

SOME OBSERVATIONS ON MICROBIAL ACTIVITIES IN TWO SUGARCANE SOIL GROUPS

By J. R. ANDERSON

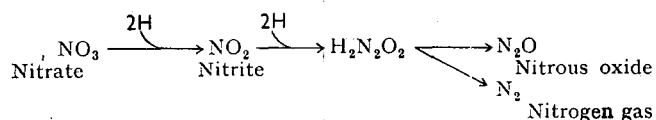
Introduction

The nature of the processes reported here should not be regarded as occurring independently of other reactions for the soil has a universe of its own in which a great number of different reactions are occurring simultaneously with one another. The type of investigations conducted below are thus only a part of a great number which can and do occur, and have been investigated in the hope that the trends noted will stimulate interest in a fresh line of approach and aid the grower in understanding the microbial universe of his soil.

The investigation is a preliminary probe into the all important problem of conserving soil nitrogen. The formation and destruction of nitrogenous materials is almost entirely accomplished by micro-organisms and it is felt that the investigation is particularly warranted in South African sugarcane soils, since little if any work of this nature has been carried out under such or even similar, agricultural conditions.

Preliminary Observations on Denitrification and Nitrification in Two Sugarcane Soil Groups

Denitrification is the term reserved to describe the microbial process whereby nitrates are reduced to gaseous nitrogen products such as nitrous oxide and nitrogen gas. A very simplified scheme of the current concept is shown below.



The actual process is not as straightforward as shown and was formerly thought to be of minor economic importance. In the light of experiments conducted by Broadbent (1951), however, denitrification is now accepted to be of significant economic importance, particularly in soils of moderate to high carbon content which are prone to high moisture conditions. Wagner and Smith (1958), for example, have estimated losses of up to 85 per cent of the nitrogen-form added and designate such losses to be in the form of nitrous oxide, nitrogen gas and nitrite. This latter form comprises only a small

percentage of the loss and has the possibility of being converted, microbiologically, to ammonia or chemically to gaseous waste products. The process of ammonification of the nitrite is unfortunately slow and uncertain, particularly in neutral to alkaline conditions and it appears more likely that the nitrite will react with the humic acid fraction of the soil and so become converted to the gaseous forms of nitrogen which are so readily lost.

In order to evaluate as closely as possible the general trends in nitrogen loss from some sugarcane soil-types, the following group of experiments were conducted:-

Experiment No. 1

To determine the differences in rate of nitrate loss from two dissimilar soil groups.

Materials and Method

A red doleritic soil and a recent sand (grey) were chosen on the grounds of being sufficiently dissimilar in the following aspects.

Table A: FEATURES OF THE TWO SOIL GROUPS INVESTIGATED

Soil Group	pH	Nitrate-Nitrogen	Carbon %	Water holding capacity
Red Dolerite	5.8	2.00 ppm	2.1%	45.00%
Recent Sand (grey) ...	5.2	0.50 ppm	0.4%	25.00%

One hundred grams of each soil were placed in separate tumblers and replicated five times for each treatment. The soils were maintained at 10 per cent, 50 per cent, and 100 per cent of their water holding capacities and in addition, waterlogged to a depth of 5 mms. above soil level.

The levels of nitrate application were obtained by addition of the equivalent of 10 ppm. nitrate-nitrogen (as potassium nitrate) in the water necessary to bring each soil to the requisite moisture level. The soils were then thoroughly mixed in the tumblers to ensure uniform distribution of water and nitrate and incubated at 25°C, the temperature most likely to simulate normal field conditions.

Five-gram samples were removed from each of the replicated treatments to make a composite 25 gram sample per treatment (every second day) and analysed for nitrate by the phenoldisulphonic acid method bearing in mind the possible interference of nitrites with the method as pointed out by

Metson (1956). In the absence of facilities to analyse by the modified Olsen method (Bremner and Shaw, 1957) the figures obtained and shown below in Table I are intended only to show the general trend of denitrification in these soil groups.

Table 1: RATE OF NITRATE LOSS FROM TWO DISSIMILAR SOIL GROUPS

Soil	Red Dolerite (pH 5.8)				Recent (grey) sand (pH 5.2)			
	10	50	100	W	10	50	100	W
Moisture level %								
N-form ppm	Nitrate-Nitrogen ppm				Nitrate-Nitrogen ppm			
Days 0	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
2	9.00	8.50	8.00	8.00	10.00	10.00	9.00	8.50
4	8.00	7.80	7.50	7.00	9.00	9.00	8.00	7.60
6	7.40	6.80	6.50	5.00	8.50	8.60	7.00	6.50
8	7.00	6.20	5.60	4.50	8.00	7.80	6.60	6.20
10	6.50	6.00	5.00	3.80	8.00	7.00	6.20	5.80
Final pH	5.8	5.8	6.2	6.2	5.0	5.2	5.2	5.5

W=Waterlogged

For graphs of the above results, see appendix figures 1a and 1b

It will be seen from these figures that the trend in the red dolerite is more pronounced than that of the grey sand. The rate of nitrate loss attributed to denitrification in the red dolerite is thought to be due to the greater carbon content of that soil, a factor which fits the findings of Bremner and Shaw in that, as carbon percentage increases (in the presence of moisture and nitrate) the rate of nitrate loss increases, accompanied usually by a decrease in acidity. Normally fermentations of (say) glucose and other carbon compounds lead to an *increased* acidity of the medium but in the case of denitrification, the nitrate, nitrite, etc., act as acceptors of hydrogen either from some organic substrate or molecular hydrogen (Kluyver and Verhoeven 1954). In the complete scheme for nitrate reduction (see Appendix, Diagram 1) it will be seen that a considerable quantity of hydrogen ions are required and the implication likely to arise is that acid soils (excess H⁺ ions) will supply this hydrogen more rapidly than a neutral soil, but this is not the case since the hydrogen is obtained from the utilisation by the bacteria of an energy substrate. It is possible however that denitrification will occur in acid soils (pH 4.5 to pH 6.8) but at a slower rate than in neutral to alkaline soils (pH 7.0 to pH 8.5) due possibly to—

- (1) low pH values favouring ammonia production from nitrate
- (2) the immobilisation of nitrate by micro-organisms.

The rate of nitrate loss observed for the red dolerite is considered to be fairly rapid and that for the grey sand, slow. The change in pH value at the completion of the experiment is considered not to be very

great and may possibly be explained by the release of potassium ions from the potassium nitrate during denitrification. Whether or not the effect is a lasting one, cannot as yet be commented upon in the absence of further investigations.

The energy derived for denitrification is thus obtained from the carbon substrate and in order to test whether increased carbon content would affect the loss of nitrate in the soils concerned, an experiment similar in all ways to the first, except for the addition of energy material, was set up. Sampling was carried out daily in anticipation of a more rapid loss.

Experiment No. 2

This experiment was made to determine the differences in rate of nitrate loss from two dissimilar soil groups with added energy source.

Thirty ppm. carbon in glucose form was added with the ten ppm. nitrate in the water necessary to bring each soil to the requisite moisture level. Incubation was as before and composite samples were removed daily for nitrate analysis.

The results are shown in Table 2, below.

Table 2. RATE OF NITRATE LOSS FROM TWO DISSIMILAR SOIL GROUPS WITH ADDED ENERGY SUBSTRATE.

Soil	Red dolerite (pH 5.80)				Recent (grey) sand (pH 5.20)			
	10	50	100	W	10	50	100	W
Moisture level %								
N-form ppm.	Nitrate-Nitrogen ppm.				Nitrate-Nitrogen ppm.			
Days 0	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
1	8.00	7.50	7.50	6.20	9.00	9.00	8.50	7.80
2	6.00	5.40	4.00	3.00	8.20	7.80	7.00	5.20
3	3.40	3.00	1.00	0.50	7.60	7.00	4.50	2.60
4	3.00	2.00	0.60	0.10	7.00		1.60	0.60
5	2.20	1.60	0.10	N.D.	6.20		1.00	0.10
Final pH	5.90	6.00	6.20	6.4	5.20		5.50	5.80

W=Waterlogged ND=Not detectable

For graphs of the above results see appendix figures 2a and 2b

The addition of energy material in this experiment had the effect of accelerating the loss of nitrate and this loss is attributed to denitrification. As a means of estimating bacterial activity, the method of Lenhard (1955) was employed. The principle involved is the addition of triphenyltetrazolium-chloride to a known quantity of soil and observation colorimetrically of the resultant red triphenylformazan which results from reduction of the tetrazolium salt by means of hydrogen ions released from an added carbon substrate. The method is thus a measure of bacterial dehydrogenase activity. The results showed that the bacterial activity in the soils before treatment was lower than after experi-

ments one and two. The activity in the soils after experiment one was lower than the activity after experiment two.

The increase of carbon-source as a means of increasing denitrification has been found by Bremner and Shaw to have an additional effect in that the carbon may be increased to a point where denitrification will be followed by nitrogen fixation—the non-symbiotic fixation of atmospheric nitrogen by bacterial forms. Hence, the ratio of added carbon and nitrogen was maintained at 3, the figure which Bremner and Shaw found to give optimum results. For example these two workers added nitrogen at the rate of 1,000 ppm. and carbon at the rate of 3,000 ppm. (7,400 ppm. glucose) and obtained optimum conditions for denitrification, but when the amount of carbon was increased to 10,600 ppm. (25,000 ppm. glucose), the process of nitrogen loss was followed by nitrogen fixation, viz. the amount of nitrogen increased. When wheat straw was used, the amount of carbon necessary was found to be 16,000 ppm. (40,000 ppm. wheat straw) and for nitrogen fixation to follow denitrification, 32,000 ppm. carbon (80,000 ppm. wheat straw). Thus with 1,000 ppm. nitrate and 32,000 ppm. carbon, a C:N ratio of 32 is obtained. In terms of total carbon and total nitrogen of the soils used, and taking into account the carbon and nitrogen added, the C:N ratio at which nitrogen fixation followed denitrification, is found to be approximately 20. The findings of Pinck, Allison and Sherman (1950) that less significant loss of nitrogen occurred at C:N ratios of 18 to 100 than at C:N of 3 to 15, tends to confirm this observation.

With the normal C:N ratios of most sugarcane soils lying between approximately 10 and 17.6 (Beater 1957), the chances of nitrogen fixation occurring, or even of less nitrogen losses occurring, will be greatly impaired. In describing the draining ability of the two soils in question, Beater (1957) designates the red dolerite as "free to drain" and the grey sand as "free to excessive, but sometimes imperfect to waterlogged on flat lands". The moisture level however, in the writer's opinion need not be in the waterlogged state for considerable losses of nitrogen to occur.

In the foregoing experiments no indication as to the type of nitrogen loss has been given, thus experiment No. 3 was carried out to determine the type of nitrogen loss as far as possible. This, in the absence of very costly apparatus, has been limited to nitrite and ammonia measurement.

Experiment No. 3

To determine the type of nitrogen loss in two dissimilar soil groups.

Materials and Method

For this experiment, a modified Lees and Quastel (1946) apparatus was employed. The apparatus, briefly, consists of a closed perfusion circuit with a soil sample contained between a reservoir of the nitrogen-carrying solution and a capillary tube which delivers in droplet form the nitrogen-carrying solution. After passage through the soil, the perfused solution drains back into the reservoir. The rate of flow is adjustable to facilitate aeration and saturation of the soil sample. In the experiment the soil was maintained at a saturated level but not waterlogged.

Each of the two soils were replicated in duplicate and perfused with an 0.002 per cent nitrate-nitrogen solution (as KNO_3) containing 60 ppm. carbon (as glucose). The experiment was conducted at room temperature for five days and samples removed daily for analysis. Due allowance has been made for initial nitrate-nitrogen content. The results obtained are presented in Table 3, below.

Table 3: NITRATE, NITRITE AND AMMONIA IN PPM. AS FOUND IN THE PERFUSATE OF TWO DISSIMILAR SOIL GROUPS

Soil	Red dolerite (pH 5.8)			Recent (grey) sand (pH 5.2)		
	NO_3^-	NO_2^-	NH_4^+	NO_3^-	NO_2^-	NH_4^+
N-form ppm	20.00	—	—	20.00	—	—
Days 0	20.00	—	—	20.00	—	—
1	13.00	4.60	0.10	14.40	3.50	—
2	9.50	6.00	0.40	10.00	6.50	0.70
3	2.60	7.20	0.60	4.50	8.40	1.00
4	0.80	0.50	1.00	2.20	2.50	1.40
5	ND	ND	0.90	0.80	1.00	1.00
Moisture level	Saturated			Saturated		

ND=Not detected

For graphs of the above results see appendix, figures 3a and 3b

The rate of nitrate loss is approximately the same as found in the preceding experiment, and it will be noticed that both nitrite and ammonia are detectable in considerable amounts. Whereas the nitrite disappears ultimately from the red dolerite, it tends to persist in the recent sand. The amount of carbon found initially in the soils may be the deciding factor in the persistence of nitrite since in soils of low carbon content there is not the same possibility of the nitrite reacting with humic substances to form further nitrous oxide and nitrogen losses. Furthermore, the higher initial acidity factor in the grey sand may cause the persistence of nitrite and also have a depreciating effect on the rate of denitrification.

With regard to the presence of the ammonia Bremner and Shaw have shown that this is likely in soils with an initial pH of 5.8 and less. The ammonia in all probability is lost ultimately by volatilisation, but in any event the ammonia detected in these experiments together with the nitrite do not offset the loss of nitrate, thus it is concluded that the greater part of the loss is in the form of nitrogenous gases.

As to the small amounts of nitrate-nitrogen added to the soils in the above experiments in comparison with that added by other workers, Bremner and Shaw have shown, that the quantitative aspect has no bearing on the actual outcome. These two authors have added amounts varying from 1,000 ppm. to 10 ppm. of nitrate-nitrogen and found that the rate is not affected, and that the effect is always the same under ideal conditions, viz: complete loss of nitrate.

In terms of pounds/acre the amount of 1,000 ppm. nitrate-nitrogen is equivalent to 1,800 lbs. nitrate-nitrogen. This amount can be supplied by the addition of approximately 3,000 lbs. potassium nitrate/acre which is felt to be excessive. Three hundred pounds will supply 180 lbs. nitrate-nitrogen. The equivalent in ammonium nitrate is 257 lbs./acre of which 180 lbs. will be nitrate-nitrogen.

The figure supplied by Bremner and Shaw for wheat straw application was 40,000 ppm. straw which supplied 16,000 ppm. carbon, necessary for optimum denitrification and 80,000 ppm. straw (32,000 ppm. carbon) for nitrogen fixation to follow denitrification. With the carbon and nitrogen already present in the soil, however, the overall ratio of C:N was found to be approximately 20, thus to ensure nitrogen fixation a C:N ratio of 20 *and above* is necessary. Since the carbon percentage in wheat straw and trash are approximately the same, it is calculated that at least 6,000 lbs./acre of trash is needed with the 257 lbs. of ammonium nitrate if the losses due to maximum denitrification are to be countered by gains from induced nitrogen fixation. The implications are thus in favour of trashing, but there are more aspects to be considered. Firstly, the pH for optimum nitrogen fixation is between pH 7.00 and 8.50, a figure usually considered high for sugarcane soils, and secondly the moisture level should not be excessive (i.e. waterlogged). Whereas the process of denitrification raises the pH, the increase may not be sufficient or sufficiently persistent to be beneficial, thus nitrogen fixation in acid soils has less chance of becoming established after denitrification if the increased pH value is insufficient for optimal fixation, and particularly if the soil remains in a saturated or waterlogged condition for any length of time.

Experiment No. 4

This was to determine the effect of raising the pH level on nitrate loss in two dissimilar soil groups.

The perfusion technique was again employed using a solution containing 20 ppm. nitrate-nitrogen (as KNO_3) and 60 ppm. carbon (as glucose). Calcium hydroxide was added to bring the pH to approximately 8.0. Incubation was at approximately 25°C for five days with aliquots removed daily for analysis of nitrate, nitrite and ammonia. The results are presented in Table 4, below.

Table 4: NITRATE, NITRITE AND AMMONIA DETECTED IN THE PERFUSATE OF TWO DISSIMILAR SOIL GROUPS WITH INCREASED pH VALUES

Soil	Red dolerite			Recent (grey) sand		
	NO ₃	NO ₂	NH ₄	NO ₃	NO ₂	NH ₄
N-form ppm						
Days 0	20.00	—	—	20.00	—	—
1	12.00	5.00	ND	14.00	4.00	ND
2	8.00	6.00	ND	9.60	6.50	ND
3	2.40	7.60	ND	4.50	7.60	ND
4	0.20	1.00	0.3	1.00	2.50	0.2
5	ND	ND	0.3	0.50	0.4	0.3
Moisture level	Saturated			Saturated		

ND=Not detected

For graphs of the above results see appendix, figures 4a and 4b

The effect of increasing the pH factor has been to accelerate loss of nitrate and to suppress ammonia formation in the red dolerite. The grey sand has been affected in a like manner but the rate of nitrate loss is not very different from that in the previous experiment. The depressing effect on ammonia formation substantiates the findings of other workers and draws attention to the fact that in the complete scheme of denitrification (appendix, diagram 1) the chances of accumulating a part at least of the reduced nitrogen as ammonia are even less in alkaline conditions. Thus, in brak soils for instance, nitrification probably occurs at a very fast rate with little or no ammonia formation or accumulation, and in addition nitrites will probably accumulate, particularly if ammonium nitrate is applied, since the ammonium fraction and high alkalinity favour nitrite accumulation (Metson 1956).

When the carbon content only was increased a different state of affairs was again noticed.

Experiment No. 5

This experiment was to determine the effect on nitrate losses in two dissimilar soil groups with an increased C:N ratio.

One hundred grams of each soil were placed in tumblers as in experiments one and two, with the equivalent of 20 ppm. nitrate-nitrogen added in the water necessary to bring each soil to a water holding capacity of 100 per cent. The treatments per soil

were replicated five times and incubated at 25°C for twenty days. Composite samples were removed from the replications every five days and analysed for nitrate-nitrogen only. The carbon content was raised from 60 ppm. C (150 ppm. glucose) to 600 ppm. C (1,500 ppm. glucose).

The results are shown in Table 5 below.

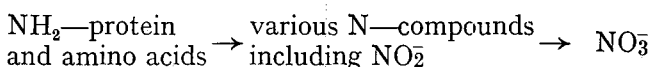
Table 5: NITRATE PPM. IN TWO SOIL GROUPS WITH INCREASED C:N RATIOS

Soil	Red dolerite (pH 5.9)	Recent (grey) sand (pH 5.2)
	Nitrate-nitrogen ppm	Nitrate-nitrogen ppm
N-form ppm		
Days 0	20.00	20.00
5	0.40	0.70
10	0.1	ND
15	0.2	0.4
20	2.6	1.8
Moisture % ...	100 %	100 %

ND=Not detected

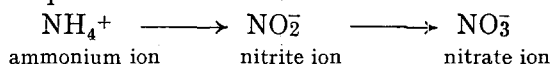
For graphs of the above results see appendix figures 5a and 5b.

This experiment showed clearly that not only was nitrate-nitrogen lost, but nitrate was being formed. In nitrogen fixation, atmospheric nitrogen is fixed in a form which is probably tied up as ammoniacal nitrogen in proteins or amino-acids. On the decomposition of this protein, nitrogen is released but not as nitrate-nitrogen. The figures obtained in the above experiment indicate that nitrate is formed as such. The only explanation offered for this is that as the fixed nitrogen is released it is immediately nitrified to nitrate, probably in the following manner:



This brings us to the second phase of the investigation conducted in this series of experiments, namely, nitrification in soil. Nitrification is the term reserved for describing the process whereby ammonia is oxidised to nitrates by micro-organisms.

The process can be outlined as follows:



It is to be expected that this process occurs at different rates in different soils and that, again, pH, organic matter, moisture, and in addition, clay fraction, play a major role. Nitrification may be observed over a wide range of pH (viz. 5-13), the optimum being in the region of pH 7.0 to 8.5. The presence of organic matter in the form of simple amino-acids is no hindrance to nitrification (Jenson, 1950) but certain types of organic matter, for example dl-methionine, secreted by roots of growing plants and other bacterial forms, have a depressing effect. Lees and Quastel (1946) have shown that the process of nitrification is a slow one and that the

rate of nitrification of a given quantity of ammonium sulphate is a function of the degree to which the ammonium ions are absorbed on, or combined in, the soil in the form of the soils base exchange complexes. i.e. the greater the amount of adsorption, the faster is the nitrification. The interpretation of Quastel and Scholefield (1951) is that the nitrifying bacteria grow on the surface of the soil crumbs at the sites where ammonium ions are held in base-exchange combination and proliferate there at the expense of the adsorbed ammonium ions. Thus when all these sites have been filled further growth of the organism will not occur except to replace dead cells. Crumb structure and base-exchange capacity are therefore essential in a soil for the formation of nitrate from ammonia.

Just as oxidation of the carbon substrate in *denitrification* resulted in the release of hydrogen ions (which were immediately utilised) so in nitrification the substrate, in this case ammonia, is oxidised with a resultant acid H⁺ ion release. This increase of H⁺ ions will, in nitrification, cause a decrease in the pH of the soil unless the soil is well buffered. One of the most important aspects of nitrification then is the necessity for good soil buffering and one of the factors controlling this is the amount of calcium carbonate present. A soil's buffering ability may be judged from the clay fraction it contains, and hence in selecting soils for comparison it was decided to employ the red dolerite and recent (grey) sand to obtain as much difference as possible. The following table shows the relative calcium contents base-exchange capacity and clay fractions of these two soil groups.

Table B: CALCIUM, TOTAL BASE-EXCHANGE AND CLAY FRACTION OF TWO DISSIMILAR SOIL GROUPS

(Figures from Beater, 1957)

Soil	Ca ppm.	Total base exchange	Clay fraction 70.002 mms.
Red dolerite	7,300 ppm.	13.20	54.4%
Recent (grey) sand	220 ppm.	0.7-5.0	5.7%

Experiment No. 6

This experiment was to determine the relative rate of nitrification of two dissimilar soil groups.

Materials and Method

One hundred grams of each soil perfused with 0.002 per cent (20 ppm.) ammonia (as ammonium sulphate) for seven days at room temperature ($\pm 25^\circ$ C) under conditions of optimum aeration with water saturation but no waterlogging. Samples were removed daily and analysed for the presence of nitrates and nitrites. The results are shown in Table 6.

Table 6: NITRATES AND NITRITES FOUND IN THE PERFUSATE OF TWO DISSIMILAR SOIL GROUPS PERFUSED WITH AMMONIUM SULPHATE

Soil	Red dolerite (pH 5.8)			Recent (grey) sand (pH 5.2)		
	NH ₄ ⁺	NO ₂ ⁻	NO ₃ ⁻	NH ₄ ⁺	NO ₂ ⁻	NO ₃ ⁻
N-form ppm						
Days 0	20.00	—	—	20.00	—	—
1	19.50	ND	—	19.80	ND	ND
2	13.50	0.40	2.70	19.50	0.10	ND
3	10.00	1.00	3.20	17.00	1.00	0.50
4	7.00	0.10	6.50	16.50	1.20	2.00
5	5.60	ND	11.40	15.80	0.40	3.40
6	5.00	ND	12.60	15.20	ND	4.00
7	4.20	ND	14.00	14.60	ND	4.70
Moisture % ...	100%			100%		
Final pH... ..	5.6			pH 4.8%		

ND=Not detected

For graphs of above results see appendix, figures 6a and 6b

The differences in the two rates are very apparent and a major part of the explanation must lie in the differences of calcium content and base-exchange buffering ability (see Table 6) of the two soils.

Not only would the above-mentioned difference and pH differences affect the rate, but also the fact that the red dolerite drains more freely than the grey sand (under these experimental conditions), is a fact which is best accounted for by the better crumb structure of the dolerite. The greater organic matter content of the dolerite may have resulted in a slower nitrification rate but in any event the rate is still much faster than in the grey sand.

In all such experiments on nitrification lag-phase is an important feature. The lag-phase is best described as the time taken for adaptation by the soil's micro-organisms to the process of nitrifying a given source of ammonium ions. Thus, if the lag-phase is long, it indicates that the nitrifying organisms are in the minority and require time to proliferate, or that the organisms are ill-adapted to conversion of ammonia and require time in order to re-adapt themselves to the substrate. In experiment No. 6 the lag-phase for the red dolerite was estimated to be approximately four hours, whereas that for the grey sand was approximately eight hours.

At the completion of the experiment, the soil samples were "flushed" with distilled water and removed to storage for five days after which they were re-perfused with ammonium sulphate. The lag-phases in this case were found to be one hour and two hours for the dolerite and grey sand respectively. In other words, once the population of nitrifiers had been stimulated to the point of saturation of soil particles, the adaptation to any further additions of ammonia was almost immediate.

When large applications of ammonium sulphate are made, the initial lag-phase is followed by a

nitrification process, which, in the absence of adverse conditions, continues until the peak conversion percentage has been reached. Thereafter, and also during the process, the plant is given access to nitrate-nitrogen but at the same time great competition for the nitrate is present, due to the existence of millions of other micro-organisms, many of which require the nitrate for their own particular purpose. Hence nitrification may be followed or paralleled by denitrification and consequently much nitrate will be lost. If no competition exists from the plant—that is if ammonium sulphate is applied at planting—the nitrate would in all probability be very greatly reduced—especially under ideal conditions for denitrification. If the ammonium sulphate could be applied in three smaller applications, the possibility exists that the soil would become adapted to the process of nitrification which would continue over a longer period of time. The growing plant would have access to part of the nitrate produced, the soil would have an induced population of nitrifiers ready for the next application and hence could convert to nitrate more readily.

Experiment No. 7

To test the effect of successive applications of ammonia to two dissimilar, previously perfused, soil groups.

The soil samples from experiment No. 6 were wrapped in cloth and suspended in a tank of running water in order to remove as much of the nitrate formed during the previous experiment as possible. The samples were stored under cool, moist, conditions for five days and re-perfused with ammonium as ammonium sulphate. The results are shown in Table 7.

Table 7: NITRATE AND AMMONIA DETECTED IN RE-PERFUSED SOIL SAMPLES

Soil	Red dolerite (pH 5.8)		Recent (grey) sand (pH 5.0)	
	NH ₄ ⁺	NO ₃ ⁻	NH ₄ ⁺	NO ₃ ⁻
N-form				
Days 0	20.00	—	20.00	—
1	15.00	1.00	19.20	—
2	10.00	2.50	18.00	0.20
3	8.60	8.00	16.00	2.50
4	7.50	10.20	15.40	3.20
5	4.60	14.00	14.50	3.80
6	4.00	14.00	13.80	4.50
... 7	3.80	14.50	12.60	4.80
Moisture % ...	100%		100%	
Final pH... ..	5.8		4.7	

For graphs of the above results see appendix figures 7a and 7b

The increased rate on re-perfusion is obvious by comparison of Tables 6 and 7. The fact that the grey sand did not produce very much more nitrate than

in experiment No. 6 indicates the lack of adequate base-exchange ability and crumb structure in this soil. A further point arising from the two experiments is that the pH of the grey sand was markedly affected, indicating a lack of adequate buffering in this soil. The rate of nitrification, even had there been a better crumb structure and hence better retention of viable cells, would in all probability still have been slower by virtue of the somewhat lower pH.

Conclusions

In all the experiments conducted in this series the general trends show that nitrification of applied ammonium salts is a comparatively slow process requiring adequate soil buffering, which in turn requires adequate calcium content. The pH aspect is variable, a considerable range sufficing for the process to occur, but since the process is essentially a surface phenomenon, it is essential that a good crumb structure be present. The organisms bringing about nitrification require higher oxygen tensions than are normally found at depths exceeding ten inches and thus nitrification occurs mainly in the top six inches provided the structure of the soil is such as to allow for adequate aeration. Nitrification of ammonia to nitrates may be followed by denitrification particularly if the soil should become saturated or waterlogged together with the presence of low C:N ratios.

It is also possible that under high C:N ratios this denitrification will be followed by a certain amount of nitrogen fixation, providing conditions are suitable. The entire process of nitrate formation and loss is thus seen in the light of a continuous reaction with reductions following oxidations and fixations. The delicate balance of conditions favouring one or the other of these reactions need only be altered slightly in order to stimulate the cycle one way or the other. Part of the answer in aiding the lowering of nitrogen losses would seem to lie in increasing the carbon-nitrogen ratio, in creating adequate drainage to the best of our ability and in maintaining a pH less favourable to denitrification. These aspects are not always practical propositions, as for example in grey sands where by increasing the pH to about 8.0 nitrification would not necessarily be greatly facilitated since increases in the clay fraction and soil structure would be necessary in order to allow the organisms to proliferate to a point where nitrification would be beneficial. As such changes are not practical propositions, an alternative would be to reconsider the type of fertiliser applied. Here again there are difficulties, since by applying ammonium salts to a soil of low nitrifying ability, little benefit can be obtained since the buffering ability will probably be inadequate to the extent of allowing the pH to decline still further, and with the slow rate of nitrification liable to occur in such

soils, much loss of ammonia by volatilisation could occur.

In the case of soils with adequate buffering and base-exchange qualities but where no build up of organic matter is allowed for, the application of ammonium sulphate would probably be more beneficial than nitrate application since in burned or untrashed lands the C:N ratio is likely to be low enough to fall within the ratio favouring denitrification.

The trends as shown in the experiments conducted in this series indicate far more the need for a greater, full scale investigation into *all* the soil groups, in particular such soils as mist belt Table Mountain Sandstones and soils with high pH values. Studies on the cultural practices of trashing, burning, green manuring, spray irrigation, flood irrigation and fallowing are also indicated if a more complete picture of the microbiological processes of our soils is to be obtained.

Summary

1. Comparative rates of denitrification in two dissimilar soil groups have been studied and the effect of increased energy substrate, increased pH and increased C:N ratio values, noted.
2. Attempts to determine the type of nitrogen lost were carried out and the conclusion made that the loss probably occurs as gaseous nitrogen forms.
3. Relative rates of nitrification in the same two soil groups were also studied and the effect of re-perfusion of the soils noted.
4. It has been concluded that denitrification is very likely to occur in sugarcane soils with low C:N ratios and that nitrogen fixation after denitrification is possible in more significant quantities only if the C:N ratio is increased.
5. Nitrification has been found to be higher in the sugarcane soil with high calcium content, high base-exchange capacity and adequate buffering potential than in the sugarcane soil with low calcium, base-exchange and buffering values.
6. Whereas no policy or change of policy has been advocated, suggestions are made which may lead to less nitrogen losses.
7. Further microbiological investigation into sugarcane soils and agricultural practices is advocated.

Acknowledgements

The author wishes to thank Dr. B. E. Beater and Mr. J. L. du Toit for their advice and encouragement, and the South African Sugar Association Experiment Station Committee in granting permission and time for this preliminary investigation to be made.

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Mt. Edgecombe.

APPENDIX

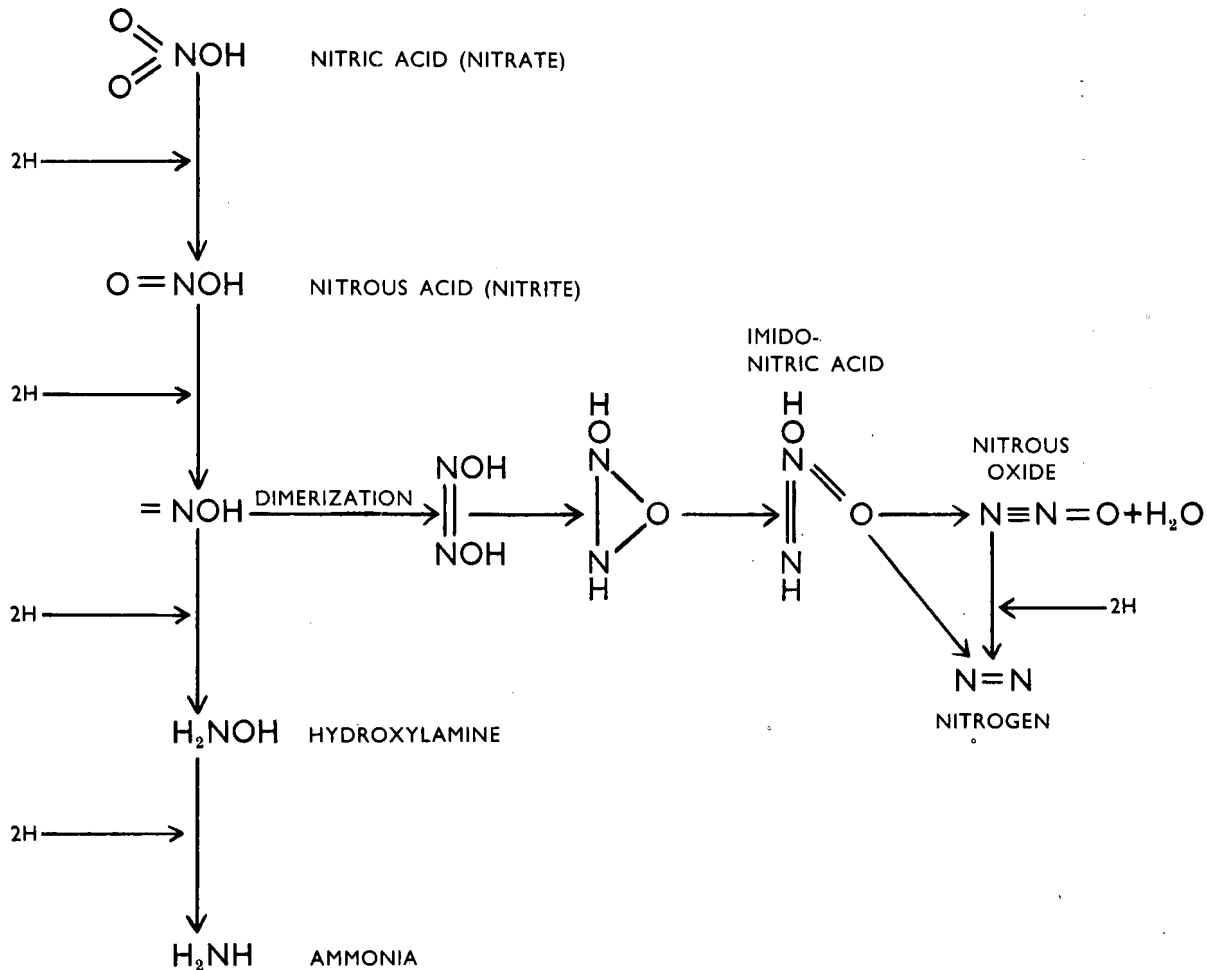


DIAGRAM I

(Kluyver and Verhoeven, 1954)

CONCEPT OF DISSIMILATORY NITRATE REDUCTION

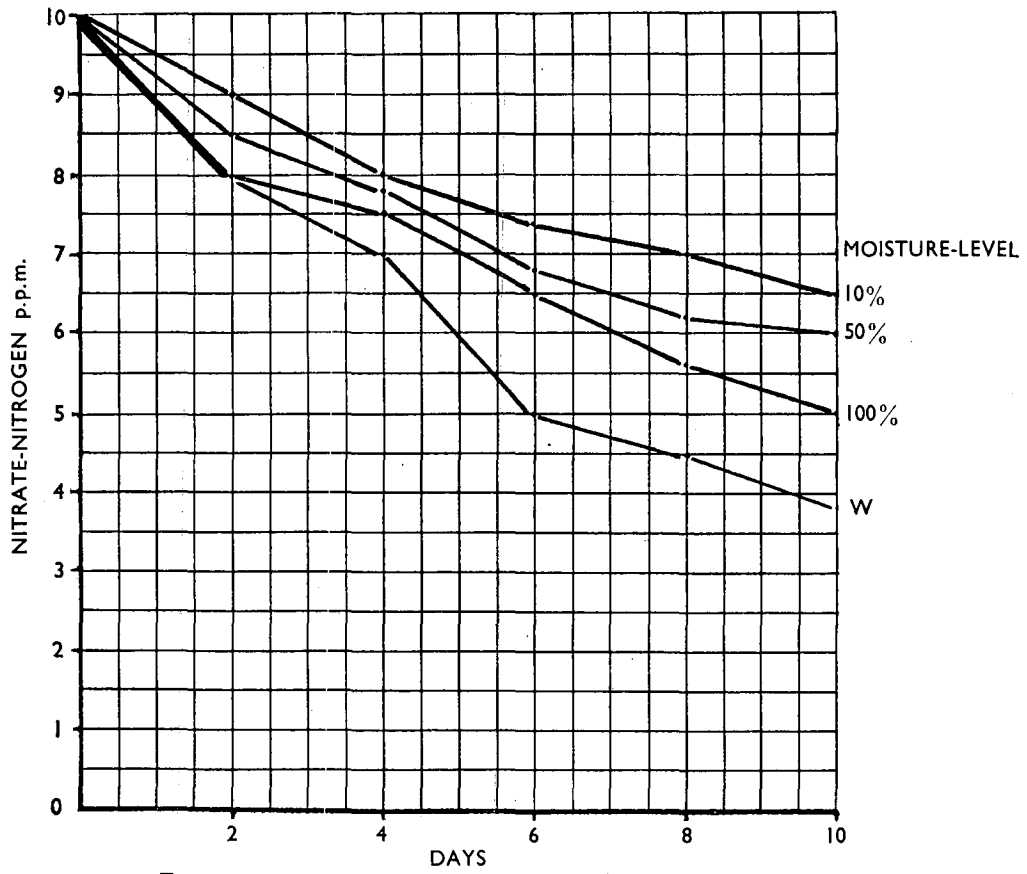


FIG. 1A: RATE OF NITRATE LOSS FROM RED DOLERITE

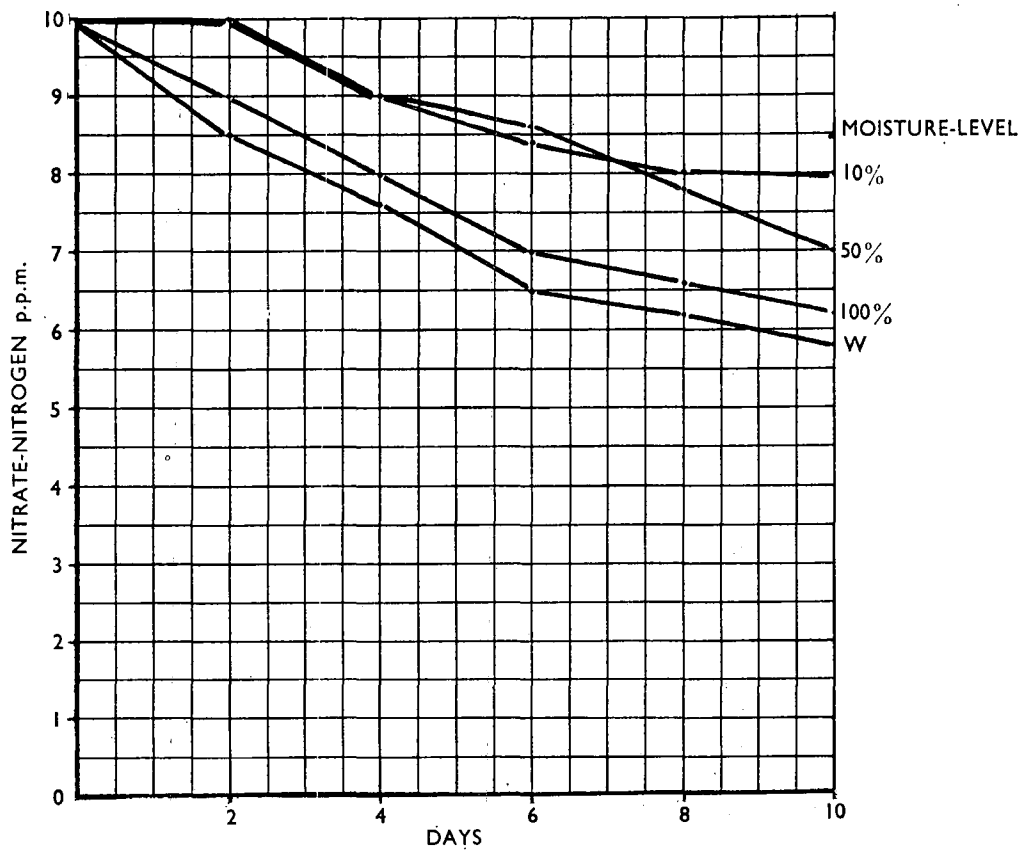


FIG. 1B: RATE OF NITRATE LOSS FROM RECENT (GREY) SAND

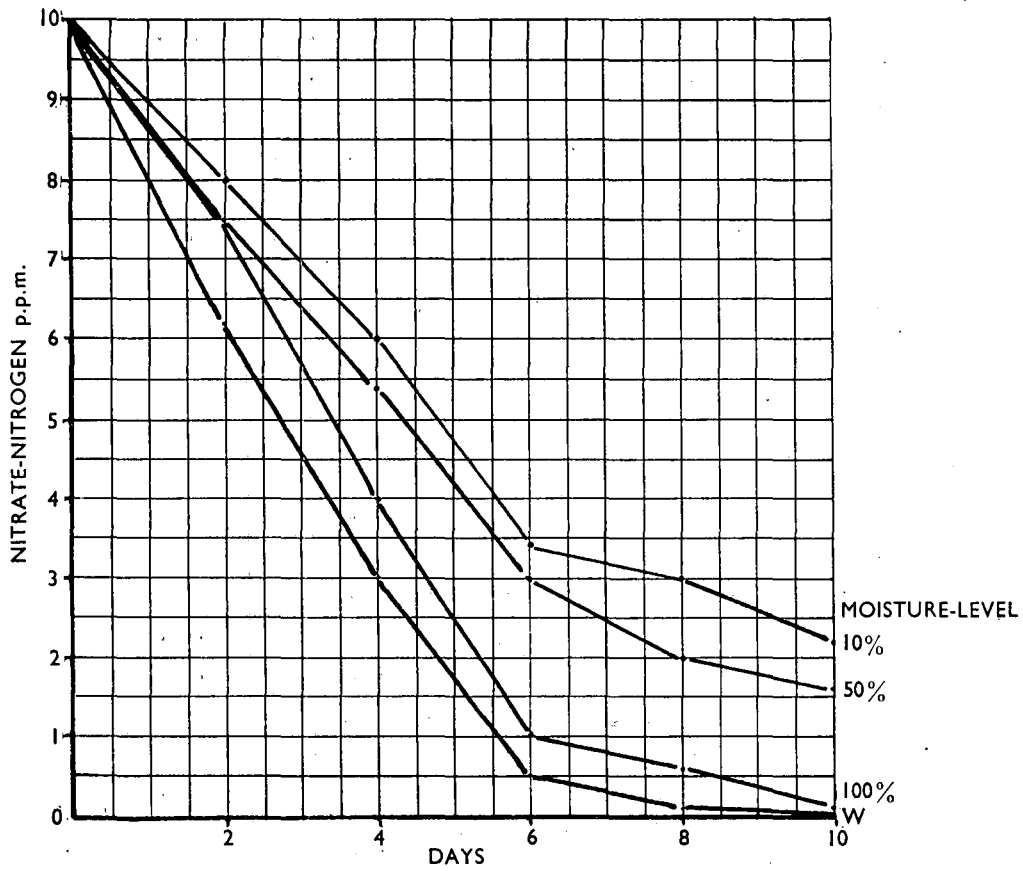


FIG. 2A: RATE OF NITRATE LOSS FROM RED DOLERITE AFTER ADDITION OF INCREASED ENERGY SUBSTRATE

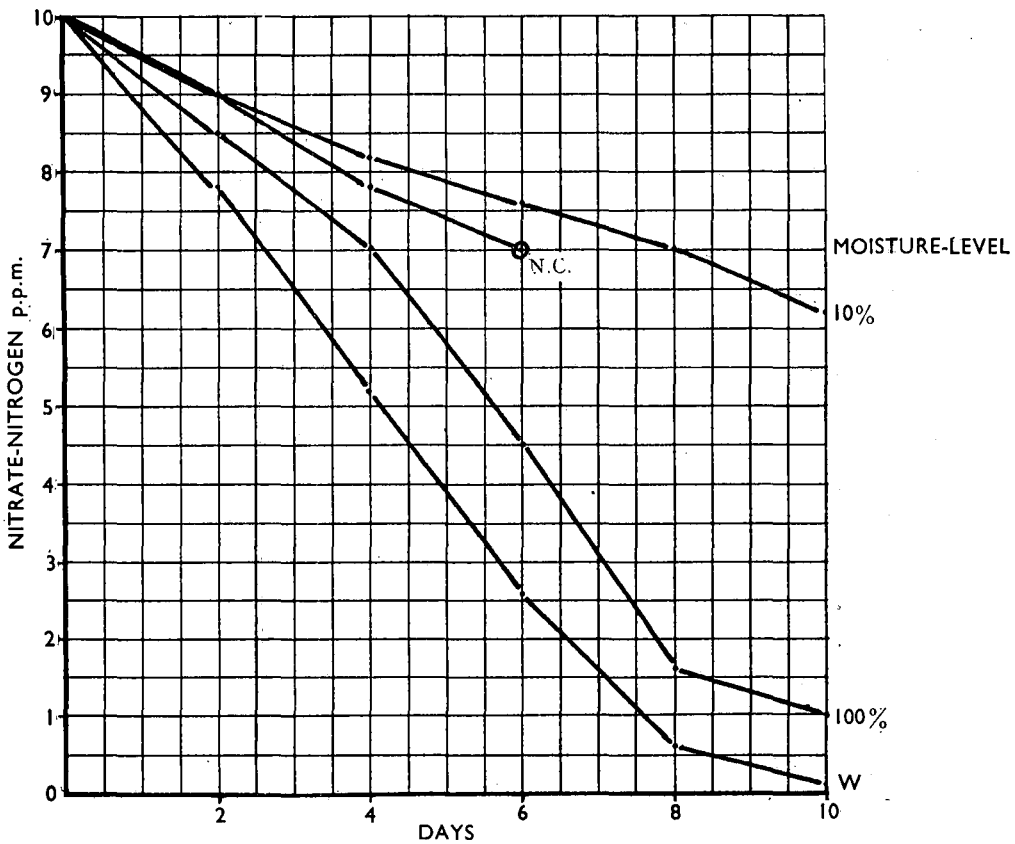


FIG. 2B: RATE OF NITRATE LOSS FROM RECENT (GREY) SAND AFTER ADDITION OF INCREASED ENERGY SUBSTRATE

N.C. = Not continued.

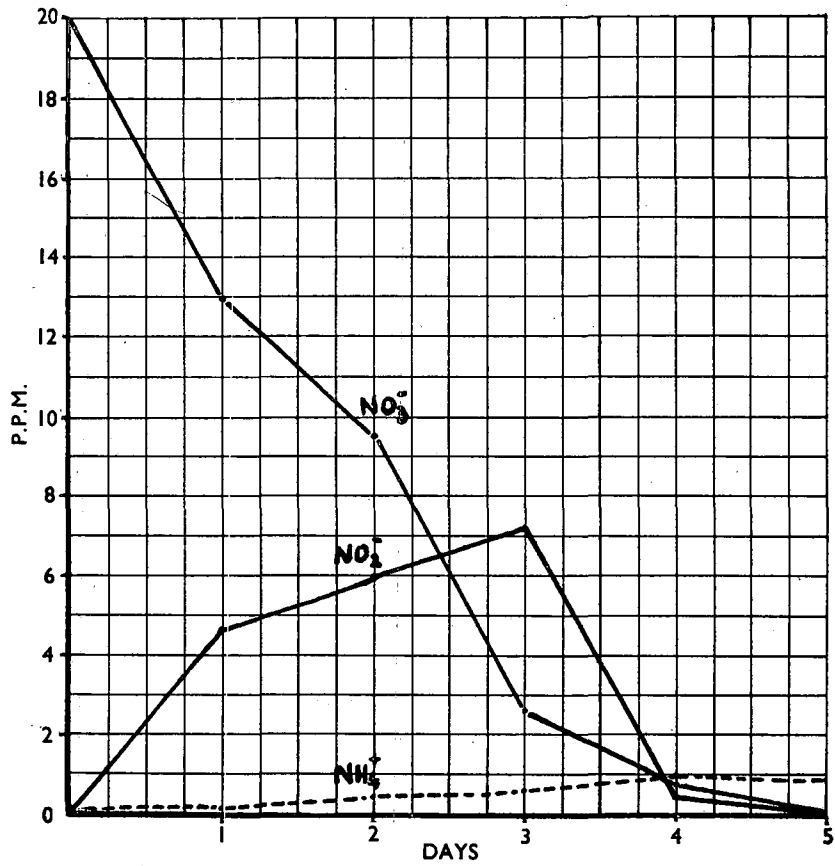


FIG. 3A: NITRATE (NO₃⁻), NITRITE (NO₂⁻), AMMONIA (NH₄⁺), IN RED DOLERITE WITH A C:N RATIO OF APPROXIMATELY 3

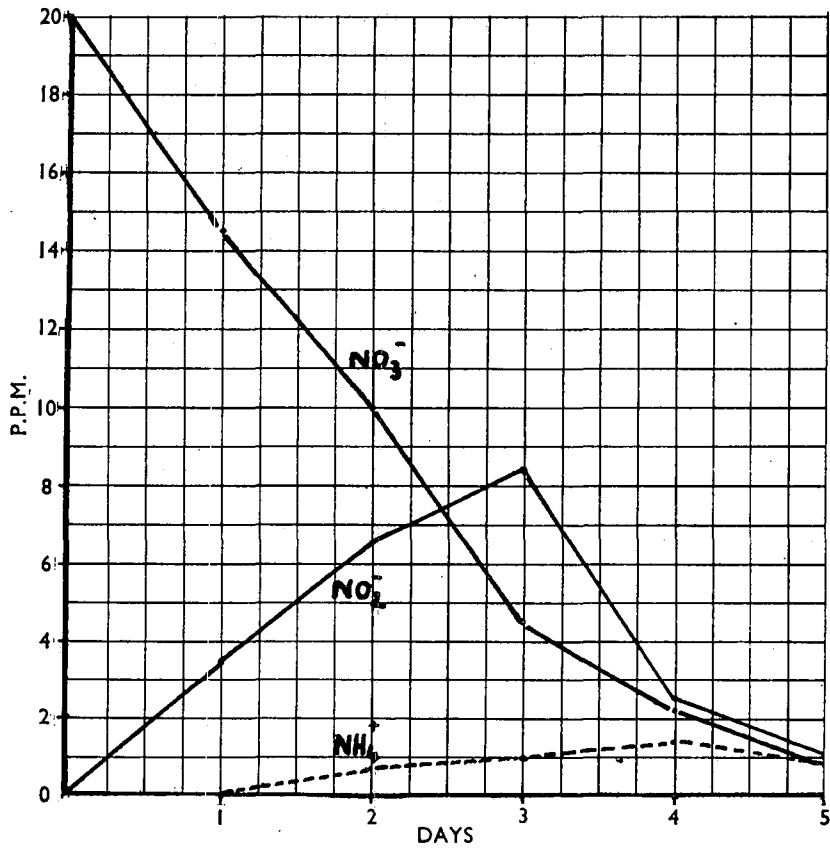


FIG. 3B: NITRATE; NITRITE, AMMONIA IN RECENT (GREY) SAND WITH A C:N RATIO OF APPROXIMATELY 3

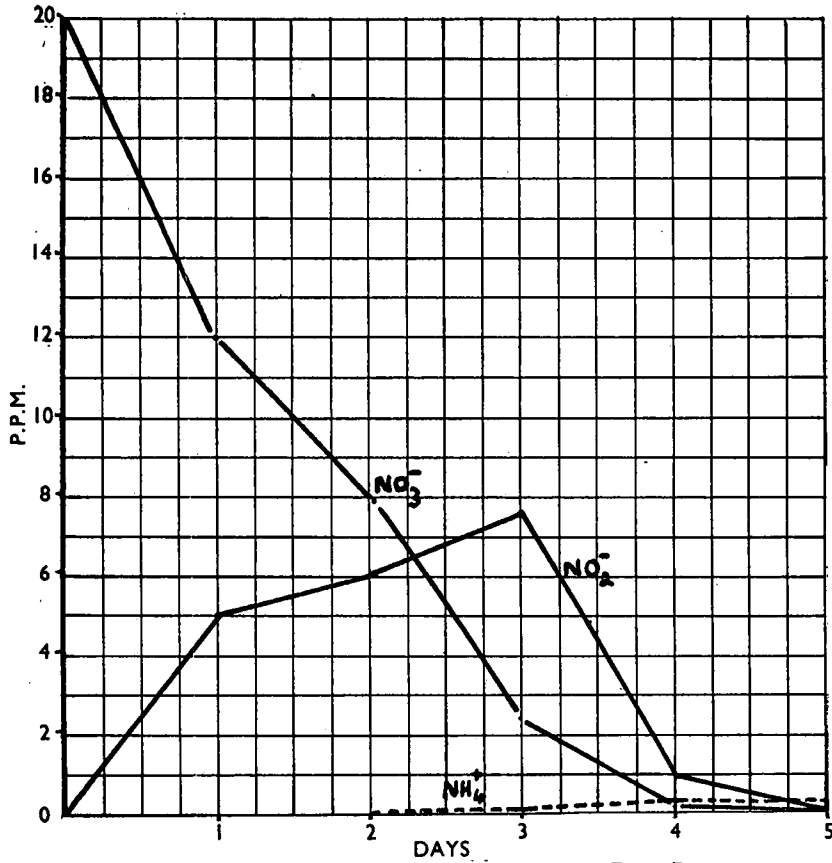


FIG. 4A: NITRATE, NITRITE, AMMONIA IN RED DOLERITE AFTER INCREASING THE pH VALUE

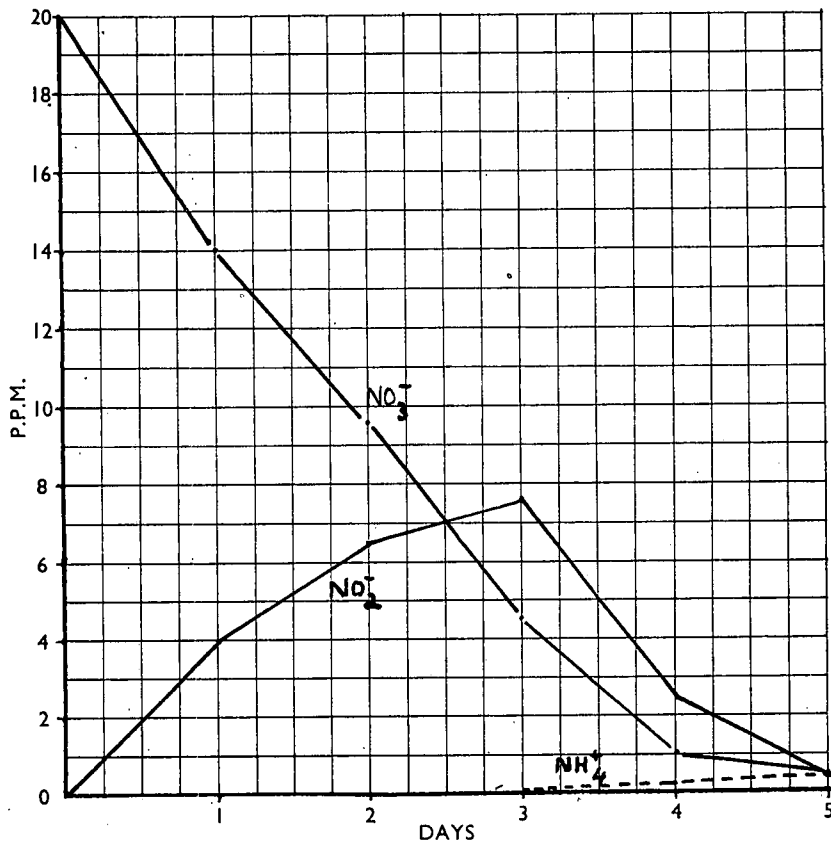


FIG. 4B: NITRATE, NITRITE, AMMONIA IN RECENT (GREY) SAND AFTER INCREASING THE pH VALUE

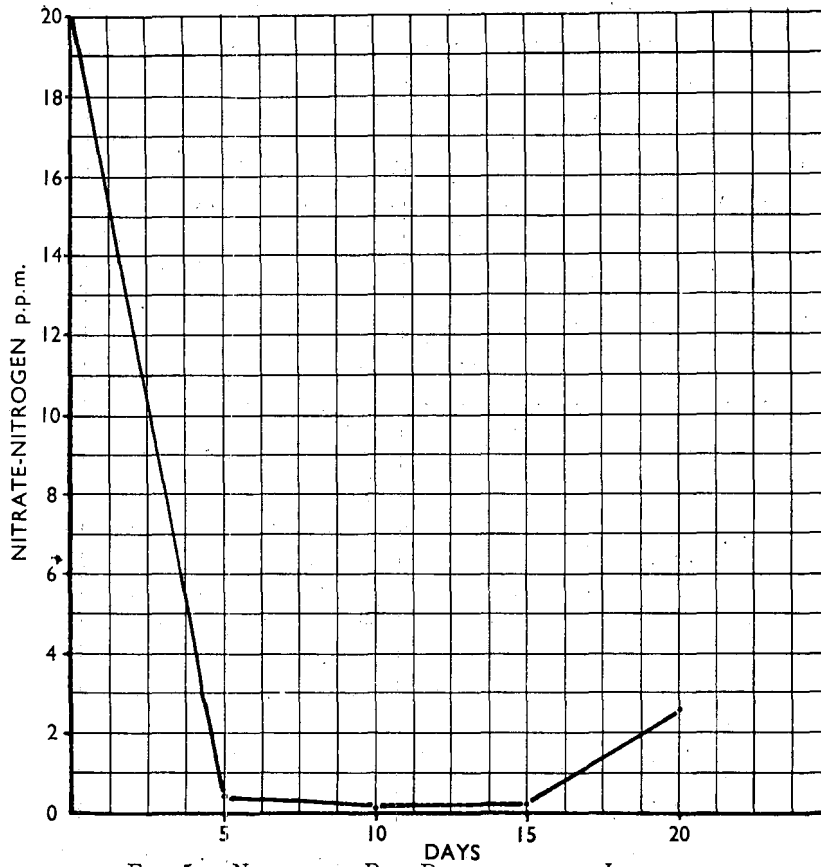


FIG. 5A: NITRATE IN RED DOLERITE AFTER INCREASING THE C:N RATIO

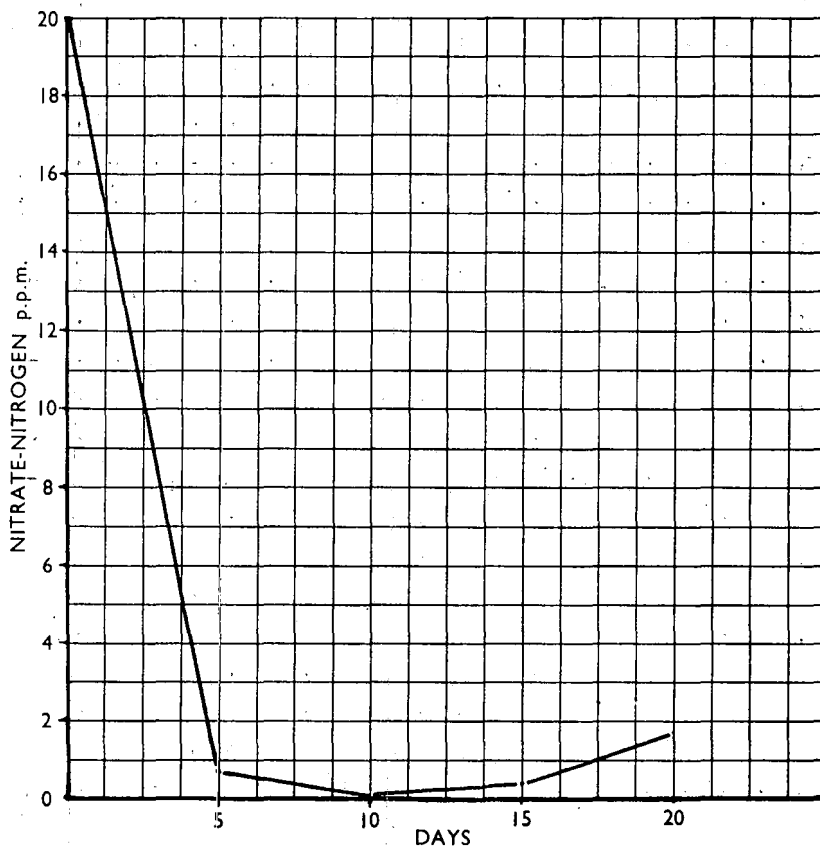


FIG. 5B: NITRATE IN RECENT (GREY) SAND AFTER INCREASING THE C:N RATIO

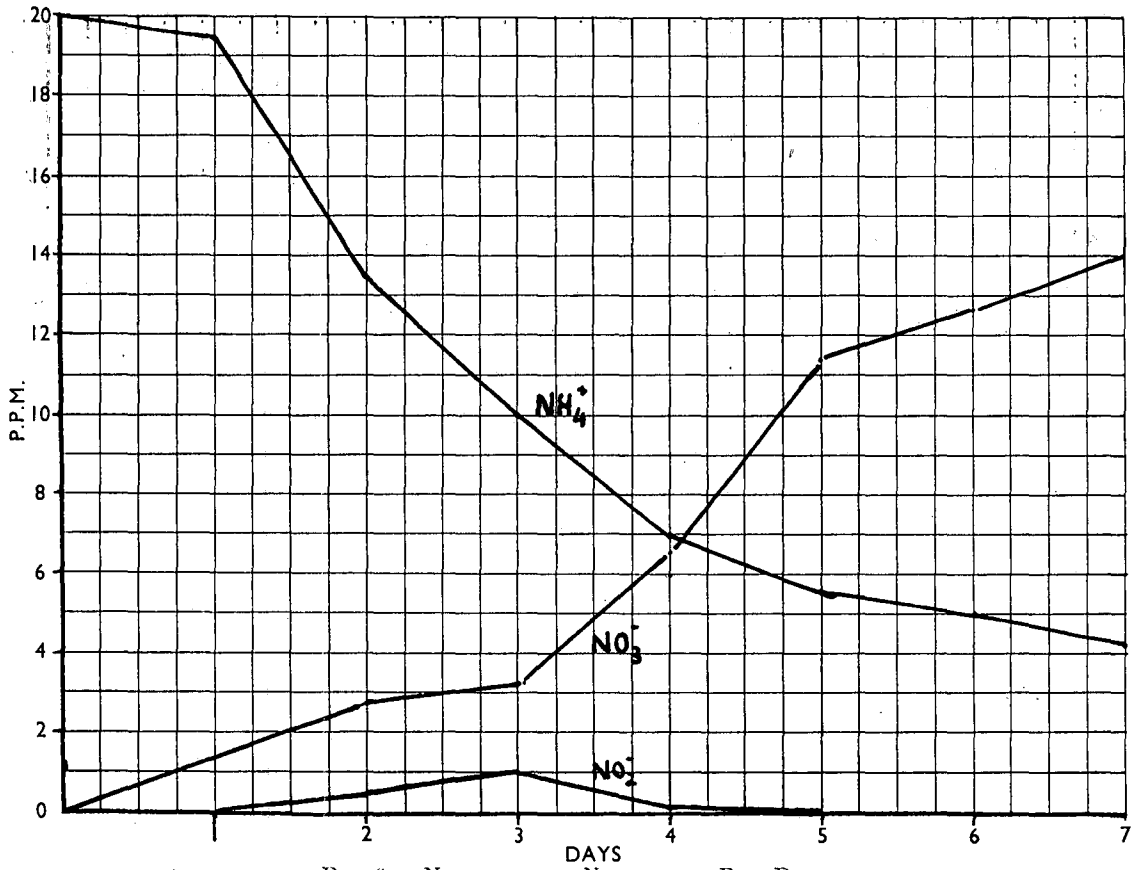


FIG. 6A: NITRATES AND NITRITES IN RED DOLERITE
PERFUSED WITH AMMONIUM SULPHATE

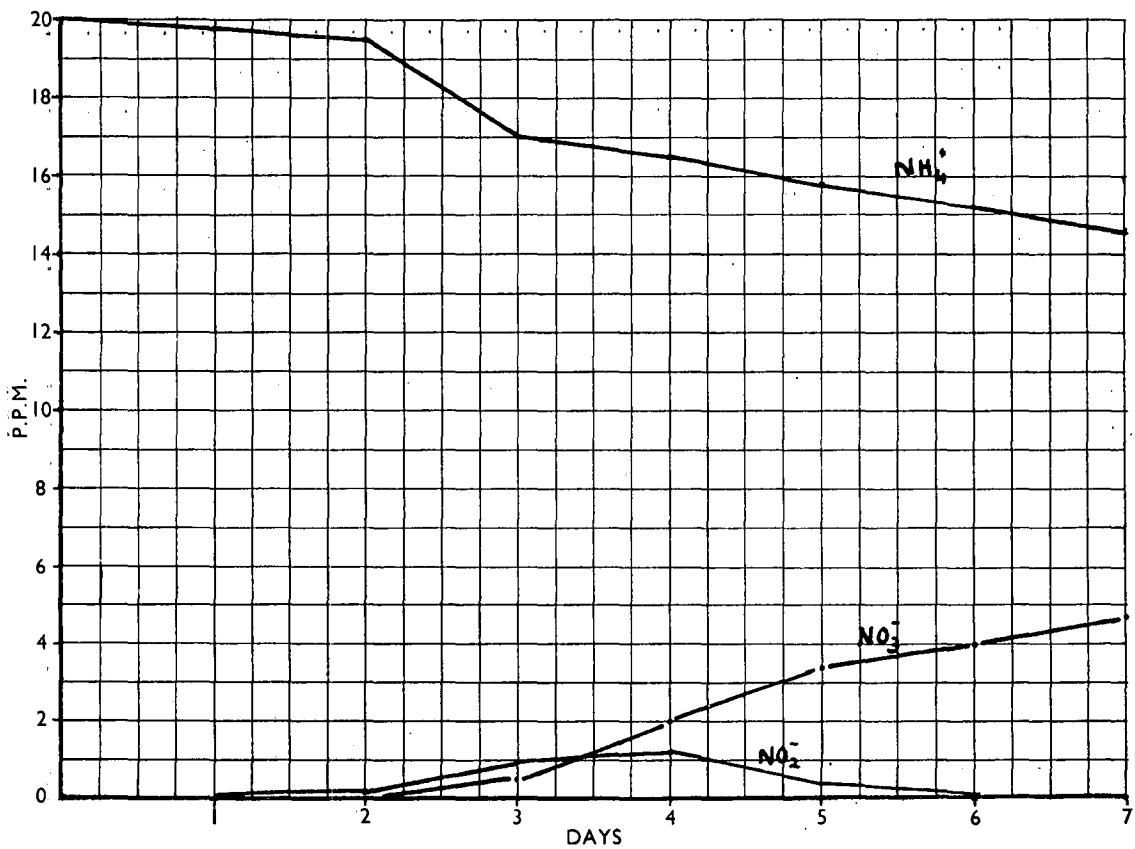


FIG. 6B: NITRATES AND NITRITES IN RECENT (GREY) SAND
PERFUSED WITH AMMONIUM SULPHATE

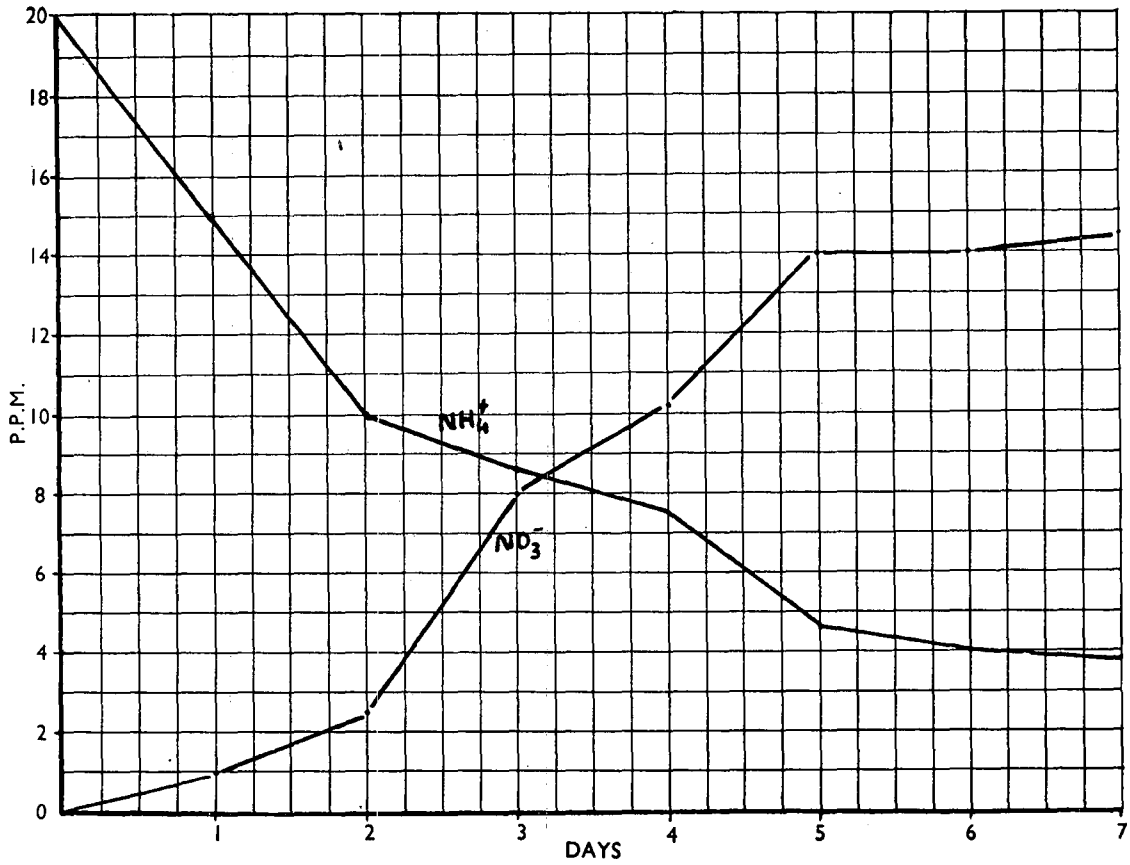


FIG. 7A: NITRATE AND AMMONIA IN RE-PERFUSED RED DOLERITE

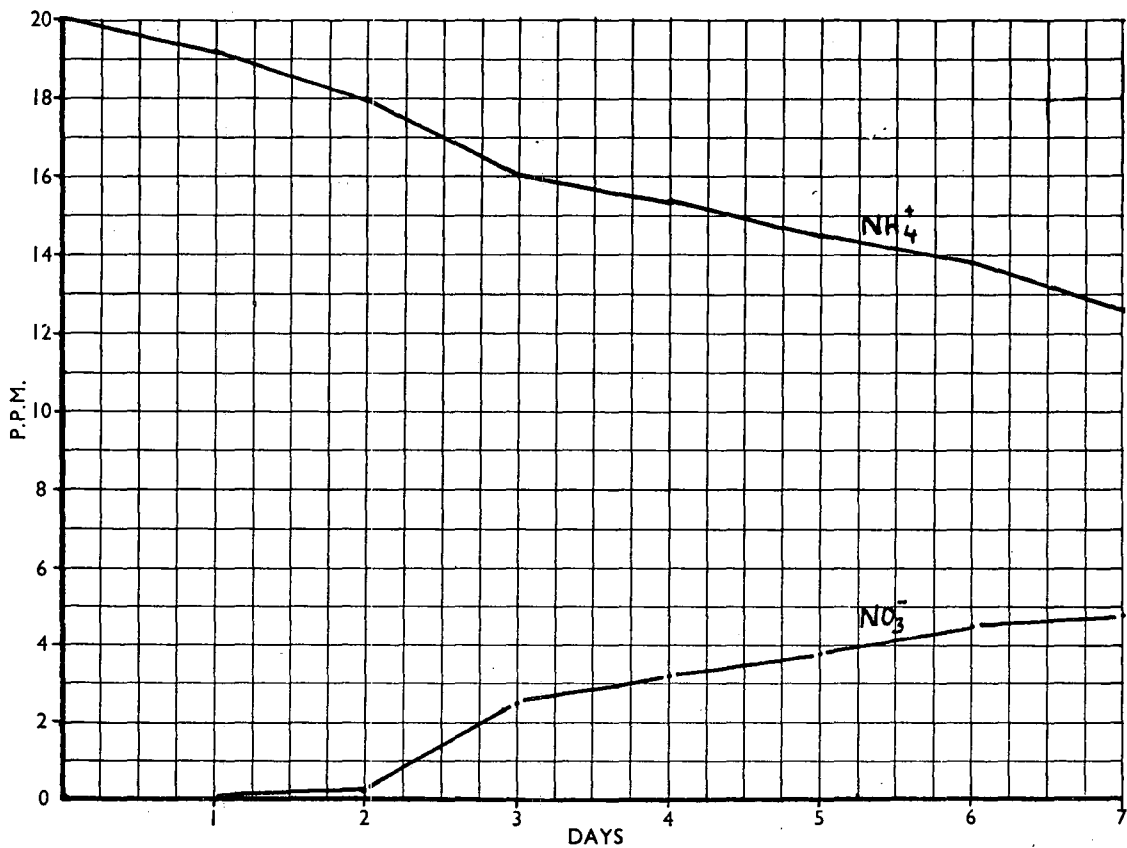


FIG. 7B: NITRATE AND AMMONIA IN RE-PERFUSED RECENT (GREY) SAND

The Chairman, Mr. du Toit, congratulated Mr. Anderson, not only for the contents of his paper, but the way in which he delivered it. The paper was a very difficult one to discuss and it required much study, but it threw great light on the behaviour of nitrogen in the soil. One must consider not only leaching but also the microbiological activities which might result in greater losses than leaching. If this study were extended to cane fields as such it might help to explain why we sometimes got responses and sometimes did not. He asked Mr. Anderson if he had studied the losses likely to occur with the different forms of nitrogen which he applied. He also wanted to know what the effect of the various other fertilizers would be in the mist belt soils with their high carbon content and high carbon-nitrogen ratio. It might be possible that phosphate and potash were deficient and he wanted to know if the addition of phosphate with potash could influence the availability and losses from the various types of nitrogenous fertilizer used.

Mr. Anderson said there seemed to be big differences in the rate of loss depending on the fertilizer form and the soil type. In the case of urea applied to the recent sands and ammonium nitrate in soils with low carbon-nitrogen ratio little response could be expected judging by results already obtained. In the case of urea in recent sands much nitrogen is lost, but further study was required to adequately explain what happened regarding this fertilizer form.

Mr. Sherrard said that with regard to flooding in the alluvial flats, the growth on the flats was hindered; even that of weeds. He would like to know the nitrogen content of the soil prior to and after flooding. A knowledge of bacterial action before and after flooding would be interesting.

Mr. Anderson said he had had no practical field experience of alluvial flats areas.

Mr. King said that he felt that the paper was just a start on this type of work. We had in the past had

results of fertilizer experiments and trashing and burning trials. We did not know why we got increased yield, but if we did know this, it would be possible to foreshadow what we would gain by the application of fertilizer. He thought the work might be extended to using plants growing in the soil itself.

Mr. Anderson said that it was his intention to go further with these experiments using growing plants.

Dr. McMartin said that this paper was a good start on something new. The microbiological study of the soil and the question of the loss of nitrogen is one of first class economic importance. He was under the impression that microbiological activity and its results had never been fully explained. One specific point was the question of azatobacter activity in our soil. The statement had been made that azatobacter was absent from our soils. He thought that it might not actually be absent but that there was an absence of suitable conditions for its development.

Mr. Anderson said that he had detected azatobacter in several soils. In virgin soils such as recent sands he had found a greater count than in mist belt T.M.S. soils. He had investigated the occurrence of azatobacter in various soils from month to month. There were other types besides azatobacter which were capable of nitrogen fixation in the soil. As far as he could see we had these various types present as well in our soil. The response to nitrogenous fertilizers in mist belt soils need not be due to the absence of azatobacter, though this might be a contributory factor.

Dr. Dodds said that this type of work, dealing with the application of nitrogen in the soil, was new to most of us. It caused new aspects to be considered; one for example, that the C:N ratio could be affected by pH, and that this had an effect on nitrification in soil. It was obvious that further study was needed.