EXPERIMENTS ON THE ACCUMULATION AND UTILIZATION OF ATMOSPHERIC NITROGEN IN THE SOIL.

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OBJECT OF THE EXPERIMENTS.

These experiments were arranged so as to bring out the relation of leguminous crops, such as cow peas, to the soil nitrogen; and to determine as far as practicable, the value of this leguminous crop as a source of nitrogen to subsequent, non-leguminous crops.

With this end in view, a soil was employed that contained an abundance of phosphoric acid and potash; and was, moreover, rather light in character, a condition that would favor the decay of organic substances. This soil originally consisted of equal parts by weight, of shale and quartz sand, and contained 0.09842 per cent. of nitrogen in the air-dry state. It was previously used for vegetation experiments in square wooden boxes during three seasons, and at the end of that time, the soil nitrogen had diminished very considerably. After these experiments, the soils from the several boxes were thoroughly mixed, and 160-pound portions were weighed off, and placed in each of the several boxes, hence there was in each box, a medium sandy soil, rich in mineral plant-food. Nitrogenous manures were added or withheld according to the following plan of study :

(1) The study of the source of nitrogen to cow peas under the following conditions:

(*a*) The addition of no nitrogen.
(*b*) The addition of different amounts of nitrate-nitrogen.
(*c*) The addition of different amounts of dried blood nitrogen.
(*d*) The addition of different amounts of ammonia-nitrogen in ammonium sulphate.
(*e*) The addition of different amounts of cow manure-nitrogen.

(2) The availability of cow-pea nitrogen, as compared with nitrate, organic (in dried blood), ammonia, and manure-nitrogen for the growth of the non-legumes.

(3) The possible accumulation of nitrogen in cultivated, but uncropped soils.

The wooden boxes used here were numbered from 1 to 54, inclusive, and only the first thirty were employed during the first season. Accordingly they received:

Boxes	1,	2,	3......	Nothing.
"	4,	5,	6......	1 gram of nitrogen as nitrate of soda.
"	7,	8,	9......	2 " " " " " " "
"	10,	11,	12......	1 " " " " dried blood
"	13,	14,	15......	2 " " " " " "
"	16,	17,	18......	1 " " " " ammonium sulphate.
"	19,	20,	21......	2 " " " " " "
"	22,	23,	24......	1 " " " in solid and liquid manure.
"	25,	26,	27......	2 " " " " " " " "
"	28,	29,	30......	Nothing, and were kept bare.

The fertilizers and manure were applied to the respective soils July 5, 1902; 20 seeds of black-eyed cow pea were planted in each box, with the exception of 28, 29 and 30, and 8 quarts of water were added to supply the initial moisture.

The growth during the season was fairly uniform. At the end of the summer the cow peas were harvested, dried and ground, and after aliquot portions were taken for analysis, the ground material was kept dry in the laboratory. In the spring of 1903, millet was planted in the several boxes, which had been treated as follows:

Boxes 1 to 27, inclusive, received each the corresponding ground cow-pea crop of 1902.

Boxes	28,	29,	30....	received no application of nitrogen.
"	31,	32,	33....	" 1 gram nitrogen in nitrate of soda
"	34,	35,	36....	" 2 " " " " " "
"	37,	38,	39....	" 1 " " " dried blood.
"	40,	41,	42....	" 2 " " " " "
"	43,	44,	45....	" 1 " " " sulphate of ammonia.
"	46,	47,	48....	" 2 " " " " " "
"	49,	50,	51....	" 1 " " " solid and liquid manure, fresh.
"	52,	53,	54....	" 2 " " " solid and liquid manure, fresh.

Each box received May 29, 1903, 1 teaspoonful of barnyard millet seed, but the germination being poor, another teaspoonful of millet seed was added, about ten days later.

THE NITROGEN IN THE COW-PEA CROP.

The following table shows the amount and composition of the cow-pea crop obtained from the several boxes.

The analytical data presented in the above table show that the application of nitrogen resulted in every case (with the exception of 17), in a slight increase in the nitrogen content of the crop. At the same time, even the greatest increase, was but silght. To be sure, a portion of the nitrogen applied was apparently utilized, if we assume that the fixation was the same in each soil series—an assumption scarcely supported by the complete analytical data. At best, the addition of the ready, available nitrogen did not encourage the plants to make a more extensive use of the soil nitrogen, which is also shown in the soil analytical data obtained. A reference to the cylinder experiments on non-leguminous crops carried on by this Station,[1] will show that at least 70 per cent of the nitrate nitrogen applied became available to the crop in most cases, and the proportion available often reached 85 to 90 per cent. With the same methods of comparison, this does not seem to hold good with plants of the legume family. The averages of each series, as given in Table IV, show that only 30.3 per cent. of the nitrate nitrogen were utilized when 1 gram of it was applied, and only 18.9 per cent. when 2 grams were applied. The same is true of the highly available ammonia nitrogen. Where 1 gram of it was applied in 16, 17 and 18, only 23.7 per cent, and where 2 grams were applied, only 20 per cent. became available. The results are somewhat different where the organic nitrogen, either as dried blood or as manure, were employed. In 10, 11 and 12, where 1 gram of dried blood nitrogen had been applied, the availability reached 66.2 per cent., but with the double portion in 13, 14 and 15, the availability was reduced to 19.8 per cent., and, moreover, the absolute amount of nitrogen was less in the latter case than it was in the former. This would indicate that even large quantities of high-grade organic materials are a disadvantage to the fixation of nitrogen by leguminous crops. Where manure was added, the utilization of the applied nitrogen was apparently more thorough. With an application of 1 gram, there was an availability of 33.9 per cent., and with an application of 2 grams of manure-nitrogen, there was an availability of 37.1 per cent. In other words, the slowly available manure-nitrogen yielded

[1] N. J. Station Reports, 1889-1904.

TABLE I.

Box.	Fertilizer.	Nitrogen applied. Grams.	Weight of crop. Grams.	Nitrogen in crop. Per cent.	Nitrogen in crop. Grams.	Nitrogen increase over check. Grams.	Per cent. of nitrogen available.	Weight of residue of crop. Grams.	Nitrogen in residue. Grams.
1....	Check	..	147	2.207	3.244	.....	....	122	2.69
2....	"	..	137	2.467	3.380	.....	....	112	2.76
3....	"	..	146	2.218	3.238	.....	....	121	2.68
4...	Nitrate of soda	1	157	2.278	3.576	0.289	28.9	132	3.01
5...	" "	1	159	2.225	3.538	0.251	25.1	134	2.98
6...	" "	1	161	2.271	3.656	0.369	36.9	136	3.09
7...	" "	2	158	2.193	3.465	0.178	8.9	133	2.92
8...	" "	2	189	2.106	3.980	0.693	34.7	164	3.45
9...	" "	2	156	2.274	3.547	0.260	13.0	131	2.98
10...	Dried blood	1	164	2.401	3.938	0.651	65.1	139	3.34
11...	" "	1	161	2.426	3.906	0.619	61.9	136	3.30
12...	" "	1	170	2.355	4.004	0.717	71.7	145	3.41
13...	" "	2	177	2.120	3.752	0.465	23.3	152	3.22
14...	" "	2	161	2.176	3.503	0.216	10.8	136	2.96
15...	" "	2	167	2.271	3.793	0.506	25.3	142	3.22
16...	Ammonium sulphate	1	158	2.310	3.650	0.363	36.3	133	3.07
17...	" "	1	139	2.352	3.269	0.018	....	114	2.68
18...	" "	1	153	2.376	3.635	0.348	34.8	128	3.04
19...	" "	2	152	2.397	3.643	0.356	17.8	127	3.04
20...	" "	2	145	2.324	3.370	0.083	4.2	120	2.79
21...	" "	2	183	2.239	4.097	0.810	40.5	158	3.54
22...	Barnyard manure	1	154	2.369	3.648	0.361	36.1	129	3.06
23...	" "	1	151	2.278	3.440	0.153	15.3	126	2.87
24...	" "	1	152	2.493	3.789	0.502	50.2	127	3.17
25...	" "	2	167	2.338	3.904	0.617	30.9	142	3.32
26...	" "	2	185	2.525	4.671	1.384	69.2	160	4.04
27...	" "	2	159	2.208	3.511	0.224	11.2	134	2.96

more to the cow-pea crop than the concentrated and highly available nitrate and ammonia-nitrogen; while with non-legumes the order of availability of the different materials is usually nitrate, ammonia, blood and manure-nitrogen, the order in this instance was blood, manure, nitrate and ammonia-nitrogen. If we consider the boxes where two grams of nitrogen were used, the order becomes manure, ammonia, blood and nitrate-nitrogen. The probable cause of these results will be discussed in connection with the soil analytical data.

TABLE II.

	Average percentage of nitrogen in crop.	Average nitrogen in crop. Grams.	Average percentage of applied nitrogen available.
1			
2	2.282	3.287	——
3			
4			
5	2.257	3.590	30.3
6			
7			
8	2.185	3.664	18.9
9			
10			
11	2.393	3.949	66.2
12			
13			
14	2.188	3.683	19.8
15			
16			
17	2.345	3.518	23.7
18			
19			
20	2.314	3.703	20.8
21			
22			
23	2.380	3.626	33.9
24			
25			
26	2.365	4.029	37.1
27			

This table shows that the cow-pea crop was proportionately richer in nitrogen in the series where only 1 gram of nitrogen was applied, as compared with the corresponding series where 2 grams were applied. Thus in 4, 5 and 6, the average percentage of nitrogen in the crop was 2.257 per cent., while in 7, 8 and 9, it was only 2.185 per cent. Similarly in 10, 11 and 12, it was 2.393 per cent.; and in 13, 14 and 15, it was 2.188 per cent.; in 16, 17 and 18, it was 2.345 per cent., and in 19, 20 and 21, —2.314 per cent.; in 22, 23, 24, —2.380 per cent., and in 25, 26 and 27, —2.365 per cent. In 1, 2 and 3, where no nitrogen was applied, the plant substance was proportionately richer in nitrogen than was the plant substance in 4, 5, 6, 7, 8 and 9, where nitrate was applied.

The total nitrogen harvested in the crop was, with one exception, greater in the series where two grams of nitrogen were applied, as compared with the corresponding series where only 1 gram had been applied. The total nitrogen in the crop from 22, 23 and 24, where 1 gram of manure-nitrogen was applied, was greater than that in 4, 5 and 6, where 1 gram of nitrate-nitrogen was applied, and also greater than that in 16, 17 and 18, where 1 gram of ammonia-nitrogen was applied.

THE NITROGEN CONTENT OF THE BOX SOILS.

The analysis of the crops gave definite information as to the relation of the fertilizer applied to the yield. It was found with the quantities applied, that the soluble and easily available forms were not as advantageous to the cow-pea crop, as the slowly available, organic nitrogen. In other words, the relations here were different than in the case of non-legumes. In order to explain these differences, it becomes necessary to render a complete account, if possible, of the income and outgo of nitrogen, to determine the amount of nitrogen in the soil before and after the season's growth, and also the nitrogen added in the seed and in the water.

As stated above, each box contained at the beginning of the experiment, 160 pounds of air-dry soil, with 2.19 per cent. of hygroscopic moisture, an equivalent in round numbers to 71,000 grams of water-free soil. In the analysis of this soil according to the Kjeldahl method, 20 grams were taken for each determination, and five or six determinations were made of each sample. In each case there were at least three determinations, which agreed within 0.1 cc. of N/10 standard ammonia solutions, so that in most instances the limit of error due to the analytical operations was rather less than 200 mg. of nitrogen in the 71,000 grams of soil. Eight analyses were made of the soil, as it was at the beginning of the cow-pea experiment. It was found to contain on the water-free basis, 0.07741 per cent. of nitrogen, or 54.96 grams of total nitrogen in each of the boxes. The twenty cow-pea seeds planted in boxes 1 to 27, inclusive, contained 71 mg. of nitrogen, and the water added during the season of growth contained 40 mg. of nitrogen.

After the cow-pea crop was removed, the soils were allowed to dry out in the boxes. A representative sample of each was then obtained by means of a soil-sampling tube, the samples were taken to the laboratory, air-dried, ground to pass through a 1 mm. sieve and their hygroscopic moisture determined. The several samples were found to contain the following amounts of hygroscopic moisture:

ORIGINAL SOIL, 2.19 PER CENT.

	Per cent.		Per cent.
1	1.19	16	1.17
2	1.20	17	1.20
3	1.26	18	1.20
4	1.20	19	1.19
5	1.32	20	1.19
6	1.10	21	1.11
7	1.16	22	1.08
8	1.25	23	1.03
9	1.08	24	1.06
10	1.16	25	1.59
11	1.19	26	2.61[1]
12	1.22	27	2.73[1]
13	1.29	28	1.33
14	1.19	29	1.03
15	1.24	30	1.00

THE NITROGEN CONTENT OF THE WATER-FREE BOX SOILS.

	Per cent.	Grams.		Per cent.	Grams.
1	0.08661	61.48	16	0.07964	56.54
2	0.08795	62.44	17	0.08596	61.02
3	0.08534	60.58	18	0.08396	59.60
4	0.08568	60.82	19	0.08228	58.42
5	0.08505	60.38	20	0.07962	56.52
6	0.08021	56.94	21	0.08088	57.42
7	0.08592	61.00	22	0.08552	60.70
8	0.08567	60.82	23	0.08148	57.84
9	0.08252	58.58	24	0.08534	60.58
10	0.08192	58.16	25	0.08630	61.26
11	0.08428	59.82	26	0.08754	62.14
12	0.08233	58.44	27	0.09103	64.62
13	0.09104	64.62	28	0.07906	56.12
14	0.08028	56.98	29	0.08182	58.08
15	0.08532	60.56	30	0.07956	56.48

[1] A slight amount of water got into the sample jar during the fire in the Experiment Station building, April, 1903.

The above figures show that the soil contained considerable quantities of nitrogen in proportion to the amounts applied. They also show that there was a gain of nitrogen in every soil, not excluding 28, 29 and 30, which were left uncropped. The gains are brought out more graphically in the following table, where the average amount of nitrogen in the crop and in the soil is given for each series.

The analytical results tabulated above show that the application of 1 gram of nitrogen resulted in a greater gain than the corresponding application of 2 grams. Thus, in 4, 5, and 6, there was an average gain of 6.97 grams of nitrogen in the soil and crop, while in 7, 8 and 9, where double the amount of nitrogen was applied, the total gain was only 6.72 grams of nitrogen. Similarly in 16, 17 and 18 there was a total average gain of 6.50 grams, and in 19, 20 and 21 there was a total average gain of 4.08 grams of nitrogen. In all of the soils, where either nitrate or ammonia was applied, the gain was much less than in the soils where no nitrogen salt was applied. This taken together with the fact that the application of the double portion of either nitrate or of ammonia, resulted in a smaller yield as compared with the single portion, shows clearly that the soluble nitrogen exerted a depressing effect on the process of fixation.

On the other hand, we find that the total average gain in 10, 11 and 12 was smaller than that in 13, 14 and 15, the corresponding amounts being 6.69 grams, and 7.33 grams. Similarly, in 22, 23 and 24, the average total gain was 7.26 grams, and in 25, 26 and 27, the corresponding gain was 9.63 grams. Here we find that the less soluble organic nitrogen was not detrimental to the fixation of atmospheric nitrogen, when applied in double the amounts.

Since the dried blood nitrogen is more readily available, that is, it becomes soluble sooner than the manure nitrogen, it should be of less advantage in the double portion to the leguminous crop than the corresponding double portion of the manure. As a matter of fact, we find that in the case of the dried blood, the increase from the double portion over the single portion is 0.64 gram of nitrogen, while the corresponding increase from the manure is 2.37 grams of nitrogen. The average gain in 10, 11 and 12 was 2.74 grams in the soil, and 3.95 grams in the crop, while in 13, 14 and 15 the average gain was 3.65 grams in the soil, and only

TABLE III.—THE INCOME AND OUTGO OF THE NITROGEN IN THE BOX SOILS.

Series.	Originally present. Grams.	Nitrogen in seed and water. Gram.	Nitrogen in fertilizer or manure. Grams.	Total nitrogen present at the beginning of the first season. Grams.	Total nitrogen present in soil at the end of the first season. Grams.	Average. Grams.	Gain in soil. Grams.	Nitrogen in crop. Grams.	Total gain. Grams.
1	54.96	0.115	......	55.07	61.48				
2	54.96	0.115	......	55.07	62.44	61.50	+6.42	3.29	9.71
3	54.96	0.115	......	55.07	60.58				
4	54.96	0.115	1.00	56.07	60.82				
5	54.96	0.115	1.00	56.07	60.58	59.45	+3.38	3.59	6.97
6	54.96	0.115	1.00	56.07	56.94				
7	54.96	0.115	2.00	57.07	61.00				
8	54.96	0.115	2.00	57.07	60.82	60.13	+3.06	3.66	6.72
9	54.96	0.115	2.00	57.07	58.58				
10	54.96	0.115	1.00	56.07	58.16				
11	54.96	0.115	1.00	56.07	59.82	58.81	+2.74	3.95	6.69
12	54.96	0.115	1.00	56.07	58.44				
13	54.96	0.115	2.00	57.07	64.62				
14	54.96	0.115	2.00	57.07	56.98	60.72	+3.65	3.68	7.33
15	54.96	0.115	2.00	57.07	60.56				
16	54.96	0.115	1.00	56.07	56.54				
17	54.96	0.115	1.00	56.07	61.02	59.05	+2.98	3.52	6.50
18	54.96	0.115	1.00	56.07	59.60				
19	54.96	0.115	2.00	57.07	58.42				
20	54.96	0.115	2.00	57.07	56.52	57.45	+0.38	3.70	4.08
21	54.96	0.115	2.00	57.07	57.42				
22	54.96	0.115	1.00	56.07	60.70				
23	54.96	0.115	1.00	56.07	57.84	59.70	+3.63	3.63	7.26
24	54.96	0.115	1.00	56.07	60.58				
25	54.96	0.115	2.00	57.07	61.26				
26	54.96	0.115	2.00	57.07	62.14	62.67	+5.60	4.03	9.63
27	54.96	0.115	2.00	57.07	64.62				
28	54.96	0.04	......	55.00	56.12				
29	54.96	0.04	......	55.00	58.08	56.89	+1.89	......	1.89
30	54.96	0.04	......	55.00	56.48				

3.68 grams in the crop. The relations of the nitrogenous materials applied to the content of nitrogen in the soil are brought out in the following tabulation:

Nitrogen applied.	Nitrogen in the soil.
Nothing.	61.50 grams.
1 gram nitrogen in $NaNO_3$	59.45 "
2 " " " "	60.13 "
1 " " " dried blood.	58.81 "
2 " " " " "	60.72 "
1 " " " $(NH_4)_2SO_4$	59.05 "
2 " " " "	57.45 "
1 " " " manure.	59.70 "
2 " " " "	62.67 "

This tabulation also shows that after the removal of the crop the soils in the series where 1 gram of nitrogen was applied were poorer in nitrogen than those in the series where 2 grams were applied. Also in this case there is one exception, namely, in the case of the ammonia-nitrogen. Here the application of 2 grams of readily available ammonia-nitrogen, while it did not reduce the yield, discouraged the utilization of atmospheric nitrogen. The soil here contained a slightly greater amount of nitrogen after the removal of the crop, than it contained immediately after the application of the fertilizer, and while, on the whole, there was utilization of atmospheric nitrogen even in this case, the utilization was more limited, and the behavior of the cow pea was more like that of a non-leguminous crop. Apparently, the formation of tubercles and the utilization of the atmospheric nitrogen did not begin here until the readily available ammonia nitrogen had been largely used up. It will also be noticed, that there was a gain in the nitrogen content of the soil in the several series. The greatest gain was in 1, 2 and 3, 6.42 grams, almost double the amount contained in the crop. Hence the greater gain in the soil, 1, 2 and 3, more than offsets the increase in the nitrogen content of the crop from the other series. The following table brings out the relation of the several series in this respect :

Series.	Nitrogen in crop. Grams.	Increase over Series I. Grams.	Gain over nitrogen in soil. Grams.	Decrease over Series I. Grams.	Total decrease over Series I. Grams.
1	3.29	......	6.42	......	......
2	3.59	0.30	3.38	3.04	2.74
3	3.66	0.37	3.06	3.36	2.99
4	3.95	0.66	2.74	3.68	3.02
5	3.68	0.39	3.65	2.77	2.38
6	3.52	0.23	2.98	3.44	3.21
7	3.70	0.41	0.38	6.04	5.63
8	3.63	0.34	3.63	2.79	2.45
9	4.03	0.74	5.60	0.82	0.08

The above results go far to show that the application of readily available, and concentrated nitrogenous materials to the growth of cow peas (and probably of other legumes) is very poor economy, indeed. Such applications may result in a slightly greater yield of crop, but they leave the soil poorer in nitrogen, and, therefore, less suitable for the growth of non-leguminous plants. The coarser organic materials seem to be much better suited for the purpose, and leave the soil in better condition. Hence, it would appear that where the supply of initial nitrogen for the growth of cow peas is desirable, the application of manure would be more advantageous than that of nitrate or ammonium salts.

It remains to note the gain of nitrogen in the uncropped soils, 28, 29 and 30. After subtracting the amount of nitrogen added in the water, namely, 0.040 gram, there still remains a gain of 1.89 grams. The gain is apparently due to nitrogen-fixing bacteria living in the soil independently of leguminous plants. The fixation of atmospheric nitrogen by such soil bacteria is discussed elsewhere[1], and need not be considered here. Without wandering too far into the realm of speculation, it would be of interest to consider, in this connection, whether these soil bacteria may add to the store of soil nitrogen, while leguminous plants are growing in this soil. If this should be so, the gain noted in the other series may be due, in part, to such bacteria, and a direct answer to this question can be obtained only by the use of pure cultures in these soil studies.

THE MILLET CROP OF 1903.

As stated above, the millet crop followed the cow peas in boxes 1 to 27, and was also seeded in the remaining boxes, 28 to 54, whose soils had been kept dry during the first season. The

[1] See N. J. Station Reports, 1903 and 1904.

TABLE IV.—MILLET, 1903.

Box No.	Fertilizer.	Nitrogen applied. Grams.	Air-dry weight of crop. Grams.	Nitrogen in air-dry matter. Per cent.	Nitrogen in crop. Grams.	Average. Grams.	Average increase over check. Grams.
1	Cow-pea vines	2.69	210	1.328	2.669		
2	" " "	2.76	213	1.448	3.084	2.860	0.754
3	" " "	2.68	221	1.279	2.827		
4	" " "	3.01	208	1.328	2.762		
5	" " "	2.98	206	1.170	2.410	2.639	0.543
6	" " "	3.09	205	1.339	2.745		
7	" " "	2.92	217	1.557	3.379		
8	" " "	3.45	204	1.242	2.534	2.773	0.677
9	" " "	2.98	191	1.260	2.407		
10	" " "	3.34	190	1.283	2.438		
11	" " "	3.30	201	1.275	2.563	2.570	0.474
12	" " "	3.41	187	1.448	2.708		
13	" " "	3.22	194	1.208	2.344		
14	" " "	2.96	197	1.144	2.254	2.398	0.302
15	" " "	3.22	195	1.332	2.597		
16	" " "	3.07	205	1.201	2.462		
17	" " "	2.68	164	1.309	2.147	2.377	0.281
18	" " "	3.04	191	1.320	2.521		
19	" " "	3.04	204	1.305	2.662		
20	" " "	2.79	203	1.140	2.314	2.352	0.256
21	" " "	3.54	163	1.276	2.080		
22	" " "	3.06	231	1.422	3.285		
23	" " "	2.87	233	1.253	2.919	3.018	0.922
24	" " "	3.17	200	1.425	2.850		
25	" " "	3.32	195	1.455	2.837		
26	" " "	4.04	203	1.388	2.818	2.679	0.583
27	" " "	2.96	162	1.471	2.383		

TABLE IV (*Continued.*)—MILLET, 1903.

Box No.	Fertilizer.	Nitrogen applied. Grams.	Air-dry weight of crop. Grams.	Nitrogen in air-dry matter. Per cent.	Nitrogen in crop. Grams.	Average. Grams.	Average increase over check. Grams.
28	Check	....	167	1.021	1.705		
29	"	....	216	1.118	2.415	2.096	.....
30	"	....	202	1.073	2.167		
31	Nitrate of soda	1.00	186	1.617	3.008		
32	" "	1.00	201	1.673	3.363	3.323	1.227
33	" "	1.00	197	1.827	3.599		
34	" "	2.00	200	1.718	3.436		
35	" "	2.00	230	1.707	3.926	3.652	1.556
36	" "	2.00	228	1.576	3.593		
37	Dried blood	1.00	215	1.564	3.363		
38	" "	1.00	217	1.422	3.086	3.179	1.083
39	" "	1.00	219	1.410	3.088		
40	" "	2.00	236	1.778	4.196		
41	" "	2.00	230	1.632	3.754	3.919	1.823
42	" "	2.00	221	1.722	3.806		
43	Sulphate of ammonia	1.00	254	1.823	4.630		
44	" "	1.00	220	1.684	3.705	4.403	2.307
45	" "	1.00	242	2.014	4.874		
46	" "	2.00	257	1.789	4.598		
47	" "	2.00	213	1.808	3.851	4.189	2.093
48	" "	2.00	244	1.688	4.119		
49	Solid and liquid manure	1.00	232	1.666	3.865		
50	" " " "	1.00	237	1.609	3.813	3.848	1.752
51	" " " "	1.00	193	2.003	3.866		
52	" " " "	2.00	196	2.134	4.183		
53	" " " "	2.00	258	2.329	6.009	5.297	3.201
54	" " " "	2.00	247	2.307	5.698		

Uncertainty as to identity of crop of boxes 7 and 54.

millet was harvested in the fifty-four boxes on August 1st, the crop dried, ground and analyzed in the customary manner. The amounts of dry matter and of nitrogen given in Table IV were found.

The facts brought out by these analytical data, are very instructive. It will be noted that there was a greater average yield, both of dry matter and of nitrogen in the second half of the entire series. Evidently the millet plants were able to secure more nitrogen from the fertilizer and the manure than from the ground cow-pea vines, even though the latter contained a greater amount of it. Moreover, the proportionate content of nitrogen in the millet crop was also greater in the second half of the series, thus showing again that there was more available nitrogen at the disposal of the crop in boxes 31 to 54 during the season's growth. Nothwithstanding the fact that the cow-pea vines had been finely ground before their incorporation into the soil, that the latter is light and well-aerated, and that plenty of moisture was provided, conditions which are all favorable to decay, the decomposition of the crop turned under was not rapid enough to furnish maximum amounts of soluble nitrogen. The subjoined tabulation shows the average yields in each series of three boxes.

TABLE V.—AVERAGE YIELDS IN THE MILLET CROP OF 1903.

	Dry matter. Grams.	Average of all. Grams.	Average per cent of nitrogen.	Average per cent of all.	Average amounts of nitrogen. Grams.	Average of all. Grams.
1	215		1.330		2.860	
2						
3						
4	206		1.281		2.639	
5						
6						
7	204		1.359		2.773	
8						
9						
10	193		1.331		2.570	
11						
12						
13	195		1.229		2.398	
14						
15		200		1.314		2.629
16	187		1.271		2.377	
17						
18						

	Dry matter. Grams.	Average of all. Grams.	Average per cent of nitrogen.	Average per cent of all.	Average amounts of nitrogen. Grams.	Average of all. Grams.
19	190		1.238		2.352	
20						
21						
22	221		1.365		3.018	
23						
24						
25	187		1.432		2.679	
26						
27						
28	195		1.075		2.096	
29						
30						
31	195		1.704		3.323	
32						
33						
34	218		1.667		3.652	
35						
36						
37	217		1.465		3.179	
38						
39		221		1.704		3.767
40	229		1.711		3.919	
41						
42						
43	239		1.842		4.403	
44						
45						
46	238		1.760		4.189	
47						
48						
49	221		1.741		3.848	
50						
51						
52	234		2.261		5.297	
53						
54						

The above figures show that the average production of air-dry plant substance in boxes 28-54 was 221 grams as against 200 grams in boxes 1-27, or an increase of rather more than 11 per cent. Similarly, the average yield of nitrogen was 3.767 grams in boxes 28-54, as against an average yield of 2.629 grams in boxes 1-27. Furthermore, with one exception, the yield of air-dry plant substance and of nitrogen in boxes 1-27 was the greatest in Series *I* (1, 2, 3), where no nitrogenous manure had been applied at the beginning of the experiment. Evidently, the previous manuring of the cow peas had no marked effect on the millet crop of 1903 and the nitrogen of the nitrate, dried blood and ammonium sulphate, applied in 1902, was altogether wasted. Conditions are rather different in boxes 28-54, where these materials were applied directly to the millet. Thus in boxes 28, 29 and 30, where no nitrogenous manure was used, the average yield of nitrogen in the millet crop was 2.096 grams, whereas, in boxes 31, 32 and 33, with one gram of nitrate nitrogen, the average yield was 3.323 grams, and in boxes 34, 35 and 36, with 2 grams of nitrate nitrogen, the average yield was 3.652 grams. Similarly, the yield with 1 gram of dried blood nitrogen was 3.179 grams, and with 2 grams of dried blood nitrogen it was 3.919 grams. With 1 gram of ammonium sulphate nitrogen, it was 4.403 grams, and with 2 grams of the same, only 4.189 grams; with 2 grams of manure nitrogen, it was 5.297 grams, as against 3.848 grams with 1 gram of manure nitrogen. The significance of these results will be discussed more fully in the following pages.

THE MILLET CROP OF 1904.

After the millet crop of 1903 was harvested, the soils in the several boxes were allowed to dry out, and were all kept under cover during the winter. In the spring of 1904, the soils were carefully stirred, moistened thoroughly, and on June 6th, millet was again seeded in each box without the further application of fertilizer materials. The seed germinated well, and the young plants grew rapidly. On August 8th, the millet was harvested and after air-drying was subsequently analyzed. Following are the analytical data.

TABLE VI.—MILLET, 1904.

Box No.	Air dry weight of crop. Grams.	Nitrogen in air-dry matter. Per cent.	Nitrogen in crop. Grams.	Average. Grams.	Average increase over check. Grams.
1	128	1.126	1.431		
2	147	1.158	1.702	1.538	0.560
3	138	1.074	1.482		
4	124	1.090	1.352		
5	129	0.983	1.268	1.288	0.309
6	109	1.142	1.245		
7	116	1.190	1.380		
8	112	1.202	1.346	1.428	0.449
9	130	1.198	1.557		
10	135	1.090	1.472		

Box No.	Air-dry weight of crop. Grams.	Nitrogen in air-dry matter. Per cent.	Nitrogen in crop. Grams.	Average. Grams.	Average increase over check. Grams.
11	129	1.011	1.304	1.419	0.440
12	128	1.158	1.482		
13	120	1.221	1.465		
14	120	1.074	1.298	1.367	0.388
15	120	1.114	1.337		
16	112	1.043	1.168		
17	105	1.214	1.275	1.246	0.267
18	120	1.078	1.294		
19	99	1.194	1.182		
20	109	1.106	1.206	1.082	0.103
21	80	1.074	0.859		
22	121	0.991	1.199		
23	114	1.186	1.352	1.240	0.261
24	105	1.113	1.169		
25	107	1.186	1.269		
26	113	1.186	1.340	1.362	0.383
27	116	1.273	1.477		
28	91	1.059	0.964		
29	93	0.999	0.929	0.979	....
30	102	1.023	1.043		
31	112	1.301	1.457		
32	122	1.122	1.369	1.473	0.494
33	150	1.062	1.593		
34	142	1.178	1.673		
35	122	1.110	1.354	1.487	0.508
36	132	1.087	1.435		
37	118	1.349	1.592		
38	116	1.190	1.380	1.390	0.411
39	115	1.042	1.198		
40	157	1.210	1.900		
41	115	1.070	1.230	1.425	0.446
42	104	1.102	1.146		
43	122	1.090	1.330		
44	125	0.955	1.194	1.807	0.828
45	164	1.767	2.898		
46	134	1.178	1.579		
47	133	1.341	1.784	1.604	0.625
48	119	1.218	1.449		
49	144	1.309	1.885		
50	131	1.178	1.543	1.928	0.949
51	146	1.614	2.356		
52	182	1.699	3.092		
53	207	2.499	5.173	4.397	3.418
54	182	2.706	4.925		

In comparing the above with the corresponding results for 1903, it becomes at once apparent that the yields in the earlier year were not only larger, but that the plant substance was richer in nitrogen than the corresponding material in 1904. Remembering that all other plant food constituents were present in abundance in 1904, as well as in 1903, the higher yields in the latter season were due to the greater amounts of available nitrogen, a fact brought out even more clearly in the following tabulation of the average yields in 1904.

TABLE VII.—AVERAGE YIELDS OF MILLET IN 1904.

	Dry matter. Grams.	Average of all Grams.	Average per cent. of nitrogen.	Average per cent. of all.	Average amounts of nitrogen. Grams.	Average of all. Grams.
1	138		1.115		1.539	
2						
3						
4	121		1.064		1.288	
5						
6						
7	119		1.200		1.428	
8						
9						
10	131		1.083		1.419	
11						
12						
13	120		1.140		1.367	
14						
15		118		1.127		1.330
16	112		1.111		1.246	
17						
18						
19	96		1.127		1.082	
20						
21						
22	113		1.097		1.240	
23						
24						
25	112		1.216		1.362	
26						
27						
28	95		1.030		0.979	
29						
30						
31	128		1.151		1.473	
32						
33						

	Dry matter. Grams.	Average of all Grams.	Average per cent. of nitrogen.	Average per cent. of all	Average amounts of nitrogen. Grams.	Average of all. Grams.
34	132		1.127		1.487	
35						
36						
37	116		1.198		1.390	
38						
39						
40	125		1.140		1.425	
41						1.832
42		132		1.388		
43	137		1.319		1.807	
44						
45						
46	129		1.236		1.604	
47						
48						
49	140		1.377		1.928	
50						
51						
52	190		2.314		4.397	
53						
54						

Also in 1904, the second half of the entire series (boxes 28-54), gave a higher average yield of dry matter and of nitrogen. At the same time there was a falling off in the yield throughout the whole series, due to the depletion of the more readily available nitrogen by the exhaustive crop of millet in 1903. In comparing the two, we find that there was a falling off in boxes 1-27, from 200 grams to 118 grams in the yield of dry matter, and a falling off from 2.629 grams of nitrogen to 1.338 grams. In boxes 28-54 there was a similar falling off from 221 grams of dry matter in 1903 to 137 grams in 1904, and a falling off from 3.767 grams of nitrogen to 1.832 grams. It will also be noted that the average yield of nitrogen in boxes 1, 2 and 3, where no nitrogen manure was applied in 1902, was, without exception, the largest for the first half of the series. The other yields seem to correspond in general with those of the preceding season. Thus, the average yield of nitrogen in boxes 4, 5 and 6, was greater than that in boxes 7, 8 and 9, in 1903, as well as in 1904. The same may be said of boxes 10, 11, 12 and 13, 14 and 15, and of boxes 16, 17, and 18 and 19, 20, and 21. Similar relations are brought out in the second half of the series.

THE AVAILABILITY OF THE NITROGEN IN THE GROUND COW-PEA VINES.

In boxes 1-27, where the ground cow-pea vines were applied in the spring of 1903, the succeeding crop of millet secured a part of its nitrogen from the decay of this ground material, and also from the decay of the cow-pea roots and stubble left in the ground. The amount of nitrogen thus obtained may be estimated by subtracting from the average yield in any series of three boxes, that obtained in boxes 28, 29 and 30 which had been kept bare in 1902, and were then used as checks in 1903 by being seeded to millet, without application of nitrogen. The following table shows the increase over the check plot in each series.

TABLE VIII.—INCREASE OVER CHECK PLOT.

Series.		1903. Grams.	1904. Grams.	Total. Grams.
1	1	0.764	0.559	1.323
	2			
	3			
2	4	0.543	0.309	0.852
	5			
	6			
3	7	0.677	0.449	1.126
	8			
	9			
4	10	0.474	0.440	0.914
	11			
	12			
5	13	0.302	0.388	0.690
	14			
	15			
6	16	0.281	0.267	0.548
	17			
	18			
7	19	0.256	0.103	0.359
	20			
	21			
8	22	0.922	0.261	1.183
	23			
	24			
9	25	0.583	0.383	0.966
	26			
	27			

Without exception, the largest increase in 1903, from the cow-pea vines and residues, was obtained in Series 1. In Series 3, with an initial application of 2 grams of nitrate-nitrogen, the increase was greater than in Series 2, where the corresponding application was only 1 gram. The increase in Series 4 was greater than that in Series 5, and the increase in Series 6 was greater than in Series 7. In Series 8, where only 1 gram of manure nitrogen was applied in 1902, the increase was greater than that in Series 9, where 2 grams of manure nitrogen were applied. Comparing the yields in 1904 with those of 1903, we find in Series 1, a greater increase than in any other series. We also find that the increase in Series 3 was still greater than that in Series 2; the increase in Series 4 still greater than that in Series 5; and the increase in Series 6 still greater than that in Series 7. On the other hand, the increase in Series 9 was here greater than that in Series 8, while the reverse held true in 1903. Taking the total increase for the two years over the check plot, we find that Series 1 produced the greatest increase, and that Series 7 produced the smallest increase. The total average increase was highest for the manure plots, and then for the nitrate, dried blood, and ammonium sulphate plots in the order named.

AVAILABILITY OF THE NITROGENOUS MATERIALS APPLIED IN BOXES 31–54.

Series.	Boxes	Nitrogen applied. Grams.	1903. Increase over check plot. Grams.	Nitrogen recovered. Per cent.	1904. Residual nitrogen recovered. Grams.	Total recovery. Per cent.
11	31, 32, 33	1	1.227	122.7	0.494	172.1
12	34, 35, 36	2	1.556	77.8	0.508	103.2
13	37, 38, 39	1	1.083	108.3	0.411	149.4
14	40, 41, 42	2	1.823	91.2	0.446	113.5
15	43, 44, 45	1	2.307	230.7	0.828	313.5
16	46, 47, 48	2	2.093	104.7	0.625	136.0
17	49, 50, 51	1	1.752	175.2	0.949	270.1
18	52, 53, 54	2	3.201	160.1	3.418	331.0

The data given here show that the nitrogen secured by the millet crop of 1903, was equivalent to 77.8–230.7 per cent. of that applied in the several nitrogenous materials. In only two cases was the recovery less than that contained in the fertilizer applied. On the other hand, the average amount of nitrogen contained in the crop from boxes 43, 44 and 45, was more than twice that applied. In the following year, another crop of millet was harvested without further application of nitrogen, and the amounts removed in this second crop, while by no means as large as in the previous season, were still very considerable. But the most striking feature of the crop of 1904, is the very large amount of nitrogen recovered in Series 18. The total average yield in this case was 4.397 grams of nitrogen as compared with 5.297 grams in 1903, yet the increase over the check plot was even greater in 1904 than it was in 1903, namely, 3.418 grams and 3.201 grams, respectively. Instead of becoming reduced in their nitrogen content, the soils in this series, unlike the soils in other series, actually supplied almost as much soluble nitrogen to the crop of 1904, as they supplied to the crop of 1903. Remembering that millet belongs to the crops incapable of utilizing the gaseous atmospheric nitrogen, it becomes possible to account for these extraordinary yields in two ways. Either the millet crop of 1904 was enabled in soils 52, 53 and 54, in some way, to utilize more thoroughly the nitrogen contained in the soil itself, than was true of the other soils, or atmospheric nitrogen was made accessible to it by nitrogen-fixing bacteria. A study of the following table, rendering a full account of the income and outgo of nitrogen in the several soils, will make this point clear.

The table contains a record of the nitrogen originally present in the soil, of that added from time to time in the seed, water and manure and fertilizer, of that added from the atmosphere by the cow peas and otherwise, of that removed in the two crops of millet, of that calculated to have remained in the soil, and of that actually present in the soil at the end of the three years' experiment. The initial amount of soil nitrogen, namely, 54.96 grams, was increased by the 100 mg. contained in the cowpea seed, by the 1 to 2 grams added in the fertilizer or manure, by the 100 mg. contained in the double quantity of millet seed in 1903, by the 50 mg. contained in the millet seed of 1904 and by the 120 mg. contained in the water used during the three seasons.

NITROGEN-BALANCE IN THE BOX SOILS.

Series.	Original in soil. Grams.	1902. In seed and water. Gram.	1902. In fertilizers and manures. Grams.	Added from the atmosphere. Grams.	1903. In fertilizers and manures. Grams.	In seed and water. Gram.	1904. In seed and water. Gram.	Total initial and added. Grams.
1	54.96	0.115	....	9.71–0.577	....	0.140	0.090	64.44
2	54.96	0.115	1.00	6.97–0.563	....	0.140	0.090	62.71
3	54.96	0.115	2.00	6.72–0.547	....	0.140	0.090	63.48
4	54.96	0.115	1.00	6.69–0.599	....	0.140	0.090	62.40
5	54.96	0.115	2.00	7.33–0.549	....	0.140	0.090	64.09
6	54.96	0.115	1.00	6.50–0.588	....	0.140	0.090	62.22
7	54.96	0.115	2.00	4.08–0.580	....	0.140	0.090	60.80
8	54.96	0.115	1.00	7.26–0.592	....	0.140	0.090	62.97
9	54.96	0.115	2.00	9.63–0.589	....	0.140	0.090	66.34
10	54.96	0.040	....	1.89	....	0.140	0.090	57.12
11	54.96	.....	....	....	1.00	0.140	0.090	56.19
12	54.96	.....	....	....	2.00	0.140	0.090	57.19
13	54.96	.....	....	....	1.00	0.140	0.090	56.19
14	54.96	.....	....	....	2.00	0.140	0.090	57.19
15	54.96	.....	....	....	1.00	0.140	0.090	56.19
16	54.96	.....	....	....	2.00	0.140	0.090	57.19
17	54.96	.....	....	....	1.00	0 140	0.090	56.19
18	54.96	.....	....	....	2.00	0.140	0.090	57.19

NITROGEN REMOVED.

Series.	1903. Millet. Grams.	1904. Millet. Grams.	Total. Grams.	Calculated nitrogen in soil. Grams.	Actual nitrogen in soil. Grams.	Gain. Grams.
1	2.860	1.539	4.399	60.04	60.05	+ .....
2	2.639	1.288	3.927	58.78	60.52	+ 1.74
3	2.773	1.428	4.201	59.28	59.59	+ .....
4	2.570	1.419	3.989	58.41	59.32	+ 0.91
5	2.398	1.367	3.765	60.33	59.04	— 1.29
6	2.377	1.246	3.625	58.60	59.13	+ .....
7	2.352	1.082	3.434	57.37	59.22	+ 1.85
8	3.018	1.240	4.258	58.71	60.15	+ 1.44
9	2.679	1.362	4.041	62.30	59.13	— 3.17
10	2.129	0.979	3.108	54.01	55.03	+ 1.02
11	3.323	1.473	4.796	51.39	55.12	+ 3.73
12	3.652	1.487	5.139	52.05	56.43	+ 4.38
13	3.179	1.390	4.569	51.62	59.31	+ 7.69
14	3.919	1.425	5.344	51.85	56.05	+ 4.20
15	4.403	1.807	6.210	49.98	58.28	+ 8.30
16	4.189	1.604	5.793	51.40	58.01	+ 6.61
17	3.848	1.928	5.776	50.41	60.89	+10.48
18	5.297	4.397	9.694	47.50	66.44	+18.94

By the conditions of the experiment, the treatment of the first half of the entire series, consisting of soils 1-30, was different from that of the second half of the series, which included boxes 28-54. In the former, there were three growing seasons accompanied by the various chemical, mechanical and biological changes, while in the latter, there were only two growing seasons. Besides, in boxes 1-27, the soil and manure nitrogen of 1902 became accessible to the millet crops of 1903 and 1904, only in so far as the cowpea vines, into which it was incorporated, yielded it up again through the various processes of decay. The larger amounts of organic matter in these soils produced, of necessity, rather different soil conditions not merely by modifying the bacterial equilibrium in the soil, but also by affecting the solubility of the mineral constituents of the soil. As was already indicated, the cow peas converted the soluble soil and fertilizer nitrogen, as well as the atmospheric nitrogen, into insoluble proteid nitrogen, and the subsequent crops of millet could secure a portion of the latter only through a complicated series of bacterial changes. It should be remembered, however, that in such bacterial changes a part of the combined nitrogen is almost always transformed into free, gaseous nitrogen, and that the proportion of organic nitrogen, that may become available through decay, is usually less than one-half, and but seldom more than that. While it is not known how much nitrogen was set free through rapid oxidation of the organic matter in the different box soils, its amount in each case was probably not inconsiderable. This view is strengthened by the fact that the box soils are light and open, that the moisture conditions were always favorable for rapid decay, and that the temperature conditions were also favorable during the growing season. But whatever the amounts thus liberated, the final analyses show that, with two exceptions to be noted later, there was throughout a gain of soil nitrogen. Manifestly, then, by some process, or processes, in the soil itself, the loss of nitrogen was more than made up by the formation in the soil of nitrogen compounds at the expense of the free nitrogen of the atmosphere.

As to the amounts thus gained in the different soils, an examination of the above table will supply some interesting information. It will be noted that in Series 1 (boxes 1, 2 and 3) there was an average addition by the cow peas, and otherwise, of more than 9 grams of nitrogen per box. Hence, at the end of the first season and at the beginning of the second season the average nitrogen content of these soils was rather more than 64 grams. The two crops of millet which followed the cow peas removed, in all, 4.398 grams of nitrogen, and the soil should have contained, at the end of the experiment, 60.04 grams of nitrogen. By actual analysis it was found to contain 60.05 grams, hence there was apparently neither a loss nor a gain of nitrogen in these soils, and the 4.399 grams of nitrogen secured by the two crops of millet were derived from the atmosphere by the legumes (cow peas), and with the decay of the latter the millet was enabled to find in the soil a quantity of nitrogen indirectly derived from the air. Nevertheless, in drawing this conclusion we should not overlook the fact already considered above, that in all processes of decay more or less of free nitrogen is returned to the atmosphere. For this reason there is much justification for the belief that the final analyses showing neither a gain nor a loss of nitrogen in Series 1, while they tell the truth, do not tell the whole truth. As a matter of fact, there was probably both a gain and a loss in these soils, and the two were so nearly balanced as not to affect the final results. Accepting this as a true interpretation of the conditions as they were, it would seem that the decay of the cow-pea residues and of the soil humus, involved nitrogen losses of unknown extent, losses that were offset by the fixation of atmospheric nitrogen in the soils in question.

In Series 2 (boxes 4, 5 and 6) there was, at the beginning of the second season, an average content of 62.48 grams (62.64 less 0.230 gram) of nitrogen made up of the initial nitrogen, and that added in the seed, water and cow-pea vines, stubble and roots. The two crops of millet withdrew 3.927 grams, leaving a theoretical balance of 58.78 grams of nitrogen. By actual analysis, however, the average amount found in this series was equivalent to 60.52 grams, thus showing a gain of 1.74 grams of nitrogen. Applying the above reasoning also in this case, it would appear that in this series the fixation of nitrogen counterbalanced the loss caused by decay, and even provided an excess of 1.74 grams of nitrogen. A similar comparison in Series 2, 3, 4, 5, 6, 7, 8 and 9 will show that there were gains in most instances and losses in two series. In seeking an explanation for the losses in these two series under conditions that led in the other series to distinct gains, we find that in Series 9, where the greatest loss had occurred, there were present at the beginning of the second season more than 66 grams of nitrogen. In other words, the greatest loss occurred where the accumulation of nitrogen was greatest. Now, since the soil must be regarded as a culture medium where hundreds of bacterial species come into competition with one another in the struggle for food, it becomes clear that the decay bacteria will predominate in a soil well provided with nitrogenous organic matter, and that by this predominance they will either partly or wholly suppress the nitrogen-fixing bacteria. Such is undoubtedly the tendency in our arable soils, and the possibility, or even the probability, is not excluded, that the explanation given here is the true one, and more specific evidence in this direction is offered in the general discussion.

Before passing on to the consideration of Series 10-18 we should note again the effect of the manures and fertilizers in Series 2-9 on the final results. Considering that the average yield of 4.399 grams of nitrogen in the two millet crops on Series 1 was greater than the corresponding yield in any other of the first nine series, it becomes evident enough that the nitrogenous materials applied to the cow peas were more than wasted. From what we know of symbiotic fixation we are justified in the conclusion that the soluble nitrogen salts discouraged fixation, and hence led to a smaller gain of nitrogen in the soil. Taking the gains and losses in the final balance we find that in Series 2, where 1 gram of nitrate nitrogen was applied in 1902 there was a final gain of 1.74 grams of nitrogen, while in Series 3, where 2 grams of nitrate-nitrogen were applied, the final gain was only 0.31 gram. Similarly, there was a final gain of 0.91 gram in Series 4, and a corresponding loss of 1.29 grams in Series 5; a final gain of 1.44 grams in Series 8, and a corresponding loss of 3.17 grams in Series 9. The fact that there was not a greater final gain in Series 1 is probably due to the addition of such a large amount of nitrogen and of organic matter in the soils of this series by the cow-pea crop, and the fact that the final gain in Series 7 was greater than that in Series 6 is probably due to the comparatively small addition of nitrogen and of organic matter in the soils of this series by the cow-pea crop of 1902.

Passing on to the consideration of the other series, we find that in Series 10, comprising boxes 28, 29 and 30, where the soils were kept moist and bare during the first season, there was a very distinct gain of combined nitrogen. In the following season this was made the check series, and it raised a good crop of millet without addition of combined nitrogen. Another fairly good crop was raised on this series in 1904. The two crops contained 3.108 grams of nitrogen, while the soil itself was found to contain as much as was present at the beginning of the experiment, or even a little more. Allowing for the nitrogen added in the seed and in the water, we find that in these soils, kept bare throughout the first season and covered with exhaustive crops of millet in the following seasons, there was a gain of almost 3 grams of nitrogen without resort to symbiotic fixation by legumes. The amount thus gained is very considerable, as compared with the total initial amount of 54.96 grams. But, however important these gains, they are greatly exceeded by those made in the following series in the course of only two seasons. Taking, for instance, Series 11 and 12, we find that the initial nitrogen was increased in 1903 by 1 and 2 grams, respectively, of nitrate-nitrogen and also by that contained in the seed and water for the two years. These additions increased the initial and added nitrogen content to 56.19 grams and 57.19 grams, respectively. The two crops of millet raised contained 4.796 grams of nitrogen in Series 11, and 5.139 grams of nitrogen in Series 12. In other words, the additional gram of nitrate-nitrogen led to an increase of 0.379 gram of millet nitrogen, and assuming that this increase was entirely due to the greater quantity of nitrate the latter showed a recovery of almost 38 per cent. in the additional gram of nitrogen. Taking into account the amounts originally present in the soil, those subsequently added in one way or another, and those removed in the millet crops, there should have been left in the soil 51.39 and 52.05 grams of nitrogen in Series 11 and 12, respectively. The corresponding amounts found by actual analysis were 55.12 and 56.43 grams, respectively, leaving a gain of 3.73 grams of nitrogen in Series 11, and a gain of 4.38 grams of nitrogen in Series 12. Similarly, in Series 13, where 1 gram of dried blood nitrogen had been applied, there was a gain of 7.69 grams, and in Series 14, with an application of 2 grams of dried blood nitrogen, there was a final gain of 4.20 grams. The greatest gains, however, were found in Series 17 and 18, where 1 and 2 grams, respectively, of manure nitrogen were applied. Beginning with an initial quantity of nitrogen in the soil, seed and water of somewhat over 57 grams, there was harvested in Series 18 an average quantity of nitrogen equivalent to 5.297 grams in 1903 and an additional quantity equivalent to 4.397 grams in 1904. The total yield in the two crops was, therefore, equivalent to 9.694 grams of nitrogen, or almost one-sixth of that supplied in the soil, manure, seed and water. Assuming that the transformation of the insoluble humus nitrogen into nitrate involved no liberation of uncombined nitrogen there should have been left in the soil at the end of the experiment 47.50 grams of nitrogen. By actual analysis it was found to contain 66.44 grams of nitrogen, or 9.25 grams more than was initially supplied. Adding to that the 9.694 grams found in the crops we obtained a total gain of 18.99 grams. This remarkable increase, almost one-third of that originally present or supplied, deserves careful study.

Assuming the nitrogen content of a fair arable soil to be 5,000 pounds per acre to a depth of one foot, an increase by one-third of this amount would mean more than 1,600 pounds of nitrogen per acre, and that in the course of only two short growing seasons. Further comparison reveals the fact that the fixation was greatest in the series where the greatest amount of manure was applied, and that the next greatest amount of nitrogen occurred in the series where the smallest quantity of manure was applied. Furthermore, the final gains thus secured represent an amount less than that actually present, for there probably was a more or less extensive volatilization of nitrogen from these soils in the course of decay. The final gain of 18.94 grams in Series 18, or the corresponding gain in any other series, thus becomes the algebraic sum of the gains and losses that had actually occurred.

Before proceeding to the discussion of the general significance of the facts established in this experiment, at least one other point should be mentioned. It will be noted in examining the detailed yields for 1903 that the parallels in each series agreed within fairly narrow limits. There are a few exceptions, however, wherein the discrepancies could only be ascribed to biological causes. Thus in the check Series 10 (boxes 28, 29 and 30), the yield in box 28 was only 1.705 grams of nitrogen, as compared with 2.4115 grams in 29 and 2.167 grams in 30. Similarly, in Series 15, the nitrogen yield in box 44 was only 3.705 grams, as compared with 4.630 and 4.874 grams, respectively, in boxes 43 and 45. Discrepancies of a like nature occurred also in some of the other series. These discrepancies are, however, greatly exceeded in some series for the following season (1904). Thus in Series 14, we find that the yield in soil 40 was 1.900 grams of nitrogen, or more than a third in excess of that yield either in soil 41 or soil 42. In Series 15 soil 45 yielded in the millet crop 2.898 grams of nitrogen as compared with 1.330 grams and 1.194 grams in soils 43 and 44, respectively; and in Series 18 soil 52 yielded in the millet crop 3.092 grams of nitrogen, as compared with 5.173 and 4.925 grams in soils 53 and 54, respectively. All these differences show that even though the same conditions of temperature and moisture prevailed in each case, very striking variations in the yield occurred, and since the soils themselves showed no loss, but rather a gain of nitrogen, these variations could very properly be ascribed to bacteriological differences more or less far-reaching in their character.

DEFINITIONS.

In order to add clearness to the deductions drawn here it is deemed advisable to define some of the terms employed in this paper. The term *fixation* implies the transformation of the uncombined elementary nitrogen of the air into organic nitrogen compounds by the activities of certain bacteria known as nitrogen-fixing, or nitrogen-gathering bacteria. The fixation of nitrogen may be *symbiotic* or *non-symbiotic*. The term *symbiotic* is derived from the noun *symbiosis* and denotes the dwelling together of two distinct forms of life with mutual benefit resulting. This term is most frequently applied to the fixation of nitrogen by members of the legume family, such as clovers, peas, beans, etc., with the aid of certain bacteria, which form tubercles on the roots of these plants. Some investigators have claimed, however, that the bacteria inhabiting the legume tubercles represent an instance of parasitism rather than of symbiosis. The bacteria inhabiting the tubercles of the legumes enable them to make use of the elementary nitrogen of the air, whereas in the absence of these bacteria no tubercles are formed, and no fixation of nitrogen by the legumes can take place. It is believed that these tubercle bacteria when living in the soil outside of the legume roots can also fix nitrogen, but to a much more limited extent, and when thus living outside of the legume roots they become *non-symbiotic*.

Besides the legume tubercle bacteria there are two important groups of soil bacteria which can fix very considerable quantities of atmospheric nitrogen. The first of these includes well-defined species of butyric ferments, which are *anaerobic*, that is, develop in the absence of oxygen. *Clostridium Pastorianum*, isolated by Winogradsky, belongs to this group. The second group include the genus *Azotobacter*, large bacilli possessing a pronounced nitrogen-fixing power and are *aerobic* in character (that is, requiring a plentiful supply of oxygen for their growth). The fixation of nitrogen by the members of these two groups is usually referred to as *non-symbiotic*, although a certain amount of symbiosis occurs also here. Unlike that of the legumes and tubercle bacteria, the symbiosis in this case is either with other bacteria or with algae, the latter being one-celled green plants.

GENERAL CONSIDERATIONS.

The facts established in this experiment are of very considerable practical importance, even though the experiment itself was carried out under conditions more or less artificial. They demonstrate, in no uncertain manner, that the bacterial activities that concern the gain and loss of nitrogen in the soil are manifold and complex, and yet susceptible of differentiation. Evidently, there was a gain of nitrogen in the box soils by means of symbiotic fixation where the cow-pea crop was grown. It is scarcely necessary in this place to review in detail the vast amount of work that has been devoted to the study of this phase of the subject, since Hellriegel and Wilfarth's publications,[1] but it may be said in general that the practical application of their discovery has been comparatively limited thus far. To be sure, leguminous crops were grown intelligently for the accumulation of nitrogen in the soil, even before their discovery,[2] and more extensive use has been made of legumes in crop rotation since their discovery. Nevertheless, as the recent studies of Hiltner[3] indicate, there is great promise of remarkable development in the production of *virulent* cultures of legume bacteria for purposes of soil inoculation. It will thus be seen readily that symbiotic fixation of nitrogen in the soil is not a constant quantity even under the same climatic conditions. As living things, the legume bacteria are affected

1 "Untersuchungen über die Stickstoffnahrung der Gramineen und Leguminosen," *Z. des Vereins fur Rubenzuckerindustrie* d. D. R., November, 1888.

2 Schultz-Lupitz, "Die Kalidüngung auf leichtem Boden," Berlin, 1883.

3 *Centr. f. Bact. u. Par.*, Part II, **12**, 497.

by changing conditions within the plant, as well as outside of it, and their nitrogen-fixing power may be very considerably modified after they find their way out of the tubercles and back into the soil. When once there they may be weakened in the competition for food with other bacteria, and thus rendered incapable of as vigorous fixation as formerly. In this connection it is interesting to note that the tubercle bacteria may be able to fix atmospheric nitrogen outside of the legume tubercles, as was demonstrated, for instance, by Maze,[1] or recently by Chester.[2] It is not known whether in the present experiment there was any addition to the store of soil nitrogen by such action of the tubercle bacteria, but in the absence of defintie information on this point, and because of the fact that there was a nitrogen gain in soils 1-27, even after the removal of the cow-pea crop, it cannot be claimed that this *possibility* was excluded. With this reservation it must be admitted, however, that the *probability* of extensive fixation, having taken place in this manner, is not very great, and for the following reason: While there were some nitrogen gains in the cow-pea soils in 1903 and 1904, the gains were not as great as they were in boxes 31-54, where no cow peas were grown, and hence were not provided with as many tubercle bacteria.

As to the bacteria capable of fixing atmospheric nitrogen when growing alone in the soil, reference need only be made to the work of Berthelot,[3] Winogradsky,[4] Beyerinck,[5] Gerloch and Vogel,[6] Freudenreich,[7] and Lipman[8] to show their universal distribution. These bacteria, occurring, as they do, in most arable soils, have undoubtedly certain functions to perform there. In other words, since they are adapted to survive and to develop under conditions where the vast majority of ordinary decay bacteria would perish, they undoubtedly add to the combined nitrogen in the soil under such conditions. In the fine-grained, compact soil the anaerobic *Clostridium Pastorianum* and allied species play the more important rôle; in the lighter, open soils the members of the *Azotobacter* group are of greater importance. But it should be re-

[1] "Fixation de l'azote libre par le bacille des nodosites de legumineuses," *Ann. de l' Inst. Pasteur*, **11**, 1897.

[2] Bull. 66, Delaware Agr. Expt. Station.

[3] Chimie Vegetale et Agricole, p. 69.

[4] *Archives des sciences biologiques*, **3**, and *Centr. f. Bact. u. Par.*, Part II, **9**, 43.

[5] *Ibid.*, **7**, 561, and **9**, 3.

[6] *Ibid.*, **8**, 669.

[7] *Ibid.*, **10**, 514.

[8] N. J. Expt. Station Reports 1903 and 1904.

membered in this connection that the nitrogen-fixing bacteria, whether aerobic or anaerobic, must have food, and like other bacteria they rapidly disappear from soils devoid of organic matter. It is for this reason that in the box soils 52, 53 and 54 there was a large fixation of nitrogen. The organic matter of the manure, by offering the food, and hence the source of energy, to these bacteria, made possible their rapid development, and, therefore, also an intense fixation of atmospheric nitrogen. But it will be asked in this connection why it was that the organic matter of the cowpea vines and residues did not offer a similar source of food and energy. To this it may be answered that the bacterial equilibrium in the soil may be shifted in one direction or another, and no better illustration of this is needed than the studies of Hiltner and Störmer.[1] When the nitrogen content of the soil passes beyond a certain limit the decay bacteria increase rapidly and in the struggle for existence they are able, with the advantages at their disposal, to suppress the more slowly growing *Azotobacter* species. Hence, where large quantities of legume material are incorporated into the soil, as was done in the case of boxes 1-27, the nitrogen-fixing bacteria cannot develop as freely, and in the algebraic sum of the products the gaseous nitrogen liberated from the soil frequently more than offsets that combined and fixed in the soil by the nitrogen-gathering bacteria. It thus becomes evident that the best results from non-symbiotic nitrogen-fixation may be obtained in open soils, provided with adequate quantities of moisture, and with fairly large amounts of organic matter having a rather small nitrogen content. These conditions are most suitable for the development of the aerobic *Azotobacter* species.

It would not be out of place here to express the hope that with our broadening knowledge we shall find means to provide for a better conservation of the soil nitrogen, whether it be by soil inoculation with vigorous cultures of symbiotic and non-symbiotic nitrogen-fixing bacteria, or by an intelligent maintenance of the nitrogen balance in the soil, so as to prevent the unnecessary dissipation of combined nitrogen through the one-sided activities of the decay bacteria.

As to the practical application of the information already in our possession, this much should be said: We should remember

[1] Studien über die Bacterienflora des Ackerbodens, etc., Berlin, 1903.

that our knowledge of the bacteriological conditions in the soil is still so limited that a general and successful inoculation with non-symbiotic nitrogen-fixing bacteria, with the decay bacteria, or with nitrifying bacteria is entirely out of the question for the present. The only direction in which soil inoculation has been rendered more or less practicable is the inoculation with cultures of various tubercle bacteria. Such inoculation can, therefore, be applied to legumes and *legumes* only. By so doing we may make possible the formation of tubercles and, therefore, the fixation of nitrogen by leguminous crops in such soils where the proper bacteria are naturally absent, but it should also be remembered that in most soils the failure of leguminous crops to grow satisfactorily is due not to the absence of the proper soil bacteria, but to general unfavorable soil conditions, to absence of a sufficient amount of lime, of a sufficient amount of humus, or of sufficient aeration. The inoculation of such soils without previous improvement would be a waste of effort and money. There is ample justification, therefore, to utter here a warning against misconception and unjustifiable expectation. Ignorance in this direction will be exploited, as ignorance in other directions has been exploited, by attempts to sell to farmers, improperly informed, cultures of soil bacteria advertised as the panacea for all soil-ills. Let the man who wishes to inoculate his soil remember that it is not yet practicable to inoculate it for wheat or potatoes, or melons and while it is practicable to inoculate it for alfalfa, or soy beans, or other legumes, he should inform himself as to the real facts before proceeding with his inoculation.

[CONTRIBUTION FROM THE FOOD DIVISION OF THE BUREAU OF CHEMISTRY, U. S. DEPT. OF AGRICULTURE. SENT BY H. W. WILEY.]

EXAMINATION OF LARD FROM COTTONSEED-MEAL-FED HOGS, BY PHYTOSTEROL ACETATE METHOD OF BÖMER.

BY L. M. TOLMAN.

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THE fact that lard prepared from hogs fed on cotton-seed meal will give positive tests with the Halphen and the Becchi reagents has been known for some time. Consequently, a question of