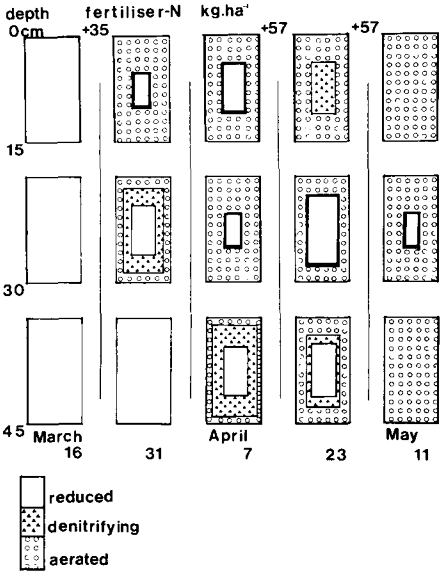

Fig. 3. Aerated and denitrifying soil volumes at 3 depths in the Denchworth series soil profile on 5 occasions, March–May 1982: cross-hatched = aerated volume, solid = denitrifying, open = reduced volume. The numbers indicate the percentage in each volume as estimated from 10 Eh electrodes at each depth.

Nitrous-oxide flux

The fastest flux of nitrous oxide per se from the soil surface was observed in the autumn (Table 4). It declined through the winter to negligible values in March, but responded to nitrogen fertilisers in the spring. The loss of nitrous oxide was probably less than 0.5 kg N ha^{-1} between October and March and less than 0.2 kg N ha^{-1} in the spring.

Nitrous-oxide flux after acetylene infusion

In the presence of acetylene, the flux of nitrous oxide from the soil surface was much faster on all occasions during the autumn and winter (Fig. 1, Table 4). In the autumn, the rate of loss was more than 6 times greater than nitrous oxide alone. Hence, the greater part of the nitrous oxide produced by denitrifiers was reduced to nitrogen gas. The total denitrification loss approximated to 5 kg N ha^{-1} in the autumn/winter period. In the spring, there was no reduction of N_2O to N_2, on the contrary, the N_2O flux was depressed by acetylene (see N_2O ratios in Fig. 1), probably because it inhibited nitrification. A similar effect had been observed in earlier studies (Colbourn et al., 1984). The response to acetylene was significant ($P<0.01$) on all occasions from October to April except in March. In early April there was a significant response in the

TABLE 4

Statistical analysis of nitrous-oxide flux data: reduction of nitrous oxide to nitrogen gas; undrained, direct-drilled Denchworth series clay soil, 1981-1982

Period	N_2O-N	$(N_2O+N_2)-N$	t^1	P	n
	($\mu g\ N\ m^{-2}\ h^{-1}$)				
1981					
October	75	450	2.934	0.05, *	7
November	20	260	3.618	0.05, *	5
1982					
January	0.7	47	6.036	0.01, **	6
February	0.2	25	3.850	0.05, *	5
March	−0.5	8.5	1.697	0.1, NS	4
March/April	8	33	5.340	0.001, ***	12
April	12	6	2.004	0.1, NS	6
May	6	1.3	1.417	0.1, NS	4
May/June	10	8	1.143	0.1, NS	7
June	52	41	1.537	0.1, NS	6
June/July	29	37	0.987	0.1, NS	11

[1] t = Student's t paired comparison test; P = probability.

nitrous-oxide flux but not in the concentration of the gas in the soil atmosphere (Tables 4 and 5).

The denitrification rate declined steeply from October to March and the association with season was highly significant ($P<0.01$). A regression could be fitted to total denitrification $(N_2O+N_2)-N$ from October to March (Table 5). There was also a significant relationship with rainfall in the spring, but apparently not with temperature alone (Table 5).

Nitrous-oxide flux, after acetylene treatment of the soil, was correlated with the rainfall for the previous n days. The number of days, n, was adjusted by reference to the prevailing temperature (see footnote, Table 5). This adjustment of the number of days contributing effective rainfall improved the relationship significantly. The relationship was improved further by including factors for wet (or dry) days, and for the number of days since fertiliser had been applied. Simply adjusting the rainfall value by dividing it by the number of dry days in the previous 20 days improved the value of the regression coefficient from 0.526 to 0.674. A more subtle adjustment of the rainfall figure improved r further to 0.704:

$$y=0.5R+0.1F+0.7W-0.24$$

where

y, nitrous oxide flux (μg N m^{-2} h^{-1});

R, rainfall (mm), in previous n days;

F, $(30-N)$;

N, number of days since fertiliser was applied;

W, number of wet days (rain > 1 mm) in previous 20 days.

TABLE 5

Regression analyses for autumn/winter, spring/summer, spring temperature and spring rainfall

Periods	N_2O-N[1]		$(N_2O+N_2)-N$	
	r	P	r	P
Regression for autumn/winter periods vs. time				
October-March	−0.870	0.1, NS	−0.961	0.01**
Regression for spring/summer periods vs. time				
April-July	0.632	0.1, NS	0.277	0.1, NS
Regression on spring temperature:				
Mean, March-May	n.d.[2]		0.697	0.1, NS
Regression on spring rainfall:				
Daily rates	n.d.		0.526	0.001***

[1] r = regression coefficient; P = probability.

[2] n.d., not determined.

**Highly significant regression of denitrification flux (y) vs. time from the beginning of October (x): $y=-3.6x+400$. ***Very highly significant regression of denitrification flux (y μg N m^{-2} h^{-1}) on rainfall in the previous n days (x, mm), 20 March-3 June 1982: $y=0.32x+6.88$, where $n=7-0.2T$ and T is the daily (10.00 h) temperature (°C) NS = not significant.

TABLE 6

The response to irrigation. Irrigated with 20-mm water on 7 June

Period	N_2O-N	$(N_2O+N_2)-N$	t[1]	P	n
	(μg N m^{-2} h^{-1})				
8 June	207	160	0.638	0.1, NS	4
9 June	51	50	0.088	0.1, NS	4
11 June	19	13	1.491	0.1, NS	4
14 June	18	12	2.572	0.05, *	4
15 June	14	7	2.248	0.1, NS	4
16 June	2.4	2.1	1.874	0.1, NS	4

t = Student's t paired comparison test; P = probability.

In all these relationships the values for the nitrous-oxide flux (μg N m^{-2} h^{-1}) were smoothed using a 3-day moving average technique.

Irrigation

Neither irrigation of the soil nor rainfall in May and June had much effect on the N_2O:N_2 balance, although denitrification was stimulated. Some of the results for a period in June illustrate the initial increase in nitrous-oxide flux and the subsequent decline following irrigation (Table 6).

DISCUSSION

Annual nitrogen loss from the soil

The total denitrification loss was estimated as greater than 5 kg N ha^{-1}. The majority of this loss occurred before spring fertiliser applications. Related to the seedbed fertiliser dressing of 24 kg N ha^{-1} it represents more than 20% of the application. This was much lower than the corresponding losses measured at the same site in the previous year, which had been estimated as 14 kg N ha^{-1} (Colbourn and Harper, 1987). Rates of emission were similar in the autumn, 260–270 μg N m^{-2} h^{-1} measured in December 1980 and 1981, but were much faster, 120-960 μg N m^{-2} h^{-1}, in February, April and May 1981 than in the corresponding periods in 1982 (Table 4). In contrast, leaching losses on the same field site were greater in 1982 than in the previous year, especially in October and from December to March, as a result of greater rainfall (Dowdell et al., 1987).

Release of N_2O to the atmosphere

The release of 0.5 kg N ha^{-1} to the atmosphere as N_2O between October and March, represents a significant contribution. The mean rate of loss over this 6 month period was 3.2 ng N m^{-2} s^{-1} attaining the maximum monthly mean rate of 20.8 ng N m^{-2} s^{-1} in October/November. These are faster rates than those observed from grassland in southern England at that time of the year (Ryden, 1981). The release of N_2O to the atmosphere represents about 2% of the autumn applied N, but taken over the whole year it was only 0.4% of the total fertiliser-N application.

Seasonal pattern and soil conditions

The overall seasonal pattern of denitrification was related to nitrate availability, soil wetness and temperature. Maximum rates were observed in the autumn when nitrate was available both from nitrification and fertiliser and the warm soil had returned to field-capacity moisture content. In early March 1982, denitrification was limited by lack of nitrate in the anaerobic soil. By April and May, aeration, with the concomitant drying of the soil, was limiting.

In the autumn, significant amounts of denitrification were indicated by the high N_2O concentrations found in deeper soil horizons (e.g. 40–60 cm; Table 3). In November 1981, aerated rainwater entering the soil was observed to interrupt denitrification. In the spring, N_2O was the dominant product. These two facts both suggest process structures in the soil with small physical dimensions: the first because the denitrification process was disrupted by rainwater percolating in macropores; the second because N_2O was able to escape the process site before further reduction. The Denchworth soil has relatively large structure with large physical dimensions compared with effective gas diffusion distances in wet soil.

So, it appears that the denitrification process is most active at or near the surfaces of the soil structural units, rather than at the centre of the peds. Very short diffusion pathways may be all that are needed for complete depletion of oxygen in the capillary porosity near the ped surface, where there are frequent roots and an active microbial population with a high oxygen demand. Here, nitrification and denitrification may proceed shoulder to shoulder, especially during a period of stability (stagnation), but aerated water washing over the surface of the ped would break the steep oxygen gradient, on which the denitrification process depends.

Acetylene distribution

The acetylene distribution was adequate in the autumn and measurements of denitrification were reliable. Later in the winter, when soils were more or less saturated with water, the diffusion of the gas to the denitrifying sites must have been considerably delayed. The response to acetylene in the field often took 1 or 2 days to develop. The observed delay in the response can be readily accounted for.

Ryden et al. (1979) discussed probable diffusion times and extrapolations of his figures applied to the system used in this study, suggest times of about 3–4 h when the soil had 10% air-filled pore space, 12 h at 5% and longer than 40 h in a completely saturated soil. Soil water-content data shows that this Denchworth series soil had less than 5% air-filled pore space throughout the top 20 cm, continuously from mid-December to mid-March (see Fig. 2). Air-filled porosities greater than 10% were only found, at 5-cm depth, during November and after the end of March and at 20-cm depth only after the beginning of May. Hence, diffusion times were probably longer than 12 h for much of the period of the study. This time must be doubled to allow time for the nitrous oxide to diffuse out to the soil surface. The observed delay in response to the acetylene treatment, often a delay of 1 day and sometimes of up to 2 or 3 days, can therefore readily be accounted for.

CONCLUSIONS

(1) The estimated denitrification loss was 5 kg N ha^{-1} between October 1981 and June 1982. This was lower than expected and may be an underestimate. The input of nitrous oxide to the atmosphere was estimated at 0.7 kg N ha^{-1}. The largest losses, in the autumn, were equivalent to 20% of the fertiliser-N.

(2) The distribution of acetylene in the soil was probably adequate in the drier soils in autumn and spring, but diffusion was slow in wet soils in the winter. The measurable response to acetylene was delayed by 2 or 3 days on some occasions. The large discrepancy between the expected loss and the measured loss of nitrogen leaves some doubt, which must be resolved in future studies.

(3) Measurement of redox potentials in the soils provided a useful insight into the volume of soil involved in denitrification and related well to measurements of the oxygen concentration in the soil macropores and soil water content.

(4) Denitrification in spring, after fertiliser applications, was closely correlated to rainfall and significant regressions could be calculated involving rainfall, wet (or dry) days and days since fertiliser was applied.

(5) Nitrous oxide was the major product in the warmer soils of April and May. This was attributed to denitrification taking place at the base of water films on aggregate surfaces beneath an oxygen sink comprising a layer of oxygen-respiring cells.

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