THE *AZOTOBACTER* TEST OF SOIL FERTILITY APPLIED TO THE CLASSICAL FIELDS AT ROTHAMSTED.

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(With Plate IV.)

The sensitiveness of the *Azotobacter* group of organisms to soil acidity and to lack of available phosphate has been long recognised and has been the basis of microbiological tests of both these soil conditions, devised especially by Christensen and Niklas. Such methods have lately (10, 12) been criticised by Winogradsky. The *Azotobacter* cells are often placed under semi-anaerobic conditions in a liquid medium, where many may perish from lack of oxygen or from competition with anaerobic acid-producers such as *Clostridium*. The author (5) in fact found that the Christensen-Niklas method sometimes failed to detect the presence of *Azotobacter* in the soil and consequently, as a test of lime or phosphate deficiency led to misleading results. In 1926–7 a new *Azotobacter* test was developed by Winogradsky and the author (1, 2), who termed it *la méthode des plaques moulées*. It has since been applied to French soils by Guittonneau (3), to Russian soils by Krjutshkova (4), to Polish soils by the author (5, 6, 7, 8), and to some American soils by Sackett and Stewart (13). The method was successful, although slight modifications were needed for different soil types.

Technique.

In principle the kneaded-plate method consists in observing the development of *Azotobacter* colonies upon the surface of the soil itself, suitably moistened and kneaded. About 150 gm. of the fresh sifted soil are mixed with 1 per cent. mannitol or organic acid salts in the case of light soil, or with 5 per cent. starch in the case of heavy soil. In light soils lacking in colloids 10–20 per cent. of sterilised kaolin is added to increase the plasticity. The mixture is then divided into four portions. The first portion is moistened with distilled water, the second with a solution containing 0·67 gm. Na_2HPO_4 + 0·33 gm. NaH_2PO_4 per litre, giving a 0·1 per cent. solution of phosphate having a reaction of *p*H 7·0. (In rare instances a stronger phosphate solution is needed.) The third portion is mixed with $CaCO_3$ (at least 2 per cent.) and moistened with distilled

water, and the fourth portion is mixed with $CaCO_3$ and moistened with the phosphate solution. This gives four treatments as follows:

0 = control,
+ P = phosphate added,
+ Ca = calcium carbonate added,
+ P + Ca = calcium carbonate and phosphate added.

Each portion is moistened to the point of saturation and is then kneaded with a pestle and mortar until a fine paste is obtained. Petri dishes of 3–4 cm. internal diameter are filled with the pastes and the surfaces smoothed by means of a glass slide moistened with sterile water. The plates are left uncovered and incubated in a moist chamber at 30° C. After 40 hours to 3 days the *Azotobacter* growth becomes visible in the form of milky drops or compact white growth. In the early stages of incubation such colonies consist of nearly pure cultures of *Azotobacter*. A quantitative estimation of the growth can be made by counting the colonies on a square centimetre of surface, using a lens. When a good growth of *Azotobacter* takes place on all the plates it is inferred that the soil contains sufficient lime and available phosphate for the needs of the organisms. A shortage of available phosphate or lime in the original soil is revealed by *Azotobacter* growth being visible only on the portions to which the deficient constituent has been supplied (see Plate IV).

It is sometimes found that no *Azotobacter* growth takes place with any of the treatments. This may mean that *Azotobacter* is absent from the original soil or that it has been suppressed by competition with other soil organisms. This suppression is especially liable to occur in soils rich in available nitrogen, which encourages the competition. On plates of such soils the *Azotobacter* is often replaced by an organism producing a vitreous sticky growth (the *Bacille gommeux* of Winogradsky) which appears after 30–48 hours' incubation and is followed by a growth of moulds. The test for lime and available phosphate by the above method will fail where *Azotobacter* is absent or suppressed in the original soil, so that abundance of available nitrogen may conceal the presence or absence of phosphate or lime. For such soils, the method may be modified by supplying *Azotobacter* from a fresh culture which may be grown either on silica jelly plates or in sterilised soil to which a carbohydrate has been added. The addition of *Azotobacter* to the sample is a valid proceeding, because the *plaque moulée* method aims at testing whether the soil is suitable for the growth of *Azotobacter* and not whether it was originally present.

The density of the *Azotobacter* population in the original soil may be estimated by the method of silica jelly plates, devised by Winogradsky (10). Silica jelly is prepared by adding 100 c.c. potassium or sodium silicate solution of specific gravity 1·06–1·08 to 100 c.c. HCl specific gravity 1·10[1] in a beaker. The solution is well mixed and poured into petri dishes 20 cm. in diameter. In about 48 hours the gel will have set and the HCl is washed out of it with flowing tap water, the washing being finished off with boiled distilled water. 1 gm. of mannitol or 0·5 gm. of the more selective calcium lactate is sprinkled over the surface of each plate, which is then watered with 10 c.c. of the following solution:

KH_2PO_4	...	...	...	0·5 gm.
$MgSO_4$	...	...	...	0·3 ,,
NaCl	...	...	...	0·3 ,,
$FeSO_4$	...	...	...	0·01 ,,
$MnSO_4$	...	...	...	Trace
Water	...	...	...	100 c.c.

The *p*H adjusted to 7·0–7·2 with 2 per cent. KOH.

Where mannitol is used, 0·5 gm. $CaCO_3$ should also be added to each plate. The supernatant solution on the plates is evaporated at 40° C., and 50 mg. (dry weight) of the fresh sifted soil is evenly distributed over the surface of the jelly. The amount of soil may be increased to 1 gm. where a thin population of *Azotobacter* is expected. The plates are incubated for 48 hours at 30° C. and the *Azotobacter* colonies are counted. The advantages of this method over the dilution plating technique are elsewhere discussed (5).

APPLICATION OF THE *AZOTOBACTER* TEST TO THE BROADBALK WHEAT PLOTS.

The classical fields at Rothamsted and Woburn afford a unique opportunity for testing methods of microbiological soil analysis, since on them the results can be correlated with manurial treatment and with yield data extending over a longer period than is obtainable elsewhere. The methods described above were therefore applied to the wheat plots on Broadbalk, to the portion of Broadbalk left as wilderness, to the barley plots on Hoos Field, to the mangold plots on Barnfield and to the rotation plots on Agdell field. For comparison with these heavy soil fields, some tests were also applied to sandy soil plots under permanent wheat and barley at Woburn, and to an acid loamy soil from a pasture in Cheshire. The samples were taken from surface soil, to a depth of about 5 in., each sample being a composite of 3 or 4 cores. The soils were passed

[1] Made by adding 400 c.c. distilled water to 600 c.c. HCl specific gravity 1·19.

through a 1 mm. sieve. The tests were applied one day after sampling, with the exception of samples collected in 1930 which were sent to the Soil Research Laboratory in Poznań University, Poland, for the examination, which took place some weeks after sampling. In making the kneaded plates, mannitol was added to the soils, as it was found to be more satisfactory than starch. In addition to the *Azotobacter* tests, the water-soluble phosphate was estimated by the field method of Spurway, as developed by Terlikowski and Królikowski (9), and in some cases the *p*H was determined.

Samples from Broadbalk were taken on 2nd August, 1930, on 11th September, 1931 and on 13–14th October, 1931. The results obtained are shown in Tables I–III. The best conditions for *Azotobacter* growth on all three occasions were found in Plot 5, which received complete minerals but no nitrogen. On this plot very numerous *Azotobacter* colonies developed on the kneaded plates; their number was not increased by the addition of either phosphate or lime, indicating no shortage of either constituent. The control Plot 3 gave *Azotobacter* growth on the kneaded plates only where phosphate was supplied, indicating a deficiency in this element. The dunged Plot 2 B showed no evidence of phosphate deficiency but the number of *Azotobacter* colonies on the kneaded plates was much reduced, and was even lower than the control plot when the latter's phosphate deficiency was made up.

The most striking feature of Broadbalk samples, however, was the repression of *Azotobacter* in plots receiving mineral nitrogen. This effect of nitrogen can be well studied in Plots 6, 7, 8, 9 and 16, where different nitrogen dressings have been given to a uniform dressing of minerals which Plot 5 shows to afford excellent conditions for *Azotobacter* growth. It can also be seen in the samples from Plots 10 to 14, where the nitrogen supply is kept constant and the minerals varied. *Azotobacter* colonies were absent or scarce on uninoculated kneaded plates made from all these plots which receive mineral nitrogen, and this scarcity was unaffected by the addition of phosphate or lime to the plates. It was clearly related to the paucity of *Azotobacter* cells in the original soil, as is shown by the counts made on silica jelly plates, which show an inverse relationship between the *Azotobacter* numbers and the nitrogen dressing.

The reduction of *Azotobacter* population in the soil of these plots can be explained as being due to competition with other organisms which thrive on the nitrogen supplied. It has been shown (2) that if soil rich in *Azotobacter* is added to a medium containing mannitol and varying doses of nitrate, the development of *Azotobacter* becomes weaker with in-

creasing nitrate until it stops completely when the C : N ratio reaches 100C : 0·4N. At this point the *Bacille gommeux*, moulds, and other organisms replace it. It is evident therefore that *Azotobacter* can compete for the energy supply only under conditions of nitrogen shortage. It was

Table I. *Broadbalk.*

Soil samples taken on 15th July, 1930, examined 2nd August, 1930. Manures applied: Dung, September; Minerals and Rape cake, October; Ammonium sulphate, October and March; Nitrate, March (and on Plot 16, half in April).

Soil sample no.	Plot	Plot treatment	Water soluble P_2O_5 mg. per kg.	Uninoculated kneaded plates. Number of *Azotobacter* colonies on 1 cm.²	
				0	+P
1	2B	Dung, 14 tons per acre	>15	50	50
2	3	Control	6–10	0	50–100
3	5	Complete minerals*	>15	80–100	80–100
4	8	129 lb. N per acre as amm. sulph. + complete minerals	>15	1–2	1–2
5	16	86 lb. N per acre as nitrate of soda + complete minerals	>15	10	10

* Complete minerals = 3½ cwt. superphosphate 200 lb. sulph. potash, 100 lb. sulph. soda, 100 lb. sulph. magnesia.

Table II. *Broadbalk.*

Soil samples taken and tested on 11th September, 1931. Manures applied: Dung, October; Minerals and Rape cake, October; Ammonium sulphate, October and March; Nitrate, March (and on Plot 16, half in May).

Soil sample no.	Plot	Plot treatment	Yields in 1930. Grain, bushels per acre	Water soluble P_2O_5 mg. per kg.	Number of *Azotobacter* colonies from 1 gm. soil on duplicate silica plates	Uninoculated kneaded plates. Number of *Azotobacter* colonies on 1 cm.²			
						0	+P	+Ca	+P+Ca
6	2B	Dung, 14 tons per acre	34	11–15	(*a*) 1479 (*b*) 945	10–15	15	—	15
7	3	Control	12	0–5	(*a*) 592 (*b*) 936	0	20	—	20–25
8	5	Complete minerals	14	11–15	(*a*) 8048 (*b*) 8752	50–70	50–70	—	—
9	8	As 5 + 129 lb. N per acre as amm. sulph.	35	11–15	(*a*) 31 (*b*) 40	0	0	2–3	2–3
10	16	As 5 + 86 lb. N per acre as nitrate	31	11–15	(*a*) 276 (*b*) 326	10–15	10–15	—	10–15
11	19	As 5 + rape cake	22	11–15	(*a*) 155 (*b*) 209	1–2	3–4	3–5	3–5

Note. In this and the following tables a dash, —, means that the particular test was not made, a nought, 0, that the test was negative.

Table III. *Broadbalk.*

Soil samples taken on 13–14th October, 1931. For times of manure application see Table II.

Soil sample no.	Plot	Plot treatment	Yields in 1930. Grain, bushels per acre	pH	Water soluble P_2O_5 mg. per kg.	Number of *Azotobacter* colonies from 1 gm. soil on duplicate silica plates	Kneaded plates: Uninoculated soil. Number of *Azotobacter* colonies on 1 cm.² 0	+P	+Ca	+P+Ca	+K	Soil inoculated with *Azotobacter* culture 0	+P	Remarks
12	2B	Dung, 14 tons per acre	34	—	*ca.* 15	(*a*) 118	20	20	—	—	—	—	—	—
13	3	Control	12	8·0	*ca.* 5	(*a*) 844	0	30	0	—	—	—	—	—
14	5	Complete minerals	14	—	>15	(*a*) 2268 (*b*) 1842	30–40	30–40	—	—	30–40	+ + +	—	—
15	6	As 5+43 lb. N per acre as amm. sulph.	22	7·1	>15	(*a*) 852 (*b*) 1113	15–20	15–20	15–20	15–20	15–20	+ + +	+ + +	*Azotobacter* well developed but mixed with *Bac. gommeux* and moulds
16	7	As 5+86 lb. N per acre as amm. sulph.	31	7·4	>15	(*a*) 253 (*b*) 253	0	0	0	0	0	+ + +	+ + +	*Azotobacter* present, but formed no colonies. Thick growth of *Bac. gommeux* and moulds
17	8	As 5+129 lb. N per acre as amm. sulph.	35	6·5	>15	(*a*) 27 (*b*) 0	0	0	0	0	0	+ + +	+ + +	Thick growth of *Bac. gommeux* and moulds. No *Azotobacter* development
18	9	As 5+43 lb. N per acre as nitrate of soda	25	7·4	11–15	(*a*) 1862 (*b*) 1324	10–15	10–15	—	—	—	+ + +	+ + +	—
19	10	86 lb. N per acre as amm. sulph. *No* minerals	19	—	6–10	(*a*) 195 (*b*) 322	0	5–10	0	+K+P+Ca 5–10	0	0	++	—
20	11	As 10+P	21	—	>15	(*a*) 187 (*b*) 252	3–5	3–5	3–5	+K+P+Ca 3–5	3–5	—	—	—
21	12	As 10+P+Na	28	—	>15	(*a*) 54	5–10	5–10	5–10	+K+P+Ca 5–10	5–10	—	—	—
22	13	As 10+P+K	30	—	>15	(*a*) 393 (*b*) 381						—	—	Counts not possible. *Azotobacter* mixed with *Bac. gommeux* and moulds
23	14	As 10+P+Mg	27	—	>15	(*a*) 75 (*b*) 100	8	6	6	—	6	—	—	—
24	15	As 5+86 lb. N per acre as amm. sulph. All applied in Oct.	28	—	>15	—	0	0	0	—	—	+ + +	+ + +	Thick growth of *Bac. gommeux*
25	16	As 5+86 lb. N per acre as nitrate	31	—	>15	(*a*) 130 (*b*) 109	2–3	2–3	—	—	—	+ + +	—	—
26	17	Complete minerals Oct. 1930. Amm. sulph. 9th Oct. 1931	29	—	11–15	—	40	40	—	—	—	- —	—	—
27	18	Amm. sulph. Oct. 1930 and March, 1931. Complete minerals 9th Oct. 1931	14	—	11–15	—	15	15	—	—	—	—	—	—
28	19	Rape cake equivalent to 86 lb. N	22	—	11–15	—	0	15	1	—	—	—	—	Moulds on all plates

observed that the samples from Broadbalk Plots 6, 7 and 8 showed a progressive increase in spore-forming bacilli and moulds as well as a progressive reduction in *Azotobacter* colonies, corresponding with the increase in nitrogen dressing. The standard kneaded-plate method thus fails to detect phosphate or lime deficiency in the presence of such mineral nitrogen dressings as are given to Broadbalk, owing to the reduced numbers or lessened viability of *Azotobacter* in the original soil. Kneaded plates inoculated with *Azotobacter* were therefore made with some of the soil samples. When this was done a normal development of *Azotobacter* colonies was always found where the necessary phosphate and lime were present.

The kneaded-plate method was also applied to that portion of Broadbalk which has been left as wilderness since 1882. Half of this piece is now woodland and half is kept in grass and weeds. The test (Table IV) showed phosphate deficiency in both portions of the wilderness, but no acidity. The woodland portion contained a far smaller population of *Azotobacter* than the herbaceous half, but even the latter contained fewer *Azotobacter* than the unmanured Plot 3 in the cultivated portion of the field.

Table IV. *Broadbalk Wilderness since* 1882.

Soil samples taken on 11–12th November, 1931.

Soil sample no.		Water soluble P_2O_5 mg. per kg.	Number of *Azotobacter* colonies from 1 gm. of soil on silica plates	Kneaded plates. Number of *Azotobacter* colonies on 1 cm.²					
				Uninoculated				Inoculated	
				0	+P	+Ca	+PCa	0	+P
30	No trees	*ca.* 5	(*a*) 317 (*b*) 358	0	2–3	0	—	0	++
31	Wood	0–5	(*a*) 139 (*b*) 100	0	2–3	0	—	0	++

THE HOOS FIELD BARLEY PLOTS.

Samples from Hoos Field were taken 15th July, 1930, 16th September, and 2nd November, 1931 (see Tables V, VI, VII). Repetition of the same mineral dressings with various forms of nitrogen makes this field particularly suitable to the application of microbiological analysis. As with Broadbalk, the best *Azotobacter* growth on kneaded plates and the highest number of colonies on silica plates were given by the plot receiving complete minerals but no nitrogen, while the control Plot 1-O gave a lower colony count on silica plates and showed phosphate deficiency on the kneaded plates. The dung Plot 7-2 again gave rather

fewer colonies on the kneaded plates than did the no-nitrogen plots where phosphate was present, although the silica plate counts made on 16th September from this plot gave high figures. Plot 7-1, which received dung from 1852 to 1871 and has since been unmanured, showed a phosphate deficiency like the unmanured Plot 1-0, but gave a somewhat higher silica plate count than did the latter (Table VI).

The plots receiving nitrate, ammonia or rape cake showed a depression in the *Azotobacter* count on silica plates and a corresponding failure or weak development of colonies on the kneaded plates. In the case of

Table V. *Hoos Field.*

(*a*) Soil samples taken 15th July, tested 2nd August, 1930. Manures applied in March.

Soil sample no.	Plots	Plot treatment	Water soluble P_2O_5 mg. per kg.	Uninoculated kneaded plates	
				0	+P
32	1-0	Control	0–5	0	Numerous
33	4-0	Complete minerals	>15	>50	>50
34	4-A	As 4-0 + 43 lb. N per acre as amm. sulph.	>15	0	0
35	4-AA	As 4-0 + 43 lb. N per acre as sodium nitrate	11–15	0	0
36	7-1	Dung in 1852–71, since then unmanured	6–10	0	50–100

Table VI. *Hoos Field.*

(*b*) Soil samples taken on 16th September, 1931. Manures applied in March.

Soil sample no.	Plots	Plot treatment	Yields in 1930. Grain, bushels per acre	Water soluble P_2O_5 mg. per kg.	Number of *Azotobacter* colonies from 1 gm. soil on silica plates	Uninoculated kneaded plates			
						0	+P	+Ca	+P+Ca
37	1-0	Control	14	5–10	(*a*) 1368 (*b*) 1245	0	50	—	50
38	4-0	Complete minerals	20	11–15	(*a*) 3244 (*b*) 2062	100	100	—	100
39	4-A	As 4-0 + 43 lb. N per acre as amm. sulph.	41	>15	(*a*) 100 (*b*) 36	0	0	1–5	1–5
40	4-AA	As 4-0 + 43 lb. N as nitrate of soda	39	>15	(*a*) 554 (*b*) 558	50	50	—	—
41	4-C	As 4-0 + rape cake	39	>15	(*a*) 117 (*b*) 190	0	0	0–1	0–1
42	7-1	Dung only in 1852–71, since then unmanured	24	0–5	(*a*) 1859 (*b*) 1739	0	35–40	—	—
43	7-2	Dung every year	46	>15	(*a*) 2098 (*b*) 2007	15–20	20	—	—

Plot 4-A this seemed to be associated with acidity. In spite of the depression in *Azotobacter* population in the soils with nitrogen manuring, the uninoculated kneaded plates gave a correct diagnosis of phosphate and lime deficiency more often than was the case on Broadbalk (see soil samples 40, 48, 49, 50, 51, 53, 55, 56 and 57). This can perhaps be

Table VII. *Hoos Field.*

(*c*) Soil samples taken on 2nd November, 1931.

Soil sample no.	Plots	Plot treatment	Yields in 1930	*p*H	Water soluble P_2O_5 mg. per kg.	Number of *Azotobacter* colonies from 1 gm. soil on silica plates	Kneaded plates: Uninoculated 0	Uninoculated +P	Uninoculated +Ca	Uninoculated +P+Ca	Inoculated 0	Inoculated +P
44	1-O	Control	14	7·6	6–10	—	0	25	> 0	—	—	—
45	2-O	P	20	—	11–15	—	50	50	50	—	—	—
46	3-O	Na, K, Mg	15	—	0–5	—	(30–40)*	30–40	—	—	—	—
47	4-O	Complete minerals	20	—	11–15	—	40–50	40–50	—	—	—	—
48	1-A	43 lb. N per acre as amm. sulph.	25	8·0	0–5	—	0	15	0	—	—	—
49	2-A	As 1-A + P	37	7·9	11–15	—	30	30	30	—	—	—
50	3-A	As 1-A + Na, K, Mg	27	7·2	0–5	—	0	20–25	0	—	—	—
51	4-A	As 1-A + complete minerals	41	5·5	6–10	—	0	0	20	—	—	—
52	1-AA	43 lb. N per acre as nitrate	25	7·8	6–10	(*a*) 462 (*b*) 400	0	0	—	0	0	+++
53	2-AA	As 1-AA + P	40	7·4	>15	—	15	15	—	—	—	—
54	3-AA	As 1-AA + Na, K, Mg	26	7·1	0–5	(*a*) 250	0	0	—	—	0	+++
55	4-AA	As 1-AA + complete minerals	39	7·1	>15	—	25	25	—	—	—	—
56	1-C	49 lb. N per acre as rape cake	37	7·2	6–10	—	0	20–25	0	—	—	—
57	2-C	As 1-C + P	39	7·1	11–15	—	20	20	—	—	—	—
58	3-C	As 1-C + Na, K, Mg	35	6·0	0–5	(*a*) 16	0	0	0	—	+	+++
59	4-C	As 1-C + complete minerals	39	7·2	11–15	(*a*) 116	0	0	0	—	+++	+++
60	6-1	Unmanured since 1852	15	—	0–5	—	0	20	—	—	—	—
61	6-2	Coal ashes	16	—	6–10	—	0	20	—	—	—	—
62	7-1	Dung in 1852–71, since then unmanured	24	—	6–10	—	0	25	—	—	—	—
63	7-2	Dung every year	46	6·8	11–15	—	20	20	—	—	—	—

* *Azotobacter* growth very weak on kneaded plate 0, sample 46, but very strong on plate + P of this sample.

attributed to the lower nitrogen dressings given to most plots on Hoos Field. Tests with inoculated kneaded plates were made from Plots 1-AA, 3-AA, 3-C and 4-C, *i.e.* where the uninoculated kneaded plates failed to show growth (samples taken 2nd November (Table VII)). These inoculated plates gave good growth where phosphate was present in the original soil or supplied in the test.

The Barnfield mangold plots.

Samples from some of the Barnfield plots were taken and tested in 1930 and 1931 (Table VIII). The kneaded plates showed no phosphate or lime deficiency, but the *Azotobacter* growth on them was weak. It is possible that the ploughing in of the mangold leaves on these plots may have reduced the *Azotobacter* population, by supplying nitrogenous energy material to organisms competing with the *Azotobacter* in the soil.

Table VIII. *Barnfield.*

(*a*) Soil samples taken 15th July, tested 2nd August, 1930. Dung applied November; Minerals and Rape cake, May; Ammonia and Nitrate, May and July.

Soil sample no.	Plots	Plot treatment	Yields in 1930. Mangolds, tons per acre	*p*H	Water soluble P_2O_5 mg. per kg.	Number of *Azotobacter* colonies from 1 gm. soil on silica plates	Uninoculated kneaded plates. Number of *Azotobacter* colonies on 1 cm.[2]			
							0	+P	+Ca	+P+Ca
64	1-O	Dung only	17·8	—	11–15	—	15	15	—	—
65	4-O	Minerals	4·8	—	>15	—	25	25	—	—
66	4-A	86 lb. N per acre as sulph. amm. +minerals	14·8	—	>15	—	20	20	—	—
67	4-N	86 lb. N per acre as nitrate +minerals	17·8	—	>15	—	20	20	—	—
68	8-O	Control	3·5	—	>15	—	10	10	—	—

(*b*) Samples taken 7th September, 1931. Dung applied November; Minerals and Rape cake, April; Ammonia and Nitrate, April and July.

69	1-O	Dung	17·8	7·6	>15	—	10	15–20	—	—
70	4-O	Minerals	4·8	—	>15	(*a*) 300 (*b*) 214	20	20	—	—
71	4-A	86 lb. N per acre as amm. sulph. +minerals	14·8	7·2	>15	—	5–10	10	10	10
72	4-N	86 lb. N per acre as nitrate +minerals	17·8	—	>15	(*a*) 712 (*b*) 744	15–20	15–20	—	15–20
73	4-C	184 lb. N as rape cake + minerals	21·1	—	>15	—	10	10	—	—
74	8-O	Control	3·5	8·0	11–15	—	10	10	—	—

The Agdell rotation plots.

Agdell field affords an opportunity of studying the effect of long continued manurial treatment under rotation cropping. Plots 1 and 2 (Table IX), which receive sulphate of ammonia, are very acid and show no *Azotobacter* either by the kneaded-plate method or in counts made on silica plates. When these soils were inoculated with *Azotobacter*, however, the kneaded-plate test was successful in indicating lack of both phosphate and lime. Plots 3 and 4 receiving minerals with the swedes, and the control Plots 5 and 6, all show a phosphate deficiency by the kneaded-plate test. The silica plate gave more *Azotobacter* colonies from Plots 3

Table IX. *Agdell Field.*

Soil samples taken 18th November, 1931. Manures applied 1928.

Soil sample no.	Plots	Plot treatment and rotation		*p*H	Water soluble P_2O_5 mg. per kg.	Number of *Azotobacter* colonies from 1 gm. soil on silica plates	Kneaded plates. Number of *Azotobacter* colonies on 1 cm.²						
							Uninoculated			Inoculated with *Azotobacter*			
							0	+P	+Ca (P−Ca)	0	+P	+Ca	+P+Ca
75	1	Complete minerals + 43 lb. N per acre as amm. sulph. +98 lb.	fallow side	5·2–5·6	0·5	0	0	0	0	0	0	+	+++
76	2	N. per acre as rape cake	clover side	5·2–5·6	0–5	0	0	0	0 (P−Ca)	0	0	0−+	+++
77	3	Complete minerals (only to swedes)	fallow side	7·0–8·0	6–10	(*a*) 509 (*b*) —	0	35	0	—	—	—	—
78	4		clover side	7·0–8·0	6–10	(*a*) 390 (*b*) 439	0	15–20	0	—	—	—	—
79	5	Control	fallow side	8·0	6–10	(*a*) 340 (*b*) 300	0	20	0	—	—	—	—
80	6		clover side	7·8–8·0	0–5	(*a*) 83 (*b*) 66	0	*ca.* 5	0	—	—	—	—

and 5 which have a fallow included in their rotation, than from Plots 4 and 6 in which this fallow is replaced by clover.

As a contrast to the heavy Rothamsted soil, about ten samples of the light sandy soil from the Woburn plots were examined. *Azotobacter* was not found to be present in these soils, probably on account of their acidity. The kneaded-plate test was also applied to a sample of acid loamy soil from Cheshire, which was known to lack phosphate. This sample was remarkable in that no *Azotobacter* growth appeared on the kneaded plates even when the soil was previously inoculated with *Azotobacter* and when ample phosphate, $CaCO_3$ and mineral salts were supplied.

CONCLUSION.

The results of the phosphate test from seventy-nine soil samples from Rothamsted plots are summarised in Table X. The kneaded-plate test affords an indication of presence or absence of phosphate in those Rothamsted plots receiving little or no mineral nitrogen, but where soils have received 86 lb. or more of mineral nitrogen per acre, the size or viability of the *Azotobacter* population has usually been so depleted that they do not show up on the kneaded plates even when sufficient phosphate and lime are present. In sixteen of these samples the test was modified by adding *Azotobacter* from culture to the kneaded plates and, when this was done, the test gave a correct indication of phosphate supply or deficiency.

Table X. *Summary of results with the kneaded-plate test for phosphate deficiency.*

Water-soluble P_2O_5 mg. per kg.	P_2O_5 deficiency shown	No deficiency shown	Total tests	P_2O_5 deficiency shown	No deficiency shown	Total tests	P_2O_5 deficiency shown	No deficiency shown	Total tests	P_2O_5 deficiency shown	No deficiency shown	Total tests	Total samples tested
	0–5			6–10			11–15			>15			
Kneaded plates not inoculated	15	1	16	12	1	13	2	17	19	0	31	31	79
Inoculated with *Azotobacter*	6	0	6	2	0	2	0	2	2	0	6	6	16

Summary and abstract.

1. The kneaded plate (*plaque moulée*) method of detecting deficiency in lime and available phosphate was applied to seventy-nine soil samples taken from the classical Rothamsted arable plots, and the *Azotobacter* population from some of these samples was estimated by counts on silica jelly.

2. The silica jelly counts showed that *Azotobacter* cells were very much reduced in number, or even absent in soil receiving 86 lb. per acre or more of mineral nitrogen. It is suggested that this is due to competition with other organisms whose growth is stimulated by added nitrogen compounds.

3. The kneaded-plate test correctly indicated whether phosphate had been applied in soils receiving little or no nitrogen manures.

4. In those soils receiving 86 lb. or more of mineral nitrogen, the kneaded-plate test usually showed little or no *Azotobacter* growth even in the presence of phosphate and calcium carbonate. This failure was probably due to the paucity of *Azotobacter* cells originally present in such soil samples. In some cases the test was modified by inoculating the sample with a culture of *Azotobacter* and it then gave correct indications as to phosphate content.

5. In general, *Azotobacter* when present was found to develop on kneaded plates, if the soil contained at least 10 mg. of water soluble P_2O_5 per kilogram of soil, but below this limit little growth occurred.

6. The requirements of *Azotobacter* and of crop plants are similar as regards the need for soluble phosphate, but mineral nitrogen, although necessary to most crops, is detrimental to *Azotobacter* growth in field soil while soil acidity is more harmful to *Azotobacter* than to most crops. Consequently there was no correlation between crop yield and *Azotobacter* activity.

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APPENDIX.

APPLICATION OF WINOGRADSKY'S METHOD TO THE NITRIFICATION TEST.

The silica-plate method for estimating relative numbers of ammonia-oxidising organisms recently developed by Winogradsky (11) was applied to some of the soil samples collected in September 1931.

Method.

Petri dishes 10 cm. diameter, containing silica jelly, were prepared as described above, the gel impregnated with mineral salts and ammonium sulphate, and its surface covered with finely powdered $CaCO_3$. 10–20 mg. of finely sifted soil was distributed over the surface of each plate which was incubated at 27° C. After about 2 weeks,

Table XI. *Nitrification in Rothamsted Fields, September*, 1931.

Soil sample no.	Plot no.	Treatment	Number of ammonia oxidisers: colonies in 1 gm. soil	Yields in 1930. Grain, bushels per acre
Broadbalk:				
7	3	0	(*a*) 157 (*b*) 347	12
8	5	Minerals	(*a*) 500 (*b*) 312	14
9	8	Minerals + amm. sulph.	(*a*) 1922 (*b*) 1976	35
6	2	Dung every year	(*a*) 2243 (*b*) —	34
11	19	Rape cake + minerals	(*a*) 1428 (*b*) 1243	22
Hoos Field:				
37	1-O	0	(*a*) 245 (*b*) 457	14
38	4-O	Minerals	(*a*) 217 (*b*) 490	20
39	4-A	Minerals + amm. sulph.	(*a*) 353 (*b*) 150	41
40	4-AA	Minerals + nitrate	(*a*) 1333 (*b*) 640	39
41	4-C	Minerals + rape cake	(*a*) 3687 (*b*) —	39
42	7-1	Dung in 1852–71	(*a*) 664 (*b*) 168	24
43	7-2	Dung every year	(*a*) 3198 (*b*) —	46

colonies of ammonia oxidisers became apparent owing to their formation of clear haloes due to solution of the calcium carbonate.

In this work counts were made on duplicate plates which sometimes did not agree very well. Wherever examined, the ammonia-oxidising colonies were found to consist of cells resembling *Nitrosomonas*.

Results (Table XI).

Among the Broadbalk samples tested the highest counts of nitrifying organisms were obtained from Plot 2 receiving dung and from Plot 8 receiving sulphate of ammonia, though the figures from the rape cake Plot 19 were also high. On Hoos Field, dung and rape cake plots gave the highest counts, but here the low figure from the sulphate of ammonia plot 4-A, was probably due to soil acidity. On the nitrate plot two parallel plates disagreed but both gave higher figures than were obtained from Plot 4-A.

The plots with no nitrogen dressings both from Broadbalk and Hoos Field gave low counts of nitrifiers. In a neutral soil the population of nitrifying organisms thus seems to be dependent upon the nitrogen supply in the soil rather than upon its content of minerals. Thus, one would expect a better correlation with crop yield than was obtained with the *Azotobacter* tests. Table XI shows that there is indeed some relation between yield of crop and numbers of nitrifying bacteria, particularly in the case of Broadbalk.

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