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THE EFFECT OF VARIOUS TREATMENTS ON MICRO-ORGANISMS IN THE SOIL UNDER A FIVE-YEAR ROTATION

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It is generally conceded now that microorganisms play a large part in the transformations through which various plant food constituents pass, in the soil. They are the agents which are chiefly responsible for the production of available plant food. They break down organic matter, liberating the plant food constituents contained therein. The products of this decomposition react with the complex mineral soil constituents and change them into soluble, available compounds. Certain microorganisms also have the ability of utilizing the free nitrogen of the atmosphere, fixing it in the soil, to serve later for the feeding of plants.

Among the various functions of bacteria in the soil, then, the production of available plant food and the fixation of atmospheric nitrogen may be listed as the most important.

Because of these activities of soil organisms and because of other functions which special groups exert under special conditions, there is quite evidently a rather definite relationship existing between bacterial activities in soil and their crop-producing power. Many attempts have been made to show the existence of such a relationship and in a number of instances, the results have been quite conclusive. It has come to be believed, therefore, that any soil treatments which would have a beneficial effect on crop yields must necessarily have a similar stimulative influence on the development of bacteria and especially on certain important bacterial activities. In fact it seems probable that the effects of various treatments of soils on bacterial action may serve to indicate the crop effects which may be expected.

With these things in mind, many investigators have studied the influence of a wide variety of treatments on the soil flora and on the activities of the better-known groups of soil organisms. The literature along this line is rather extensive and no attempt will be made to review it here. Very excellent reviews of the work with manure, lime and phosphate fertilizers have been given in a number of publications by Waksman [1,2], Brown [3,4], Greaves and Carter [5] and others.

The experiments reported in this paper were planned to throw further light on the problem of the influence of certain field treatments of soils on biological activities.

The work was carried out on the soils from a series of plots at the Agronomy Farm of the Iowa Agricultural Experiment Station. The soil is typical of the Wisconsin drift soil area and a mapping on a scale of one inch per one hundred feet shows the plots on the livestock end of the series (plots 924-928) to be mostly on Carrington loam or a fine sandy loam variation while the plots at the other end of the series are on Webster silty clay loam or loam. Thus absolute comparisons cannot be made throughout the series but for each group of plots the soils may be compared quite accurately.

The plots are one-tenth of an acre in size and have been under treatment for 13 years. A five-year rotation of corn, oats, clover, wheat and alfalfa is followed, the alfalfa remaining on the land for five years. The series was in corn when these tests were begun in the fall of 1926.

The treatments employed were as follows:

PLOT NO.	TREATMENT
924	Check (No treatment)
926	Manure + Limestone
927	Manure + Limestone + Rock Phosphate
928	Manure + Limestone + Superphosphate
929	Check
931	Crop Residues + Limestone
932	Crop Residues + Limestone + Rock Phosphate
933	Crop Residues + Limestone + Superphosphate

Manure is applied to these soils at the rate of 10 tons per acre per five-year rotation. Rock phosphate is added at the rate of 2500 pounds per acre each five-year period and superphosphate is employed at the rate of 200 pounds per acre annually. Limestone is supplied in sufficient amounts to neutralize the acidity of the soil as determined by the Truog qualitative test. The crop residues treatment consists in the return to the land of the stover and straw and the plowing under of the second crop of clover.

1 Waksman, S. A. 1922. Microbiological Analysis of Soil as an Index of Soil Fertility. III. Influence of Fertilization Upon the Numbers of Miroorganisms in the Soil. Soil Science 14: 321.

2 Waksman, S. A. 1923. Microbiological Analysis of Soil as an Index of Soil Fertility VI. Nitrification. Soil Science 16: 55.

3 Brown, P. E. 1911. Some Bacteriological Effects of Liming. Iowa Agr. Exp. Sta. Rsch. Bul. 2.

4 Brown, P. E. 1913. Bacteriological Studies on Field Soils. III. The Influence of Barnyard Manure. Iowa Agr. Exp. Sta. Rsch. Bul. 13.

5 Greaves, J. E. and Carter, E. G. 1916. Influence of Barnyard Manure and Water Upon the Bacterial Activities of the Soil. Jour. Agr. Rsch. 6: 889.

Six samplings were made from these plots, four in the early fall of 1926 and two in the spring of 1927. The samples were secured by removing the top 2 inches of soil from an area about 6 inches square and the soil thoroughly mixed to a depth of about 7 inches. A sample was then drawn and mixed with 19 other similar samples taken elsewhere in the plot, to form a composite sample. The studies were all made on these composite samples from each plot.

At each sampling the numbers of bacteria were determined, the nitrifying power of the soil was measured and the non-symbiotic nitrogen-fixing power of the soil was determined.

The number of bacteria were determined by the usual dilution method, plating on Brown's albumen agar [6] and incubating 10 days at room temperature.

The results shown in table I, indicate that the soil treatments

Table I — Number of Bacteria Per Gram of Dry Soil

Plots	Date Sampled					Average
	Oct. 6	Oct. 12	Nov. 9	Mar. 19	Apr. 2	
924	4,600,000	2,500,000	4,000,000	7,000,000	6,000,000	4,820,000
926	6,300,000	4,250,000	4,800,000	6,900,000	7,500,000	5,950,000
927	6,000,000	4,600,000	7,700,000	9,200,000	10,800,000	7,660,000
928	9,400,000	6,600,000	7,400,000	7,600,000	9,600,000	8,120,000
929	4,600,000	4,130,000	3,600,000	5,200,000	6,700,000	4,840,000
931	7,100,000	5,000,000	7,300,000	5,200,000	8,700,000	6,660,000
932	11,500,000	4,400,000	6,400,000	4,000,000	5,800,000	6,420,000
933	7,600,000	7,300,000	6,300,000	5,300,000	6,875,000	6,680,000

have a pronounced effect on the numbers of organisms present. Manure and lime increased the numbers and the rock phosphate and superphosphate gave further increases. The crop residues and lime had a large effect and the superphosphate with the residues and lime gave a further increase. The rock phosphate had little effect, on the average, but showed greater influence than the superphosphate at some samplings.

There were some variations at the different dates of sampling but the effects of the treatments were always shown, the variations occurring in the relative effects of the different treatments.

The numbers of organisms present varied at the different samplings, the largest number being present in March and April in the livestock or manure-treated series (plots 924-928) while in the grain or crop residue series the greatest numbers occurred at the early fall sampling.

In general the treatments exerted large effects on these soils in

6 Brown, P. E. 1913. Methods for the Bacteriological Analysis of Soil. Iowa Agr. Exp. Station, Rsch. Bul. 11.

spite of some soil differences. The manure treated plots showed higher numbers on the average and greater effects from treatment although the soil is a Carrington or perhaps because it is a Carrington, a soil less fertile than the Webster, which occurs on the grain system end of the series. Often, however, the Webster soils will respond to quite as large an extent as the Carrington to applications of a phosphate fertilizer. Rock phosphate usually does not give as large effects with residues as with manure and superphosphate often brings about greater effects than rock phosphate when crop yields are measured. Apparently these bacteriological tests are quite in accord with general field observations on the effects of these soil treatments on crop growth.

The nitrification tests were carried out by the method of Waksman [7] using 100 gram quantities of soil in duplicate, without addition, with 30 mgs. of ammonium sulfate added, and with 30 mgs. of ammonium sulfate and 210 mgs. of calcium carbonate supplied. The moisture content was brought up to the optimum and the cultures incubated for 4 weeks at room temperature, water being added every week to keep up the content. The nitrates present were determined by the phenoldisulfonic acid method. The results are given in table II.

When no additions were made to the soil in the test, the differ-

Table II — Nitrification (Nitrates Present in p.p.m.)

Plots	Dates of Sampling					Average
	Oct. 6	Oct. 26	Nov. 9	Nov. 19	Apr. 2	
	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	
Nitrification of the Soils Own Nitrogen						
924	---	7	12	35	16	18
926	---	26	15	34	21	24
927	---	27	22	42	44	34
928	---	17	18	30	44	27
929	---	13	16	34	22	21
931	---	22	17	56	38	33
932	---	22	24	56	33	36
933	---	22	24	41	44	33
Nitrification of Ammonium Sulfate						
924	55	42	74	15	83	53
926	80	118	144	26	178	109
927	106	212	236	21	239	163
928	155	184	176	147	263	185
929	100	98	84	17	131	106
931	154	167	163	103	156	149
932	651	355	325	1072	657	612
933	651	284	433	770	657	559

7 Waksman, S. A. 1923. Microbiological Analysis of Soil As an Index of Soil Fertility V. Methods for the Study of Nitrification. Soil Science 15: 241.

NITRIFICATION OF AMMONIUM SULFATE IN THE PRESENCE OF LIME

924	111	74	140	17	263	81
926	153	153	289	122	357	215
927	206	275	306	168	405	272
928	413	275	273	538	478	396
929	230	131	216	89	202	174
931	400	237	325	450	329	348
932	425	284	347	1072	585	543
933	391	317	400	898	478	497

ences in nitrate content were too small to be significant. It does seem, however, that the treatments showed some effect. When ammonium sulfate was added in the test, the effects of the treatments with manure, limestone, crop residues and the phosphates are very well shown, especially in the average figures. There are some variations at the different samplings but in general the averages reflect the differences shown at the various dates. With the addition of ammonium sulfate and calcium carbonate in the tests, the treatment effects are again quite evident.

Manure and limestone evidently stimulate the nitrifying power of the soil to a considerable extent. This result is in accord with previous observations along this line. Rock phosphate and superphosphate with the manure and lime have pronounced effects. The superphosphate seems to be greater in influence than the rock phosphate, in all the tests. Where crop residues and lime constituted the basic treatment, however, the rock phosphate is about as effective as the superphosphate. This is the reverse of the results of the determinations of numbers.

It may be noted that the soils in the crop residue plots are affected to a greater extent by the treatments, especially plots 932 and 933 which is undoubtedly due in part at least to the difference in soil types, these plots being on the Webster loam while the manure series plots are mainly on the Carrington loam.

In general these results are in line with the numbers of organisms present in the soils, showing that the effects of the various treatments are very definite on the nitrifying power of the soils.

The non-symbiotic nitrogen-fixation tests were carried out in Lipman's modified Azotobacter medium, using dextrose. One hundred cc. portions of the medium were sterilized in flasks, inoculated with 5 gms. of soil and incubated for ten days at room temperature. The nitrogen fixed was determined by the regular Kjeldahl method, deducting the amount in the soil from the total content in the culture at the end of the incubation period. The results are given in table III.

The results of these tests are not so definite as in the case of

Table III — Non-Symbiotic Nitrogen Fixation
(Nitrogen fixed per gram of dextrose)

Plots	Date Sampled						Average
	Oct. 6	Oct. 12	Oct. 26	Nov. 9	Mar. 19	Apr. 2	
	mgms.	mgms.	mgms.	mgms.	mgms.	mgms.	
924	2.1	1.9	2.9	1.6	3.4	3.0	2.50
926	3.8	2.5	1.6	2.6	4.1	3.4	3.00
927	4.6	3.7	5.1	3.2	3.8	2.8	3.90
928	4.6	4.6	3.6	4.7	3.8	2.5	4.00
929	3.5	2.5	1.3	2.9	3.7	4.0	3.00
931	2.1	4.2	4.4	4.3	2.7	3.0	3.50
932	3.7	5.4	3.6	4.2	3.9	3.5	4.05
933	4.3	2.7	3.6	3.8	4.2	5.6	4.05

the nitrification studies but the effects of the treatments of the soils are evidenced in the average figures and in the data secured at each sampling. The application of manure, limestone, rock phosphate, superphosphate and crop residue evidently increases the fixation of nitrogen by the non-symbiotic organisms, stimulating their activities to an appreciable extent. With a more exact method of measuring the nitrogen-fixing power of soils, the results would undoubtedly be more definite.

CONCLUSIONS

These studies on a typical Wisconsin drift soil, under a five-year rotation system indicate very definitely that the application of manure and limestone stimulates bacterial development to a very pronounced extent and increases in the nitrifying power and nitrogen-fixing power of the soil are also noted.

The addition of rock phosphate and superphosphate with the manure and limestone gives further increases in numbers of microorganisms and in nitrification and nitrogen fixation. The superphosphate generally seems to have a greater stimulative effect than the rock phosphate which may be due to the solubility of the phosphate or the presence of calcium sulfate in the superphosphate or to both.

With the crop residues and limestone treatment increases were noted in numbers and in the nitrifying and nitrogen-fixing activities of microorganisms. The phosphate applied with the residues and limestone caused increases which in some cases were greater than with the manure and lime. However, the soil on the crop residue plots was a Webster loam to silty clay loam while that on the manured plots was a Carrington loam to fine sandy loam. The differences were probably due to the variations in the soils on the two series of plots. In general the treatments affected the bacterial numbers, nitrification and nitrogen-fixation without regard to the variation in soil type. This is due to the fact that the soils were very similar in the manure series of plots and in the crop residue series, and varied only between these series.

It seems evident from this work that such normal soil treatments as manuring, liming, the use of rock phosphate, superphosphate and the addition of crop residues will stimulate bacterial development and such desirable activities as nitrification and non-symbiotic nitrogen-fixation. The beneficial effects of such treatments on crop yields, as so often and in fact, usually noted may be due, therefore, in part at least to effects on the soil microorganisms and their activities. In some cases the most important effects of certain soil treatments are undoubtedly bacterial.

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