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DENITRIFICATION STUDIES ON SOME SOUTH AUSTRALIAN SOILS

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INTRODUCTION

Denitrification is now generally accepted as a potential biological process in soils in which aeration is restricted. Therefore it might be assumed that reduction and denitrification of nitrate leached from surface horizons could occur in deep subsoils where low oxygen tensions may exist during part of the year. This has been supported to some extent by evidence of evolution of nitrous oxide in the subsurface horizons of field soils [1], and the presence of denitrifying micro-organisms in the lower horizons of many of the great soil groups [6 11 12]. However, denitrification in anaerobic soil horizons is dependent not only on the presence of nitrate and nitrite as electron acceptors in the oxidative breakdown of organic matter by soil micro-organisms, but also on the content and nature of the decomposible organic matter itself [3 7].

Recently Bremner and Shaw [2], working with a range of soils of varying organic carbon content, were unable to detect denitrification under their experimental conditions when the levels of native soil organic carbon were less than 1 per cent. When glucose was added to these soils, however, denitrification proceeded rapidly. This observation suggests that the levels of soil organic carbon which occur in surface soils in sub-humid regions may be too low to provide sufficient energy material for active metabolism of denitrifying organisms even if all other conditions for the denitrification process are optimal. In addition, it would appear

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that the deeper horizons of the soil profile with even lower soil organic carbon levels would be most unlikely sites for denitrification. Studies were therefore undertaken to determine the extent and nature of denitrification in soil samples from the genetic horizons of two sub-humid Australian great soil groups.

A modification of the manometric method proposed by Gilmour, Bollen and Damsky [5] was used to determine denitrification in the present studies. A description of the method is included and also data indicating its reliability. The effects of such substrate factors as water, nitrate, and glucose levels have been examined in the appraisal of the method.

MATERIALS

Soil samples from the red brown earth and black earth great soil groups [13] were collected at the Waite Institute from five sites with different histories of utilization.

The red-brown earth, (Urrbrae loam) at the Waite Institute is characterized by a red-brown, fine sandy loam to loam, soft granular surface soil, changing gradually to a red prismatic clay subsoil, with free calcium carbonate appearing at depths of 40 to 50 inches. Soil reaction in the surface is pH 5.6, rising gradually to pH 8.4 in the zone of free lime. The black earth (Claremont clay loam) has a dark-brown to black, granular to sub-angular blocky, clay loam surface soil, with a dark-brown, massive clay subsoil showing large columnar cracking and containing concretions and streaks of free lime. Reaction ranges from pH 7.0 at the surface, to pH 8.4, in the lime horizons which appear at a shallow depth.

A surface sample 0 to 1″ of red-brown earth under 14 years pasture of Wimmera rye grass and sub-clover was used in initial experiments. Samples were taken from shallow pits on May 11 and July 31, 1959. In the intervening period the content of NO_3-N fell from 60 ppm to 13 ppm. These samples contained 3.06 per cent and 3.18 per cent organic carbon respectively. The samples were dried in a forced-draft oven at 45°C. Aggregates of 2 to 5 mm size were obtained by seiving and stored in jars until required.

Three profiles of red-brown earth were sampled from the Waite Institute permanent-rotation plots established in 1927. The rotation treatments were continuous wheat, permanent pasture, and fallow-wheat-pasture-pasture at present in fallow (FWPP). Further details may be found in the Waite Institute Report [14]. The soils were sampled in 3″ layers to a depth of 12″, with the exception of permanent pasture where samples of 0–3″, 3–6″, and 6–12″ were collected. A Jarrett 4″ post-hole type soil auger was used to sample 6″ layers of soil from 12 to 48″. As the genetic horizons were diffuse it was not generally possible to determine the exact location of the horizon

boundaries. For this reason the 6″ layers may comprise whole or part of a complete genetic horizon. Precautions were taken in sampling to prevent microbiological contamination of soil samples.

The organic-carbon content at 0–3″ in permanent pasture sample was 2.63 per cent; in FWPP 2.12 per cent; and in continuous wheat, 1.65 per cent, reflecting the differences in utilization. Other analytical data are reported in the tables of results.

The black-earth profile under Wimmera rye grass sub-clover permanent pasture was sampled as above. A heavy $CaCO_3$ horizon commencing below 18″ became so impenetrable as to make sampling impossible below 36″. The organic-carbon content of the surface horizon was 5.24 per cent.

METHODS

Manometric method

The quantitative determination of small nitrogen losses from soils (less than 100 ppm) has usually depended on the determination of evolved gases by N^{15} tracer techniques [8 9 15], or on the difference between the initial total-nitrogen level and the final level in the soil [2]. The latter method can yield satisfactory results only when the naturally occurring field soil is subject to an intensive pretreatment of grinding and mixing to ensure uniform distribution of the total soil nitrogen, thereby eliminating sampling variation. This pretreatment must alter many of the physical, chemical, and microbial characteristics of the soil sample.

In the study reported here, denitrification in soils was followed using the Warburg manometric method. Other workers have indicated the possibility of using this method for soil denitrification studies under anaerobic conditions [5], and the results reported herein confirm the value of this approach. Apart from eliminating the need for extensive pretreatment of the soil, the manometric method affords a simple means of measuring changes in the rate of denitrification over comparatively short time intervals.

Respiration vessels of approximately 50 ml capacity were used. They contained a slightly off-centre inside well in which KOH solution was placed for the absorption of CO_2 (and H_2S). A vented sidearm permitted gassing of the vessels and their contents during the period of equilibration in the constant-temperature bath. Appropriate substrate additions of KNO_3 or glucose solutions were applied uniformly and dropwise using a hypodermic syringe to 10 g of air-dry soil aggregates contained in the respiration vessels. A measured quantity, usually 0.6 ml of 5 or 20 per cent KOH, was pipetted into the center well which contained a piece of folded filter paper.

The respiration vessels were closed with a tightly fitting rubber bung, the side-vent plug was opened, and the vessels were then placed in a vacuum desiccator. The vessels and their contents were evacuated to 1 mm (Hg) pressure and back flushed to room pressure with argon *. This procedure was repeated four times. In the final flushing with argon, the pressure was increased to slightly in excess of room pressure, the desiccator lid was removed, and the side-vent plugs immediately turned to the closed position. The respiration vessels were then attached to the manometers, placed in the constant-temperature bath (30°C), and gassed with purified argon for 30 minutes. This period sufficed for temperature equilibration, and the whole operation of adding substrates and gassing was completed in 90 minutes. The argon used for the final gassing was passed through a train of alkaline pyrogallol, sulphite-anthraquinone, KOH, and distilled water to absorb traces of oxygen. Check determinations using heat-sterilized soil, and pyrogallol and KOH reagents in the center wells of respiration vessels have shown that no uptake of gas attributable to O_2 can be detected manometrically using this evacuation and gassing procedure.

The duration of experiments varied from 144 to 400 hours and manometric readings were made at frequent intervals, generally every 4 hours. Evolution of gases other than CO_2 was indicated by the development of positive pressures in the respiration vessels. On the assumption that the gases measured were N_2 and N_2O, gas evolution has been reported as micrograms of N per gram of oven-dry soil.

Analytical methods

Chemical and physical properties of the soils were determined by the following procedures: Soil organic carbon by dry combustion [10]; total soil nitrogen by the modified Olsen digestion method [2]; and NO_2-N and NO_3-N on soil extracts by the α-napthylamine–sulphanilic acid and phenol-disulphonic acid methods respectively.

Saturation capacity was determined by measuring the water content of aggregates held at zero water tension for 24 hours on a sintered-glass Buchner funnel. For subsoil aggregates which broke down badly on wetting, the approximate water content at zero tension was estimated by multiplying the water content at 100 cm tension by a factor of 2. This factor was established from the water contents of non-disintegrating subsoil aggregates determined at the two tensions.

EXPERIMENTAL RESULTS

The relationship of gas evolution and soil-nitrogen loss

The efficacy of the manometric method for denitrification studies depends on the assumption that any positive pressure developed in the respiration vessels after all the CO_2 produced

* Commonwealth Industrial Gases argon containing 5 ppm oxygen.

by microbial metabolism has been absorbed by KOH is due to the evolution of either N_2 or N_2O. If N_2 and N_2O are the gases evolved, a close quantitative relationship should exist between gas evolution measured by the manometric method in the presence of KOH, and loss of NO_3-N and total soil nitrogen. Accordingly, the following experiment was conducted to determine the validity of this hypothesis.

Red-brown-earth aggregates containing 13 μg/g NO_3-N from the surface soil (0–1″) under 14-year pasture (July samples) were used in this experiment. KNO_3 equivalent to 101 μg N/g oven-dry soil (ods) was added to the air-dried soil in the respiration vessel and the water content adjusted to approximately 90 per cent of saturation capacity. Treatments numbered 1 to 5 were set up. After gassing and equilibration as described above, manometric readings were taken at 3- to 4-hourly intervals.

With Treatment 1, the respiration vessel was removed after manometric readings indicated that approximately half the theoretical denitrification of the nitrate had occurred. Determinations of NO_3-N, NO_2-N and total soil nitrogen were made on portions of the soil contained in the respiration vessel immediately after removal of the vessel from the manometer and bath. The remaining treatments were similarly analysed when a rapid fall in rate of gas evolution indicated near completion of denitrification, (Table 1). With Treatment 5, manometric readings were continued for a further 60 hours after completion of gas evolution, (Figure 1).

The level of initial total nitrogen was determined also on five replicates of aggregates to which 101 μg N/g as KNO_3 had been added. The results have been included in Table 1, and a nitrogen balance has been drawn up using average values.

The data show a close relationship of increasing total gas production coupled with an increasing loss of NO_3-N and total nitrogen. This same relationship of NO_3-N disappearance and gas production was found in all subsequent experiments. While traces of other gases such as H_2, CH_4, NH_3, H_2S may be evolved, there is little doubt that this method measures essentially the rate and course of conversion of NO_3-N and NO_2-N into nitrogenous gases.

TABLE 1

Nitrogen loss and gas evolution from Urrbrae loam using the manometric method						
Treatment	Final level in respiration vessel μg N/g					Initial total soil N †
	NO_2-N	NO_3-N	Total soil N *	Gaseous N **	total N	
1	20	20	2730	45	2775	2738
2	1	8	2726	103	2829	2779
3	1	10	2709	111	2820	2835
4	1	7	2710	119	2829	2837
5	1	22	2746	94	2840	2845
Average:						
Final	5	14	2724	94	2819	
Initial *	0	114	2807	0	2807	

* Includes NO_2-N and NO_3-N.

** Calculated from manometric readings.

† Determination on independent soil samples containing initial addition of 101 μg NO_3-N/g.

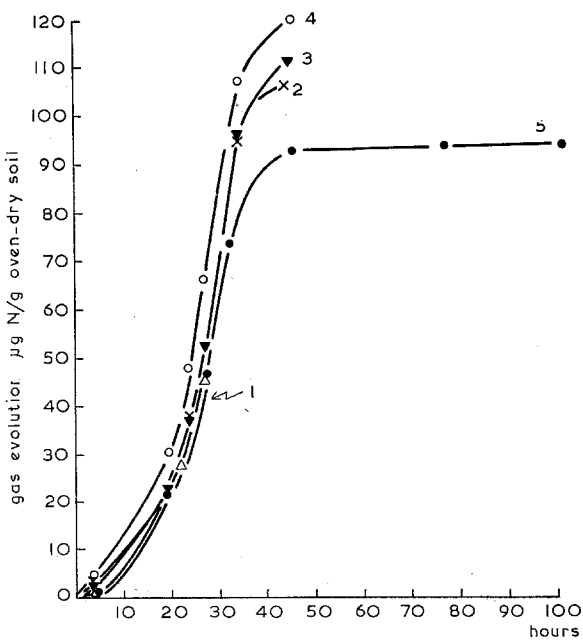

Fig. 1. Gas evolution from Red Brown Earth containing added NO_3–N. Numbered graphs refer to treatments in Table 1.

Factors influencing denitrification

(a) Water content. The effect on denitrification of varying soil water contents was determined using the manometric technique. Soil aggregates from the 14-year-pasture samples collected in May and containing 60 μg NO_3-N/g were used in these experiments.

Gas evolution was followed manometrically with 11 treatments containing 10 g of soil aggregates to which a further 103 μg NO_3-N/g of soil and varying quantities of water had been added. The center-well contained KOH, and the usual gassing procedure was followed. Water content, NO_2-N, and NO_3-N were measured at the end of each experiment. The final water content is reported, and represents a content lower by 2 to 4 per cent than the calculated initial level. Typical results are graphed in Figure 2.

The gas-evolution curves indicate an initial short lag period followed by a steady rate of gas evolution until the NO_3-N substrate is depleted. This pattern applies to all treatments except that with the lowest water content, (near wilting point). The uniformity of the pattern of the curves enables the denitrification

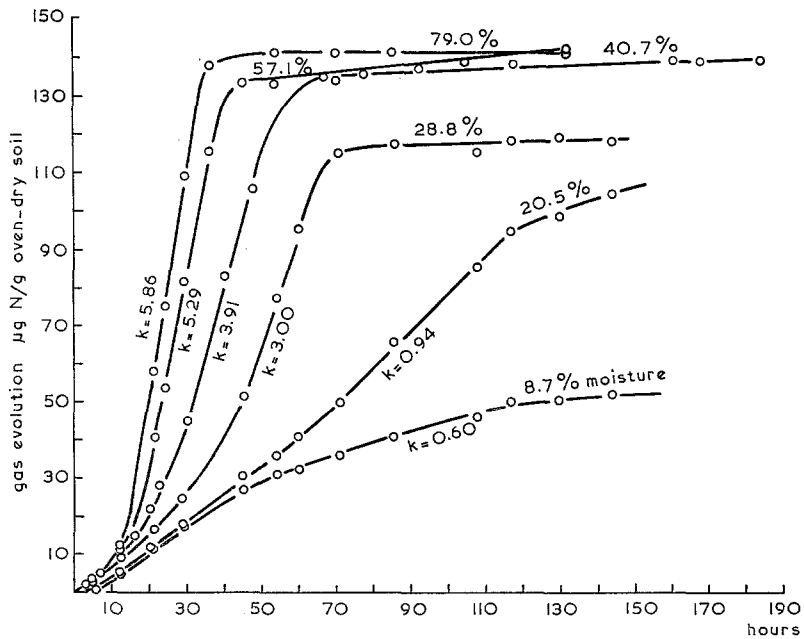

Fig. 2. Effect of soil water content on denitrification.

process to be characterized by the rate of gas evolution (k) as μg N/g oven-dry soil per hour over the linear part of the curve. In this experiment the k-values are also the maximum rates of gas evolution. The results under anaerobic conditions show that increased rate of denitrification is related to an increase in soil water content. The total denitrification also increases from the lower moisture range under these experimental conditions up to 40.7 per cent H_2O or approximately 80 per cent of saturation capacity.

Other experiments were performed on the same soil sample as used above. In one such experiment glucose was added and the water content was varied. In another experiment the level of nitrate addition was varied within a narrow range of soil water levels. These experiments are described below, but the results in relation to water content are considered here. Since the pattern of denitrification with this soil is characterized by the maximum

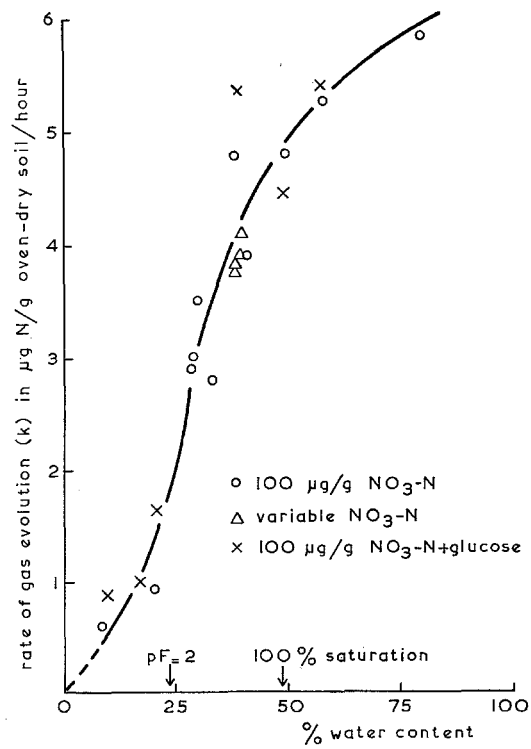

Fig. 3. The effect of water content on rate of denitrification.

rate of denitrification, this rate has been graphed against water content, (Figure 3).

The graph shows that under anaerobic conditions the rate of denitrification increases as the content of soil water increases within the range of wilting point to 165 per cent saturation capacity. It follows that for the purpose of comparative denitrification experiments, it is important to standardize the water content.

(b) Content of nitrate nitrogen. Red-brown-earth 14-year-pasture soil aggregates (0–1″) containing 60 μg N/g were used in an experiment to determine the effect of different contents of NO_3-N on denitrification. The soils were brought to approximately 85 per cent of saturation capacity with solutions of KNO_3 which added NO_3-N equivalent to 0, 52, 103, 206, and 412 μg N/g. Gassing and manometric procedure were as previously described.

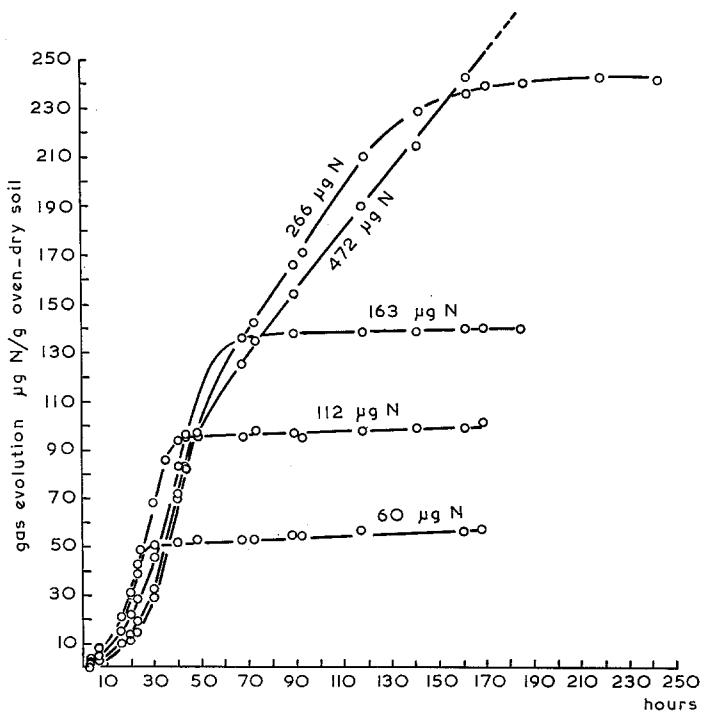

Fig. 4. The effect of initial nitrate content on denitrification.

The results indicate that between 60 μg N/g and 472 μg N/g the level of NO_3-N does not influence the maximum rate of denitrification (Figure 4). However, with levels above 112 μg N/g, a lag in the onset of the maximum rate is evident with increasing concentrations of NO_3-N. This lag does not alter the pattern of denitrification and in subsequent experiments 100 μg NO_3-N/g of soil was added as the usual substrate irrespective of the level of native soil NO_3-N.

(c) Effect of organic-matter addition. The importance of a high level of decomposible organic matter as a substrate to supply the energy needs of denitrifying micro-organisms is well known. As a preliminary study, denitrification was followed in four soils containing different quantities of native soil organic carbon.

The soils were red-brown-earth 0–3″ permanent pasture (2.63 % org. C), 0–3″ continuous wheat (1.65 % org. C), 0–3″ FWPP (2.12 % org. C), and 30–36″ permanent pasture (0.38 % org. C. NO_3-N equivalent to 100 μg N/g was added to each, and the water

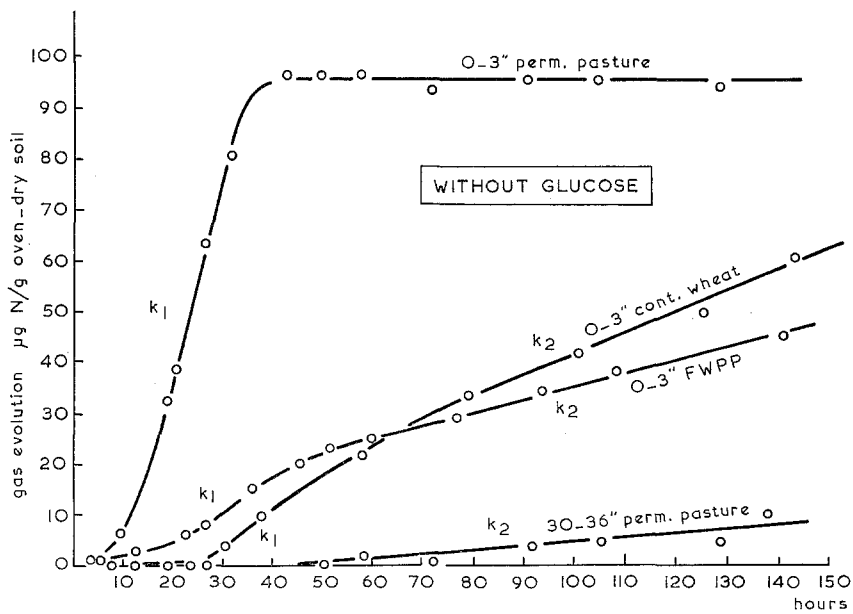

Fig. 5. Patterns of denitrification in 3 surface soils and 1 subsoil of red-brown earth without added glucose substrate.

content brought to 90 per cent saturation. Treatments without glucose and with 0.1 per cent glucose were set up.

The results of treatments without glucose indicate three distinct patterns of gas evolution, (Figure 5). The 0–3″ permanent pasture shows a short lag followed by rapid attainment of a steady and high rate of gas production, which eventually ceases as the NO_3-substrate is depleted. The second pattern, represented by the continuous-wheat and FWPP treatments, also shows a short lag followed by at least two different rates of gas production: An initial higher rate, k_1, followed by a lower rate, k_2. A third pattern is represented by the 30–36″ permanent pasture in which a long lag is followed by an extremely slow rate of production, k_2.

In the treatment in which glucose was added, all soils showed a more rapid rate of denitrification, (Figure 6). This increase in rate was least in the 0–3″ permanent pasture containing a high level of native organic matter. In the 0–3″ continuous wheat, 0–3″ FWPP, and 30–36″ permanent pasture samples, the pattern of the gas evolution was changed to a pattern similar to that of the 0–3″ permanent pasture. The length of the lag phase was also

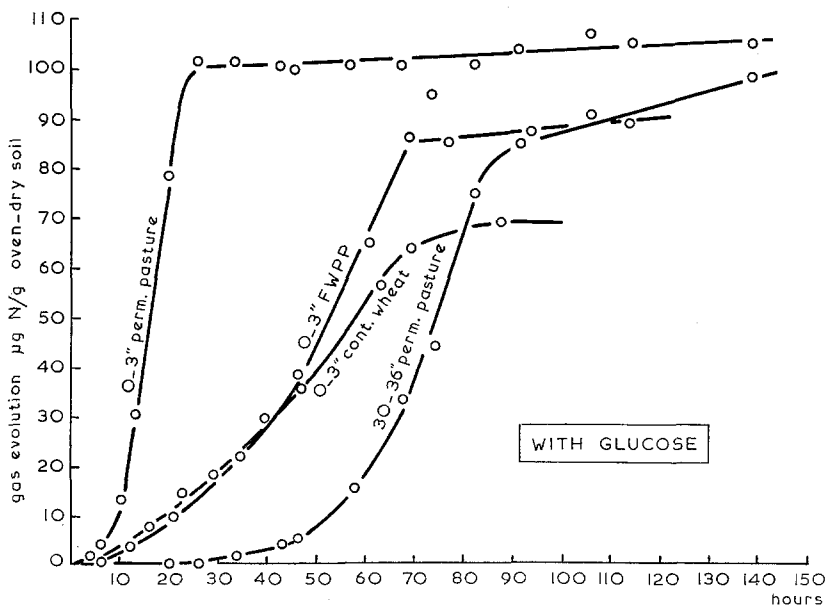

Fig. 6. Patterns of denitrification in 3 surface soils and 1 subsoil of red-brown earth with added glucose substrate.

shortened by the addition of glucose. These results indicate that the level of assimilable organic carbon is a major factor determining the pattern and maximum rate of gas evolution.

A further experiment was carried out to determine the pattern of response to low levels of glucose addition. Aggregates from the 0–3″ continuous-wheat rotation were placed in the respiration vessels, and a substrate of KNO_3 (100 μg N/g) and glucose (0, 0.005, 0.01, 0.05, 0.1, and 0.2 per cent) was added. The soils were brought to a water content of 90 per cent saturation capacity.

The addition of 0.01 per cent glucose (0.004 per cent carbon) is sufficient to change the rate of gas evolution quite markedly (Figure 7). With increasing amounts of glucose above the 0.05 per cent level, very little change in the rate and course of gas evolution occurs. This suggests that a limit to the rate of denitrification is imposed by factors other than the content of readily utilizable energy material.

This is also demonstrated by the results (Table 2) of a further experiment with 0–1″ permanent pasture (May), which contained a high level of organic carbon. Addition of glucose (0.1 per cent) at varying moisture levels did not stimulate any greatly increased

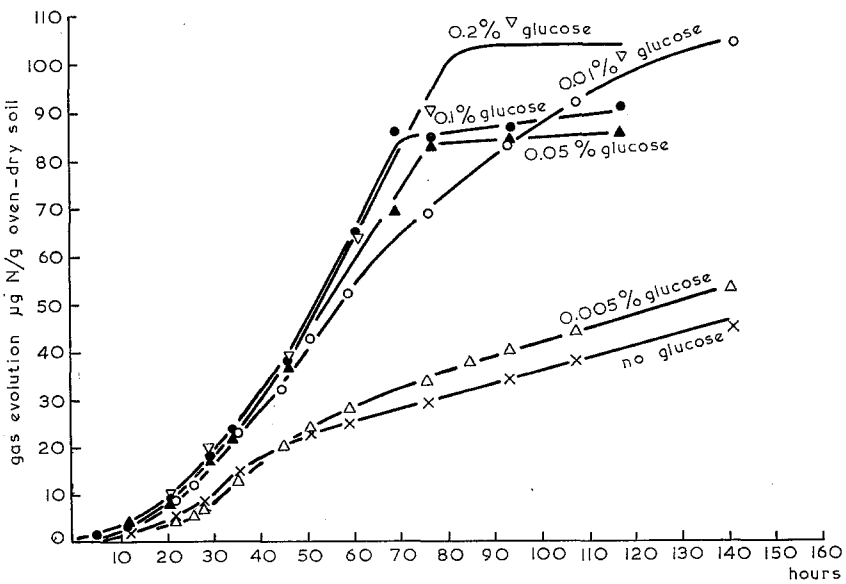

Fig. 7. The effect of glucose addition on denitrification.

rate of gas production. The patterns were of similar type to those depicted in Figure 2.

TABLE 2

Effect of water content and glucose on rate and quantity of nitrogenous-gas production

No Glucose			Glucose (0.1%)		
% H_2O	Rate (k) *	Total N gas **	% H_2O	Rate (k) *	Total N gas **
8.7	0.60	52	9.4	0.87	63
20.5	0.94	110	16.6	1.00	92
28.8	3.00	121	27.5	2.83	106
40.7	3.91	132	38.7	5.35	111
48.8	4.83	147	49.0	4.45	104
57.1	5.29	136	57.1	5.40	100

* μg N/g oven-dry soil per hour.
** μg N/g oven-dry soil after 150 hours or completion of gas evolution.

It is obvious that once a certain level of assimilable organic carbon is reached, then additional amounts do not increase the maximum rate of denitrification appreciably although it may prolong the period during which the maximum rate of denitrification is maintained. This is suggested by the shape of the curve for 0.01 per cent glucose in Figure 7, and also by the shape of the curves in Figure 4 of the highest levels of NO_3-N addition. In Table 2, the amount of gas produced is less with glucose than without. Apparently more NO_3-N is immobilized as microbial protein in the presence of glucose.

(d) Distribution of denitrifying organisms. To test for the presence of denitrifying organisms in the surface and subsoil, selected depth samples (Table 3) of red-brown-earth permanent pasture were placed in respiration vessels and the pattern of denitrification determined over 144 hours. One set of soils was supplied with glucose (0.1 per cent), and NO_3-N (100 μg/g), and the other set with NO_3-N (100 μg/g) only; all soils were brought to 90 per cent saturation.

It is evident that denitrifying organisms are present to the depth studied, although the longer lag period in denitrification for subsoils suggests that the numbers may be considerably lower than in the surface and sub-surface horizons. It is apparent from

TABLE 3

Denitrification in red-brown earth under permanent pasture								
Depth (inches)	Initial soil NO_3-N (μg/g)	Glucose substrate *	Gas evolution (μg N/g) at stated hours				Final soil	
			36	72	108	144	NO_2-N (μg/g)	NO_3-N (μg/g)
0–3	129	–	92	95	95	95	1	12
		+	101	100	104	104	0	13
3–6	120	–	17	41	56	67	12	37
		+	22	68	89	93	0	1
6–12	117	–	16	32	47	62	18	17
		+	40	89	91	88	0	2
18–21	103	–	19	36	54	68	18	21
		+	45	79	90	89	0	0
30–36	107	–	0	1	5	12	17	69
		+	2	40	90	98	0	0
42–48	113	–	1	0	3	13	9	76
		+	2	9	58	113	0	3

* – No glucose
\+ 0.1% glucose

the two sets of data that the lower rate of denitrification in the subsoils is the consequence of low availability of native organic substrates rather than the absence of organisms, for once given a source of decomposable organic matter, gas evolution increased rapidly.

Denitrification in the genetic horizons of red-brown earth and black earth

The foregoing studies indicate that denitrification of NO_3-N added to soil can be estimated by the manometric technique with an accuracy within the limits of soil and biological variation. Neither the differences in concentration of NO_3-N substrate produced when 100 μg NO_3-N/g soil is added to the level of native NO_3-N in the soil, nor slight variations in the water content near saturation capacity produces any marked effect on either the rate or the extent of denitrification. The major factor influencing

TABLE 4

Denitrification in the genetic horizons of black earth and red-brown earth

Depth (inches)	Genetic * horizon	% Org. carbon	Initial NO_3-N ** µg/g	Gas evolution † µg N/g				Final	
				36	72	108	144	NO_2-N µg/g	NO_3-N µg/g
Black earth – permanent pasture									
0–3	$A_{1.1}$	5.24	105	73	94	93	94	0	11
3–6	$A_{1.1}$	4.30	106	55	93	93	94	0	11
6–9	$A_{1.2}$	2.77	103	42	96	100	100	0	8
9–12	$A_{1.2}$	2.49	105	47	95	98	95	0	10
12–18	AB	1.61	105	40	91	101	100	0	10
18–24	BC_{ca}	0.83	104	24	41	51	61	19	21
24–30	BC_{ca}	0.52	102	9	24	45	52	22	26
30–36	BC_{ca}	0.38	102	4	10	13	15	32	43
Red-brown earth – permanent pasture									
0–3	$A_{1.1}$	2.63	129	92	95	95	95	1	12
3–6	$A_{1.1}$	2.26	120	17	41	56	67	12	37
6–12	$A_{1.2}$	1.82	117	16	32	47	62	18	17
12–18	AB	1.40	—	—	—	—	—	—	—
18–21	AB	1.05	103	19	36	54	68	18	21
21–24	B_1	0.42	103	4	11	12	14	8	62
24–30	B_1	0.27	104	2	9	10	13	12	70
30–36	$B_{2.1}$	0.33	107	0	1	5	12	17	69
36–42	$B_{2.2}$	0.40	--	—	—	—	—	—	—
42–48	$B_{2.2}$	0.36	113	1	0	3	13	9	76
Red-brown earth – continuous wheat									
0–3	A_p	1.65	116	9	30	45	61	6	7
3–6	A_p	1.06	131	6	16	25	27	12	47
6–9	A_p	0.53	102	6	15	19	23	16	59
9–12	$A_{1.2}$	0.41	104	8	15	21	21	10	53
12–18	AB	0.33	102	3	12	12	16	16	57
18–24	B_1	0.31	102	2	12	19	20	8	64
24–30	$B_{2.1}$	0.33	102	1	9	22	25	3	69
30–36	$B_{2.1}$	0.35	103	0	4	6	18	0	79
36–42	$B_{2.1}$	0.32	103	0	4	10	17	0	77
42–48	B_{ca}	0.26	103	1	4	10	15	3	68
Red brown earth – FWPP									
0–3	A_p	2.12	136	15	28	38	45	11	49
3–6	A_p	1.67	177	6	12	25	31	9	94
6–9	A_p	0.71	147	2	5	12	13	6	63
9–12	$A_{1.2}$	0.71	103	3	3	7	6	5	73
12–18	B_1	0.49	108	3	7	13	16	3	52
18–24	$B_{2.1}$	0.50	105	0	1	5	3	0	92
24–30	$B_{2.1}$	0.51	110	1	5	9	12	1	69
30–36	$B_{2.1}$	0.43	106	0	6	14	17	0	68
36–42	$B_{2.2}$	0.41	106	2	6	7	14	5	74
42–48	B_{ca}	0.38	104	1	3	2	6	15	42

* According to U.S.D.A. Soil Survey Manual Handbook 18; 1951.
** No NO_2-N in initial samples.
† Gas Evolution at various periods in hours.

denitrification under these experimental conditions is the level of assimilable organic carbon.

In the following series of experiments, the denitrifying ability of soil aggregates from the genetic horizons of the black earth under permanent pasture, and the red brown earths under permanent pasture, continuous wheat, and FWPP was determined by the described manometric technique. NO_3-N equivalent to 100 μg N/g was added to each soil in the respiration vessel and the water content was adjusted to 90 per cent saturation. As no organic matter was added, the denitrifying process was made completely dependent on the content of native soil organic matter as an energy-supplying substrate.

The gas evolution values at 36, 72, 108, and 144 hours have been obtained by interpolation from curves based on approximately four-hourly readings (Table 4). In some soils the gas evolution was still slowly continuing at the end of the 144-hour period.

Initial NO_3-N data have been included in Table 4 as well as the final NO_2-N and NO_3-N contents. The average recovery of NO_2-N, NO_3-N, and gaseous N, based on initial NO_3-N, was for the black-earth profile 97.8 per cent; and for the red-brown-earth permanent pasture 91.0 per cent, continuous wheat 87.5 per cent, and FWPP 74.3 per cent. The difference between the recovered nitrogen and initial NO_3-N may be ascribed to either assimilation of nitrogen by micro-organisms, or conversion in the pathway of denitrification to nitrogen compounds which have not been estimated, or both.

Of significance, however, is the finding that denitrification is possible in the subsoil B-horizon, even at depths of three to four feet, given conditions of anaerobiosis and a supply of NO_3-N. Although both initiation and rate of denitrification in soils from these depths is slow compared with the surface horizons, it is nevertheless a distinct and measurable process.

The relationship of denitrification to the genetic horizons is apparently a function of the organic carbon entity. This is shown in Figure 8, where the total denitrification at 144 hours is directly related to the level of total soil organic carbon. It should be noted that the horizontal portion of the graph for the black earth reflects the almost complete conversion of substrate NO_3-N addition to gas in horizons containing more than 1.61 per cent organic carbon.

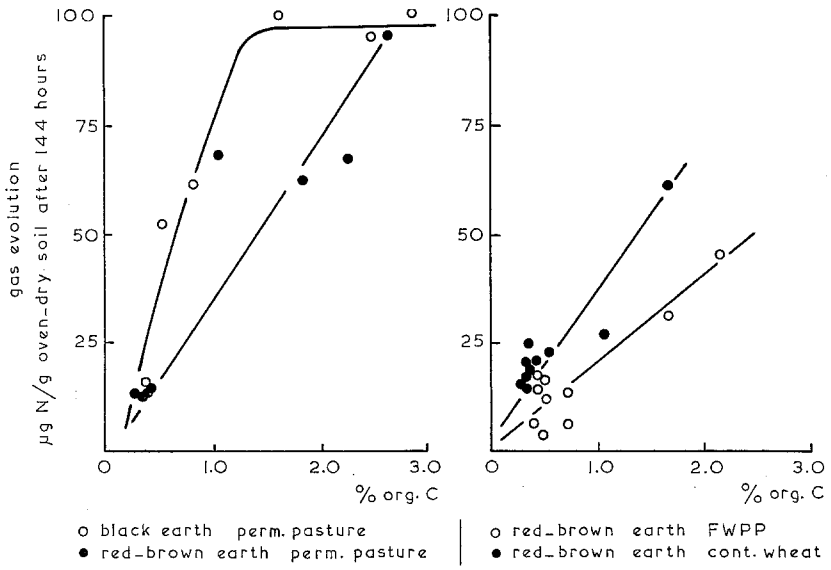

Fig. 8. The relationship of denitrification and native organic carbon content (in oven-dry soil) in profiles of 3 red-brown earths and 1 black earth.

The rate of evolution of gas differs between the profiles, and is greater per unit of native organic carbon for the black-earth permanent pasture, than for the red-brown-earth permanent pasture, (Figure 8). Of the red-brown earths, the FWPP rotation shows the lowest gas production per unit organic carbon. The reasons for these differences in rate of denitrification have not been investigated, but for the red-brown earths they may relate to the proportion of readily utilizable organic carbon in the total organic matter, which in turn is probably a reflexion of differences in the rotation treatments.

DISCUSSION AND CONCLUSIONS

Although various workers have suggested the possibility that gases such as H_2, CH_4, NH_3, H_2S may be evolved during denitrification experiments in closed anaerobic vessels [5 9], these gases have not been recorded even when total gas analyses of the vessel atmospheres were made by scan mass spectrometry [8 9]. The consistent relationship of NO_3-N disappearance to gas evolution

calculated as nitrogen, supports the view that nitrogenous gases are the only gases measured by the described manometric technique. It has not been possible to distinguish between N_2O and N_2 in the present work. It is noteworthy that the use of argon has effectively prevented anaerobic nitrogen fixation by free-living organisms, as shown by the stable level of gas pressure maintained in vessels after denitrification had ceased. The manometric technique reported here can therefore be regarded as a rapid and effective means of investigating many of the factors influencing denitrification in soils.

The effect of increasing soil water content on denitrification yielded similar data to those of other workers [2 9]. Bremner and Shaw [2] have shown that there is an increase in rate of denitrification in flasks open to air with increasing water contents from 50 per cent water-holding capacity to beyond saturation. They attributed this to a decrease in the rate of diffusion of oxygen into soil at higher moisture levels, and consequent increase in anaerobiosis. Some other explanation is needed for the results of the experiments reported here, (Figure 2). It would appear that the activity of the denitrifying organisms is controlled by the volume of water available for proliferation, and that the limit to increased rate of denitrification with increased water content is probably determined by complex nutritional factors.

The effect of readily utilizable organic carbon on denitrification in the red-brown earths is demonstrated by experiments in which glucose additions were made (Figure 7, Table 3 *etc.*).

From these experiments it appears that the utilizable organic carbon present in these soils determines to a large extent the patterns of gas evolution. The slow evolution of gaseous nitrogen from subsoil samples containing low levels of total organic carbon may therefore be attributed to a slow release of energy materials suitable for utilization by the denitrifying organisms. These utilizable materials could be derived from the breakdown of more complex soil organic matter by other groups of organisms. However, the greater part of the total denitrification in the initial 144 hours appears to result from use by the denitrifying organisms of the more decomposible energy material already present in the soil. The direct relationship of denitrification to total organic carbon in the soils examined suggests that the decomposible

fraction utilized by the denitrifying organisms must also be directly related to the total carbon content. Since denitrification is essentially a respiration process these results may be compared with a similar relationship linking total soil organic matter to respiration which has been found under aerobic conditions with the general soil microbial population [4].

The differences in rate ot denitrification, and total denitrification, shown between profiles and between the various horizons of each soil profile may therefore be ascribed to differences in levels of utilizable organic carbon. It would appear also that soil organic carbon is more readily utilized in the black-earth profile than in the red-brown earth, and that organic matter in the permanent pasture and continuous wheat is more available for utilization than in FWPP.

This study indicates that the amount of organic carbon in the subsoils of the black earth and red-brown earths is far below the level which is needed to give high rates of denitrification, even over comparatively short time periods and with the favourable conditions of these experiments. Nevertheless a content of soil organic carbon as low as 0.3 per cent can still supply energy substrate to denitrifying organisms, thus producing measurable gas evolution. While the surface horizons have a much higher potential denitrifying activity, the subsoils also possess a potential activity which could lead to significant agronomic losses of nitrogen. The question remains, however, of the extent to which denitrification occurs in the field; the present study indicates that neither lack of organic carbon content nor lack of organisms should be factors limiting denitrification in these soils.

SUMMARY

A manometric technique based on a method proposed by Gilmour *et al.* [5] was shown satisfactory for the study of the rate and pattern of denitrification in soils under anaerobic conditions. Using this method increases in water content in the soil were found to increase the rate of denitrification. Additions of glucose markedly increased the rate of denitrification in soil with low content of native organic carbon but the increase was only slight in soils with high native levels. The content of NO_3-N in the range of 60 to 472 μg/g of soil did not affect the denitrification rate or pattern. Denitrification was not prevented by absence of micro-organisms in any of the surface or subsoils investigated.

Experiments with the genetic horizons of three red-brown earths and one black earth showed that denitrification of added NO_3-N took place in these soils to a depth of at least 36″, under conditions of anaerobiosis. Surface-soil samples containing the highest level of native soil organic carbon had the greatest denitrifying activity measured over a 144-hour period. The deep subsoil with low levels of organic carbon (0.3%) showed a much weaker denitrifying activity; nevertheless the potential activity in these horizons under field conditions could have significance in the soil nitrogen cycle. The total denitrification in 144 hours was directly related to the level of organic carbon in these experiments.

The denitrifying activity of red-brown earths from rotation plots was greatest in the order of permanent pasture, continuous wheat, fallow-wheat-pasture-pasture. Differences not only in total content of soil carbon, but also in the availability of the soil carbon as an energy substrate are considered to account for this order.

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