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Lees, H. and Quastel, J. H. 1946. Biochemistry of nitrification in soil. 2. The site of soil nitrification. *Biochemical Journal*. 40 (5-6), pp. 815-823.

The publisher's version can be accessed at:

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Biochemistry of Nitrification in Soil

2. THE SITE OF SOIL NITRIFICATION

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(Received 14 February 1946)

Chick, in her interesting paper (1906) on the study of nitrification with reference to sewage purification, states that it had been long upheld that a most important preliminary to nitrification both in soil and in sewage filters was an absorption of NH_4^+ or ammonium salts on surfaces of soil particles or filtering medium. In the case of soil, a long controversy had taken place as to whether a physical or chemical process was involved, and the former view on the whole prevailed. Chick, however, concluded against an absorption theory, since nitrification took place in sewage filtering through coke, although no absorption of NH_4^+ took place on the coke.

The problem of the site of nitrification in soil has not been seriously considered for many years, and it has been probably taken for granted that since nitrification takes place in isolated culture media, soil nitrification also occurs at the expense of NH_4^+ held in solution in the soil moisture. Albrecht & McCalla (1937), however, have obtained evidence that NH_4^+ held in the base-exchange complex of clay may be oxidized by bacteria, indicating that NH_4^+ need not be in the aqueous phase in order to be attacked by the nitrifying bacteria. McCalla (1941) has suggested that the bacteria themselves act as base-exchangers and can exchange cations with those taken up in base-exchange complexes (such as clay). This theory is a development of the recent views of Jenny & Overstreet (1940) who postulate a base-exchange between plant roots and soil complexes.

Waksman, Renszer, Carey, Hotchkiss & Renn (1933), Peele (1936), Rubentschik, Roisin & Bieljinsky (1936), Conn & Conn (1940) and ZoBell (1943) have all either shown that bacteria can be adsorbed on various surfaces or have investigated the effects of surfaces on bacterial activity. Waksman *et al.* (1933), for example, found that nitrifying bacteria of the sea water in the Gulf of Maine were largely adsorbed on to bottom-mud.

Little, however, has been done to determine whether nitrification as it occurs normally in soil takes place wholly or partly at soil surfaces, whether NH_4^+ in soil solution or adsorbed in the base-exchange complexes of soil forms the actual substrate of the nitrifying bacteria in soil, or whether the kinetics of nitrification in soil are dependent on the physico-chemical conditions obtaining in soil.

We have attempted to answer these questions by experiments carried out with the soil-perfusion technique described previously (Lees & Quastel, 1946). The following paper describes the results obtained.

EXPERIMENTAL

Methods

The analytical methods are fully described in our previous paper (Lees & Quastel, 1946).

Soil solution

The evidence shows that in comparison with the nitrification that takes place on the soil little nitrification takes place in the soil solution. This was

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shown by aeration of perfusate taken from a rapidly nitrifying soil-perfusate system. Results of experiments carried out in slightly different ways are shown in Table 1. As the results in the tables show that

Table 1. *Nitrification in soil perfusates*

Exp. A. 50 g. soil were placed in each of four perfusion units. These were perfused with 200 ml. $\text{N}/70\text{-(NH}_4\text{)}_2\text{SO}_4$ for 4 days at 70° F. After this period the soils were removed from two of the perfusion units. The other two units were left intact. Perfusion was then continued in all four units.

Days after removal of soil from two of the perfusion units	$\mu\text{g. nitrate-N produced/ml. perfusate}$			
	In apparatus where soil was not removed		In apparatus where soil alone was removed	
	Exp. 1	Exp. 2	Exp. 3	Exp. 4
0	42	42	40	42
6	176	186	52	51

Exp. B. Perfusates from rapidly nitrifying soils (nitrate-N increasing at the rate of 20 $\mu\text{g. N/ml. per day}$) were removed from the perfusion apparatus, and gently aerated at the same temperature (i.e. 70° F.). The rate of increase of nitrification was then estimated in this solution.

Time in days (after start of aeration of perfusate)	Ammonium-N ($\mu\text{g./ml. perfusate}$)	Nitrite-N ($\mu\text{g./ml. perfusate}$)	Nitrate-N ($\mu\text{g./ml. perfusate}$)
0	28	1	156
2	29	1	156
5	29	1	156
7	28	1	157

nitrification does not take place at an appreciable rate in the solution present in a rapidly nitrifying soil, it is reasonable to deduce that nitrification is in some way connected with the soil surface.

As it is clear that the presence of the soil itself is necessary if nitrification is to be appreciable, the question arises whether the soil surface acts merely as a mechanical support for the nitrifying bacteria held there, or whether the soil surface acts in a more specific manner.

It will be shown that the soil surface acts in the nitrification process specifically by the supply of

NH_4^+ held in base-exchange complexes, and that nitrification probably takes place at those sites on the soil surfaces where base-exchangeable NH_4^+ is located. If these conclusions are correct, the rate of soil nitrification is in part a function of the base-exchange capacity of the soil, and the process is restricted to specific sites of the soil surface.

The effects of addition of calcium ions

Preliminary experiments were carried out to see whether the addition of calcium ions to a perfusate containing ammonium sulphate would affect the rate of nitrification. If nitrification in soil takes place at the expense of adsorbed NH_4^+ in preference to that of the NH_4^+ in solution, then any treatment that reduces the amount of adsorbed NH_4^+ in a soil-perfusate system should automatically reduce the rate of nitrification. The addition of calcium ions, by reducing the quantity of adsorbed NH_4^+ (by base-exchange), should therefore reduce the rate of nitrification.

In the experiments whose results are recorded in Table 2, 100 g. lots of Rothamsted garden soil were perfused with 250 ml. lots of $\text{M}/50$ ammonium sulphate solution containing various concentrations of CaCl_2 . It will be seen that as the calcium-ion concentration is increased, the rate of nitrification is decreased. The velocity constant, K , calculated from the autocatalytic equation, which holds for soil nitrification (Lees & Quastel, 1946), falls from a value of 0.194 where no calcium was added to 0.136 in presence of $\text{M}/25$ CaCl_2 . The fraction of the total ammonium ions in the system adsorbed on the soil, expressed by the term r , also drops as would be expected from the exchange reaction between NH_4^+ and Ca^{++} . It will be seen, however, that the ratio K/r is approximately constant, indicating that the rate of nitrification is proportional to the fraction of ammonium ions adsorbed on the soil.

It is not possible to conclude from these experiments that nitrification in soil takes place at the expense of adsorbed NH_4^+ , because calcium ions are known to inhibit the rate of nitrification of isolated cultures of nitrifying bacteria (Meyerhof, 1917), and chloride ions also have inhibitory effects on the

Table 2. *The effect of calcium chloride on the rate of nitrification in a garden soil*

Conc. of calcium chloride in perfusate	Nitrate-N formed ($\mu\text{g./g. soil}$)		
	Nil	$\text{M}/62.5$	$\text{M}/25$
Duration of exp. (days)			
4	180	180	103
6	311	232	193
8	472	287	263
11	518	453	389
'K' calculated from the autocatalytic equation	0.194	0.165	0.136
'r' (adsorbed ammonium/total ammonium)	0.64	0.56	0.50
K/r	0.303	0.295	0.273

metabolism of these bacteria (Hahn, Olsen & Roberts, 1942). While the inhibitory effects described by these authors are only considerable at relatively high concentrations of calcium chloride, it was felt that some attempt to repeat the experiments of Table 2 with lower concentrations of CaCl_2 might give results less open to question. The results given in Fig. 1, showing effects of perfusion of 30 g. air-dried garden soil with 200 ml. ammonium sulphate (M/280), in presence of M/200-calcium chloride show a definite inhibitory effect of calcium chloride on the rate of nitrification at relatively low concentration.

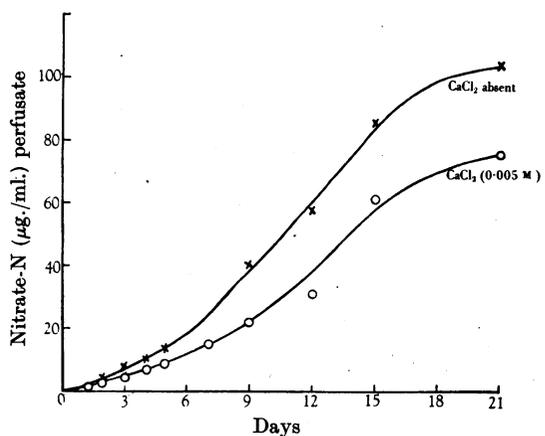


Fig. 1. Rates of nitrification at 70° F. of M/280 ammonium sulphate solution by 30 g. soil in presence and absence of calcium chloride.

It is obvious, however, that the experiments with calcium are not conclusive. The proof that nitrification in soil takes place only at the expense of the

adsorbed NH_4^+ comes from the following consideration and experiments.

Ratio of adsorbed NH_4^+ to total NH_4^+

The most direct way of studying the problem of whether the nitrifying bacteria metabolize adsorbed-ammonium cations or solution-ammonium cations is to compare the rates of nitrification in two perfusion units each containing the same total ammonium ion but different proportions of adsorbed ammonium ion. If the adsorbed ammonium ions are preferentially nitrified, then a greater rate of nitrification should obtain in the unit containing the greater proportion of adsorbed ammonium ion. A prerequisite to the validity of this method, however, is that if (say) the first unit has, at the start of the experiment, a greater proportion of its total ammonium adsorbed on the soil than has the second unit, then this relation should continue throughout the experiment, i.e. at any given percentage completion of nitrification the first unit should always have a greater proportion of its ammonium adsorbed than has the second. The 'adsorption ratio' (r) expressed as

$$r = \frac{\text{ammonium-N adsorbed in the soil exchange-complex}}{\text{total ammonium-N in the system}}$$

should remain constant. If this ratio does not remain constant then no direct correlation of adsorbed-ammonium with rate of nitrification will be possible.

The adsorption ratio, r , has, however, been found by experiment to remain reasonably constant throughout a normal nitrification experiment. In Table 3 are shown the complete data for three

Table 3. The adsorption ratio, r

$$r = \frac{\text{Ammonium-N adsorbed in the soil exchange-complex}}{\text{Total ammonium-N in the system}}$$

Weight of garden soil; 4.0-1.0 mm. fraction (g.)	30	100	100
Initial vol. perfusate (ml.)	300	250	250
Initial ammonium-N conc. of perfusate (µg./ml.)	280	400	280
Duration of exp. (days)	1 5 9 12 14	1 4 6 8 10	1 4 6 8 11
Total (nitrate + ammonium)-N (µg./ml. perfusate)	280 268 256 247 241	400 391 385 379 373	280 271 265 259 250
Ammonium-N in solution in perfusate (µg./ml.)	164 144 100 36 0	217 176 128 65 0	100 79 51 22 0
Rise in nitrate-N (µg./ml. perfusate)	0 20 101 190 241	0 61 151 241 373	0 76 133 206 250
Total ammonium-N available in system (µg./ml. perfusate)	280 248 155 57 —	400 330 234 138 —	280 195 132 53 —
Ammonium-N adsorbed in soil exchange-complex (µg./ml. perfusate)	116 104 55 21 —	183 154 106 73 —	180 116 81 31 —
Adsorption ratio (r)	0.41 0.42 0.36 0.37 —	0.46 0.47 0.45 0.53 —	0.64 0.60 0.61 0.58 —

experiments from which adsorption ratios have been calculated as

$$r = \frac{T - N - A}{T - N}$$

where T = total (ammonium and nitrate)-N, expressed as $\mu\text{g./ml.}$ perfusate, i.e. = total inorganic N available,

N = nitrate-N, $\mu\text{g./ml.}$ perfusate, i.e. = nitrified ammonium-N,

A = ammonium-N in solution in perfusate.

It is assumed that there is no adsorption of NO_3^- on the soil. This expression is identical with

$$\frac{\text{ammonium-N adsorbed in soil exchange-complex}}{\text{total ammonium-N in system}}$$

If the nitrification process were always 100% efficient and were uncomplicated by other processes, T would be constant from start to finish of any one experiment. In point of fact, however, T falls slightly throughout a normal experiment because of utilization of soluble nitrogen by the various microorganisms in the soil. In the calculation in Table 3 it is therefore assumed that T falls regularly from the $\mu\text{g./ml.}$ perfusate ammonium-N added at the start to the $\mu\text{g./ml.}$ nitrate-N produced at the end. It will be seen from Table 3 that r remains reasonably constant throughout an experiment. The values of r are not accurately determinable very near the end of an experiment because the percentage accuracy of the ammonium-N determinations is not very high at very low ammonium-N concentrations. Nevertheless, the r values of one experiment seem sufficiently close throughout to justify the conclusion that r does in fact remain constant.

Relation between adsorbed NH_4^+ and nitrification rate

The constancy of r throughout an experiment makes it possible to set up a series of perfusion units containing soils having different quantities of adsorbed NH_4^+ (but the same initial total NH_4^+), to study the rate of nitrification in each unit, and to correlate this rate with the quantity of the adsorbed NH_4^+ .

A technique for preparing the required series of units is described in the next paragraph. The description applies only to a pair of units, but a graded series may be prepared by adding a higher or a lower concentration of ammonium sulphate solution at the first stage.

In the detached soil tubes of each of two units A and B 50 g. soil were placed. On to the soil in tube A was placed 25 ml. distilled water and on to the soil in tube B 25 ml. $\text{M}/10$ ammonium sulphate solution. After about half-an-hour's standing to allow some replacement of some of the cations in the soil-exchange complex by the ammonium ions in the added ammonium sulphate, the soils were washed at 5 min. intervals with 25 ml. lots of distilled water and the washings

collected. After 15 washings the total ammonium washed out of each tube was estimated; in tube A of course it was negligible. The amount retained by the soil in tube B was calculated by subtracting the total ammonium-N washed out from the known quantity put in. The tubes were then assembled into their units. Tube B was perfused with 200 ml. distilled water and tube A with 200 ml. ammonium sulphate solution containing the same total ammonium-N as was retained initially on soil B. The whole operation from the start to finish including the time for ammonium analyses required not more than 3 hr. This period was clearly too short to allow the ammonium ions added to tube B markedly to stimulate the nitrifying bacteria therein, and one could therefore assume that the bacterial population of tubes A and B differed, at the start of perfusion, and of the experiment proper, by a degree no greater than was accountable for by soil-sampling variance.

After perfusing overnight, the perfusates of the two units were sampled and their ammonium-N concentrations determined. As the volumes of these perfusates were equal and known (200 ml. + the volume of water retained by the soil after washing), and as the total quantities of ammonium-N contained by the units were equal and known, it was possible to calculate how much ammonium-N was adsorbed in each unit and thence the adsorption ratio, r . On the basis of the results of Table 3, r was assumed to remain constant throughout the experiment—after equilibration had been attained initially by overnight perfusion.

Some of the added ammonium-N in unit A always enters by cation exchange into the base-exchange complex of the soil and as washing is never perfect, some ammonium-N yet remains in solution in unit B. Nevertheless, the equilibrium proportion of ammonium-N adsorbed in B is always higher than that adsorbed in A. Apart from this difference in the values of r , however, the only difference between the two units is that the total non-ammonium cation in B is slightly less than in A, some having been displaced by the initially added ammonium sulphate and removed in the washing process.

The desired condition of having two units containing the same total ammonium-N at different levels of r is thus established, and if the adsorbed ammonium ions are preferentially nitrified, the soil in unit B should nitrify more rapidly than that in unit A. If, however, the solution ammonium ions are preferentially nitrified then the soil in unit A should nitrify more rapidly than that in unit B.

In Table 4 are given the relevant figures from three different series of perfusion units prepared in the manner just described. The three series involved altogether fifteen different units each containing 50 g. of the allotment soil (4.0–1.0 mm. fraction). The perfusate of each unit was analyzed three times during each experiment. The following analyses were performed:

(i) *After overnight perfusion.* The ammonium-N concentrations of these initial samples were used to calculate the ratio, adsorbed ammonium-N/total ammonium-N (r), for each unit.

(ii) *After 8 days' perfusion.* The nitrate-N concentrations of these samples, after conversion to $\mu\text{g.}$ nitrate-N formed/g. soil, were used to calculate the percentage nitrification of

Table 4. *The relation between ammonium adsorption and rate of nitrification in a garden soil*

Ammonium-N added ($\mu\text{g./g. soil}$)	Nitrate-N finally formed ($\mu\text{g./g. soil}$)	Nitrate-N formed after 8 days		Ammonium-N adsorbed in base-exchange complex (%)
		As $\mu\text{g./g. soil}$	As percentage of final value	
694	660	446	67.5	50.5
694	679	504	74.0	62.0
810	685	413	60.5	46.5
810	675	474	70.0	57.5
810	726	595	82.0	77.0
783	730	435	59.5	47.0
783	787	485	61.5	56.0
783	770	563	73.0	62.0
783	786	615	78.0	67.0
720	641	402	62.5	48.5
720	661	414	62.5	47.5
720	649	468	72.0	55.0
720	634	492	74.5	60.5
720	661	469	71.0	58.0
720	633	490	77.5	64.5

Square of coefficient of correlation between percentage ammonium adsorption and percentage nitrification at 8 days = 0.83.

Table 5. *The relation between ammonium adsorption and rate of nitrification in a Kent (Romney Marsh) soil*

Ammonium-N added ($\mu\text{g./g. soil}$)	Nitrate-N finally formed ($\mu\text{g./g. soil}$)	Nitrate-N formed after 7 days		Ammonium-N adsorbed in base-exchange complex (%)
		As $\mu\text{g./g. soil}$	As percentage of final value	
1380	1322	628	47.5	37.5
1380	1349	689	51.0	45.0
1380	1353	716	53.0	52.5
540	485	316	65.0	48.0
540	472	336	71.0	64.0
540	460	306	66.5	62.0
540	497	355	71.5	68.0
540	490	349	71.0	68.0
1120	1108	516	46.5	47.0
1120	1162	572	49.0	52.0
1120	1124	582	52.0	53.0
1120	1143	604	53.0	55.0
1120	1135	594	52.5	54.0

Square of coefficient of correlation between percentage ammonium adsorption and percentage nitrification at 7 days = 0.68.

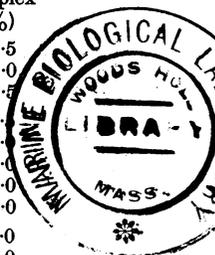
ammonium sulphate. This value was not based on the ammonium-N initially added, but on the nitrate-N finally formed. The reasons for the adoption of this mode of expressing percentage nitrification are discussed below.

(iii) *After 16-21 days when nitrification was complete.* The nitrate-N concentrations of these samples, after conversion to $\mu\text{g. nitrate-N formed/g. soil}$, were used as bases for obtaining the percentage nitrification at 8 days.

The choice of 8 days as a time at which to differentiate between the degrees of nitrification attained in the different units is based simply on experience. It has been found that at 8 days after the start of an experiment the difference between the percentages of nitrification in the different units of one series is at its greatest. After 8 days the difference becomes smaller and smaller until finally, at the end of an experiment, all the units show the same degree of nitrification. The expression of nitrification at 8 days as the 'percentage of final amount of nitrate-N formed' rather than as

'the percentage of initially added ammonium-N' compensates for the effect of one nitrification unit containing slightly more ammonium-N at the start than another. It is experimentally impossible to obtain exact replication of the initial ammonium-N additions throughout one series of units because the original estimate of the small amount of ammonium-N retention from the large ammonium-N addition is not easy. Furthermore, the final efficiency of conversion of ammonium-N to nitrate-N is not 100%, nor can it be assumed to be absolutely constant from unit to unit. However, by expressing the percentage nitrification at 8 days as a percentage of the final nitrification the effects of these inter-unit variations is minimized, and the percentage nitrification so expressed is a valid index of the relative speed of nitrification obtaining in each unit.

Table 4 shows the fairly high correlation between 'percentage nitrification at 8 days' and 'percentage



ammonium adsorption' obtained experimentally. The correlation coefficient between the experimental values of these two expressions is $\sqrt{0.83}$. Therefore only $(1 - 0.83) \equiv 17\%$ of the variance in one column of figures is not accountable for by parallel variance in the other.

The same correlation has been found in similar experiments conducted on an arable clay loam from Kent (Romney Marsh). The experimental data for these experiments are given in Table 5. The Kent soil nitrified ammonium sulphate somewhat faster than did the garden soil; the operative nitrification percentages were therefore calculated on the basis of the amount of nitrification after 7 days, not 8 days. The correlation coefficient between the values for 'percentage nitrification at 7 days' and the corresponding values for 'percentage ammonium adsorption' is $\sqrt{0.68}$. Therefore 32% of the variance in one column is not accountable for by a parallel variance in the other.

The data contained in Tables 4 and 5 show that the rate of nitrification of a given quantity of ammonium sulphate by a given quantity of soil varies with the degree to which the ammonium ions of the ammonium sulphate are adsorbed into the soil base-exchange complex; the greater the adsorption, the faster the nitrification, or conversely, the greater the solution ammonium, the slower the nitrification. The most obvious explanation of these results is that the nitrifying bacteria in soil preferentially oxidize the adsorbed NH_4^+ .

Independence of the velocity of nitrification in soil of the concentration of solution ammonium ions. Effects of varying the volume of perfusate containing a constant quantity of ammonium ions.

Soils were perfused with varying volumes of ammonium sulphate solution each containing initially the same total amount of NH_4^+ . Two soils

Table 6. Rates of nitrification in soil perfused with various concentrations of NH_4^+

Exp. 1. Air-dried Rothamsted garden soil, 30 g.							
Perfusion units		A		B		C	
Volume of perfusate (ml.)		200		150		100	
Total ammonium-N initially present (mg.)		20		20		20	
Concentration ammonium-N initially present ($\mu\text{g./ml.}$ perfusate)		100		133		200	
Concentration ammonium-N ($\mu\text{g./ml.}$ perfusate) present at equilibrium (24 hr. from start of perfusion)		56		74		104	
Adsorption ratio (r)=fraction of total NH_4^+ adsorbed on soil		0.44		0.44		0.48	
Time (days) from start of perfusion	Nitrate-N ($\mu\text{g./ml.}$ perfusate)			Nitrate-N formed (mg.)			
	A	B	C	A	B	C	
0	Trace	Trace	Trace	0	0	0	
2	4	2	.4	—	—	—	
3	7	7	7	—	—	—	
5	7	9.5	12	1.40	1.42	1.20	
7	16	25	38	3.20	3.75	3.80	
10	25	35	50	5.00	5.25	3.80	
13	60	78	110	12.00	11.70	11.00	
19	96	126	190	19.20	18.90	19.00	
Exp. 2. Air-dried Romney Marsh soil, 40 g.							
Perfusion units		A		B		C	
Volume of perfusate (ml.)		300		200		150	
Total ammonium-N initially present (mg.)		30		30		30	
Concentration ammonium-N initially present ($\mu\text{g./ml.}$ perfusate)		100		150		200	
Concentration ammonium-N ($\mu\text{g./ml.}$ perfusate) at equilibrium (24 hr. from start of perfusion)		62		87		109	
Adsorption ratio (r)=fraction of total NH_4^+ adsorbed on soil		0.38		0.42		0.45	
Time (days) from start of perfusion	Nitrate-N ($\mu\text{g./ml.}$ perfusate)			Nitrate-N formed (mg.)			
	A	B	C	A	B	C	
1	0	0	0	—	—	—	
4	12	24	35	3.60	4.80	5.25	
7	34	50	72	10.10	10.00	10.80	
10	85	115	185	24.50	23.00	27.75	
12	108	162	210	32.40	32.40	31.50	
17	104	165	212	31.20	31.00	31.80	

were used, a Rothamsted garden soil and a Romney Marsh soil.

The amounts of NH_4^+ adsorbed on the soils were estimated when ionic equilibrium between soil and perfusate had been established (i.e. about 24 hr. after the start of the perfusion). These figures, together with values of the volumes of perfusate etc. are shown in Table 6 (Exps. 1 and 2). In this table also will be seen the rates of formation of nitrate in the various perfusion units which were all run at 70° F.

It is clear from these results that the rate of nitrification in either soil, expressed in terms of the total nitrate-N produced, is independent of the volume of the perfusate and of the concentration of NH_4^+ in the perfusate. The rates of nitrification in the three tubes of one experiment are approximately equal, and this fact is to be correlated with the observation (Table 6) that the fractions of total NH_4^+ adsorbed on the soil are also approximately equal. These results lead to the conclusion that the nitrification process is, other things being equal, dependent on the amount of NH_4^+ adsorbed upon the soil surface.

The velocity of nitrification as a function of the base-exchange capacity of the soil system

The previous findings all agree with the view that nitrification proceeds at the expense of adsorbed NH_4^+ in the soil and is a function of the total surface area on which NH_4^+ is adsorbed. The truth of this view can be checked in yet another way. The addition of sterile base-exchange material to the soil-perfusate system (containing free NH_4^+ in the perfusate) should cause an increase in the rate of nitrification of the whole system by virtue of adsorbing more NH_4^+ and thus increasing the total surface area of adsorbed NH_4^+ . The nitrifying cells should spread by proliferation on to the new surface and attack the NH_4^+ adsorbed there. The addition of material having no base-exchange capacity should not increase the rate of nitrification since no extra ad-

sorbed NH_4^+ is formed and no suitable surface for activity of the nitrifying bacteria is there presented.

Experimentally some care is necessary in the choice of material to ensure that a positive result really is valid. It is essential that the sterile base-exchange material to be added should comply with the following conditions:

(i) It must supply as little extra nutrient as possible to the perfusing fluid. This condition is necessary in order to minimize any effect the added material may have over and above its base-exchange effect.

(ii) It must be sterile initially in order to avoid complications due to addition of extra micro-organisms.

(iii) It must be non-toxic and capable of supporting bacterial proliferation. If this condition is not fulfilled the nitrifying bacteria may be unable to utilize the ammonium ions adsorbed by the added base-exchange material.

All three conditions are admirably fulfilled by a non-fertile clay or sandy soil which has been autoclaved. For this reason two such soils were autoclaved for 2 hr. at 15 lb./sq.in. pressure, and used immediately after autoclaving as sterile base-exchange material. The soils were:

(a) Soil from the unmanured plot on Barnfield, Rothamsted Experimental Station. This is a heavy red clay of high base-exchange capacity; cropped but unmanured for a century.

(b) Soil from Stackyard at Woburn Experimental Station, Beds. This is a poor sandy soil of medium base-exchange capacity.

The questions at issue were: Will the addition of any of these sterile materials (Barnfield soil, Woburn soil, sand) to a nitrifying system increase the rate of nitrification of that system, and will the increase, if any, have any relation to the increase in adsorbed NH_4^+ in the system?

The experimental technique used to provide the answer to the questions was as follows: A given amount of allotment soil was mixed with about twice its weight of freshly autoclaved Barnfield or Woburn soil, or quartz sand, and the mixture perfused with ammonium sulphate solution. The quartz

Table 7. *The effect of added base-exchange material on the rate of nitrification in soil*

AQ=autoclaved sand; AW=autoclaved Woburn Stackyard soil; AB=autoclaved Barnfield soil.

Weight of allotment soil (g.)	Addition	Weight of addition (g.)	Volume perfused (ml. N/35 ammonium sulphate)	r for whole system	Duration of exp. (days)	Nitrate-N formed in whole system (mg.)	Nitrate-N formed divided by 100r
30	—	—	200	0.33	13	39	1.18
30	AQ	70	200	0.33	13	37	1.12
30	AW	70	200	0.54	13	57	1.05
30	AB	70	200	0.64	13	76	1.19
50	—	—	250	0.54	10	54	1.00
50	AW	100	250	0.69	10	78	1.13
50	AQ	100	250	0.55	10	55	1.00
30	—	—	250	0.36	12	45	1.25
30	AB	70	250	0.69	12	73	1.06

r=ammonium-N adsorbed in base-exchange complex/total ammonium-N in system.

sand was used as a control material having (presumably) no base-exchange capacity. The nitrification in a system containing one or other of these autoclaved additions was compared with the nitrification in a system containing the original weight of allotment soil alone, perfused with the same quantity of the ammonium sulphate solution. This comparison was made at a time when the nitrification in the unit containing the allotment soil alone was roughly half-completed. This period was chosen simply because, at this time, differences between different units are displayed most satisfactorily. The results of nine such experiments are given in Table 7. The results in this table are not amenable to exact mathematical treatment, because too many unknown factors are involved, e.g. the rate of movement of bacteria from the fresh soil on to the autoclaved addition, the suitability of the autoclaved surface as a bacterial nidus, the amount of chemical stimulation or inhibition due to products from the autoclaved additions. Nevertheless with all known complications reduced to a minimum, there remains a real correlation between the percentage ammonium-N initially adsorbed in a system and the degree of nitrification at the arbitrary time. The results of Table 7 show that sand neither increases the adsorption of ammonium ions nor increases the nitrification rate. Autoclaved Barnfield soil markedly increases the adsorption of ammonium ions and correspondingly increases the nitrification rate. Woburn soil falls midway between sand and Barnfield soil on both counts. This is what would be expected on the basis of the hypothesis that the nitrification takes place at the expense of adsorbed ammonium ions. Clearly other conditions must also influence the activities of the bacteria, at the NH_4^+ receptor sites of the soils and these will be the subject of future investigations.

Bacterial saturation of soils

It follows from the above conclusions that the nitrifying organisms will ultimately spread over the entire soil surface where NH_4^+ is adsorbed if other essential nutritional conditions are also satisfied at these surfaces. It would be expected that when this condition obtains and when there is an excess of NH_4^+ in the system, the rate of nitrification will become constant and proportional to the 'base-exchange' area covered by the bacteria. Experiment has verified this prediction.

Six lots of 40 g. air-dried garden soil were perfused with 200 ml. of $m/200$ -ammonium sulphate solution (28 mg. ammonium-N) until nitrification was complete. This took about 15 days at 70° F. The soils were washed with water to remove the nitrate formed during the nitrification and then reperfused with a total of 200 ml. ammonium sulphate solution

containing 20 mg. ammonium-N. The initial rate of nitrification and the subsequent rate on re-perfusion are shown in Fig. 2.

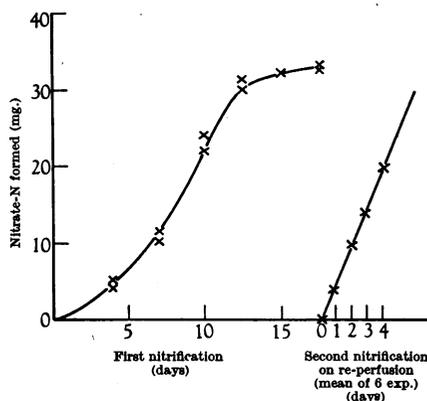


Fig. 2. Nitrification of 200 ml. ammonium sulphate solution (containing 30 mg. ammonium-N) by 40 g. air-dried garden soil.

The results show that the second rate of nitrification, during the perfusion of the soil enriched with nitrifying organisms, is constant. The initial lag period, typical of the course of nitrification in a fresh soil that has not been bacterially enriched by perfusion, is absent.

In another series of experiments, in which varying quantities of soil were used, estimations were made of the rates of nitrification, after preliminary enrichment of the soils by perfusion with ammonium sulphate solution. The results are shown in Fig. 3; the subsequent rates of nitrification are linear and again there is no initial lag period.

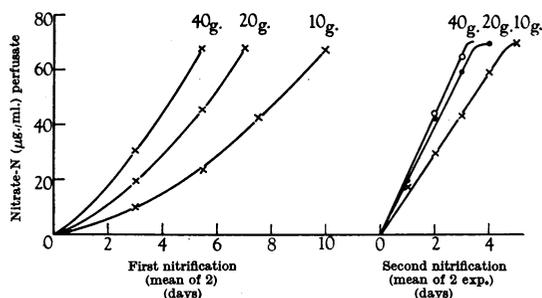


Fig. 3. Nitrifications of 200 ml. NH_4Cl (at 70 $\mu\text{g./ml.}$ ammonium-N) on perfusion through three lots of soil (40, 20, 10 g.).

It can be shown that the linear rates of nitrification are proportional to the adsorption ratios r , i.e. to the fractions of total NH_4^+ in the system which is adsorbed on the soil. The following experimental findings of Table 8 demonstrate this fact.

Table 8. *Relationship of nitrification rates to r*

Quantity of soil (g.)	Rate of 'subsequent' nitrification ($\mu\text{g. nitrate-N/ml. perfusate/day}$)	Adsorption ratio, r , determined experimentally	Rate of nitrification divided by r
10	15	0.45	33
20	19	0.50	38
40	22	0.63	35

DISCUSSION

The results of all our experiments designed to test the view that nitrification in soil takes place at the soil crumb surfaces strongly support the truth of this hypothesis, and lead to the conclusion that the nitrifying organisms necessarily carry on their metabolism at soil surfaces. Moreover, it becomes clear that the rate of nitrification in soil is a function of the base-exchange capacity of the soil and that the process is confined to specific areas of the soil surfaces.

The results have led to the concept of bacterial saturation of soils and this concept has been used by us in experiments designed to determine whether the same bacteria are responsible for the breakdown of more than one substance in soil.

The results of these experiments will be given in a further paper.

SUMMARY

1. When nitrification takes place in soil perfused with solutions of ammonium sulphate, little or no nitrification takes place in the perfusate or soil solution.

2. Nitrification in soil takes place wholly at the soil surfaces. It occurs at those areas of the soil surface where NH_4^+ is combined or adsorbed.

3. The addition of calcium ions to a perfusate containing ammonium ions diminishes the rate of nitrification in soil. The rate of nitrification is diminished in proportion to the amount of NH_4^+ displaced from the soil surface by base-exchange with the Ca^+ .

4. The ratio of soil-adsorbed NH_4^+ to the total NH_4^+ in a soil-perfusate system remains approximately constant throughout the course of nitrification.

5. The rate of nitrification in soil is (a) proportional to the fraction of the total NH_4^+ which is adsorbed or combined in the base-exchange complexes of the soil, (b) apparently independent of the concentration of NH_4^+ in the soil solution, and (c) increases with the amount of soil on which the NH_4^+ is adsorbed.

6. The rate of nitrification in a soil is increased by the addition of sterile soil, the amount of increase being roughly proportional to the base-exchange capacity of the added sterile soil. Addition of sand leads to no increase in the rate of nitrification. It is concluded that the rate of nitrification in soil is a function of the base-exchange capacity of a soil.

7. These results are in harmony with the view that nitrifying bacteria nitrify on the surfaces of soils, in the vicinity of the receptor sites where NH_4^+ is combined or adsorbed. Conditions which lead to an increase or diminution of these receptor sites likewise lead to an increase or diminution of the rate of nitrification.

8. Soils which have been enriched with nitrifying bacteria by preliminary perfusion with ammonium sulphate solutions show, subsequently, linear courses of nitrification. Such soils are termed bacteria-saturated soils and they do not show the familiar time lag of a bacterial proliferation curve.

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