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THE NITRATE ASSIMILATING POWER OF THE SOIL AND SOME NITRATE ASSIMILATING SOIL BACTERIA

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INTRODUCTION

The ultilization of such crop residues as wheat and oat straw presents a perplexing problem, especially to the grain farmer who would maintain the organic matter content of his soil. It has been generally observed that such materials added to the soil decrease the yields of the following crops.

Thomas and Harper (2) found that straw might be applied to the soil without danger of reducing the yield of the following crop provided sufficient time had elapsed between the time the straw was plowed under and the planting of the succeeding crop. It is rather inconvenient and not always possible to make an application of straw or other crop residues to the soil several months before planting. Composting these materials with suitable reagents and thereby converting them into an artificial manure offers some possibilities and much fundamental research has been conducted along this line.

A number of investigators have noticed the decreased yields of crops following applications of straw to the soil but only one of these investigations will be referred to here. Murray (1) made applications of straw in varying amounts to the soil and observed that ammonia production in the soil was inhibited above 0.9 per cent of straw, nitrate accumulation was arrested, nitrates present in the soil were utilized and nitrogen fixation was stimulated. He also isolated and identified a number of bacteria from fresh soil and straw and from the soil to which straw had been added. However, he states that 53 weeks had elapsed from the time of application of the straw until samples of the soil were taken from which the organisms were isolated.

It has been observed in soils which received an application of two tons per acre of dry oat straw that nitrate assimilation has

1 Suggestions given by Dr. P. E. Brown and the assistance of Mr. Ray Pendleton and Mr. D. F. Breazeale with some of the analytical work are greatly appreciated.

decreased and nitrate accumulation in the soil has increased after about six weeks. This would seem to indicate that the active nitrate assimilating organisms might best be isolated during this time. It is the purpose of this paper to give the results secured in a study of the rate of nitrate assimilation, or the nitrate assimilating power of the soil and to report briefly on the nitrate assimilating bacteria isolated from the soils studied.

EXPERIMENTAL WORK

Preliminary work was conducted with solution cultures and soil cultures to determine whether the nitrate nitrogen was being assimilated or reduced. Two soils were used from the four year rotation of the fertility plots from the Agronomy Farm of the Iowa Agricultural Experiment Station. Soil 1 was a check plot and soil 2 was a crop residue plot. Ten grams of each soil were used to inoculate 150 cc. of a nitrate solution in 500 cc. erlenmeyer flasks. The cultures were incubated at 25°C for eight days. Duplicate flasks were taken at intervals of two, four, six and eight days for qualitative tests on nitrate content. These qualitative tests indicated a gradual decrease in the nitrate content of the cultural solutions from two to six days and after eight days all traces of nitrates had disappeared. Total nitrogen determinations made on the cultures after eight days showed an enormous increase in nitrogen over the nitrogen in the beginning. The results of this test are given in table I.

The data in the table show that 2.008 mgms. of nitrate nitrogen had disappeared in eight days and the total nitrogen had increased in both soils. This would seem to indicate that nitrate nitrogen was being assimilated rather than reduced. However, this is not necessarily true where the amount of nitrates disappearing is relatively small as compared to the amount of nitrogen fixed.

Table I — Nitrogen Transformation: Nitrate Assimilation and Nitrogen Fixation — 10 Grams Soil

Soil No.	Mgms. N at Beginning		Mgms. N After Eight Days		Increase in N Over the N in the Beginning	
	Total N	NO_3 N	Total N	NO_3 N	Total N	NO_3 N
1	13.86	2.008	37.05	T	23.19	-2.008
2	17.36	2.008	38.20	T	20.84	-2.008

Tumblers were used for the soil cultures. The equivalents of 100 grams of dry soil were weighed into tumblers and 3.00 mgms.

of nitrogen as KNO^3 added in solution. The moisture content was adjusted to the optimum and the cultures incubated at room temperature for eight days. Total nitrogen determinations were made by the Kjeldahl method, modified to include nitrates, and the nitrate nitrogen was determined colorimetrically by the phenol-disulfonic acid method. The soils used in this test had been treated three weeks previously in four gallon pots in the greenhouse and the moisture content maintained at the optimum. Soil 2 received an application of fresh farm manure equivalent to 10 tons per acre and soil 1 received an application of dry oat straw equivalent in nitrogen to the nitrogen contained in the fresh farm manure. Soil 3 was untreated.

The results obtained with soil cultures are given in table II and are very similar to the figures obtained with the solution cultures. The amount of nitrogen fixed, however, was not as large as was the case in the solution cultures. The soil treated with dry oat straw had assimilated all of the nitrate nitrogen and had not increased in total nitrogen in eight days. The manured soil produced a small amount of nitrates whereas the untreated soil assimilated a similar small amount of nitrates. Both the manured soil and the untreated soil made a considerable increase in total nitrogen over the amount of nitrogen in the beginning.

Table II — Nitrogen Transformation: Assimilation of Nitrate-Nitrogen and Nitrogen-Fixation

Soil No.	Mgms. N in 100 Gms. Soil at Beginning		Mgms. N in 100 Gms. Soil After 8 Days		Mgms. NO_3 N Assimilated in 8 Days	Increase in N Over N in the Beginning. Total N
	Total N	NO_3 N	Total N	NO_3 N		
1	209.2	3.00	209.1	T	3.00	- 0.1
2	206.1	4.41	217.3	5.15	-0.74	11.2
3	187.0	6.07	202.4	5.19	0.88	15.4

The effect of various materials on the nitrate assimilating power of the soil was studied in soil cultures. Soils 1, 2, 3, 4, 5 and 6 were treated with various artificial manures. Soil 7 was treated with dry oat straw and soil 8 had an application of 10 tons per acre of fresh farm manure. Soil 9 was the untreated check. The rate of nitrate assimilation as affected by the various materials was determined by incubating one set of cultures for 24 hours, another set for 48 hours and a third set for 8 days. The results of these determinations are given in table 3 and the amount of nitrate nitrogen assimilated in the various periods is shown in table 4.

It is seen from these tables that dry oat straw and fresh farm

manure affected the nitrate producing power of the soil markedly one week after application, whereas the various artificial manures had very little effect. The soil treated with dry oat straw showed 0.75 mgms. of nitrate nitrogen assimilated in 24 hours and 3.0 mgms. of nitrate nitrogen (the total nitrate nitrogen content of the culture) after 48 hours. The results indicate that a larger difference in the nitrate assimilating power of the soil would be shown if the materials were added to the soil and an excess of nitrate nitrogen were applied immediately before the cultures were incubated.

Table III — Nitrate Assimilation

Soil No.	Mgms. Nitrate-Nitrogen in 100 Grams Soil						
	At Beginning	After 24 Hours		48 Hours		8 Days	
		Control	Soil + 3.0 Mgms. NO_3N	Control	Soil + 3.0 Mgms. NO_3N	Control	Soil + 3.0 Mgms. NO_3N
1	3.02	2.86	5.65	3.31	5.75	4.30	7.21
2	2.73	2.58	5.00	3.08	5.49	3.88	6.50
3	2.85	2.88	5.00	3.11	5.55	3.90	6.79
4	3.03	3.14	5.80	3.42	5.90	3.52	6.50
5	2.98	2.58	5.35	2.81	5.52	4.20	7.04
6	2.58	2.58	5.27	3.25	5.68	4.28	7.50
7	T	T	2.25	T	0	T	0
8	1.42	1.56	4.11	----	4.59	----	5.15
9	3.07	1.72	4.70	2.22	4.75	2.59	5.19

Table IV — Nitrate Assimilation

Soil No.	Mgms. NO_3N Assimilated in 24 Hours	48 Hours	8 Days
1	0.21	0.56	0.90
2	0.58	0.59	0.38
3	0.88	0.56	0.11
4	0.34	0.52	0.02
5	0.23	0.29	0.16
6	0.31	0.25	0.22
7	0.75	3.00	3.00
8	0.45	----	----
9	0.02	0.47	0.40

Another set of soil cultures to which 0.2 per cent of the various artificial manures, dry oat straw, fresh farm manure and 30.00 mgms. of nitrogen as KNO^3 in solution were applied was incubated at room temperature (23°-25°C) for 14 days and the nitrate content determined. Table 5 shows the nitrate nitrogen content of the soil before and after incubation and the amount of nitrate nitrogen assimilated in the different cultures.

The results show a very great variation in the effect of the various materials on the nitrate assimilating power of the soil. The nitrate assimilating power of the soil treated with 0.2 per cent of

dry oat straw is larger than that of any of the other treated soils. Soil 3, treated with one of the artificial manures, exhibited a low nitrate assimilating power as compared with the other soils.

The questions of how much nitrogen as nitrates to add and how long to incubate the cultures in order to measure the nitrate assimilating power of the soil have not been answered. However, the amount of nitrogen added has a marked influence on the amount assimilated and the length of the incubation period is not without its effect. Doubtless there are other factors as the results given in tables 6 and 7 indicate.

Table V — Nitrate Assimilation

Soil No.	Mgms. NO_3N in 100 Gms. Soil			
	At Beginning	After 14 Days		Mgms. NO_3N Assimilated in Fourteen Days
		Control	Soil + 30.0 Mgms. NO_3N	
1	2.83	3.76	21.70	12.06
2	2.83	3.68	19.29	14.39
3	2.83	3.81	24.04	9.77
4	2.83	3.66	17.97	15.69
5	2.83	4.13	19.75	14.38
6	2.83	3.73	17.38	16.35
7	2.83	2.17	12.61	19.56
8	2.83	3.22	18.05	15.17
9	2.83	4.04	32.60	1.44

The amount of nitrate nitrogen assimilated after four days was the same at fourteen days with only a small amount assimilated after eight days when 5.00 mgms. of nitrogen as nitrates were added. With the 10.00 and 20.00 mgm. additions of nitrogen as nitrates the assimilation was larger but varied irregularly.

Four weeks after the soils were treated in the greenhouse samples were taken from the straw treated pots and enrichment cultures made by using about 10 grams of the moist soil and 100 cc. of a nitrate solution in 500 cc. erlenmeyer flasks. The enrichment cultures were incubated at 25°C and dilution plates poured on nitrate agar after 3 and 7 days.

Typical colonies were selected after the dilution plates had incubated at 25°C for one week and transfers made to agar slants of the same medium. A large number of cultures were selected for special study of their morphological, physiological and cultural characteristics. The physiology of these organisms, especially the utilization of compounds of nitrogen and carbon, is being studied further.

SUMMARY

This work is in agreement with many other investigations in

which a decreased nitrate accumulation has been observed in soils treated with straw, and in which it has been shown that some of this nitrate nitrogen, if not all of it, is assimilated by soil microörganisms. Soils have a "nitrate assimilating" power which can be measured. Further work is being conducted on methods of determining the nitrate assimilating power of the soil and on some of the nitrate assimilating soil microörganisms.

Table VI

Mgms. NO_3N Added	Mgms. NO_3N Per 100 Gms. Soil			
	At Beginning	After 4 Days	8 Days	14 Days
None	T	T	T	T
5.00	T	3.98	4.90	3.96
10.00	T	8.70	11.12	5.03
20.00	T	16.63	18.47	10.47

Table VII

Mgms. NO_3N Added	Mgms. NO_3N Assimilated		
	4 Days	8 Days	14 Days
5.00	1.02	0.10	1.04
10.00	1.30	-1.12	4.97
20.00	3.37	1.53	9.53

LITERATURE CITED

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