

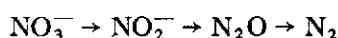
Studies on Nitrogen in Malaysian Soils. III. Denitrification

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The potential and actual changes in gaseous N products during denitrification in nine selected soils collected from different areas under rubber in Peninsular Malaysia were studied in the laboratory using gas chromatograph technique. The denitrification capacity during seven days of incubation varied from 89 µg N per gramme soil or 22% of applied nitrate-N in Segamat series soil (Oxisol) to as much as 380 µg N per gramme soil or 96% of applied nitrate-N in Linau series soil (Inceptisol). Such losses were highly significantly correlated with both soil organic and mineralisable C.

Nitrous oxide (N₂O) was the principal gaseous N produced after seven days of denitrification of high rate of nitrate-N. Results of fourteen days of monitoring gaseous N changes during denitrification of a lower rate of nitrate-N, showed an initial predominance of N₂O which was subsequently replaced by molecular nitrogen (N₂). Nitric oxide (NO) made its transitory appearance during the incubation period but disappeared rapidly.

Denitrification normally refers to biological reduction of nitrate (NO₃⁻) and nitrite (NO₂⁻) under anaerobic soil conditions to volatile nitrogenous gases (nitric oxide; nitrous oxide, molecular nitrogen). It is considered to be one of the important processes accounting for gaseous N loss from soil^{1,2}. In laboratory investigations using tagged N, Cady and Bartholomew³ observed that nitrous oxide (N₂O) and molecular nitrogen (N₂) were the principal gases evolved during denitrification in soil and the pathway or pattern of denitrification was as follows:



While considerable information on the various aspects of denitrification studies on temperate soils¹⁻⁹ is available, there are relatively few reports¹⁰⁻¹³ on this nitrogen transformation process in soils of tropical or subtropical regions of the world.

In Peninsular Malaysia, denitrification has long been recognised as a serious problem in water-logged soil of paddy field when nitrate source of nitrogenous fertiliser is used. In soils supporting dry land plantation crops (e.g. rubber, oil palm) the possibility and significance of the occurrence of this transformation process has not been fully investigated except for an earlier attempt at the Rubber Research Institute of Malaysia¹⁴ in 1956.

The lack of interest in denitrification in soils under plantation crops in Malaysia may be attributed to the fact that most of these soils are relatively well drained and rarely develop anaerobic conditions necessary for denitrification. However, biological reduction of nitrate-N may be of agronomic importance in some poorly drained soils (e.g. Linau series, Selangor series) or even in apparently well drained soils which may have anaerobic pockets in the profile.

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In view of the above, a study was conducted using gas chromatograph technique to determine the potential, the pattern and the gaseous N products formed during denitrification in nine soils collected from different areas under rubber in Peninsular Malaysia.

EXPERIMENTAL

Soils and Soil Chemical Analysis

Surface soils (0–30 cm) of nine soil series commonly found under rubber were collected from the various areas under rubber in Peninsular Malaysia. For each soil series, ten core samples were taken from the interrow areas of rubber trees and they were bulked to form one composite sample per soil series. All soil samples were air-dried and passed through a 2 mm sieve.

The exchangeable ammonium and nitrate in soil were extracted in 2 M KCL solution and they were determined by the steam distillation method described by Bremner¹⁵. The organic C was analysed by the method of Allison¹⁶. Soil pH was determined with a glass electrode (Soil: water ratio, 1:2.5).

Some relevant properties of the soils used in the present investigations are given in Table 1.

Incubation Procedures

Twenty grammes of air-dried and sieved soil sample were placed in a 1.2 litre glass bottle fitted with ground-glass joints. In studying the denitrification capacity of the nine selected soils, 20 ml of KNO₃ solution containing 8 mg of nitrogen was added to the soil (*i.e.* 400 µg N per gramme of soil). In a separate investigation on the changes in gaseous N composition during denitrification, 20 ml of KNO₃ solution containing only 1.8 mg of nitrogen was added (*i.e.* 90 µg N per gramme of soil).

After adding KNO₃ solution, the bottle was immediately sealed with a glass-stopper fitted with a ground-glass joint and glass-stopcock. It was connected to a manifold system and evacuated by a vacuum pump followed by filling the atmosphere with helium. This was repeated about ten times to ensure complete replacement of air by the helium atmosphere. The bottles were then

TABLE 1. SOME PROPERTIES OF SOILS USED IN DENITRIFICATION STUDIES

Soil series	Soil order ^a	pH	Organic C (%)	Ammonium N (µg N/g soil)	Nitrate-N (µg/g soil)	Internal drainage
Durian	Ultisol	4.4	0.64	14.7	2.3	Moderately well drained
Jerangau	Ultisol	4.8	0.88	9.1	1.6	Well drained
Batu Anam	Inceptisol	4.5	1.03	16.0	2.2	Imperfectly drained
Segamat	Oxisol	4.7	1.45	6.1	4.0	Well drained
Sogomana	Inceptisol	4.8	1.59	11.2	0.2	Imperfectly drained
Sitiawan	Inceptisol	4.6	1.62	12.6	2.3	Moderately well drained
Briah	Entisol	4.4	1.75	9.0	0.1	Poorly drained
Selangor	Inceptisol	4.2	3.48	15.1	5.0	Poorly drained
Linau	Inceptisol	3.8	6.72	16.2	1.6	Poorly drained

^aSoil classified by Chan¹⁷ according to the Seventh Approximation, U.S.D.A.

incubated at 32°C and their atmosphere sampled at intervals for the determination of NO, N₂O and N₂.

Gas Sampling and Analysis

Details on gas sampling and analysis were as described by Blackmer and Bremner¹⁸. Some important features are briefly outlined here.

The gas chromatograph used was a Tracer model MT 150G with an ultrasonic detector and dual-phase meters. The helium carrier gas flew at 50 cc per minute through the 'hot Q' column (detector side A), 'cold Q' column (detector side B) and out of the instrument through the back pressure regulator for detector side B.

After removing the bottle from the incubator, 1 cc of gas was sampled from its atmosphere and injected into the gas chromatograph. Before injection, the atmosphere in the tube extending from the top of the closed stopcock on the incubation bottle to the plug on the opposite side of the sample loop was evacuated, rinsed with helium and re-evacuated; the stopcock at the top of the manifold was then closed and the stopcock at the top of incubation bottle opened to allow gas from the bottle to flow into the evacuated tube and sample loop. After 5 s, the stopcock at the top of incubation bottle was closed and the sampling valve rotated to place the content of sample loop into the helium carrier gas stream flowing through the chromatograph.

The amount of NO, N₂O, and N₂ present in the sample removed from the atmosphere of incubation bottle was determined from the calibration curves which had been previously established by placing known amounts of the respective nitrogenous gas in the bottles of known volume and sampling these bottles with

the same injection system described above. Based on these results, the amount of gas in the incubation bottle of known volume was calculated.

Determination of Mineralisable Carbon

Mineralisable C in the soils studied was estimated by determining the amount of C evolved as CO₂ on incubation of 20 g of soil with 6 ml of water at 32°C for seven days. KNO₃ solution or other amendments were not added. The incubation procedures, gas sampling and analysis were similar to those described earlier. The CO₂ evolved was determined by the gas chromatographic procedure described by Burford and Bremner¹⁹.

RESULTS AND DISCUSSION

Denitrification Capacity of Soils and Its Relationships with Organic and Mineralisable Carbon Contents

The amounts of gaseous N evolved and nitrate-N lost from nine surface soils after anaerobic incubation for seven days are shown in *Table 2*.

The amounts of gaseous N evolved expressed as percentage of applied nitrate-N and N₂O as percentage of total gaseous N evolved are also presented in *Table 2*. Since these soils contained inherently very low nitrate-N content (*Table 1*), the gaseous N evolved can be considered as originating from the applied nitrate-N in KNO₃ solution.

The present results showed biological denitrification of applied nitrate-N was possible during anaerobic incubation in all the nine soils collected from different areas under rubber. These results therefore, support the earlier preliminary observations made at the Rubber Research Institute of Malaysia¹⁴.

In all the nine soils studied N₂O was the predominant gaseous N evolved. The

TABLE 2. AMOUNTS OF GASEOUS NITROGEN EVOLVED AND NITRATE-NITROGEN LOST AFTER ANAEROBIC INCUBATION FOR SEVEN DAYS

Soil series	Nitrate-N lost ($\mu\text{g N/g soil}$)	N evolved as NO , N_2O and N_2		N_2O as % of total gaseous-N evolved
		($\mu\text{g N/g soil}$)	(as % of applied nitrate-N)	
Linau	389	380	95	75
Selangor	262	265	66	70
Briah	201	190	48	76
Sitiawan	185	175	44	84
Sogomana	155	160	40	89
Segamat	103	89	22	80
Batu Anam	108	106	27	86
Jerangau	110	105	26	85
Durian	102	95	24	90

magnitude of total gaseous N evolved which was closely related to the amount of nitrate-N lost, ranged from 89 $\mu\text{g N}$ per gramme soil or 22% of applied nitrate-N in Segamat series to 380 $\mu\text{g N}$ per gramme soil or 95% of applied nitrate-N in Linau series soil. In comparison, Dubby and Fox¹¹ reported between 8% and 31% of applied nitrate-N was denitrified from waterlogged tropical soils of Puerto Rico after two weeks of incubation. In another similar study, as much as 80% of nitrate-N applied to tropical Ghanaian soils from savannah grassland or forest was lost in six to eight days¹⁰.

In spite of observations made by Bremner and Shaw⁴ that denitrification was markedly inhibited in temperate soils of pH 5.0, the present data shows the possibility of denitrification in acid soils from areas under rubber. This may be due to either presence of acid adapted strains of denitrifiers or the ability of denitrifying organisms to operate over a wide pH range⁵.

The relationships between denitrification capacity and organic C and mineralisable C contents of soil are shown in Figures 1 and 2 respectively. The results

show denitrification capacity was highly significantly correlated with both organic C ($r = 0.95^{***}$) and mineralisable C ($r = 0.98^{***}$). It was also found that organic C had a linear relationship with mineralisable C ($r = 0.94^{***}$). Similar results were also reported by Burford and Bremner²⁰. Since denitrifiers are heterotrophs which depend on soil organic C as an energy source²¹, it is not surprising to find denitrification capacity being governed by the availability of readily decomposable soil organic C. Greenland¹⁰

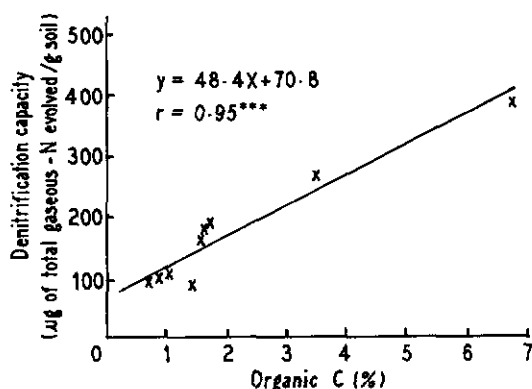


Figure 1. Relationship between denitrification capacity and organic carbon (nine soils).

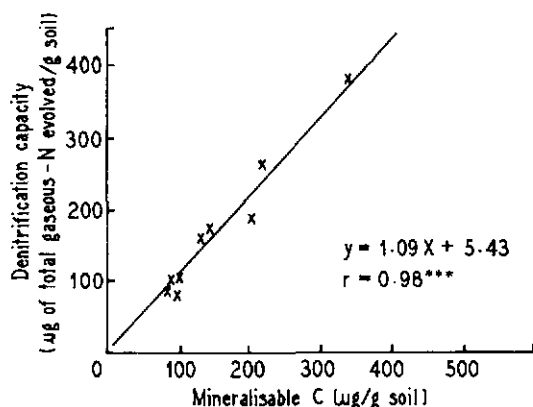


Figure 2. Relationship between denitrification capacity and mineralisable carbon (nine soils)

suggested that the denitrifiers in tropical soils were generally less demanding in their requirements for an available energy substrate than those in the temperate soils and were more efficient in utilising the available soil organic materials.

Although results of the present laboratory studies have demonstrated the capacity of soils under rubber to denitrify during anaerobic incubation, these do not imply that the same magnitude of denitrification would take place under field conditions. In the field, anaerobic waterlogged conditions may temporarily develop in some surface soils after heavy and long rainfall, but such conditions seldom persist for a long period except during the monsoon season of October to December, besides, the nitrate-N present is very likely to be leached in percolating water to the lower depths where anaerobic sites may occasionally exist which are conducive for denitrification. However, the magnitude of denitrification is likely to be restricted by the low organic C content at these depths.

In most of the better drained soils under rubber, losses of nitrogen by denitrification can be considered not

significant because they are unlikely to be anaerobic for a long period. On the other hand, in some poorly drained soils with high organic C content such as Linau and Selangor series, there is a potential danger of high denitrification losses of nitrogen from the soil system when nitrate-N source of nitrogenous fertilisers is applied to these soils.

Changes in Gaseous N Composition During Denitrification

In studying the changes in gaseous N composition during denitrification, a lower rate of nitrate-N (90 μg N per gramme soil) was incubated anaerobically with soils over a longer period of fourteen days to enable complete transformation of the added nitrate-N. The results for all the nine surface soils are illustrated in Figure 3. In the atmosphere of these soils, N₂O was the predominant gaseous N found during the initial period of incubation for at least up to four days. With increasing time, the concentration of N₂O decreased gradually and it was not detected at the end of the fourteen days incubation in all soils except Sogomana series where there were still traces of N₂O. This gradual decline and final disappearance of N₂O was accompanied by a concomitant rise in N₂ which eventually replaced all the N₂O and became the major form of gaseous N in the atmosphere. The present observations of initial N₂O predominance followed by final N₂ replacement of N₂O during denitrification was in full agreement with results of other investigators^{3,6,7}. However, they differ with the findings of Wijler and Delwiche⁸ who reported that N₂O reduction was strongly inhibited in acid soils of below pH 6 such as the soils used in the present investigations (Table 1). According to Blackmer and Bremner²², the N₂O could be taken up by soil micro-organisms

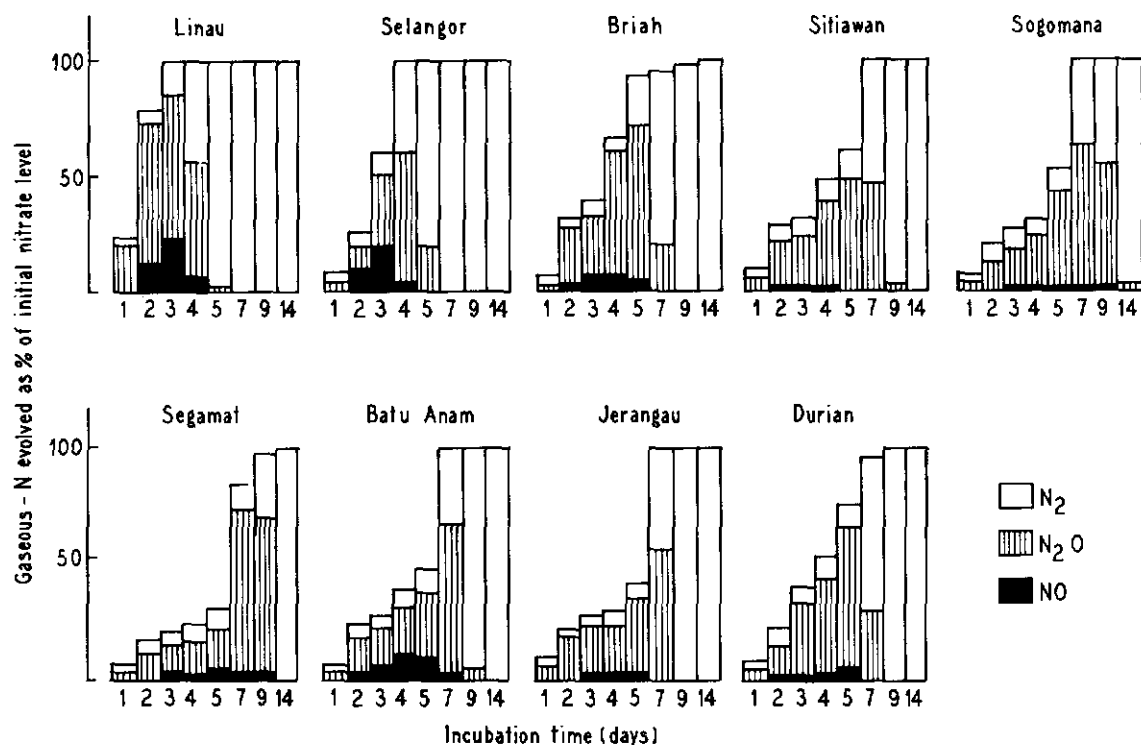


Figure 3. Changes in gaseous – nitrogen composition during denitrification.

which would make use of the oxygen in its molecule by reducing it further to N_2 . From the earlier results on denitrification capacity of the nine soils collected from under rubber and also those reported for the unamended tropical Hawaiian soils¹² there was a general agreement that N_2O was the predominant gaseous N. Balasubramaniam and Kenehiro¹² attributed their observations to the presence of nitrate-N in soil which inhibited microbial reduction of N_2O . This explanation was later confirmed by Blackmer and Bremner²² and it could also be applicable to the present observations, especially in those soils with relatively low denitrification capacity. With prolonged incubation of soils as in the present studies, nitrate-N would eventually be exhausted and all the N_2O evolved is expected to be completely reduced to N_2 in order to satisfy the

oxygen demand of expanding growth of denitrifying population.

A comparison of the different soils reveals that the time required for complete transformation of nitrate-N to N_2O and subsequently to N_2 varied from soil to soil. In the Linau and Selangor series with high denitrification capacity (Table 2) reduction of N_2O to N_2 was rapid (Figure 3) because of presumably higher oxygen demand by the denitrifiers. Transformation of nitrate-N to gaseous N was completed within three to four days, and N_2O reduction was almost completed by the fifth day of incubation. In the other soils while it took seven to nine days to denitrify all the applied nitrate-N, it required nine to fourteen days to completely reduce the N_2O to N_2 (Figure 3).

Although the present evidence show that the eventual microbial reduction

of N_2O to N_2 is in a closed incubation system, Nommik⁶ believes that under open field conditions, N_2O is the predominant gaseous N evolved. However, the recent findings of Blackmer and Bremner²² indicate a much higher capacity for N_2O uptake than release by nine Iowa surface soils under conditions favourable for denitrification of nitrate-N. This could imply that N_2 can also be an important gaseous N evolved.

Besides N_2O and N_2 as the predominant gaseous N in the atmosphere of incubated soils, NO was also detected mainly during the initial stage of incubation. The formation of NO in acid soils was either due to chemical decomposition of micro-biologically produced nitrite-N⁸ or to biological reduction of nitrite-N²³. Broadbent and Clark⁹ found NO to be an insignificant gaseous N product of denitrification except under very unusual conditions.

CONCLUSION

Results of the present investigations show all the nine surface soils from various areas under rubber in Peninsular Malaysia were capable of denitrifying the applied nitrate-N under laboratory anaerobic incubation conditions. However, the denitrification capacity of these soils varied considerably and it was found to be highly significantly correlated with both soil organic C ($r = 0.955^{***}$) and mineralisable C ($r = 0.98^{***}$) contents. The linearity of these relationships suggests that the two measurements may be used as indices of denitrification potential. Thus, in poorly drained soils with high organic C and mineralisable C contents (e.g. Linau series and Selangor series) the use of nitrogenous fertilisers containing nitrate-N should be discouraged because of their high denitrification potential.

Nitrous oxide appeared to be the principal gaseous product during denitrification of 400 μ g nitrate-N per gramme soil over a period of seven days of incubation. On the other hand, results in another study with lower rate of nitrate-N (90 μ g N per gramme soil) incubated over a longer period of fourteen days indicated N_2O was the predominant gaseous N in the ambient atmosphere of all the nine soils during the initial period of incubation. Nitric oxide also made its transitory appearance during this period but it disappeared rapidly. With prolonged incubation, however, the concentration of N_2O gradually declined and it was not detectable after fourteen days in most of the soils. This gradual fall and subsequent disappearance of N_2O was accompanied by a concomitant rise in N_2 which eventually became the major form of gaseous N in the atmosphere.

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