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A NEW FACTOR IN THE STUDY OF NITROGEN FIXATION IN SOIL

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The nitrogen fixation was found to be greater in the farmyard manured plots than the corresponding unmanured ones in both the fields, one with a heavy alkaline clay soil and the other with a light sandy acid soil. The amount of fixation showed considerable variation with the quantity of the inoculum; with a smaller quantity of the inoculum, the fixations were identical in two plots of the same field while with a larger quantity, fixations increased with the farmyard manured plots and decreased with the unmanured ones. The fixation by a small amount of the inoculum appeared to indicate the nitrogen fixing capacity of the strain while a larger amount reflected the nitrogen fixing index of a soil.

The nitrogen fixation was influenced by other micro-organisms besides the nitrogen fixing flora, whereas the physico-chemical character of soil appeared to have no effect The nitrogen fixation in soil showed variation with the change of season, the activity being low during winter months.

Introduction

The discovery of the phenomena of nitrogen fixation, ammonification, nitrification, carbon dioxide evolution and other microbiocal activities in soil gave an impetus to determine their corelation, if any, with the property of a soil or its productivity. The study of nitrogen fixation in soil as an index of soil fertility received considerable attention in this respect but the results so far had been inconclusive. Brown (1915) found a definite corelation between the nitrogen fixing property of a soil and its yield; Given and others (1916-17) obtained a similar result with one set of experimental plot but not in another; Waksman (1924) likewise could not find any definite co-ordination.

In the present paper, the nitrogen fixation in different types of soil under different manural treatment, have been examined to study the effect of the quantity of inoculum and certain other factors, and the results appear to throw a new light on certain aspect of nitrogen fixation in soil.

Materials and Methods

The soils used were obtained mainly from two different localities, *viz.*, Barnifield at Rothamsted and Stackyard at Woburn. The former is a heavy alkaline clay soil with a *p*H varying from 7.1–7.3, while the latter is a light acid soil with a *p*H range between 5.8–6.0. From each of the fields two plots, a farmyard manured and an unmanured, were selected for the investigation.

The physical character of the different plots and their annual yields are respectively given in Tables 1 and 2.

Table 1 *Physical character of soil (in mg.)*

	Barnfield		Stackyard	
	FYM	X	FYM	X
Coarse sand	13.8	8.3	50.0	58.6
Fine sand	36.4	39.3	24.3	18.8
Silt	21.7	23.7	8.5	12.0
Clay	22.3	27.5	13.3	9.5
Carbonates	0.5	0.3	0	0
Air dry moisture	3.8	3.6	1.3	1.1
Lost by solution	0.9	0.5	0.9	0.1
Difference	0.6	+3.2	1.7	−0.4
Total	100	100	100	100
Loss on ignition	7.8	5.1	3.9	3.7

Farmyard manured = FYM Unmanured = X

Table 2 *Annual yields of mangold root (Tons per acre) and barley grain (cwts. per acre).*

		Barnfield (Mangold root)		Stackyard (Barley grain)	
		FYM	X	FYM	X
Year	1929	18.14	10.85	16.87	9.19
	1930	15.39	3.37	9.50	5.13
	1931	29.10	15.80	8.98	4.02

Farmyard manured = FYM Non-manured = X

Before taking samples, the surface soil of about an inch was first scraped off and six borings to a depth of about 4 inches were taken at random from the different parts of a plot. Samples were then thoroughly mixed and passed through a 3 mm. seive.

Two methods are generally used for estimating the nitrogen fixing capacity of a soil, the soil method and the solution method. The soil method is an approach to a more natural condition, but the results are often unsatisfactory as the amount of nitrogen fixation in this method, is mainly determined by the soil reaction; soil with a *p*H value below 6.0 fixes very little or no nitrogen (Waksman, Karunakar, 1924). The following solution method was, therefore, adopted in these experiments.

50 c.c. amounts of the medium containing Mannitol 10.0 gm., K_2HPO_4 0.5 gm., $MgSO_4$ 0.2 gm., NaCl 0.2 gm., $FeCl_3$ and $MnSO_4$—a trace and distilled water 1 l., and *p*H adjusted to 7.0 were taken in 250 c.cm. Erlenmeyer flasks, sterilized and inoculated with different quantities of soil. About 0.05 gm. calcium carbonate were added to each flask, and incubated for a period of 30 days. The total nitrogen was determined by the Kjeldahl process.

Experimental

(*i*) *Nitrogen fixation in manured and unmanured plots*

A series of experiments were conducted to determine variation in nitrogen fixation between farmyard manured and unmanured plots of the two fields, Barnfield and Woburn, using 10 gm. of soil as inoculum and the results are given in Tables 3 and 4.

Table 3. *Nitrogen fixation (in mg.) in 50 c.cm. mannitol solution inoculated with 10 gm. of soil.*

(Barnfield)

Experiment	Farmyard manured				Unmanured			
	Initial nitrogen	Fixation in days			Initial nitrogen	Fixation in days		
		10	20	30		10	20	30
1.	20.31	5.22	5.92	6.49	8.74	3.23	3.10	3.54
2.	20.74	6.34	7.05	7.76	9.73	1.83	2.11	2.82
3.	16.93	6.21	7.02	7.48	8.32	2.62	2.96	4.01
Average	19.52	5.92	6.63	7.24	8.93	2.56	2.72	3.45

Table 4. *Nitrogen fixation (in mg.) in 50 c.cm. mannitol solution inoculated with 10 gm. of soil*

(Woburn)

Experiment	Farmyard manured				Unmanured			
	Initial nitrogen	Fixation in days			Initial nitrogen	Fixation in days		
		10	20	30		10	20	30
1.	12.41	2.39	3.38	3.90	8.45	1.12	2.28	2.68
2.	11.99	1.12	3.50	4.00	8.04	2.39	2.70	3.02
3.	11.85	2.39	3.52	4.23	8.18	2.39	2.25	2.41
4.	11.85	2.95	3.87	3.72	7.75	1.83	2.65	2.90
Average	12.02	2.21	3.56	3.96	8.10	1.93	2.47	2.75

It may be observed that in both the cases the nitrogen fixation is comparatively more in manured than the corresponding unmanured plots and the difference is greater at Barnfield than at Woburn. The nitrogen fixation in the manured plot at Woburn again is slightly more than the unmanured plot at Barnfield although the nitrogen fixing flora in the latter is *Azotobacter* which is known to be superior in respect of nitrogen

fixation (Lipman and Burgess, 1915) than *Clostridium* present in Woburn soil.

(*ii*) *The influence of the quantity of inoculum on Nitrogen fixation*

The effect of variation in the quantity of inoculum on nitrogen fixation was examined by using 1–10 gm. of soil from both the plots of the two fields and the results are given in Table 5.

Table 5. *Nitrogen fixation (in mg.) in presence of different quantities of inoculum*

Quantity of inoculum		Farmyard manured			Unmanured			
		10 gm.	2 gm.	1 gm.	10 gm.	5 gm.	2 gm.	1 gm.
Woburn	Initial Nitrogen	12.22	2.37	1.26	8.11	3.95	1.55	0.79
	Fixation	3.91	3.27	3.01	2.82	2.96	3.00	3.01
Barnfield	Initial Nitrogen	19.32	—	1.97	8.93	—	—	0.84
	Fixation	7.24	—	5.55	3.45	—	—	5.40

It may be observed that when 1 gm. of soil from differently treated plots of the same field is used as inoculum, the amount of fixation is identical, although the plots may differ considerably in the degree of their fertility (Table 2). With a gradual increase in the inoculum, the nitrogen fixation rises in the farmyard manured plots, but shows a regular decrease in the unmanured ones. This dissimilar behaviour between the manured and unmanured plots of the two fields in presence of larger quantity of inoculum may be due to the physico-chemical character of a soil, or its microbial flora. The following experiment was accordingly carried out to examine the effect of eliminating the biological factor.

Varying quantities of soil, from the differently treated plots of a number of fields, were sterilized, each in a 250 c.cm. flask, and 50 c.cm. of sterile media was added to each of the flasks. They were then inoculated, each with 1 c.cm. soil suspension containing 0.01 gm. of soil from Barnfield farmyard manured plot. The results of nitrogen fixation are given in Table 6.

In all cases, the amount of fixation is fairly uniform, the quantity of sterile soil, or the difference in its physical or chemical nature, does not appear to have any effect. Further, the fixation in the control flasks where no sterile soil was present, was the same as in the experimental ones. The biological nature of the soil thus appears to play the deciding role. It has been established that nitrogen fixation by *Azotobacter* is considerably increased in the presence, not only of other bacteria (Mahmoud 1932), but also of different soil micro-organisms, including Algae (Jone 1930), Mycorrhiza-fungi (Waksman 1932) and Protozoa (Nasir 1923). Further experiments appear to confirm the above observations.

Table 6. *Effect of soil on nitrogen fixation (in mg.)*

	Broadbalk		Hoosfield		Barnfield		Barnfield				Woburn	Without Soil
	FYM	X	FYM	X	FYM	X	FYM	FYM	X	X	FYM	—
Quantity of inoculum	1 gm.	1 gm.	1 gm.	1 gm.	10 gm.	10 gm.	10 gm.	1 gm.	10 gm.	1 gm.	10 gm.	0.01
Initial Nitrogen	2.11	1.05	2.25	1.19	18.23	7.05	17.93	1.76	11.28	1.12	21.28	0.017
Fixation (A)	5.78	5.57	6.21	5.99	7.48	5.23	7.63	5.33	4.95	5.25	4.1	5.38
Fixation (B)	5.5	5.5	5.51	5.85	5.64	5.52	5.41	5.32	5.51	5.3	5.39	

Fixation (A) = Nitrogen fixation in presence of sterilized soils.

Fixation (B) = Nitrogen fixation after inoculation with Branfield soil.

FYM = Farmyard manured X = Unmanured

(iii) Effect of season on nitrogen fixation

Microbial population as well as their activity is known to vary considerably during different seasons (Cutler *et al.*, 1922, Fritsch 1913, Herdman 1922) and similar behaviour is expected in respect of nitrogen fixation in soil. The preceding experiments were carried out during the summer, and in order to investigate the effect of change of season on nitrogen fixation, experiments were repeated from December to March, and the results are given in Table 7.

Table 7. *Effect of Winter Season on Nitrogen fixation (in mg.)*

Inoculum		Broadbalk		Hoosfield		Barnfield	
		FYM	X	FYM	X	FYM	X
10 gm.		5.37	3.1	3.41	3.1	3.7	4.2
„ „		3.8	4.2	4.5	3.57	4.6	3.4
	Aver.	4.58	3.6	3.95	3.32	4.1	3.8
1 gm.		5.75	5.08	4.85	4.86	5.15	5.32
„ „		5.78	5.5	5.21	5.23	5.22	5.21
	Aver.	5.76	5.29	5.03	5.04	5.18	5.26

FYM = Farmyard manured X = Unmanured

It may be observed that the amount of fixation with 1 gm. of inoculum does not show any variation with the change of season, and there is no decrease or any significant difference between the differently treated plots of the same field. With 10 gm. of inoculum, however, the nitrogen fixations decreased decidedly even in the manured plots. Repeated experiments with soils from Barnfield, Broadbalk and Hoosfield confirmed this observation.

Further, in all flasks containing 1 gm. of soil there was a heavy growth of *Azotobacter* and the characteristic grey film developed on the culture solution. But in flasks inoculated with 10 gm. of soil the film as well as the culture solution were orange red, a colour usually associated with the nitrogen fixing group other than *Azotobacter*. The microscopic examination of the film and the solution showed the presence of *Steptrothrix* and numerous other slender bacteria while the number of *Azotobacter* were very few and they were observed forming a thin film on the wall of the flask just above the surface of the culture solution.

Discussion

Except for the use of nitrogen-free nutrient solution no standard procedure was laid down for the determination of nitrogen fixation in soil. The amount of nutrient solution, the quantity of soil used and even the period of incubation varied considerably with the different workers. In view of the diversity of experimental conditions it is not feasible to draw a satisfactory conclusion from a comparative study of these data.

In the present investigation it may be observed that variation nitrogen fixation between two plots of unequal productivity is reflected when 10 g. of soil is used as inoculum. They also indicate variation in nitrogen fixation with the change of season, while the fixation with a small quantity of inoculum is identical irrespective of soil productivity or change in season.

The differential behaviour with variation in the quantity of inoculum may be explained as follows. In a soil the different groups of micro-organisms are in a state of unstable equilibrium with a number of associative and antagonistive influences at work (Waksman 1932). When a small quantity of soil is used as inoculum the initial concentration of the different groups of micro-organisms is low, and as there is no source of nitrogen other than that from the inoculum itself, the growth and activity of the heterotropic non-nitrogen fixing bacteria is considerably restricted. The nitrogen fixing flora, on the other hand, multiply raipidly and attains a large population. It is well known that in a mixed bacterial culture, the high initial concentration of a species checks considerably the growth and activity of the rest. The nitrogen fixing flora thus acts more or less as a pure culture and at any time under similar condition would show very little variation in their nitrogen fixing capacity. With a definite quantity of favourable food, the same strain will fix an identical amount of nitrogen.

This explains the uniformity of nitrogen fixation with 1 gm. of inoculum, in two differently treated plots of the same field. Further, when 0.01 gm. of inoculum from Barnfield farmyard manured plot was used in the presence of different quantities of sterile soil, of diverse physical and chemical character, the amount of fixation was not only uniform, but was also equivalent to the amount fixed by 1 gm. Barnfield soil. The fixation by a small inoculum, therefore, indicates the nitrogen fixing capacity of the strain in soil and not of the soil itself.

The condition is different when a large inoculum is used. The initial concentration of other bacterial species is high, further as there is considerable amount of nitrogen in the inoculum itself, along with the growth of *Azotobacter*, there is also the development and activity of other micro-organisms. The flora in the solution is no longer more or less exclusively of the nitrogen fixing type, but a composite group, varying both in composition and activity during the different periods in the season. The nitrogen fixation, therefore, is not constant as with a small inoculum, it may vary in either direction according to whether at any period the mixed flora is favourable or unfavourable to the process of nitrogen fixation. The result of nitrogen fixation during winter is also in agreement with and confirms the above conclusion.

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