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SEASONAL VARIATION IN DENITRIFICATION IN A CLAY SOIL UNDER A CULTIVATED CROP AND A PERMANENT PASTURE

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Summary—Biological denitrification in a clay soil was measured under the annual crop black gram (*Vigna mungo*) and the perennial pasture grass green panic (*Panicum maximum* var *trichoglume*). The soil treatments were: water, water plus C_2H_2, or water plus C_2H_2 plus glucose applied down brass tubes to depths of 7.5, 22.5, 52.5 and 112.5 cm. Soil gas samples were taken just before and 24 h after treatments were applied. Treatments and samplings were repeated every 3 months for 2 years. In untreated soil, the amounts of N_2O from crop and pasture were highest in spring and summer with approximately 75% of the total amount being measured. For black gram, this represented equal amounts in spring and summer (approx. 34 g N_2O-N ha^{-1}) whereas for green panic, 25.9 g N_2O-N ha^{-1} was found in spring and 9.2 g N_2O-N ha^{-1} in summer. Soil water was correlated with N_2O concentration beneath black gram ($r = 0.87$, d.f. = 6, $P < 0.01$) and CO_2 concentration under green panic ($r = 0.69$, d.f. = 6, $P < 0.05$). Multiple regression analysis showed that 87.8% of the variation in N_2O concentration from beneath black gram was associated with monthly rainfall and NO_3^- concentration.

Gas samples collected 24 h after the application of the treatments showed that N_2O and CO_2 concentration did not vary between treatments. However, treatment application did increase the N_2O found beneath black gram. A mean increase of 29 g N_2O-N ha^{-1} was observed in January, 1986. Concentration of N_2O changed little after the application of the treatments to green panic. N_2O due to microsite activity represented 11% of the measured loss of nitrate from the subsoil in the field.

INTRODUCTION

When fertile soils are first brought into crop production, organic matter breaks down rapidly and nitrate is produced in large quantities. Frequently, nitrate leaches below the normal effective rooting depth of the soil (Catchpoole, 1981). In semi-arid cropping systems, this subsoil nitrate may continue to accumulate over a period of years and is apparently stable. However, in wetter than normal years that occur periodically, the subsoil nitrate may be leached or denitrified or both. Such a picture has been observed in the clay soils of the brigalow lands of subtropical eastern Australia, large areas of which have been cultivated in the past 30 years. Catchpoole (1981) has reported a large build-up of subsoil nitrate in such soil. During a prolonged wet period in the winter of 1985, V. R. Catchpoole (pers. comm.) observed that most of the 275 kg ha^{-1} of nitrate accumulated in the subsoil of a cultivated clay soil, was apparently lost from the system by leaching or denitrification.

The problems that we address here are, firstly, whether denitrification is a major pathway of loss from cropped clay soil in the subtropics, and secondly, if it is then where in the soil profile is the loss process taking place. Reliable estimates of denitrification in the field have always been difficult to obtain as biological denitrification can produce both nitrous oxide (N_2O) which is easily detected and measured and dinitrogen (N_2), which is not. The technique for measuring denitrification in the field improved greatly with the discovery that C_2H_2 inhibited the conversion of N_2O to N_2 (Yoshinari and Knowles, 1976; Balderston *et al.*, 1976) so that the total denitrification loss could be measured as N_2O. Following refinement, the C_2H_2 inhibition technique is now commonly used for measuring total gas production from denitrification in the field (Burton and Beauchamp, 1984; Duxbury and McConnaughey, 1986; Ryden *et al.*, 1987).

Using this technique, estimates of denitrification from both cultivated and grassland soils have ranged from 0.7 to 8 kg N ha^{-1} (Mosier *et al.*, 1981; Webster and Dowdell, 1982; Mosier *et al.*, 1986; Myrold, 1988). The N_2O detected was believed to have come from surface horizons with no account taken of the N evolved from the subsoil. However, before the use of C_2H_2 inhibition, it was shown that losses of N_2O could occur from subsoil (Burford and Stefanson, 1973; Gilliam *et al.*, 1978; Gambrell *et al.*, 1975). The major factor limiting denitrification in subsoil horizons is probably the low energy supply, expressed usually as soluble carbon (Myrold and Tiedje, 1985; Sexstone *et al.*, 1985). Groffman *et al.* (1988) found that the role of available C in controlling denitrification in the field was poorly defined and could be improved by studying the decomposition processes in the soil.

A second factor limiting denitrification is temperature, particularly in temperate climates where waterlogged conditions necessary for the process

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frequently occur when soil temperature is low. Few seasonal studies exist. However, Bremner *et al.* (1980) found that N_2O evolution from Iowa soils was most rapid in summer with little production occurring in winter. Burford *et al.* (1981) also found that the greatest amounts of N loss (2.2–3.3 kg N ha^{-1}) were in the spring with little loss during the winter. In both cases, the higher N_2O evolution rates were associated with higher soil water contents. Weier and Doran (1987), studying clay soils of the brigalow lands, observed that subsoils did have the capacity to denitrify, but that rapid denitrification occurred only in wet soil to which available C had been added.

Our work was aimed at establishing if biological denitrification could be an important loss process for accumulated nitrate from clay soil in the subtropics and to determine the seasonal conditions under which it is most important. Endogenous N_2O concentration and total $(N_2O + N_2)$ gas concentration (the latter by C_2H_2 inhibition) were measured beneath a cultivated food legume and a perennial grass pasture.

MATERIALS AND METHODS

Experimental plots

Experimental plots of black gram (*Vigna mungo*) and green panic (*Panicum maximum* var *trichoglume*) had been established in 1975 at the CSIRO Narayen Research Station (25°41′S, 150°52′E). The area has a sub-humid, sub-tropical continental climate with a mean annual rainfall of 710 mm. The soil is a black earth of principal profile form Ug5.13 (Northcote, 1965; D. J. Ross, pers. comm.) characteristics of which are given in Table 1.

Measurements were made every 3 months or less for 2 years. No 0 h sample was obtained from the April, 1987 sampling. For sampling, each plot (40 m × 16 m) was divided into 5 strata (8 m × 16 m) with each stratum having 10 sampling areas (3.2 m × 4 m). Three strata were chosen at random within each plot and were used as replicate sampling areas. Within each sampling area, repeated sampling occurred at a randomly selected permanent site chosen from one of the 10 available areas.

Experimental procedure

For the collection of soil atmosphere samples, cylindrical brass probes were installed to depths of 7.5, 22.5, 52.5 and 112.5 cm beneath each plot with a horizontal distance of 30 cm between each probe. The probes were 6.35 mm dia (4.6 mm i.d.) containing 32 perforations (1.5 mm dia) evenly spaced over a distance of 40 mm from the closed (lower) end of the probe. Three soil treatments were used:

(1) water only—to establish endogenous N_2O concentration
(2) water saturated with C_2H_2 to measure total gaseous concentration, i.e. $(N_2O + N_2)$
(3) water saturated with C_2H_2 and containing 400 μg glucose ml^{-1}—to measure the effect of C on gaseous N concentration.

The treatments were established by delivering down the probe to the soil at each depth 2 ml of each of the solutions. Each probe was then sealed with a rubber septum and a volume of gas > 100% of the deadspace volume of the probe was withdrawn and discarded. A partial vacuum was thus created, ensuring a gas flow from the surrounding soil into the probe. The soil atmosphere at each depth was sampled before and 24 h after the application of the treatments using 10 ml venoject tubes (evacuated glass test tubes) and double ended needles. The 2 ml treatment addition after the 0 h sampling reduced the air filled porosity around the probe thereby increasing the volume of soil from which the 24 h sample was withdrawn.

Chemical measurements

Nitrous oxide (N_2O) was measured on a 0.5 ml sample using a Shimadzu GC-8AIE gas chromatograph fitted with a 320 mm × 2 m stainless steel column packed with 80–100 mesh Porapak Q and an electron capture detector (ECD). Operating conditions were: column temperature 100°C; ECD temperature 350°C; 5% CH_4/95% Ar carrier gas; flow rate 30 ml min^{-1}. CO_2 and O_2 were measured on a 1 ml sample using a Tracor MT-150G gas chromatograph with an ultrasonic detector and He as carrier gas. A 320 mm × 2 m stainless steel column packed with 80–100 mesh Chromosorb 101 maintained at 25°C was used for CO_2 and a 320 mm × 2.5 m stainless steel column packed with 60–80 mesh Molecular Sieve 5A, maintained at 80°C, was used for O_2.

Conversion of N_2O measurements to g N_2O-N ha^{-1} was accomplished by first estimating the gravimetric water content and bulk density of the soil (a particle density of 2.65 Mg m^{-3} was assumed) from the dry weight and volume of 5 cm × 5 cm soil cores respectively. The calculation of volumetric water content and total porosity was then made and the volumetric air content of the soil found. The volume of soil sampled at each depth was then calculated from the volume of the sample of soil air (12.8 ml) and the volumetric air content. The volume of soil

Table 1. Characteristics of the soil underlying the experimental plots

Plot	Depth (cm)	pH*	Total N (%)	Total C (%)	Mineral-N† (mg kg^{-1} soil)		Bulk density (Mg m^{-3})	Textural analysis (%)		
					NO_3^--N	NH_4^+-N		Sand	Silt	Clay
Black gram	5–10	8.2	0.23	2.43	21.8	3.6	1.07	19.7	17.4	58.4
	20–25	8.3	0.12	1.65	20.9	3.2	1.37	17.7	15.9	63.6
	50–55	8.7	0.06	1.73	11.9	2.7	1.50	15.7	15.5	60.8
	110–115	9.3	0.02	2.29	19.7	2.3	1.45	19.8	19.5	41.0
Green panic	5–10	8.3	0.28	3.28	0.9	3.7	1.11	15.0	18.2	59.7
	20–25	8.6	0.14	1.73	0.6	2.8	1.37	14.9	18.8	63.1
	50–55	8.8	0.05	1.19	0.7	3.1	1.43	12.7	17.3	64.5
	110–115	8.5	0.04	0.97	0.6	2.3	1.61	13.4	11.9	65.4

*1:2.5 soil/water.

†Values are the means of 8 sampling periods.

Table 2. Nitrous oxide concentration at 0 h from the four depths beneath black gram and green panic before the application of the treatments

Plot	Depth (cm)	Oct. 85*	Jan. 86	Mar. 86	Apr. 86	Jul. 86	Oct. 86	Jan. 87	Jul. 87
					$g\ N_2O\text{-}N\ ha^{-1}$				
Black gram	5–10	6.26	1.53	0.85	0.04	0.28	1.64	0.84	2.17
	20–25	4.38	1.00	0.88	0.07	0.56	3.47	3.38	2.03
	50–55	3.18	3.00	2.45	0.23	0.69	3.58	13.74	1.92
	110–115	5.46	5.03	2.35	1.32	1.62	3.91	8.02	2.70
		4.82 ± 0.34†	2.64 ± 0.49	1.63 ± 0.25	0.42 ± 0.13	0.79 ± 0.11	3.15 ± 0.33	6.50 ± 1.41	2.21 ± 0.12
Green panic	5–10	5.86	1.82	0.37	0.78	0.20	1.51	1.17	2.66
	20–25	5.61	1.69	0.18	0.48	0.08	1.10	0.66	1.92
	50–55	3.85	1.23	0.25	0.41	0.40	1.86	0.58	2.14
	110–115	3.72	1.35	0.42	0.65	0.05	2.39	0.68	1.55
		4.76 ± 0.25	1.52 ± 0.11	0.31 ± 0.04	0.58 ± 0.09	0.18 ± 0.05	1.72 ± 0.26	0.77 ± 0.11	2.07 ± 0.14

*Values are the means of 9 replications.
†Mean ± SE.

sampled varied with the % water filled pore space (% WFPS) of the soil and ranged from 31 to 587 ml and 27 to 347 ml for the four soil depths beneath black gram and green panic respectively. If we assume that the gas samples were drawn from the volume of soil that immediately surrounds the end of the probe and that the influence of any added treatments will also occur in the vicinity of the probe end, then the radius of the volume of soil sampled can be found. Once this radius is known, conversion of $\mu g\ N_2O\text{-}N\ cm^{-3}$ to $g\ N_2O\text{-}N\ ha^{-1}$ can be made.

Total N in the soil was estimated following Kjeldahl digestion using the thiosulphate treatment modification of Dalal *et al.* (1984) to include nitrate. Total C was measured by dry combustion using the procedure of Carr (1973) while water-soluble C was measured using the centrifugation and filtering steps of Burford and Bremner (1975) followed by chromic acid digestion (Heanes, 1984). Mineral N was extracted with 2M KCl (Catchpoole and Weier 1980), and the NO_3^--N and NH_4^+-N measured colorimetrically (Henzell *et al.*, 1968).

Soil temperatures were recorded for the 7.5 and 22.5 cm depths at each sampling using max-min soil thermometers inserted to these depths. Soil temperatures for the 52.5 and 112.5 cm depths were recorded initially but this was discontinued because they were within 1°C of the temperature at a depth of 22.5 cm.

RESULTS

Soil atmospheres before treatments

Nitrous oxide (N_2O) was detected at all depths beneath the cultivated crop and the permanent pasture at the initial (0 h) sampling before the application of the three treatments (Table 2). The total quantities found in the profile at a single sampling were greater from beneath black gram (1.7–$26\ g\ N_2O\text{-}N\ ha^{-1}$) than from beneath green panic (0.7–$19\ g\ N_2O\text{-}N\ ha^{-1}$). N_2O concentrations were highest in spring and summer (September to February) and lowest in autumn and winter (March to August). Distribution of N_2O over the season was estimated to be *ca.* 75% in spring-summer for both crop and pasture, with about equal amounts in spring and summer under black gram but 54% in spring and 21% in summer under green panic.

N_2O concentration under black gram tended to increase with depth at all but two samplings. On average, N_2O concentration in the third and fourth layers was double that in the two layers closer to the surface. Under green panic, concentrations showed no change or a slight decrease with depth. O_2 concentrations at the 4 depths beneath both systems approximated 17% at all sampling occasions (data not shown).

For initial CO_2 concentration, the greatest proportions were found in the summer months (December to February) (42% for black gram and 46% for green panic) with similar proportions being found in both profiles during the other seasons of the year (Table 3). Beneath black gram, concentrations of CO_2 were greater from below 50 cm, where 73% of CO_2 was found. Variations in the quantities of CO_2 beneath green panic were similar to those observed with N_2O concentration, i.e. little change.

N_2O concentrations in the black gram plot were closely related to % WFPS ($r = 0.87$, $P < 0.01$, d.f. = 6) reaching a peak mean value of $6.5\ g\ N_2O\text{-}N\ ha^{-1}$ in January 1987 when % WFPS was 82.9% (Fig. 1). No such relationship was found for green panic but in terms of water relationships, a significant correlation was observed between monthly rainfall and CO_2 concentration beneath green panic ($r = 0.69$, $P < 0.05$, d.f. = 6, Fig. 2). A mean value of

Table 3. Carbon dioxide concentration at 0 h from the four depths beneath black gram and green panic before the application of the treatments

Plot	Depth (cm)	Oct. 85*	Jan. 86	Mar. 86	Apr. 86	Jul. 86	Oct. 86	Jan. 87	Jul. 87
					$\mu g\ CO_2\ cm^{-3}$ soil				
Black gram	5–10	14.28	5.63	13.27	12.24	12.24	11.37	8.53	6.52
	20–25	12.59	14.45	14.21	3.25	4.47	4.35	13.31	5.18
	50–55	6.71	67.21	33.75	6.24	7.40	8.82	29.01	11.69
	110–115	22.09	83.77	34.00	18.24	24.24	23.48	26.89	25.48
		13.92 ± 1.24†	42.77 ± 6.58	23.81 ± 2.85	9.99 ± 1.27	12.09 ± 1.88	12.00 ± 2.26	19.44 ± 3.01	12.22 ± 1.56
Green panic	5–10	16.05	11.55	19.72	8.94	8.26	17.35	45.55	8.15
	20–25	16.75	8.68	9.45	4.41	11.87	28.10	37.45	6.77
	50–55	11.88	11.99	7.23	2.83	10.15	4.55	44.30	7.03
	110–115	11.46	31.49	20.91	10.11	6.68	7.14	38.56	12.01
		14.04 ± 0.93	15.93 ± 2.73	14.33 ± 1.41	6.57 ± 0.70	9.24 ± 0.70	14.29 ± 1.88	41.47 ± 2.39	8.49 ± 0.71

*Values are the means of 9 replications.
†Mean ± SE.

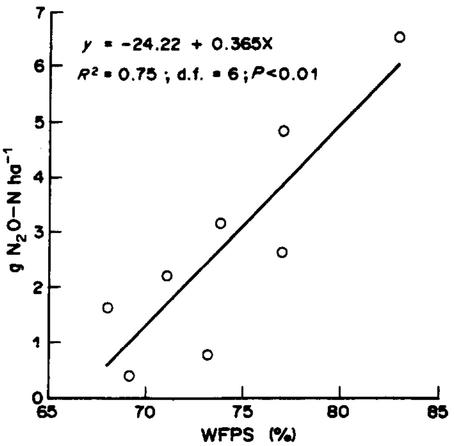

Fig. 1. The relationship between N_2O concentration from the black gram plot, before treatment application, and % WFPS during the 2 yr sampling period ($r = 0.87$).

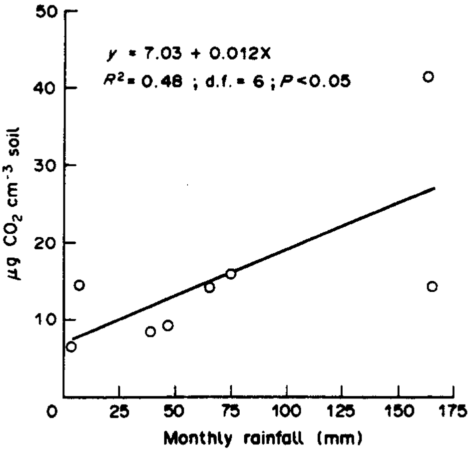

Fig. 2. The relationship between CO_2 concentration from the green panic pasture, before treatment application, and monthly rainfall during the 2 yr sampling period ($r = 0.69$).

41.5 μg CO_2 cm^{-3} soil was obtained in January, 1987 when monthly rainfall totalled 163.2 mm.

There was little difference in NH_4^+-N values between plots but black gram had *ca.* 20 times more NO_3^--N present in its profile than green panic (Table 1).

Results for soluble C were similar for the first three depths beneath both crop and pasture (mean values of 31.7 and 30.3 μg C g^{-1} soil respectively) but there was a significantly higher concentration of C present at the 110–115 cm depth beneath black gram than under green panic (mean values of 33.1 and 18.5 μg C g^{-1} soil respectively). This may have been caused by the presence of $CaCO_3$ nodules as none were found at the other three depths.

A relationship between N_2O concentration, monthly rainfall and NO_3^- concentration at all depths for the black gram plot can be inferred from the data in Fig. 3. Nitrate and rainfall both affected the concentration of N_2O. The highest N_2O concentrations were in summer when both NO_3^--N and soil water contents tended to be high. The lowest N_2O concentrations were in March and April, 1986 when rainfall had been relatively low.

A multiple regression analysis of the data from the black gram plot relating N_2O concentration to monthly rainfall and nitrate concentration (Fig. 3) for the 2 yr gave the following equation:

$$y = 9.48(\pm 2.64) + 0.17(\pm 0.03)x_1 - 0.15(\pm 0.05)x_2$$

where

y = N_2O production from the soil profile (gN_2O-N ha^{-1})
x_1 = rainfall in the month before sampling (mm $month^{-1}$)

and

x_2 = NO_3^- concentration (mg kg^{-1}).

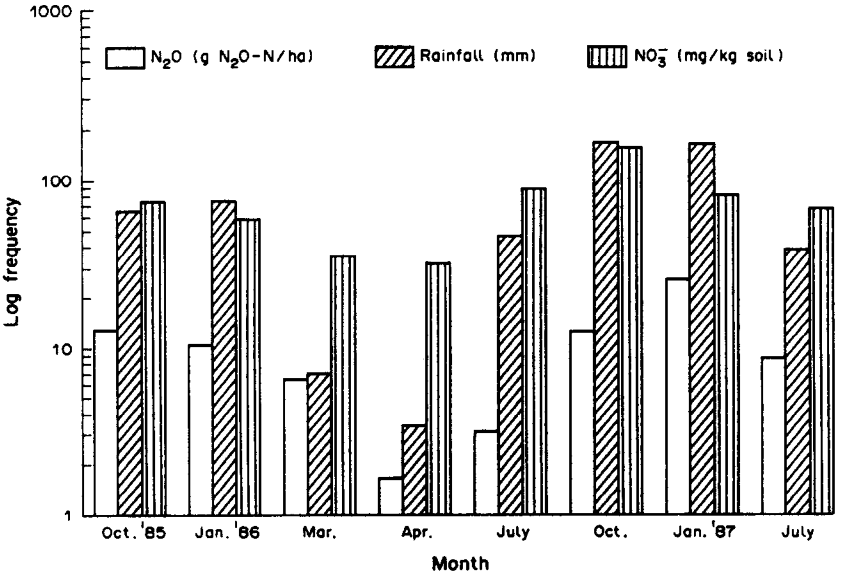

Fig. 3. N_2O concentration (profile total) from beneath the black gram plot, before the application of treatments, as influenced by rainfall in the month before sampling and NO_3^- concentration (profile total).

Table 4. Nitrous oxide concentration, 24 h after the application of the three treatments to the four depths beneath black gram and green panic

Plot	Depth (cm)	Oct. 85*	Jan. 86	Mar. 86	Apr. 86	Jul. 86	Oct. 86	Jan. 87	Jul. 87
					g N_2O-N ha^{-1}				
Black gram	5–10	5.02	1.03	0.99	1.12	0.21	0.97	3.95	1.25
	20–25	10.11	0.79	0.93	0.50	0.33	11.15	9.92	3.04
	50–55	2.78	29.03	1.42	0.98	3.64	6.11	14.19	2.28
	110–115	5.46	0.93	1.58	0.67	6.54	2.09	3.43	1.35
		5.84 ± 1.02†	7.95 ± 6.95	1.23 ± 0.11	0.82 ± 0.14	2.68 ± 1.56	5.08 ± 1.87	7.87 ± 1.76	1.98 ± 0.29
Green panic	5–10	8.57	0.16	1.57	0.56	0.02	—	1.13	3.35
	20–25	4.93	0.28	0.60	0.45	0.01	0.05	1.20	1.51
	50–55	3.11	0.26	0.56	0.24	0.02	4.56	0.89	1.21
	110–115	2.75	0.11	1.23	0.34	0.02	1.01	0.57	0.95
		4.84 ± 0.78	0.20 ± 0.04	0.99 ± 0.17	0.40 ± 0.04	0.02 ± 0.004	1.41 ± 0.61	0.95 ± 0.10	1.76 ± 0.30

*Values have been averaged across treatments.
†Means ± SE.

The multiple regression coefficient was highly significant and accounted for 87.8% of the variation in N_2O concentration ($r = 0.94$). The negative regression coefficient for x_2 is indicative of a decrease in N_2O-N concentration for values of x_2, the change being independent of the recorded rainfall. A similar relationship could not be found for the green panic pasture with only 5% of the variation explained.

Soil atmospheres one day after treatment

No significant difference was observed between treatments in either N_2O or CO_2 concentration, 24 h after the treatments were applied to the four depths (Tables 4 and 5). The profile totals of N_2O were again higher from beneath black gram (3.3–31.5 g N_2O-N ha^{-1}) than from beneath green panic (0.07–19.4 g N_2O-N ha^{-1}). The seasonal pattern of N_2O concentration observed before treament addition for black gram was again followed, but the amounts of N_2O were greater, e.g. in January 1986, a mean increase of 29 g N_2O-N ha^{-1} was observed at the 50–55 cm depth. For green panic, there was little change in N_2O concentration associated with the treatments. There are two possible reasons for this—a lack of NO_3^- in the soil and retention of N_2O in the soil solution. Values obtained for CO_2 concentration after 24 h were very similar to the 0 h values for both black gram and green panic. Only slight variation in the CO_2 concentrations occurred for black gram in January 1986 and 1987.

DISCUSSION

Differences in the N_2O concentrations between crop and pasture before the application of the treatments may have reflected differences in NO_3^- concentration in the respective soil profiles. N_2O production is enhanced if large concentrations of NO_3^- are present. However, high NO_3^- concentrations also have an inhibitory effect on the reduction of $N_2O \rightarrow N_2$ (Blackmer and Bremner, 1978). The smaller concentrations of N_2O detected beneath the pasture may have been a result of an increase in N_2 concentrations because of the lower NO_3^- concentrations (Limmer *et al.*, 1982). This was not confirmed on the measurement of total gas concentration ($N_2O + N_2$) by C_2H_2 inhibition. Published figures for N_2O losses from grasslands range from 2 to 3 g N ha^{-1} day^{-1} from a native shortgrass prairie (Mosier *et al.*, 1981) to 212 g N ha^{-1} day^{-1} from a fertilized ryegrass plot (Ryden, 1981). This suggests that the concentrations observed here above the perennial pasture (0.02–4.8 g N_2O-N ha^{-1}) are not of great significance.

The seasonal pattern in N_2O concentration observed beneath black gram was similar to that found by Bremner *et al.* (1980) for soybean in Iowa soils. The concentration of N_2O was also higher in the summer months but the quantities found in the Iowa soils were much greater. The values obtained during winter were much smaller than observed at Narayen and was attributed to lower temperatures. However, N_2O concentration beneath black gram was closely related to soil moisture with no relationship being found with soil temperature. This probably reflects the differences between tropical and temperate zones since microbial activity in tropical soils is more dependent on soil water than on soil temperature.

Table 5. Carbon dioxide production, 24 h after the application of the three treatments to the four depths beneath black gram and green panic

Plot	Depth (cm)	Oct. 85*	Jan. 86	Mar. 86	Apr. 86	Jul. 86	Oct. 86	Jan. 87	Jul. 87
					μg CO_2 cm^{-3} soil				
Black gram	5–10	12.79	7.04	14.43	11.25	11.81	13.01	15.25	5.78
	20–25	10.92	15.09	19.06	2.94	5.05	5.80	18.32	5.32
	50–55	5.31	62.13	47.25	6.40	7.10	8.06	46.85	9.93
	110–115	19.97	59.18	35.34	14.59	10.52	28.91	32.41	22.28
		12.25 ± 1.66†	35.86 ± 7.69	29.02 ± 4.21	8.80 ± 1.45	8.62 ± 1.24	13.95 ± 2.75	28.21 ± 3.95	10.83 ± 2.20
Green panic	5–10	13.82	16.29	16.16	15.40	7.61	20.26	53.06	8.40
	20–25	16.17	10.86	7.89	9.91	11.91	34.48	38.14	7.28
	50–55	10.59	11.37	6.20	4.14	9.06	5.03	42.60	7.06
	110–115	11.86	20.34	20.84	11.21	6.21	14.14	32.54	12.53
		13.11 ± 0.96	14.72 ± 1.32	12.77 ± 2.00	10.16 ± 1.29	8.70 ± 0.87	18.48 ± 3.30	41.59 ± 3.45	8.82 ± 0.84

*Values have been averaged across treatments.
†Mean ± SE.

Liberation of N_2O by nitrifying organisms in this soil varies according to the agricultural practice used. For the cultivated soil, incubation studies showed nitrification occurred at 50–55 cm and 110–115 cm after 2 and 4 weeks respectively, whereas 10 and 8 weeks were required before measurable nitrification occurred in the 5–10 cm and 20–25 cm depths respectively. Beneath the pasture, net mineralization of organic N was very slow with little or no increase observed after 12 weeks. Nitrification was only apparent at the 5–10 cm depth after 6 weeks and the 20–25 cm depth after 10 weeks (K. L. Weier, unpublished Ph.D. thesis, University of Queensland, 1990). The contribution therefore of the N_2O released during nitrification to the N_2O concentrations measured in these soils would be negligible, in the case of the pasture soil, and minimal for the cultivated soil. This is in contrast to other findings which suggest significant contributions of N_2O from nitrifying organisms, but this occurred after NH_4^+ fertilizer application in the field or use of NH_4^+-N in laboratory studies (Bremner and Blackmer, 1979; Aulakh *et al.*, 1984; Klemedtsson *et al.*, 1988).

The increase in N_2O concentration with depth beneath black gram indicated that NO_3^- may have been lost from the subsoil through denitrification. Studies on the Atlantic Coastal Plain of North Carolina by Gambrell *et al.* (1975) indicated that denitrification of residual NO_3^- in the subsoil was possible if sufficient available C was present. The amounts of soluble C at 110–115 cm beneath black gram were larger than those beneath green panic but this may have been caused by the presence of large amounts of $CaCO_3$. However, Stefanson (1972) also reported higher amounts of available C in cropped than in pasture soils while Smith and Tiedje (1979) found denitrification in planted soils to be reduced significantly when NO_3^- concentrations were low. Thus, unless there is an adequate supply of NO_3^-, the role of plant roots, in aiding denitrification through provision of C sources in the form of root exudates, is reduced.

Weier and Doran (1987) found a strong relationship between CO_2 concentration and % WFPS in these plots. Such a relationship was found again for CO_2 concentration and monthly rainfall for the green panic plot. The importance of soil water on CO_2 concentration was recognized by Buyanovsky and Wagner (1983) and Linn and Doran (1984) in field experiments in Mexico and U.S.A. respectively. Although no such relationship was evident beneath black gram in this experiment, increased CO_2 concentration did accompany the increase in N_2O concentrations. This increase in respiration of C indicated greater microbial activity at depth in the soil profile. Thus, percolation of soluble organic C to the lower depths after heavy rainfall (Volz *et al.*, 1976; Meek *et al.*, 1969) may provide substrate for the reduction of NO_3^-.

The use of the C_2H_2 inhibition technique in the field should have allowed quantitative estimates of total denitrification losses to be made. Acetylene inhibition of the N_2O to N_2 reduction would be immediate with the rapid diffusion of the C_2H_2 to the active microsites even in soils approaching saturation (Ryden *et al.*, 1979). However, the absence of any differences between the three treatments in N_2O concentrations indicates that either the 24 h sampling period was too long with the C_2H_2 block becoming ineffective or that the end product of denitrification in these soils is primarily N_2O. The latter fact was confirmed in a recent study where *ca.* 70% of the denitrifiers isolated from these soils produced only N_2O (K. L. Weier, *loc. cit.*). The failure of glucose to increase N_2O concentration implies that the available C present at 0 h was sufficient for the needs of the microbial population. The absence of an increase in respiration of C, 24 h after treatment application, supports this observation.

However, in 6 of the 8 months, an increase was observed in N_2O concentration beneath black gram in mean values across treatments. The higher rates of N_2O release may have been caused by the creation of anaerobic microsites (Tiedje *et al.*, 1984) on application of the treatments. "Hot-spots" or microsites of microbial activity associated with the presence of decaying plant material, are known to occur during measurement of natural denitrification rates and vary with time in response to changing moisture conditions (Parkin, 1987). Microsite activity was identified on at least one occasion beneath black gram where the quantity of N_2O evolved, 84.4 g N ha^{-1} (mean value 29 g N_2O-N ha^{-1}), occurred at 50–55 cm in January, 1986 which amounts to an annual loss of 30.8 kg N_2O-N ha^{-1} or 11% of the measured loss of NO_3^--N from the subsoil in the field. The large calculated standard error supports the finding of Parkin (1987) that soil microsites are a source of extreme variability in denitrification.

We conclude that denitrification cannot be regarded as a major source of loss of N from clay soils under perennial pasture. The maximum concentration of 4.8 g N_2O-N ha^{-1} is relatively low and the process is limited at all times by low NO_3^- concentrations in the soil profile. In cultivated land growing annual crops such as black gram, denitrification can be regarded as important as losses in the order of 84 g N_2O-N ha^{-1} can be expected. This extrapolates to an annual loss of 30.8 kg N_2O-N ha^{-1} yr^{-1}. The factor limiting this loss is available energy supply. N_2O concentration in the black gram plot can be predicted using multiple regression, the most useful predictors being monthly rainfall and soil nitrate concentration. N_2O liberated by nitrifying organisms does not make a significant contribution to the N_2O concentrations measured in these soils. Seasonal variation in N_2O and CO_2 concentration can be directly related to soil water.

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