

Merlewood Research and Development Paper

Number 45

A PRELIMINARY ATTEMPT TO ESTIMATE RATES OF  
AMMONIFICATION AND NITRIFICATION IN SOIL  
IN THE FIELD

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R. & D. 73/45

May 1973

## INTRODUCTION

Nitrogen is an important plant nutrient, and in unfertilized soils a large proportion of the nitrogen available to plants as ammonium-N or nitrate-N comes from the decomposition of organic matter, chiefly plant remains. In the decomposition of organic nitrogenous substances, mainly proteins and amino acids, ammonium is released by the activities of the ammonifying bacteria and the process is called ammonification. Ammonium is oxidized to nitrate by the nitrifying bacteria and the process is called nitrification. The two groups of organisms require somewhat different conditions, and the rates at which the two processes proceed are not the same in all soils. There is also some evidence to suggest that the pathways of nitrogen metabolism differ in different soil types, and that in some soils much of the nitrogen in plant remains may be locked in an unavailable form (Howard, review in prep.).

Small (1954) states that ammonification can occur within the pH range 3.5 to 7.0, with optimum rates between pH 4.5 and 5.5; nitrification can occur within the pH range 3.5 to 11, with optimum rates between pH 6.5 and 7.5. It is not clear on what evidence this is based, but it seems likely that these figures were obtained from laboratory experiments. No systematic study appears to have been made of rates of ammonification and nitrification in British woodland soils in ecologically realistic conditions. In soils, for example, low pH is accompanied by other chemical characteristics which are likely to influence the rates of these processes. Various workers (e.g. Pearsall, 1938) have failed to detect significant quantities of nitrate in freshly-collected, very acid, soil (pH <3.8 to 4.0). This failure may indicate low rates of nitrification in the very acid soils, with uptake by plants and micro-organisms consuming all of the nitrate produced. On the other hand, it may be that, in very acid soils, the conditions inhibit nitrification. There is clearly a need for more information on the rates of ammonification and nitrification, and the factors affecting these processes, in British woodland soils.

A research project was carried out at the Merlewood Research Station in 1972 in order to test and compare methods for measuring ammonification and nitrification in the field, and to try to obtain some estimate of the rates of the two processes in Meathop Wood. The chief difficulty of such studies lies in getting a realistic estimate of nitrification. It is well known that nitrification is stimulated when soils are disturbed, and so we require some method which will allow us to measure nitrogen mineralisation with a minimum of disturbance of the soil. This condition precludes laboratory incubation experiments and necessitates a field method. However, in the field observations, there are two main problems to be overcome, (a) uptake of nitrate by plants, and (b) leaching of nitrate by rain.

Most methods mentioned in the literature are unsuitable for our purpose because they entail studying disturbed soil. However, Lemeé (1967) experimented with the use of small metal boxes of

## b) Laboratory methods

In the laboratory, each soil sample was sorted, large stones and roots were removed, and the soils were weighed (fresh weight). Two subsamples of soil from each core were removed for determination of moisture content. Two further subsamples of soil from each core, each of approximately 10 g fresh weight, were weighed into 100 ml jars and 50 ml of NKCl extractant were added. The mixture was shaken vigorously for 30 minutes and filtered through a Whatman 44 filter paper. Part of each extract was immediately analyzed for  $\text{NO}_3^-$ -N content, the remainder was stored in a refrigerator overnight and  $\text{NH}_4^+$ -N was determined the following day.

$\text{NO}_3^-$ -N in the extracts was determined by the method of Sims and Jackson (1971) in which nitrate forms a yellow complex with chromotropic acid (4,5-dihydroxy-2,7-naphthalenedisulphonic acid), this yellow colour can be determined colorimetrically. Certain difficulties were experienced with this method (McNeilly and Howard, 1973) and it is not recommended for future use.

$\text{NH}_4^+$ -N was determined by a modification of the Pye Unicam Automatic Analysis method for determining  $\text{NH}_4^+$ -N in plant extracts. This method is based on the fact that  $\text{NH}_4^+$  reacts with hypochlorite to form chloramine which combines with phenol to give p-quinone-chlorimine. The latter reacts with another molecule of phenol forming blue indophenol, which is determined colorimetrically (McNeilly and Howard, 1973).

## Results

Results of analyses for ammonium-N, nitrate-N, and total (ammonium plus nitrate) N as  $\mu\text{g N per g OD soil}$  are given in Table 1 and Figure 1. The results calculated for a 10 cm core 25 mm diameter are given in Table 2.

If we let  $n_0$  be the mean nitrogen (either ammonium, nitrate, or total) content of the control sample,  $n_1$  be the mean nitrogen content of uncovered cans, and  $n_2$  be the mean nitrogen content of covered cans, then, assuming for present purposes that there is no denitrification, the major mineral nitrogen losses from the soil during the time the cans were in the soil would be as follows:

- in  $n_0$  to plants, leaching, micro-organisms
- in  $n_1$  to leaching, micro-organisms
- in  $n_2$  to micro-organisms

Hence, mineral nitrogen lost to plants would be estimated by  $n_1 - n_0$ , and nitrogen lost to leaching by  $n_2 - n_1$ .

One possible objection to this method is that the roots severed inside the cans may decompose and affect the nitrogen transformations within the cans. This is more likely to be a problem where there is a high density of fine roots, which may begin to decompose quite quickly. Hibberd (pers. comm.) estimates that, in experiments, roots of trees and shrubs decompose, on average, four percent per month. Presumably, a woody root severed by one of our sampling cans will take some days to die before decomposition begins. If the cans are in position for only a short time (less than three weeks), root decomposition is not likely to be a serious source of error. Nevertheless, the possibility should be borne in mind, as it may need to be investigated, and the likelihood of error from this source assessed, before any extensive use of the method described.

Another important consideration is that the cans which are covered to prevent leaching should not become significantly drier than the uncovered cans. This did not happen in our observations, presumably because of the shade provided by the vegetation and also the capillary rise of water into the soil in the covered cans. Nevertheless, the factor could be important in some situations, and should be borne in mind.

#### Acknowledgements

We are grateful to Mr. J. N. R. Jeffers for statistical advice.

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Table 1.

$\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  contents of 0-10 cm soil samples, per gram O.D. soil (Mean  $\pm$  SE)

	Nitrogen in $\mu\text{g/g}$ OD soil				
	28 Feb.	20 Mar.	10 April	1 May	22 May
Controls:					
$\text{NH}_4\text{-N}$	2.85 $\pm$ 0.51	5.03 $\pm$ 0.40	4.20 $\pm$ 0.28	5.46 $\pm$ 0.96	4.42 $\pm$ 0.57
$\text{NO}_3\text{-N}$	7.56 $\pm$ 0.73	2.77 $\pm$ 0.56	1.73 $\pm$ 0.43	3.52 $\pm$ 0.59	2.18 $\pm$ 1.52
total	10.41 $\pm$ 1.09	7.80 $\pm$ 0.92	5.93 $\pm$ 0.51	8.98 $\pm$ 1.53	6.60 $\pm$ 2.06
Uncovered cans:					
$\text{NH}_4\text{-N}$	-	7.07 $\pm$ 1.81	3.90 $\pm$ 0.55	7.37 $\pm$ 1.90	20.05 $\pm$ 4.50
$\text{NO}_3\text{-N}$	-	5.57 $\pm$ 1.06	2.29 $\pm$ 0.43	5.92 $\pm$ 1.81	7.31 $\pm$ 1.84
total	-	12.64 $\pm$ 2.16	6.19 $\pm$ 0.97	13.29 $\pm$ 2.17	27.36 $\pm$ 6.15
Covered cans:					
$\text{NH}_4\text{-N}$	-	-	5.67 $\pm$ 0.85	6.57 $\pm$ 1.00	18.86 $\pm$ 4.11
$\text{NO}_3\text{-N}$	-	-	5.27 $\pm$ 1.30	5.22 $\pm$ 1.33	12.77 $\pm$ 0.61
total	-	-	10.94 $\pm$ 2.12	11.79 $\pm$ 1.61	31.63 $\pm$ 3.99

Table 2.

NH<sub>4</sub>-N and NO<sub>3</sub>-N contents of 0 to 10 cm soil cores 25 mm diameter, OD basis (mean ± SE)

	Nitrogen in µg/10 cm core					
	28 Feb.	20 Mar.	10 April	1 May	22 May	
Controls:						
NH <sub>4</sub> -N	118.31 ± 12.22	196.14 ± 9.45	176.00 ± 7.79	208.34 ± 38.63	204.37 ± 20.66	
NO <sub>3</sub> -N	523.80 ± 28.96	106.77 ± 19.73	74.36 ± 20.40	130.67 ± 13.07	95.25 ± 62.24	
total	442.11 ± 29.09	302.91 ± 28.27	250.36 ± 24.35	339.01 ± 43.43	299.62 ± 80.65	
Uncovered cans:						
NH <sub>4</sub> -N	-	277.59 ± 71.08	167.31 ± 15.39	300.13 ± 78.80	889.26 ± 206.89	
NO <sub>3</sub> -N	-	219.07 ± 44.63	97.54 ± 13.88	236.49 ± 66.82	325.57 ± 83.20	
total	-	496.66 ± 89.02	264.85 ± 28.45	536.62 ± 82.89	1214.83 ± 281.04	
Covered cans:						
NH <sub>4</sub> -N	-	-	234.15 ± 30.84	271.32 ± 46.31	779.92 ± 123.18	
NO <sub>3</sub> -N	-	-	212.91 ± 47.62	210.63 ± 47.36	454.37 ± 59.79	
total	-	-	447.06 ± 75.03	481.95 ± 63.83	1234.29 ± 113.82	

Table 3.

Results of analysis of variance of the untransformed data for ammonium-N. Values are in  $\mu\text{g NH}_4\text{-N/g OD soil}$

Date	mean $\text{NH}_4\text{-N}$		Covered cans ( $n_2$ )	Effect of plants ( $n_1 - n_0$ )	Effect of leaching ( $n_2 - n_1$ )
	Control ( $n_0$ )	Uncovered cans ( $n_1$ )			
28/2/72	2.85	-	-	-	-
20/3	5.03	7.07	-	2.04	-
10/4	4.20	3.90	5.67	- 0.30	1.77
1/5	5.46	7.37	6.57	1.91	- 0.80
22/5	4.42	20.05	18.86	15.63***	- 1.19

Differences for significance

5%	5.36
1%	7.14
0.1%	9.36

Standard error of the means 1.89

Standard error of the difference between two means 2.67

This analysis agrees with that of the logarithmically transformed data



Table 4.

Results of analysis of variance of the untransformed data for nitrate-N. Values are in  $\mu\text{g NO}_3\text{-N/g OD soil}$

Date	mean $\text{NO}_3\text{-N}$			Effect of plants ( $n_1 - n_0$ )	Effect of leaching ( $n_2 - n_1$ )
	Control ( $n_0$ )	Uncovered cans ( $n_1$ )	Covered cans ( $n_2$ )		
28/2/72	7.56	-	-	-	-
20/3	2.77	5.77	-	2.80	-
10/4	1.73	2.29	5.27	0.56	2.98(*)
1/5	3.52	5.92	5.22	2.40	- 0.70
22/5	2.18	7.31	12.77	5.13(***)	5.46(*)

Differences for significance

5% 3.28

1% 4.37

0.1% 5.73

Standard error of the means 1.15

Standard error of the difference between two means 1.63

These results are very similar to those of the logarithmically transformed data. The asterisks in brackets show the levels of significance for the transformed data

Table 5.

Results from analysis of variance of the untransformed data for total (ammonium plus nitrate) N. Values are in  $\mu\text{g N/g OD soil}$

Date	mean total N				
	Control ( $n_0$ )	Uncovered cans ( $n_1$ )	Covered cans ( $n_2$ )	Effect of plants ( $n_1 - n_0$ )	Effect of leaching ( $n_2 - n_1$ )
28/2/72	10.41	-	-	-	-
20/3	7.80	12.64	-	4.84	-
10/4	5.93	6.19	10.94	0.26	4.75(*)
1/5	8.98	13.29	11.79	4.31	- 1.50
22/5	6.60	27.36	31.63	20.76(***)	4.27

Differences for significance    5%    7.15  
                                           1%    9.53  
                                           0.1% 12.87

Standard error of the means        2.51

Standard error of the difference between two means 3.55

These results are very similar to those of the logarithmically transformed data. The asterisks in brackets show the levels of significance for the transformed data

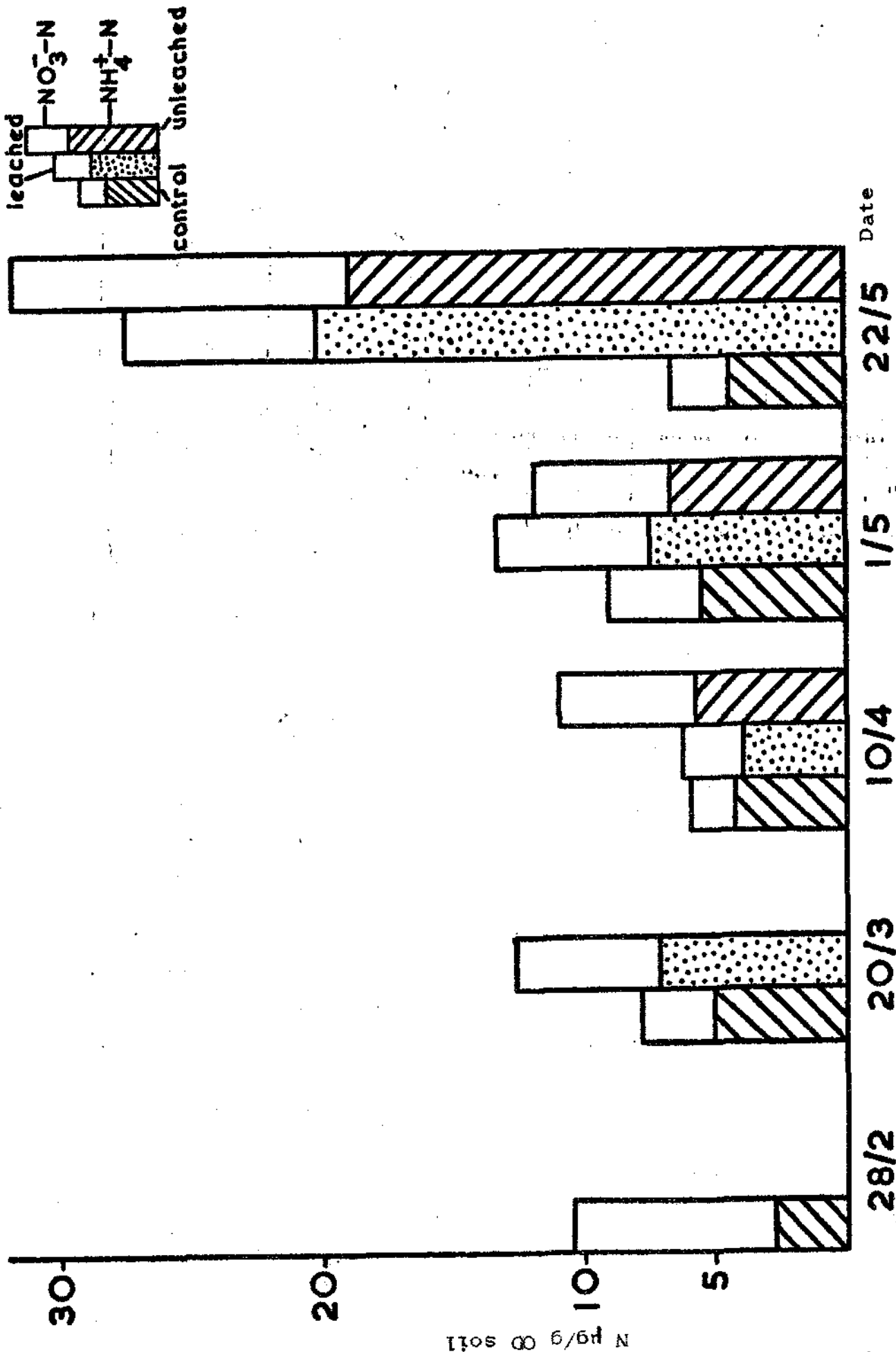


Fig. 1. Ammonium-N, nitrate-N and total (ammonium + nitrate) N in control, leached, and unleached soil samples

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