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KANSAS STATE AGRICULTURAL COLLEGE
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BACTERIOLOGICAL STUDIES OF METHODS OF PREPARING A SEEDBED FOR WHEAT

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SUMMARY

The purpose of the investigations herewith reported has been to ascertain the significance of bacterial phenomena in bringing about the variations in nitrate accumulation observed to follow different methods of preparing a seedbed for winter wheat.

Samples of soil were taken to the depth of a foot from plots showing both high and low nitrate accumulation and studied as a unit. Using the nitrate and ammonia accumulation as a criterion of bacterial activity no significant differences could be detected in the laboratory between different plots. This was true when measurements were made on soil incubated under the following varying conditions: (1) Soil as it came from field. (2) Soil with calcium carbonate added. (3) Soil with water added. (4) Soil with both calcium carbonate and water added. (5) Soil with nitrogen added in the form of ammonium sulphate, cottonseed meal, or dried blood. (6) Soil with the above forms of nitrogen and in addition calcium carbonate, moisture, or with both calcium carbonate and moisture. (7) A sterile soil capable of supporting vigorous nitrification to which ammonium sulphate had been added inoculated with soil from the plots under study. (8) A sterile cultural solution inoculated with the soils under study.

Under all these conditions there was vigorous and approximately equal ammonia and nitrate accumulation whether the soil or flora came from a high or low nitrate-accumulating plot. These experiments have shown conclusively that (1) the organisms concerned in the accumulation of nitrates are abundant in all plots and are at least capable of becoming active; that (2) the factor or factors responsible for low accumulations under field conditions are eliminated or overshadowed when the soil is transferred to laboratory conditions; also that (3) the inoculation of soil from low nitrate-accumulating plots with soil from the high nitrate-accumulating plots had little or no effect upon subsequent ammonia and nitrate accumulation.

Studies similar to these were carried out on soil from different layers of the first foot. Soil from all layers of all plots studied showed vigorous ammonia formation with but slight variations.

The accumulation of nitrate nitrogen in soil from the surface three inches of shallow-cultivated plot 1 when nitrogen was added was somewhat more rapid, as a rule, than from any other layer of the same plot or any layer of early deep-plowed plot 9. Next in order of nitrate accumulation came the stirred layers of the deep-plowed plot. As a rule the unstirred areas of all plots studied gave a much slower nitrate accumulation, the accumulation apparently decreasing as the depth from which the soil came increased. However, the formation and accumulation of nitrates in soil from all layers was far in excess of that necessary to utilize all the ammonia that would ever become available under field conditions.

Neither the inoculation of cultural solutions nor the transfer of soil from one layer or plot to soil from another layer or plot, revealed significant differences in the flora of the various layers of the different plots.

All these experiments indicate that the organisms concerned in the accumulation of nitrates are abundant in all layers of all the plots studied and if not already active are capable of becoming so.

When the organisms present in the different layers were left to act upon the native nitrogen, i. e., the nitrogen contained in the soil, large differences were observed in the accumulation of nitrate nitrogen. The accumulation in soil from the surface three inches of shallow, late-cultivated plot 1, was very much more rapid than in that from any other layer of this or any layer of deep, early-plowed plot 9. This relation held true whether or not the moisture was corrected to optimum and whether or not calcium carbonate was added. The soil from this layer was followed, in rapidity of nitrate accumulation, by that from the stirred layers of the deep-plowed plot. The nitrate accumulation in soil from the last mentioned layers was approximately only one-half as great after one year as in the surface soil of plot 1. Below the stirred area of all plots the accumulation was much slower and decreased as the depth from which the soil came increased. Similar relations were found to exist, though to a less marked degree, in the soil of plots 13 and 15.

Quantitative studies of the organic matter and nitrogen content showed that the surface soil of plot 1 contained a large quantity of nitrogen locked up in undecomposed organic mat-

ter. Apparently the field conditions of the surface soil are such that normal decomposition does not take place. Shallow cultivation tends to keep the stubble and subsequent weed growth on or near the surface. Deep cultivation ultimately brings such organic matter to a depth where the factors preventing decomposition at the surface are eliminated. Transferring the soil to laboratory conditions also eliminates the factor or factors preventing normal decomposition and with the high nitrogen content of the surface soil of shallow-cultivated plots a much larger nitrate accumulation is possible.

A study of the factors which might prevent normal decomposition in the surface soil under field conditions indicates that the moisture content is probably of paramount importance.

A study of the ammonia content of soil under field conditions indicates that in all plots nitrification was taking place as rapidly as ammonia was becoming available. In other words, if actual differences in nitrate formation are taking place it is due to differences in some of the decomposition processes preceding nitrification and not to the actual processes of nitrification.

It is not believed that the differences detected in the various processes concerned with the formation of nitrates are sufficient to explain all the observed differences in nitrate accumulation. A study of the possible factors causing the disappearance of nitrates revealed the significant fact that practically all observed differences in nitrate content between early- and late-plowed plots could be accounted for by the amount of nitrogen assimilated by the weeds growing thereon.

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BACTERIOLOGICAL STUDIES OF METHODS OF PREPARING A SEEDBED FOR WHEAT

P. L. GAINEY

INTRODUCTION

The Department of Agronomy, Kansas Agricultural Experiment Station, has for several years been investigating the influence of different methods of preparing the seedbed for winter wheat on yield, soil moisture, and nitrate nitrogen in the soil. Some very interesting results have been reported in a paper by Call (1915). It was early noted that the different treatments brought about marked differences in the accumulation of nitrate nitrogen during the late summer months.

The major points of variation in methods of preparing the seedbed have been: (1) Time at which the first cultivation was made and (2) the depth to which the soil was stirred or cultivated. As a general rule early and deep cultivations have given the highest yields and the greatest accumulation of nitrate nitrogen. The differences in the accumulation of nitrate nitrogen are very strikingly shown by Call (1915). The quantity of NO_3 in the first three feet at seeding time, for the season of 1912, varied from 22 pounds to 522 pounds per acre. Important differences have been consistently recorded during the course of the investigation. Low and high yields have almost invariably been associated with similar variations in nitrate nitrogen content.

The recurrence of these very marked variations in nitrate accumulation from year to year and its apparent correlation with yield were deemed of sufficient importance to warrant a more careful investigation of the underlying causes. Since the only probable source of the accumulation of nitrate nitrogen under existing conditions was by the activity of certain fairly well defined groups of microorganisms, the problem was necessarily associated with bacteriological phenomena. Certain phases of the investigation were, therefore, assigned to the Department of Bacteriology and to the writer in particular. Investigations were begun in the fall of 1914. The major results of these investigations are presented in the following pages.

[1] Contribution No. 33 from the Department of Bacteriology.

DESCRIPTION OF PLOTS

Eleven different methods of preparing a seedbed for winter wheat have been compared in this project. The plots are one-tenth of an acre in size, and are situated on low upland. (Fig. 1.) Five plots (Nos. 2, 5, 8, 11, and 14), distributed throughout the series, are treated similarly to act as check plots. "The soil is a dark brown silt loam about 10 inches deep, the subsoil to the depth of at least six feet being a reddish-brown, silty clay loam. The upper portion of the subsoil contains more clay and is quite plastic, but the content of fine and very fine sand increases with depth so that in the fifth and sixth foot the subsoil contains a considerable quantity of the finer grades of sand. As a rule, the soil is retentive of moisture and is not quickly affected by dry weather. The soil over the area shows some

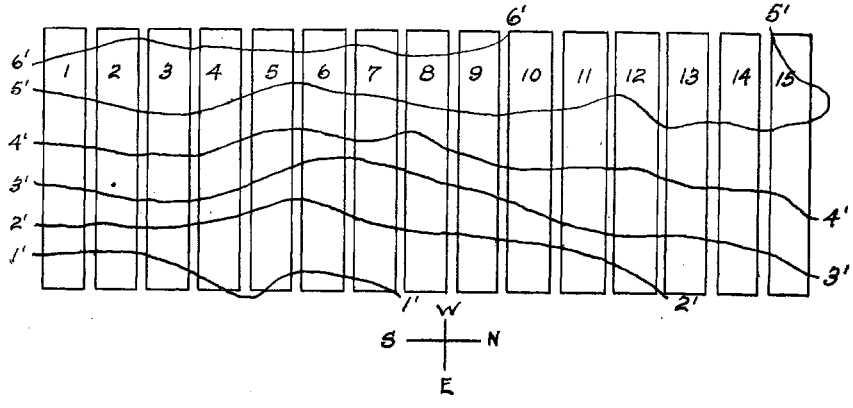

FIG. 1.—Topography of plots

variation, the south part being slightly heavier, darker, and more plastic than the ground at the north end of the area. This condition is more noticeable in the sub-surface soil.

"The difference in texture affects the moisture equivalent and wilting coefficient which, for the average of the surface four feet at the south end of the area, are 25.4 percent and 13.8 percent respectively; and for the north end of the area are 23.5 percent and 12.8 percent respectively, there being, therefore, a difference of 1 percent in wilting coefficient in different parts of the area.

"The plots slope gently to the south and east, but are comparatively level. The accompanying contour map (fig. 1) shows the topography of the area and the method of plotting." (Call, 1915, pp. 249-251.)

The methods of treatment have been as follows, the numbers here used corresponding to those in figure 1:

1. Disked sufficiently at seeding time to prepare as good a seedbed as is possible by that method.
2. Plowed three inches deep September 15.
3. Disked July 15 and plowed seven inches deep September 15.
4. Disked July 15, and plowed seven inches deep August 15.
5. Plowed three inches deep September 15.
6. Listed five inches deep July 15, ridges worked down.
7. Listed five inches deep July 15, ridges split August 15.
8. Plowed three inches deep September 15.
9. Plowed seven inches deep July 15.
10. Plowed seven inches deep August 15.
11. Plowed three inches deep September 15.
12. Plowed seven inches deep August 15. No further work until September 15.
13. Plowed seven inches deep September 15.
14. Plowed three inches deep September 15.
15. Plowed three inches deep July 15.

In all instances, except plot 12, the soil was worked sufficiently after plowing to prevent any growth of weeds.

The annual production of wheat and the nitrate content of soil at seeding time for the different plots during the past eight years are given in Table I.

TABLE I.—EFFECT OF SEEDBED PREPARATION ON NITRATE (NO_3) CONTENT OF THE SOIL AT SEEDING TIME AND ON YIELD OF WHEAT, 1909-1916

Plot No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Year	Pounds of nitrates per acre to a depth of three feet (*a*)														
1909	39	64	85	160	47	180	140	48	193	83	44	90	48	42	160
1910	69	77	38	150	53	158	63	39	141	101	38	0	44	38	88
1911	22	95	82	296	96	247	251	105	386	251	57	202	43	65	282
1912	22	76	263	385	74	372	522	77	408	256	52	243	77	57	517
1913	255	315	316	283	239	257	211	290	258	191	245	267	163	144	192
1914	60	174	492	367	130	323	382	206	340	373	132	284	135	109	280
1915	30	48	89	159	22	108	51	18	136	56	16	25	29	24	62
1916	96	122	189	342	185	427	572	100	362	237	131	166	92	113	286
Average	74	121	194	268	106	259	274	110	274	194	89	182	79	74	233
	Corrected yield 1911 to 1916, bushels per acre (*a*)														
1911	4	13	23	33	13	35	34	13	38	28	13	24	16	13	33
1912	6	9	8	8	9	5	5	9	8	12	9	9	9	9	7
1913	9	16	27	30	16	28	29	16	35	33	16	29	17	16	21
1914	22	24	27	28	24	23	25	24	23	23	24	26	24	24	23
1915	4	15	22	17	15	14	17	15	23	23	15	23	17	15	13
1916	2	5	6	6	5	8	6	5	7	7	5	6	6	5	7
Average	8	14	19	20	14	19	19	14	22	21	14	19	15	14	17

(*a*) Pounds NO_3 are *observed* data. Bushels per acre are corrected on the bases of the yields of the check plots

From the results presented in Table I, it is evident that there has been either a much more vigorous formation of nitrate nitrogen in soil under certain treatments, or a much more pronounced disappearance of nitrate nitrogen in soil under other treatments. It also appears that the factor or factors controlling the accumulation of nitrate nitrogen are variable, permitting of a large accumulation in some seasons and preventing it in others. For the season of 1913 the differences in nitrate accumulation between the various treatments were not as great as between checks. As has already been mentioned, the difference under various treatments for 1912 was as great as 500 pounds nitrate (NO_3) per acre, the extreme variation in five checks for the same season being only 24 pounds per acre.[1]

METHODS

It was deemed advisable to restrict the study of the biological phenomena to a very limited number of plots and make it intensive, rather than endeavor to study all plots. Further, it was thought best to select for study those plots which had exhibited the most marked differences in nitrate nitrogen accumulation, regardless of the system of preparation to which they had been subjected. The data given in Table I show that plots 1 and 9 fulfill these requirements.

As mentioned heretofore the major points of comparison have been time of preparation and depth of cultivation. Plot 1, was the last to receive cultivation and the depth of culture was less than for any other plot. Plot 9, on the other hand, was one of the earliest and deepest cultivated plots in the series. Plot 1, therefore, presented a combination of the two major factors in preparation, late and shallow cultivation, which tend to keep the nitrate accumulation low, while plot 9 presented a combination of the two major factors, early and deep preparation, which tend toward a high accumulation of nitrate nitrogen. It was believed that if biological differences could not be detected in soil from these two plots it would be a waste of time to look for differences in other plots. Most of the studies here reported have been made on soil from these two plots. It was thought best, however, to check results secured from these plots against results secured from at least one

[1] These data were kindly supplied by the Department of Agronomy.

other plot showing a high accumulation, and one showing a low accumulation of nitrate nitrogen. Plot 15, fulfilling the former, and plot 13, fulfilling the latter requirement, were selected.

In all cases where the surface foot of soil was studied as a unit, soil samples have been taken with an ordinary soil auger, the size of the auger depending on the amount of soil needed. Samples were drawn to a depth of 12 inches and from 12 to 15 cores taken at each sampling. In a few cases, when ammonia nitrogen only was to be determined, six cores were taken. Samples were drawn uniformly over the plots, avoiding close proximity to alleys.

When the different layers of soil mentioned later were to be studied, the number of cores required to secure sufficient soil became so large and the chances for contamination so great that the auger method of sampling was discarded and one of the following methods used: The soil was removed from around a column approximately four inches square and to the depth of the first desired layer. After shaving the sides with a clean spatula this layer was removed and the process repeated. Where this method was not employed a sharp edged cylinder five inches in diameter with the cutting edge slightly contracted was driven into the soil to the required depth. The soil surrounding this cylinder was removed and the core taken out and divided into the various layers. This same method was employed when an unbroken column was desired. Usually only four to six samples were taken when these methods were used.

The results reported in Table XII were secured from samples of soil taken under as near aseptic conditions as could be observed. In this case a hole was dug to the required depth and by means of a sterile spatula the exposed soil was removed and samples taken horizontally from the vertical side. Except in this particular case no special effort was made to observe absolute aseptic conditions. However, in order to reduce contamination to a minimum such precautionary measures as were consistent with the necessary rapidity of work were always observed. Clean or sterile containers and tools were always used and instruments, hands, etc., were always cleaned before passing from one sample to another or from one plot to another. It is believed that the chances for cross contamina-

tion have been no greater than exist naturally under field conditions. Furthermore, if the biological differences are not sufficient to overshadow such contaminations as probably occurred, it is hardly conceivable that they could be detected by methods now in use in studying soil biological phenomena. Data are submitted showing that actually transferring large numbers of organisms from one soil to another had no appreciable effect in altering the results herein reported.

The soil samples after being drawn were immediately brought to the laboratory where they were passed through a three-millimeter sieve and all those from the same locations thoroughly mixed. The ability of the soil in a freshly sieved loose condition to retain moisture was determined by placing 50 grams on a carbon filter and pouring a measured quantity of water on it. The water was allowed to percolate through and then poured on again. This was repeated several times to insure thorough moistening. From the quantity of water retained plus the quantity lost by drying other samples at 110° C. for two hours, the water holding capacity expressed in C.C. per 100 gm. of soil was calculated. It was found that the samples of soil collected from time to time varied slightly in their ability to retain moisture, consequently this factor was determined at each sampling.

When the investigations were first begun an experiment was conducted to ascertain the percent of the total water-holding capacity necessary to insure optimum moisture conditions for aerobic bacterial activity. The results of such an experiment are reported in Table II and shown graphically in figure 10. When tested in this manner the writer has always found the optimum to be near two-thirds saturation and the data in Table II show that this soil is no exception to the rule. Therefore, in all succeeding experiments if any change in the moisture content was made it was adjusted to two-thirds saturation, i. e., two-thirds of its maximum water-holding capacity when in a loose condition. After such additions as were to be made were thoroughly mixed in, and the soil placed in an incubation container, the water necessary to bring it up to two-thirds saturation was poured on top. It was found that if the water content of the soil being studied was brought to this high percent saturation, it was sufficient to insure a rapid and equal distribution of the water throughout the whole soil mass.

TABLE II.—EFFECT ON NITRATE ACCUMULATION OF VARYING THE MOISTURE CONTENT OF THE SOIL

C.c. H_2O per 100 gm. soil	Percent saturation	Mg. NO_3 per 100 gm. soil (incubation four weeks)				
		$(NH_4)_2SO_4$ (a)	$(NH_4)_2SO_4$ $+CaCO_3$	Cottonseed meal (a)	Cottonseed me l $+CaCO_3$	Average
16	33	18.0	.....	32.7	30.0	26.9
19.2	40	25.8	91.9	71.9	104.4	73.5
24	50	32.1	150.0	81.6	143.8	102.0
28.8	60	36.0	171.6	100.0	171.6	119.8
32	66	37.5	205.8	102.8	180.0	131.5
33.6	70	34.6	150.0	96.0	168.0	112.0
36	75	35.3	111.1	102.8	144.0	98.3

(a) Ammonium sulphate and cottonseed meal added at the rate of 60 mg. of nitrogen per 100 gm. of soil.

One hundred gram samples of soil were usually used. The incubation containers were, as a rule, 500 c.c. wide mouth bottles, though in some instances it was necessary to use 500 c.c. Erlenmeyer flasks and 300 c.c. bottles. Experiments have been reported by Gainey and Metzler (1917), showing that the difference in nitrate accumulation under these slightly varying conditions is negligible.

Where organic sources of nitrogen were added the quantity of the material necessary to supply the required amount of nitrogen was weighed on analytical balances, wrapped in paper, and sterilized in the autoclave. Ammonium sulphate was added in a sterilized solution, unless the moisture was to remain unchanged, in which case it was added in the form of fine powder. Calcium carbonate was added as a precipitated powder. After all additions were made and water content adjusted, the container and contents were weighed and at frequent intervals thereafter during incubation the loss in weight was restored by adding sterile water.

It was observed in some experiments where the moisture content was comparatively low that the accumulation of nitrate nitrogen seemed to be greater than the moisture content would justify. This was possibly due to an increased moisture content of the surface soil when the moisture lost by evaporation was restored, thus bringing about more or less optimum moisture relations in a limited area. To obviate this it was necessary to prevent loss of moisture. Experimentation showed that the oxygen content of a 500 c.c. bottle containing 100 gm. of soil was sufficient to insure optimum oxygen relations for some time, provided the consumption of oxygen was not stimulated by the addition of organic sources of nitrogen. In sub-

sequent experiments with low moisture content the containers were tightly stoppered and instead of restoring the loss of moisture during incubation, the air content of the container was changed at frequent intervals. Incubation in all experiments was at room temperature.

In determining nitrate nitrogen, water equal to twice the weight of the soil was added and the whole shaken vigorously on the shaking machine for 20 minutes. If the quantity of nitrate nitrogen was believed to be high the soil was further diluted. If ammonia nitrogen was to be determined by the distillation method an aliquot of the suspension was removed for nitrate nitrogen determinations and the remainder distilled in the presence of magnesium oxide. To secure a clear solution a small quantity of calcium oxide was added to the suspension, after which it was filtered through filter paper. A clear solution was always obtained by this method. An aliquot part was evaporated in duplicate and nitrate nitrogen determined by the phenol-di-sulphonic acid method, using a Schreiner colorimeter and potassium hydroxide as an alkali. Where nitrification tests were made in cultural solutions the total nitrate nitrogen was determined by the aluminum reduction method. If ammonia nitrogen only were to be determined by the magnesium oxide method the whole 100 gm. of soil was transferred to a copper distilling flask and distillation carried out in the ordinary way.

Where ammonia nitrogen was determined by the aeration method (Potter and Snyder, 1914),10 to 20 grams of accurately weighed soil was placed in large test tubes (1¼ by 10 inches) together with twice the weight of water, one gram of sodium carbonate and a few drops of paraffin oil. A current of air freed of ammonia was drawn through for six to eight hours and the ammonia nitrogen collected in $^{n}/_{100}$ acid and titrated. With the current of air available it was found that practically all removable ammonia nitrogen was drawn over in six to eight hours.

All results, unless otherwise stated, are reported as mg. nitrate (NO_3) and mg. nitrogen as NH_3 per 100 gm. of soil (soil dried at 110° C. for two hours).

In many instances qualitative tests were made for ammonia nitrogen by means of Nessler's reagent. Such tests taken in conjunction with the quantitative determinations often furnish valuable information.

Where no nitrogen was added and where nitrogen was added in the form of ammonium sulphate, the nitrate originally present was subtracted from that present at the various analyses and the difference reported as net gain. Where cottonseed meal and dried blood were added all nitrate nitrogen present at the various analyses was, for reasons previously pointed out by the writer (1914), regarded as net gain and reported as such.

DEVELOPMENT OF AMMONIA AND NITRATE IN SOIL FROM DIFFERENT PLOTS

STUDY OF THE SURFACE FOOT OF SOIL AS A UNIT

The accumulation of nitrate nitrogen in soils is, as far as is now known, conditioned upon and usually controlled by the formation of ammonia. Hence, it is essential in such a study as this to ascertain whether the soils exhibit any marked differences in their ability to liberate ammonia from organic nitrogen.

AMMONIA FORMATION

The usual method for the determination of the ammonia forming ability of a soil is to add to samples of soil various forms of organic nitrogen and make subsequent measurements of the ammonia and nitrate nitrogen present, assuming, of course, that the nitrate nitrogen has been derived from ammonia and hence must be taken into consideration. Experiments of this kind are recorded in Tables III and IV.

A study of the data here presented reveals no significant differences in the ability of soil from the various plots to bring about the formation of ammonia when suitable organic nitrogen is added. In Table III ammonia accumulation in soil from plot 9 is slightly higher when cottonseed meal was added, while soil from plot 1 shows a slightly higher accumulation when dried blood was the source of nitrogen. These differences, however, are all within the limit of experimental error. After four weeks incubation the ammonia recovered (Table IV) is, in most instances, less than at the end of one week. Similar phenomena are almost always observed and are due to the conversion of ammonia nitrogen into nitrate nitrogen. After prolonged incubation there will usually be found an inverse ratio between the quantity of nitrogen present as ammonia and that

TABLE III.—AMMONIA ACCUMULATION IN SOIL

(Incubation, seven days)

Plot No.	Mg. nitrogen as NH_3 per 100 gm. soil			
	Soil collected 10-24-'14		Soil collected 11-7-'14	
	C. S. M. added (a)	D. B. added (a)	C. S. M. added (a)	D. B. added (a)
1	26.43	15.30	28.07	7.14
9	29.01	14.76	30.69	6.36

(a) Sixty mg. nitrogen as cottonseed meal (C. S. M.) and dried blood (D. B.) added per 100 gm. soil. Moisture content in c.c. per 100 gm. soil: Plot 1, 37 and 40; plot 9, 36 and 37.

TABLE IV.—AMMONIA ACCUMULATION IN SOIL

Weeks of incubation	Mg. nitrogen as NH_3 per 100 gm. soil (a)							
	No $CaCO_3$ added				$CaCO_3$ added			
	Plot No. 1	Plot No. 9	Plot No. 13	Plot No. 15	Plot No. 1	Plot No. 9	Plot No. 13	Plot No. 15
1	32.00	30.00	29.56	28.91	30.36	29.30	29.56	28.51
4	22.90	23.76	28.31	27.72	28.16	19.00	31.08	31.35

(a) Soil collected July 24 and 27, 1915. Moisture content in c.c. per 100 gm. soil: Plot 1, 24; plot 9, 25; plot 13, 23; plot 15, 21. Sixty mg. nitrogen as cottonseed meal added per 100 gm. soil.

present as nitrates. Under such conditions the soil containing the most active nitrifying flora will contain the smallest quantity of ammonia. In the experiments here given the quantity of ammonia nitrogen present in soil from different plots varied but slightly. The same was true for nitrate nitrogen.

In the light of the above experiments any observed differences in the accumulation of nitrate nitrogen can not be attributed to differences in the ability of the respective flora to form ammonia provided other conditions are equally favorable.

NITRATE ACCUMULATION I S SOIL

A number of methods have been employed for detecting variations in the ability of soils to bring about the formation and accumulation of nitrate nitrogen. One of the more common and one which we have employed in this study is to add to the samples of soil definite quantities of various forms of nitrogen that are capable of being transformed into nitrate nitrogen. Subsequently at definite or varying intervals, determinations of the nitrate nitrogen content are made. If differences in the rate of accumulation are observed it is ascribed to differences

TABLE V.—ACCUMULATION OF NITRATES IN SOIL WHEN NITROGEN WAS ADDED

(Mg. NO_3 per 100 gm. soil)

	No $CaCO_3$ added				1 gm. $CaCO_3$ added per 100 gm. soil							
	60 mg. nitrogen as cottonseed meal added per 100 gm. soil				60 mg. nitrogen as cottonseed meal added per 100 gm. soil				60 mg. nitrogen as $(NH_4)_2 SO_4$ added per 100 gm. soil			
Plot No.....	1	9	13	15	1	9	13	15	1	9	13	15
Weeks of incubation												
1 (*a*).....	4.94	5.10	4.72	5.33	2.22	3.74	3.40	3.48	10.54	13.60	7.80	10.14
4 (*a*).....	44.20	46.10	21.14	27.20	39.80	79.00	16.60	18.20	154.86	.....	68.80	89.64
12 (*a*).....	90.00	102.00	95.00	100.00	132.00	128.40	150.00	150.00	178.84	178.40	223.00	237.64
1 (*b*).....	1.72	1.29	.....	.....	1.04	1.50	.....	.....	3.85	8.80	.....	.....
3 (*b*).....	16.00	48.00	.....	.....	21.80	109.20	.....	.....	74.05	116.00	.....	.....
10 (*b*).....	112.20	128.40	.....	.....	150.00	150.00	.....	.....	224.25	221.00	.....	.....

(*a*) Soil collected July 24 and 27, 1915. Water content in c.c. per 100 gm. soil: Plot 1, 24; plot 9, 25; plot 13, 23; plot 15, 21.
(*b*) Soil collected Sept. 15, 1915. Water content in c.c. per 100 gm. soil: Plot 1, 39; plot 9, 40.

TABLE VI.—ACCUMULATION OF NITRATES IN SOIL

Sample No.	Plot No.	Soil collected 10-8-'14				Soil collected 10-23-'14		Soil collected 11-7-'14		Average
		Nitrogen added (a)	Gm. $CaCO_3$ (a)	C.c. H_2O (a)	Gain, mg. NO_3 per 100 gm. soil	C.c. H_2O (a)	Gain, mg. NO_3 per 100 gm. soil	C.c. H_2O (a)	Gain mg. NO_3 per 100 gm. soil	
1	1	None	0	35	2.15	37	3.40	40	3.23	2.93
2	9	None	0	32	1.07	36	2.65	37	3.03	2.26
3	1	None	1	35	4.55	37	9.26	40	11.47	8.43
4	9	None	1	32	4.17	36	8.12	37	8.25	6.85
5	1	60 mg. as $(NH_4)_2 SO_4$	0	35	14.65	37	23.12	40	25.57	21.11
6	9	60 mg. as $(NH_4)_2 SO_4$	0	32	17.27	36	26.07	37	22.14	21.83
7	1	60 mg. as $(NH_4)_2 SO_4$	1	35	133.75	37	201.00	40	130.93	155.23
8	9	60 mg. as $(NH_4)_2 SO_4$	1	32	192.87	36	217.41	37	161.26	190.51
9	1	60 mg. as cottonseed meal	0	35	50.80	37	70.08	40	86.20	69.03
10	9	60 mg. as cottonseed meal	0	32	72.60	36	90.00	37	93.72	85.45
11	1	60 mg. as cottonseed meal	1	35	114.30	37	178.80	40	174.60	155.90
12	9	60 mg. as cottonseed meal	1	32	150.30	36	167.40	37	172.20	163.30
13	1	60 mg. as dried blood	0	35	20.80	37	42.12	40	29.99	30.97
14	9	60 mg. as dried blood	0	32	36.90	36	66.54	37	32.22	45.22
15	1	60 mg. as dried blood	1	35	73.50	37	145.00	40	94.50	104.33
16	9	60 mg. as dried blood	1	32	82.00	36	156.80	37	97.80	112.20
17	1	None	0	22	1.60	23	1.83	19	1.59	1.67
18	9	None	0	24	1.07	22	1.61	18	1.30	1.33
19	1	None	1	22	3.45	23	4.60	19	3.31	3.79
20	9	None	1	24	3.27	22	4.86	18	3.12	3.75
21	1	60 mg. as $(NH_4)_2 SO_4$	0	22	13.25	23	17.61	19	13.17	13.68
22	9	60 mg. as $(NH_4)_2 SO_4$	0	24	14.17	22	18.99	18	13.06	15.41
23	1	60 mg. as $(NH_4)_2 SO_4$	1	22	.....	23	73.40	19	14.53	43.96
24	9	60 mg. as $(NH_4)_2 SO_4$	1	24	.....	22	71.36	18	19.38	45.37
25	1	60 mg. as cottonseed meal	0	22	17.70	23	32.40	19	17.95	24.02
26	9	60 mg. as cottonseed meal	0	24	40.60	22	39.89	18	19.14	33.21
27	1	60 mg. as cottonseed meal	1	22	.....	23	72.45	19	18.90	45.67
28	9	60 mg. as cottonseed meal	1	24	.....	22	65.45	18	15.78	40.61
29	1	60 mg. as dried blood	0	22	16.50	23	28.26	19	18.82	21.19
30	9	60 mg. as dried blood	0	24	32.80	22	39.45	18	19.14	30.46
31	1	60 mg. as dried blood	1	22	.....	23	26.76	19	18.10	22.43
32	9	60 mg. as dried blood	1	24	.....	22	41.16	18	21.12	31.14

(a) Mg. of nitrogen; gm. of $CaCO_3$; and c.c. of water are each per 100 gm. soil.

in the relative efficiency of the flora as a whole, and the soil as a pabulum, to form nitrate nitrogen. A number of such experiments have been carried out on soil from plots 1 and 9, and, to a less extent, plots 13 and 15. Ammonium sulphate, cottonseed meal, and dried blood have been used as sources of nitrogen. The results are reported in Tables V and VI.

A large number of similar experiments have also been carried out except that no nitrogen was added. Calcium carbonate has been added to some samples and not to others. The moisture content has sometimes been left as it existed under field conditions but more often adjusted to optimum. Experiments of this nature are reported in Table VII and shown graphically in figures 2, 3, and 4.

Table VI presents the results of three series of experiments conducted on soil from plots 1 and 9 carried out, at three different dates and including all the above conditions. The incubation period in these experiments was four weeks—a period generally employed for nitrification experiments. The varia-

TABLE VII.—ACCUMULATION OF NITRATES IN SOIL

(No nitrogen added)

Weeks of incubation	Gain, gm. NO_3 per 100 gm. soil							
	No $CaCO_3$ added				1 gm. $CaCO_3$ added per 100 gm. soil			
	Plot No. 1	Plot No. 9	Plot No. 13	Plot No. 15	Plot No. 1	Plot No. 9	Plot No. 13	Plot No. 15
7 (*a*)	6.60	5.60	7.50	5.70	19.40	19.70	15.50	13.00
20 (*a*)	17.40	18.20	15.80	12.10	42.60	40.70	39.50	32.50
41 (*a*)	31.40	20.20	17.50	14.50	47.40	45.50	40.30	.....
9 (*b*)	8.60	6.30	3.40	2.30	24.80	16.60	12.20	13.00
18 (*b*)	17.50	14.60	10.80	9.80	43.80	31.10	25.20	30.50
30 (*b*)	26.60	17.70	16.80	13.00	54.60	41.70	36.80	36.00
1 (*c*)	2.06	1.60	1.40	1.64	4.19	3.36	1.70	3.34
2 (*c*)	.....	2.84	1.76	1.64	5.52	4.56	3.00	3.94
4 (*c*)	4.58	4.90	2.00	1.64	10.10	8.18	3.00	5.64
12 (*c*)	8.34	7.40	4.00	4.30	12.66	12.20	4.90	8.92
31 (*c*)	12.54	10.40	5.50	5.64	17.34	17.60	14.00	13.64
60 (*c*)	24.56	26.00	14.30	17.64	50.26	36.90	30.60	20.14

(*a*) Soil collected April 1, 1915. Moisture content of all samples, 36 c.c. per 100 gm. soil.
(*b*) Soil collected April 6 and April 12, 1915. Moisture content of all samples, 36 c.c. per 100 gm. soil.
(*c*) Soil collected July 24 and 27, 1915. Moisture content in c.c. per 100 gm. of soil: Plot 1, 24; plot 9, 25; plot 13, 23; and plot 15, 21.

tions in experimental conditions reported in Table VI were more numerous than in experiments reported in other tables.

A consideration of the data secured from the experiments in which nitrogen was added, presented in part in Tables V and VI, reveals no significant differences in the ability of soil from different plots to nitrify. This is true whether the nitrogen added was in a mineral (ammonium sulphate) or organic (cottonseed meal or dried blood) form. It is also true whether or not calcium carbonate was added in addition to the various forms of nitrogen. Soil from plots 9 and 15 usually brought about a more rapid accumulation of nitrates during the first few weeks of incubation than did soil from plots 1 and 13. This is evident both in Tables V and VI, being particularly marked at the three-week analysis of soil collected Sept. 15 and recorded in Table V. If the period of incubation were continued for from 10 to 13 weeks such differences were entirely eliminated. Soil from all four plots brought about very large and rapid accumulations of nitrate nitrogen provided other conditions were favorable. All reasonable quantities of nitrogen added either in a mineral or organic form were converted into nitrates in a comparatively short time if the moisture content and reaction were favorable.

In the absence of added nitrogen (Table VII and figs. 2, 3, and 4), either with or without the addition of calcium carbonate, the soil from plots 1 and 13 usually showed a more rapid accumulation of nitrates than did soil from plots 9 and 15.

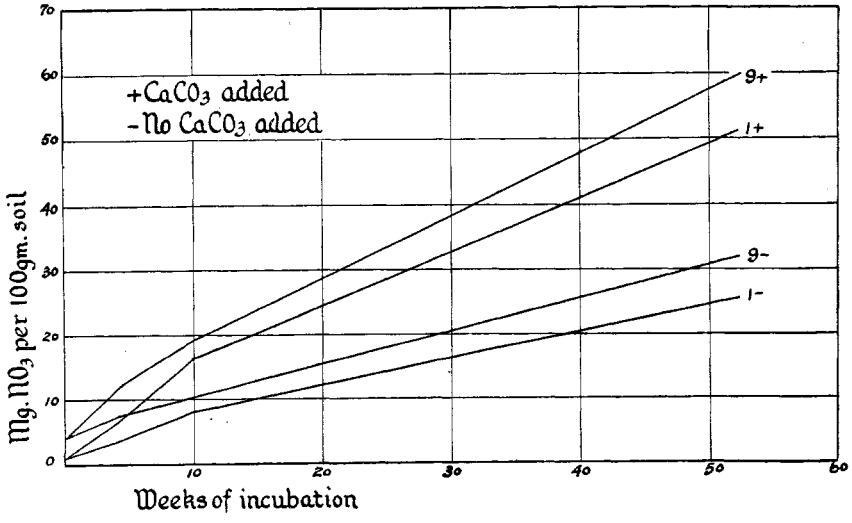

FIG. 2.—Graphs showing accumulation of NO_3 in soil from plots 1 and 9. (Soils collected Sept. 15, 1915)

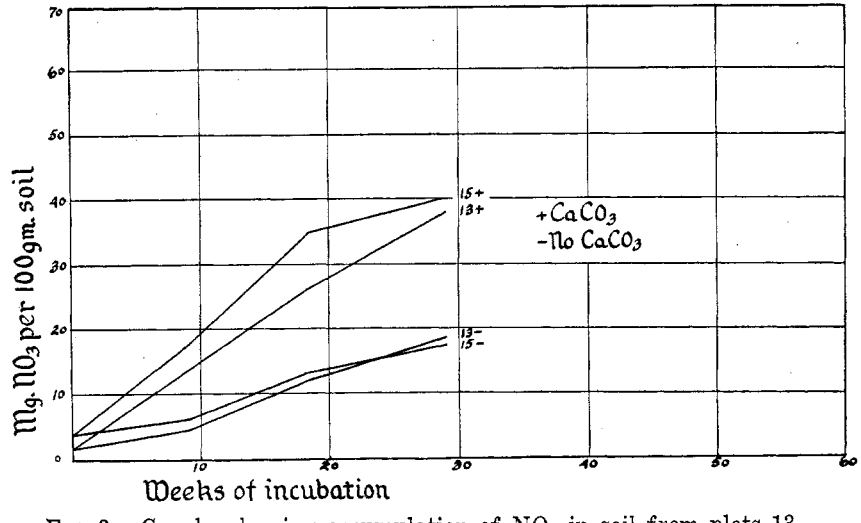

FIG. 3.—Graphs showing accumulation of NO_3 in soil from plots 13 and 15. (Soil collected April 13, 1916)

With a favorable moisture content the addition of calcium carbonate to soil from all plots increased enormously the accumulation of nitrate nitrogen either in the presence or absence of an addition of nitrogen. When the moisture content was low, below approximately 20 C.C. per 100 gm. of soil, and nitrogen was added, the addition of calcium carbonate had little effect upon the accumulation of nitrates. (See Table VI, soil collected Nov. 7.) In the absence of an addition of nitrogen, even with low moisture content, calcium carbonate still exercised a very beneficial effect upon the accumulation of nitrates. The differences in the effect of calcium carbonate here

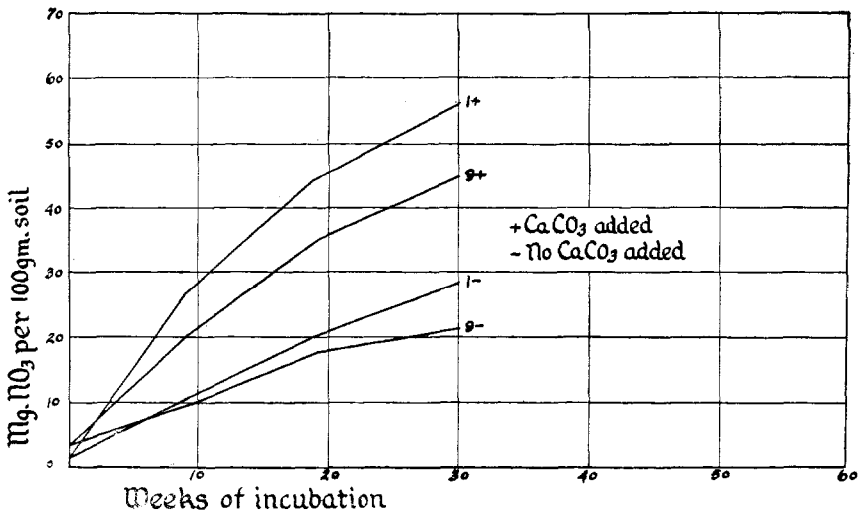

FIG. 4.—Graphs showing accumulation of NO_3 in soil from plots 1 and 9. (Soil collected April 6, 1916)

exhibited may possibly be explained upon a basis of the concentration of ammonia. A high concentration of ammonia in solution is known to be toxic to nitrifying organisms and it is possible that with a low moisture content the concentration of ammonia was such that a toxic effect screened the beneficial effect of calcium carbonate.

There is no evidence in the data here submitted to show that the soils of plots 1 and 13 are deficient in their ability to form or accumulate nitrates when placed, under favorable conditions.

TABLE VIII.—NITRATE ACCUMULATION IN INOCULATION EXPERIMENTS

(Mg. NO_3 per 100 c. c. solution or 100 gm. soil)

Inoculum, soil from plot No.	Medium	Incubation, four weeks					Incubation, twenty weeks			
		Collected 10-10-'14	Collected 10-24-'14	Collected 11-9-'14	Collected 11-14-'15	Average	Collected 10-10-'14	Collected 10-24-'14	Collected 11-9-'14	Average
1	Solution	5.40	8.97	12.13	37.70	16.05	.....	.....	.....	.....
9	Solution	32.33	9.27	19.07	38.10	24.69	.....	.....	.....	.....
1	Sterile soil (*a*)	6.30	59.10	.....	.....	32.70	42.75	248.00	228.80	173.18
9	Sterile soil (*a*)	9.00	68.10	.....	.....	38.55	52.70	240.00	224.00	172.20
1	Sterile soil (*b*)	3.69	25.60	.....	.....	14.65	145.50	207.00	221.00	191.20
9	Sterile soil (*b*)	7.20	18.30	.....	.....	12.75	157.80	200.00	226.40	194.70

(*a*) Sixty mg. nitrogen as $(NH_4)_2SO_4$ added. (*b*) Sixty mg. nitrogen as cottonseed meal added.

NITRATE ACCUMULATION IN INOCULATED SOILS AND SOLUTIONS

Another method commonly employed for detecting differences in the presence or activity of nitrifying organisms is to transfer a small quantity of the soil, or suspension of the soil to be tested to a pabulum which favors the development and activity of the nitrifying organisms and to measure the subsequent accumulation of nitrate nitrogen. Sometimes a solution and sometimes sterile soil known to possess the ability to support a vigorous nitrifying flora is inoculated. Both these methods have been used in the present study. The data secured are reported in Table VIII. In addition there are reported in Table IX the results of an experiment in which a suspension of soil from plot 9 was introduced into soil from plot 1.

The results given in Table VIII are not materially different from those given in Tables V to VII. There are fluctuations in the relative ability of the soils from the different plots to accumulate nitrates when inoculated into solution or into sterile soil; but with the exception of solution samples for October 10 and November 9 the differences exhibited are not beyond that of duplicate tests of the same soil. The same is true regarding the cross inoculating experiment reported in Table IX.

As a general summary of the work conducted upon samples of soil taken to a depth of 12 inches and treated as a unit the data are negative as far as exhibiting differences in their ability to bring about the conversion of the various forms of nitrogen used into nitrate nitrogen. This includes not only the processes of nitrification, but also those of decomposition of organic matter with the liberation of ammonia. If no nitrogen were added and the microorganisms were left to act upon the

TABLE IX.—NITRATE ACCUMULATION IN INOCULATED SOIL FROM PLOT 1

(Mg. NO_3 per 100 gm. soil)

Inoculum	$CaCO_3$ added	Nitrogen added	Weeks of incubation	
			4	10
None	None	None	3.32	8.20
Plot 1	None	None	4.00	7.50
Plot 9	None	None	4.00	8.20
None	1 gm.	60 mg. as $(NH_4)_2SO_4$	.....	225.00
Plot 1	1 gm.	60 mg. as $(NH_4)_2SO_4$	133.30	200.00
Plot 9	1 gm.	60 mg. as $(NH_4)_2SO_4$	150.00	200.00

native nitrogen, i. e., the nitrogen contained in the respective soils, the soil from plots 1 and 13 exhibited, as a rule, a more marked accumulation of nitrates than did the soil from plots 9 and 15. This is believed to be due, not to any difference in the respective flora, but to a difference in the quantity or quality of the nitrogen content of the soil.

STUDY OF SURFACE FOOT OF SOIL DIVIDED INTO DIFFERENT LAYERS

Having anticipated early in the investigation the conclusions to which the above reported data were going to lead, it became evident that if a solution of the problem were to be reached, other lines of investigation would be necessary.

As mentioned earlier, the depth to which cultivation has been practiced varies from shallow disking to plowing seven inches deep. While the depth to which the soil has been cultivated has not brought about such wide variations in nitrate accumulation as has the time of plowing, still it has apparently exercised a marked influence. It was thought that by sampling the plots under study at different depths and making studies similar to those heretofore reported, more specific information might be gained. Accordingly samples were taken from various layers down to 12 inches and studied. Samples were not taken below 12 inches because a large amount of data accumulated by the Department of Agronomy indicates that the major portion of the nitrate formation under field experimental conditions takes place in the first foot of soil. If differences could not be detected within this area it would seem useless to look for them at lower depths.

The same series of tests was conducted using the soil from different depths as was employed on the foot-column samples. The results are reported in Tables X to XXII and illustrated graphically in figures 5 to 9. In all these tables and figures, "A" refers to the surface three inches of soil; "B," "C," and "D" to the second, third, and fourth three inches, respectively. "E" refers to the layer between four and seven inches deep, and "F" to the layer between seven and twelve inches deep.

AMMONIA FORMATION

The amounts of ammonia nitrogen present after varying incubation periods when organic nitrogen was added are reported in Tables X and XI. Since cottonseed meal seemed to

give more satisfactory results than dried blood, it was used as a source of nitrogen in these experiments. In addition to the quantitative results recorded a large number of qualitative tests, which gave valuable information, were made.

There is nothing of special interest to be noted regarding the ammonia formation except the rapid and uniform production in soil from all layers of all plots studied. Owing to a more rapid transformation of ammonia nitrogen into nitrate nitrogen in certain layers of soils than in others, the uniformity in actual quantities of ammonia nitrogen present was not usually maintained for more than one or two weeks. After rapid nitrate accumulation began, usually between the fifth and fifteenth day of incubation, there was found an inverse ratio between the quantity of ammonia and nitrate nitrogen present. An examination of the data will show that where there was a marked difference in the ammonia nitrogen content those samples with the smaller quantities of ammonia nitrogen contained the higher quantities of nitrate nitrogen. (Compare

TABLE X.—AMMONIA ACCUMULATION IN SOIL FROM DIFFERENT DEPTHS

(Mg. nitrogen as NH_3 per 100 gm. soil)

Source of soil (a)	Incubation period		
	One week	Two weeks	Eight weeks
1 A	19.04	26.90	5.47
9 A	15.04	28.95	11.17
1 E	16.40	30.52	16.87
9 E	13.04	25.72	8.51
13 A	30.40	34.68	13.86
15 A	30.88	33.90	19.00
13 E	30.40	32.57	14.00
15 E	30.88	37.76	17.52

(a) Soil collected April 17 and 21, 1915.

TABLE XI. — AMMONIA ACCUMULATION IN SOIL FROM DIFFERENT DEPTHS

(Mg. nitrogen as NH_3 per 100 gm. soil)

Source of soil (a)	Incubation period			
	One week	Two weeks	Four weeks	Eight weeks
1 A	28.38	24.55	1.45	Trace
9 A	27.85	25.74	1.58	Trace
1 B	27.13	29.63	2.64	Trace
9 B	28.25	21.78	1.19	Trace
1 C	26.00	32.74	1.85	Trace
9 C	28.51	22.57	1.06	Trace
1 D	27.78	32.01	3.96	Trace
9 D	26.79	26.66	1.58	Trace

(a) Soil collected August 20, 1915.

Tables X and XI with Tables XIV and XII, respectively.) There was no evidence of a deficiency in activity of the organisms capable of transforming cottonseed meal nitrogen into ammoniacal nitrogen in soil from any plots and at any depths studied. Neither was there any indication of there being a less active ammonia forming flora in soil from plots 1 and 13 than in soil from plots 9 and 15.

NITRATE ACCUMULATION (NITROGEN ADDED)

The data for the transformation of added nitrogen into nitrate nitrogen are contained in Tables XII, XIII, XIV, and XV.

TABLE XII.—ACCUMULATION OF NO_3 IN SOIL AND IN INOCULATED CULTURAL SOLUTION

	Mg. NO_3 per 100 gm. soil (a)								Mg. NO_3 per 100 c. c. cultural solution
Weeks of incubation	1 (b)	2 (b)	4 (b)	8 (b)	1 (c)	2 (c)	4 (c)	8 (c)	4
Soil 1 A	4.00	19.50	128.40	138.00	4.98	13.88	188.18	198.58	32.80
Soil 9 A	2.73	16.00	116.40	120.00	4.40	13.00	177.20	177.20	39.20
Soil 1 B	2.00	8.16	114.00	120.00	1.42	5.96	112.76	178.76	27.40
Soil 9 B	2.73	18.50	112.80	123.60	4.56	16.36	177.66	197.66	29.40
Soil 1 C	1.60	4.17	120.00	120.00	0.76	3.26	120.60	178.76	20.60
Soil 9 C	2.10	13.20	118.00	128.40	2.78	11.58	177.58	197.58	20.40
Soil 1 D	1.00	1.50	114.00	128.40	0.18	1.58	58.76	178.76	37.70
Soil 9 D	0.84	3.60	118.00	123.60	0.04	4.00	73.40	178.40	36.60

(a) Soil collected August 20, 1915. Moisture content in c. c. per 100 gm. soil: 1 A, 1 B, 9 B, and 9 C, 37; 1 C and 1 B, 40; 9 A, 35; and 9 D, 43.
(b) Sixty mg. nitrogen as cottonseed meal and one gm. $CaCO_3$ added per 100 gm. soil.
(c) Sixty mg. nitrogen as ammonium sulphate and one gm. $CaCO_3$ added per 100 gm. soil.

TABLE XIII.—ACCUMULATION OF NO_3 IN SOIL AND IN INOCULATED CULTURAL SOLUTION

	Mg. NO_3 per 100 gm. soil (a)		Mg. NO_3 per 100 c. c. cultural solution	
Weeks of incubation	3	18	2	4
Soil 1 A	10.00	108.00	32.00	62.70
Soil 9 A	9.60	85.20	10.60	43.80
Soil 1 B	3.00	108.00	13.00	46.80
Soil 9 B	12.00	120.00	8.80	56.40
Soil 1 C	2.66	78.00	3.30	33.40
Soil 9 C	5.44	94.80	7.40	19.90
Soil 1 D	2.50	3.36	3.90	17.00
Soil 9 D	3.44	72.00	9.70	19.70
Soil 13 A	10.40	120.00	30.80	48.10
Soil 15 A	6.90	120.00	25.50	28.20
Soil 13 B	14.56	112.80	26.90	39.50
Soil 15 B	5.20	51.60	29.50	9.00
Soil 13 C	4.00	100.00	23.80	19.00
Soil 15 C	5.00	94.80	22.90	16.00
Soil 13 D	3.00	36.00	22.90	21.00
Soil 15 D	4.40	7.20	24.60	16.00

(a) Soil collected October 26, 1915. Sixty mg. nitrogen as cottonseed meal and one gm. $CaCO_3$ added per 100 gm. soil. Moisture content in c. c. per 100 gm. soil: 1 A, 1 B, 12 A, 12 C, 12 D, 13 A, 13 B, and 15 B, 23; 1 D, 9 B, 9 C, 12 B, 12 C, 12 D, 13 C, 13 D, 15 C, and 15 D, 25; 9 A, 22; 9 D, 27; and 15 A, 20.

TABLE XIV.—ACCUMULATION OF NO_3 IN SOIL

(Mg. NO_3 per 100 gm. soil)

Weeks of incubation	Source of soil									
	1 A	9 A	13 A	15 A	1 E	9 E	13 E	15 E	1 F	9 F
1 (*a*)	0.00	0.00	2.50	3.33	0.00	0.00	4.20	1.66	.....	.....
2 (*a*)	12.00	3.60	20.00	25.00	3.00	3.60	29.50	8.00	.....	.....
8 (*a*)	110.40	114.40	100.00	100.00	88.00	86.40	106.60	100.00	.....	.....
1 (*b*)	1.33	1.46	.....	.....	1.14	4.00	.....	.....	Trace	4.00
2.5 (*b*)	53.30	23.60	.....	.....	14.20	80.00	.....	.....	2.84	120.00
4.5 (*b*)	200.00	180.00	.....	.....	180.00	180.00	.....	.....	15.66	180.00

(*a*) Soil collected April 17 and 21, 1915. Moisture content, two-thirds saturation. Sixty mg. nitrogen as cottonseed meal added per 100 gm. soil.
(*b*) Soil collected May 4, 1915. Moisture content, two-thirds saturation. Sixty mg. nitrogen as cottonseed meal added per 100 gm. soil.

TABLE XV.—ACCUMULATION OF NO_3 IN SOIL

(Mg. NO_3 per 100 gm. soil)

Soil (*a*)	Nitrogen added per 100 gm. soil	$CaCO_3$ added per 100 gm. soil	Weeks of incubation			
			4		18	
			A	E	A	E
1 (*b*)	None	None	0.96	0.54	6.00	1.67
9 (*b*)	None	None	1.50	0.99	1.92	Lost
1 (*b*)	None	1 gm.	3.03	4.61	8.00	7.34
9 (*b*)	None	1 gm.	2.28	2.78	5.52	7.30
1 (*b*)	60 mg. as $(NH_4)_2SO_4$	None	9.63	6.68	31.20	20.80
9 (*b*)	60 mg. as $(NH_4)_2SO_4$	None	3.36	8.24	12.00	31.30
1 (*b*)	60 mg. as $(NH_4)_2SO_4$	1 gm.	11.91	5.66	26.50	108.20
9 (*b*)	60 mg. as $(NH_4)_2SO_4$	1 gm.	5.52	21.92	22.80	159.10
1 (*b*)	60 mg. as cottonseed meal	None	14.22	12.60	60.00	102.00
9 (*b*)	60 mg. as cottonseed meal	None	6.00	18.00	36.00	108.00
1 (*b*)	60 mg. as cottonseed meal	1 gm.	7.50	13.08	112.50	189.60
9 (*b*)	60 mg. as cottonseed meal	1 gm.	3.60	20.88	19.26	211.30
1 (*c*)	None	None	4.34	0.98	33.00	6.00
9 (*c*)	None	None	2.58	1.76	10.80	10.00
1 (*c*)	None	1 gm.	12.63	3.98	40.40	17.20
9 (*c*)	None	1 gm.	7.32	5.60	24.00	22.60
1 (*c*)	60 mg. as $(NH_4)_2SO_4$	None	25.71	8.42	74.20	46.00
9 (*c*)	60 mg. as $(NH_4)_2SO_4$	None	9.48	11.84	58.20	70.30
1 (*c*)	60 mg. as $(NH_4)_2SO_4$	1 gm.	105.63	31.58	222.70	199.20
9 (*c*)	60 mg. as $(NH_4)_2SO_4$	1 gm.	102.00	128.48	219.00	253.31
1 (*c*)	60 mg. as cottonseed meal	None	60.00	40.80	144.00	156.00
9 (*c*)	60 mg. as cottonseed meal	None	44.10	63.00	156.00	186.00
1 (*c*)	60 mg. as cottonseed meal	1 gm.	75.00	40.80	180.00	186.00
9 (*c*)	60 mg. as cottonseed meal	1 gm.	60.00	85.80	218.00	225.00

(*a*) Soil collected November 18, 1915.
(*b*) Moisture content in c. c. per 100 gm. soil: 1 A and 9 A, 13; 1 E, 18.5; 9 E, 16.5.
(*c*) Moisture content in c. c. per 100 gm. soil: 1 A, 38; 9 A, 37; 1 E, 37; 9 E, 35.

Soil from the surface three inches of shallow cultivated plots (1 and 15), as a rule, possessed the ability to bring about, a more rapid accumulation of nitrate nitrogen than did soil from the same layer of deep cultivated plots (9 and 13). This is especially evident in Table XV. The differences were usually more marked with the shorter periods of incubation. If the period of incubation was appreciably extended complete nitrification of added nitrogen usually took place in a comparatively

short time. After complete nitrification has taken place the only variation to be expected in the nitrate content is a possible difference due to a greater loss of nitrogen available for nitrification from some samples than from others. Such losses may be due to volatilization or to assimilation of ammonia. Owing to the longer period during which such action could take place, such a loss would be greater where nitrification was less active. All surface soils examined possessed very active nitrifying flora.

The second and third three-inch layers of the deep cultivated soils exhibited as great nitrate accumulating power as did the surface three inches. Below the cultivated areas of both shallow and deep plowed plots there was usually a marked decrease in the nitrate accumulating power of the soil, less marked, however, in the deep cultivated soil. The soil collected from plot 9 on May 4, 1915, and reported in Table XIV was a marked exception to this rule.

If tested in solution, the nitrate forming power, while somewhat irregular, was apparently as marked at 12 inches deep as shallower. This would indicate the possibility of some physical or chemical factor, rather than biological, being responsible for the low nitrate accumulation observed in the lower layers of uncultivated soil when tests were made in the soil itself. No appreciable differences were exhibited between the different plots.

Comparatively slow nitrate accumulations were sometimes observed in those layers of soil remaining uncultivated, the decrease in nitrate accumulating power apparently becoming more marked as the depth increased. Examples of such a phenomenon are evident in Table XIII, in soil from both plots 1 and 15.

CROSS INOCULATION EXPERIMENTS NITROGEN ADDED)

Results of an experiment of this nature are reported in Table XVI.

In the presence of an addition of nitrogen in the form of cottonseed meal the introduction of soil from the more active nitrate accumulating layers of soil into soil that within itself possessed a low accumulating power, increased somewhat the accumulations of nitrate. In view of the large differences exhibited between the various layers this increase was not as great as might be expected. A specific example will probably

serve best to illustrate this point. The accumulation in soil 1 E, a soil exhibiting within itself a very low accumulating power, when inoculated with itself was 15 mg. of NO_3. The accumulation in 9 F, a soil at this time possessing the most active accumulating, power, when inoculated with itself was 181.6 mg.[1] This soil was, therefore, apparently more than twelve times as efficient as 1E in accumulating nitrates. However, when 1 E was inoculated with 9 F the increase in efficiency was only from 15 to 33.4 mg. or only slightly more than two times. This would indicate that there was something in soil 1 E which hindered the processes of nitrification even when nitrifying organisms of a very active type were introduced.

TABLE XVI.—CROSS INOCULATING EXPERIMENTS WITH SOIL

(Mg. NO_3 per 100 gm. soil)

Soil (a)	1 A	9 A	1 E	9 E	1 F	9 F	Average
C. c. water per 100 gm. soil	40	32	35	31	40	42	
Inoculum 1 A	49.00	22.20	20.00	125.00	Not tested	181.60	79.60
Inoculum 9 A	47.60	24.10	13.80	121.20	Not tested	170.40	75.40
Inoculum 1 E	58.00	16.00	15.00	97.60	Not tested	160.00	69.32
Inoculum 9 E	57.20	18.00	15.60	119.60	Not tested	184.00	78.88
Inoculum 1 F	54.00	22.20	18.20	108.00	Not tested	186.10	77.70
Inoculum 9 F	85.20	43.50	33.40	120.00	Not tested	181.60	92.74
Average	58.5	24.33	19.33	115.23	Not tested	177.28	78.94

(a) Soil collected May 4, 1915. Sixty mg. nitrogen as cottonseed meal added per 100 gm. soil.

NITRATE ACCUMULATION IN SOIL FROM DIFFERENT DEPTHS (NO NITROGEN ADDED)

The experiments here recorded are similar to those in which the first foot of soil was used as a unit and from which it was observed that those soils which under field conditions gave low nitrate accumulations gave high nitrate accumulations under laboratory conditions. The results are presented in Tables XVII to XX and shown graphically in figures 5 to 9. The period of incubation was in many cases greatly prolonged. The figures reported, with the exception of those for samples analyzed without incubation, show actual increases over the NO_3 initially present. The graphs, however, are plotted on a basis of NO_3 actually in the soil at the times of the various analyses.

[1] Soil 9 F for some reason possessed an exceptionally active nitrate accumulating power at this date. This was exhibited both in the quantity of nitrate in the soil when sampled, and in subsequent accumulations when nitrogen was added. See Tables XIV and XVI. Soil collected May 4, 1915.

TABLE XVII.—ACCUMULATION OF NITRATES IN SOIL FROM DIFFERENT DEPTHS

(Mg. NO_3 per 100 gm. soil)

Soil (a)	1 A	9 A	13 A	15 A	1 E	9 E	13 E	15 E
C.c. water per 100 gm. soil (b)	13	13	16	13	18.5	16.5	22	21
Weeks of incubation:								
0	2.37	6.00	0.58	0.66	0 82	4.72	0.50	0.44
1	0.17	0.00	1.42	1.34	0.13	0.18	1.00	0.95
2	0.37	0.00	1.92	2.08	0.09	0.42	1.50	1.26
4	0.96	1.50	1.92	2.28	0.54	0.99	2.44	1.56
8	0.03	1.35	2.62	2.54	1.11	0.35	4.00	1.97
14	1.26	1.50	.....	.....	1.18	0.41	.....	.....
16	.....	.....	5.42	5.04	.....	.....	8.06	3.96
18	6.00	1.92	.....	.....	1.67	.....	.....	.....
31	.....	.....	6.62	6.54	.....	.....	11.50	4.96
33	6.18	0.00	.....	.....	2.18	0.87	.....	.....
52	9.63	2.70	6.02	6.34	4.38	4.38	14.40	.....
73	.....	.....	.....	6.94	.....	.....	19.50	.....
85	12.03	4.00	.....	.....	4.78	5.88	.....	.....
C.c. water per 100 gm. soil (c)	38	37	37	34	37	35	34	37
1	0.03	0.98	1.69	1.74	0.15	0.65	1.00	1.01
2	0.34	1.36	2.90	3.42.	0.21	0.86	2.72	1.74
4	4.34	2.58	5.08	5.58	0.98	1.76	6.22	2.92
8	12.63	5.62	7.62	8.78	2.83	3.46	8.70	4.00
14	22.53	7.20	.....	.....	5.36	5.72	.....	.....
16	.....	.....	11.42	17.54	.....	.....	16.10	8.06
18	33.00	10.80	.....	.....	6.00	10.00	.....	.....
31	.....	.....	13.82	22.54	.....	.....	22.00	9.86
33	44.73	15.45	.....	.....	11.18	13.43	.....	.....
52	45.23	16.10	23.92	31.74	13.18	15.28	26.50	13.96
73	.....	.....	18.62	37.84	.....	.....	.....	.....
85	57 63	21.00	.....	.....	17.18	26.98	.....	.....

(a) Soil collected 1 and 9, Nov. 19, 1914; 13 and 15, April 21, 1915.
(b) Moisture unchanged from field condition.
(c Soil moisture corrected to two-thirds saturation.

TABLE XVIII.—ACCUMULATION OF NITRATES IN SOIL FROM DIFFERENT DEPTHS

(Mg. NO_3 per 100 gm. soil)

Soil (a)	1 A	9 A	1 E	9 E
C. c. water per 100 gm. soil (b)	19	19	25	23
Weeks of incubation:				
0	0.75	0.60	0.50	0.50
1	2.00	1.72	1.34	1.30
2	4.01	2.02	2.22	2.30
4	6.83	2.84	2.60	3.22
8	11.57	4.40	4.50	5.22
16	25.91	7.64	8.30	10.16
24	32.25	10.70	11.50	13.30
53	36.45	12.60	.....	.....
C. c. water per 100 gm. soil (c)	40	39	40	39
1	4.25	1.40	2.66	2.23
2	8.13	2.50	4.30	3.64
4	15.25	5.60	7.30	6.54
8	22.65	7.40	11.50	18.50
16	43.69	14.40	19.50	23.50
24	55.65	19.40	25.20	25.30
53	59.25	21.00	29.50	.....

(a) Soil collected April 17, 1915.
(b) Moisture unchanged from field conditions.
(c) Soil moisture corrected to two-thirds saturation.

TABLE XIX.—ACCUMULATION OF NITRATES IN SOIL FROM DIFFERENT DEPTHS

(Mg. NO_3 per 100 gm. soil)

Soil (a)	1 A	9 A	1 E	9 E	1 F	9 F
C. c. water per 100 gm. soil (b)	18	19	22	21	22	21
Weeks of incubation:						
0	1.42	1.2	0.89	0.86	0.80	4.00
1	0.70	0.34	0.11	0.47	0.34	1.68
2	2.10	1.46	0.60	1.53	0.74	3.28
4	4.60	2.98	1.34	3.50	0.62	5.08
8	9.62	4.96	3.55	7.14	1.20	4.64
24	21.38	10.80	9.11	12.94	3.70	5.00
55	27.38	16.80	9.61	20.94	.....	.....
C. c. water per 100 gm. soil (c)	40	32	35	31	40	42
1	0.92	0.80	0.31	0.64	0.62	1.68
2	4.27	2.22	1.25	2.39	0.76	3.28
4	10.66	4.60	3.31	5.02	1.70	6.00
8	20.66	6.16	3.55	5.80	2.28	3.61
24	25.58	12.10	7.11	13.54	3.70	5.00
52	34.88	14.20	8.71	19.74	.....	.....

(a) Soil collected May 4, 1915.
(b) Moisture unchanged from field conditions.
(c) Soil moisture corrected to two-thirds saturation.

TABLE XX.—ACCUMULATION OF NITRATES IN SOIL FROM DIFFERENT DEPTHS

(Mg. NO_3 per 100 gm. soil)

Soil (a)	1 A	9 A	1 B	9 B	1 C	9 C	1 D	9 D
C. c. water per 100 gm. soil	37	35	37	37	40	37	40	43
Weeks of incubation:								
0	1.42	2.80	1.24	2.34	1.24	2.42	1.24	1.60
1	3.80	2.00	2.72	3.66	1.76	2.80	0.38	0.38
2	2.58	4.10	1.01	5.16	0.41	3.58	0.41	1.40
4	8.18	2.48	1.01	2.80	1.01	4.78	0.38	1.13
8	12.98	4.10	4.76	5.46	3.62	5.38	2.36	2.15
16	16.58	8.00	6.96	10.46	6.26	8.38	5.66	2.90
55	47.58	22.70	19.86	30.06	16.76	17.58	12.56	7.40
1 (b)	7.16	2.42	3.38	2.46	2.76	1.30	0.47	0.11
2 (b)	3.98	6.20	1.76	7.26	1.01	5.38	0.26	0.89
4 (b)	16.58	11.00	1.40	6.24	1.25	7.18	0.05	2.60
8 (b)	28.58	8.60	12.62	11.46	4.04	12.58	1.76	4.82
16 (b)	38.58	13.50	18.16	17.66	13.76	17.58	7.76	6.10
55 (b)	88.58	51.20	47.76	51.66	43.76	51.58	28.76	28.40

(a) Soil collected August 20, 1915.
(b) One gm. $CaCO_3$ added per 100 gm. soil.

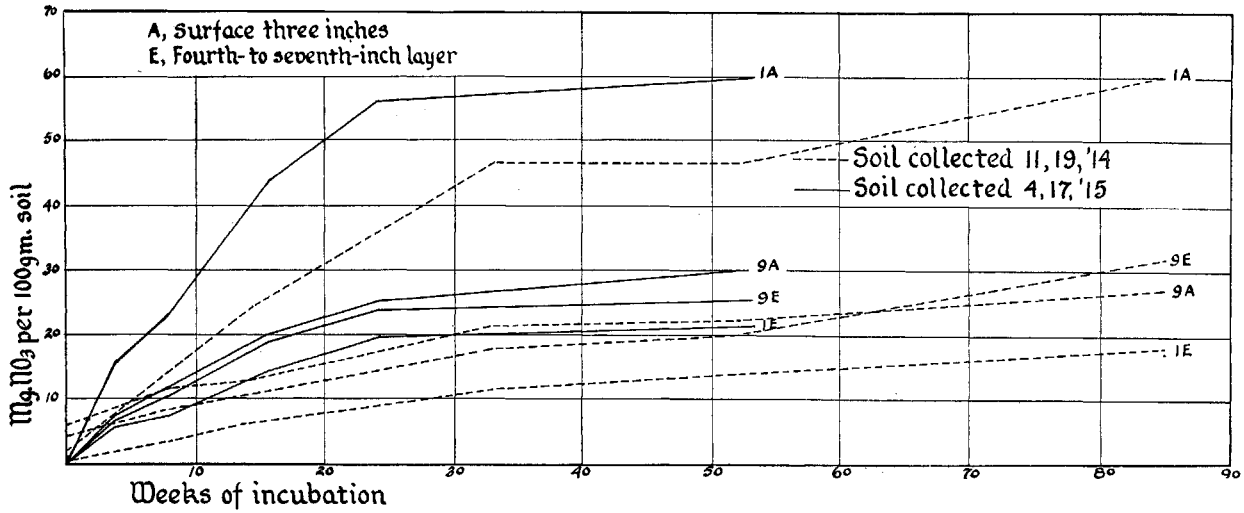

FIG. 5.—Graphs showing accumulation of NO_3 in soil from different depths

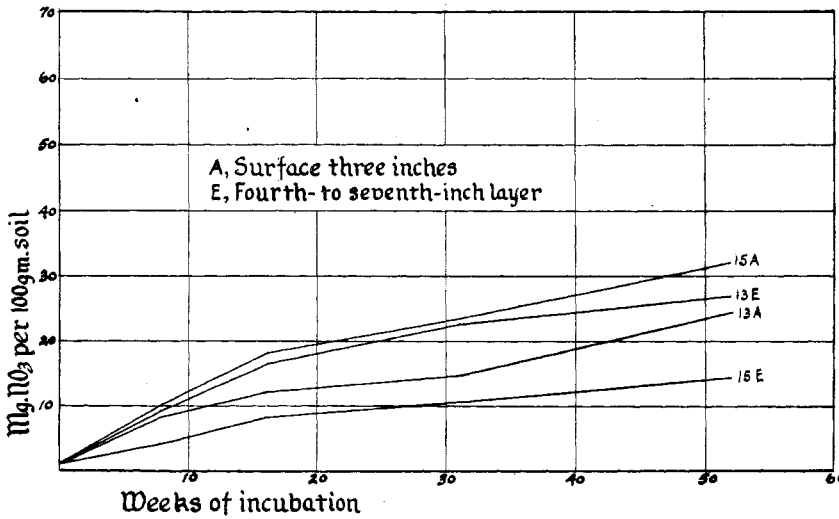

FIG. 6.—Graphs showing accumulation of NO_3 in soil from different depths. (Soil collected April 21, 1915)

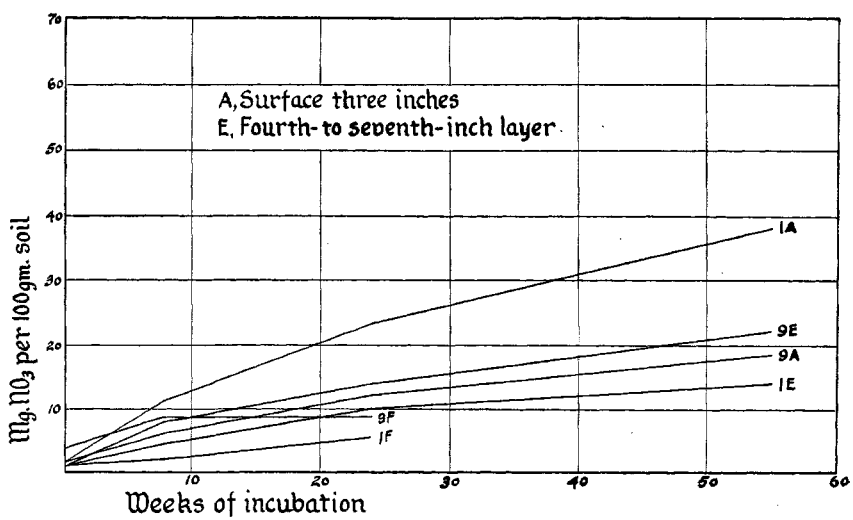

FIG. 7.—Graphs showing accumulation of NO_3 in soil from different depths. (Soil collected May 4, 1915)

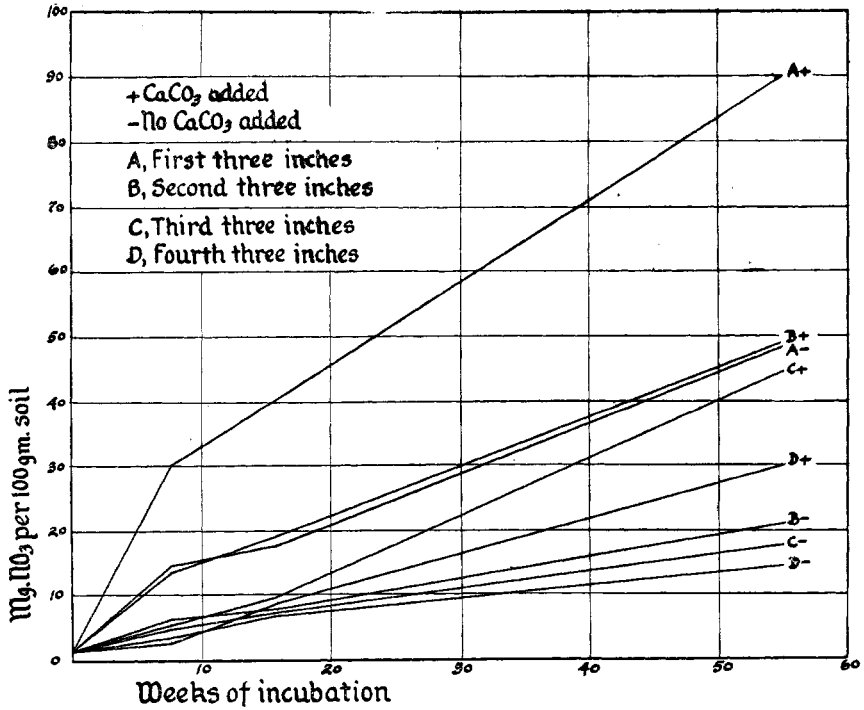

FIG. 8.—Graphs showing accumulation of NO_3 in soil from plot 1. (Soil collected August 20, 1915)

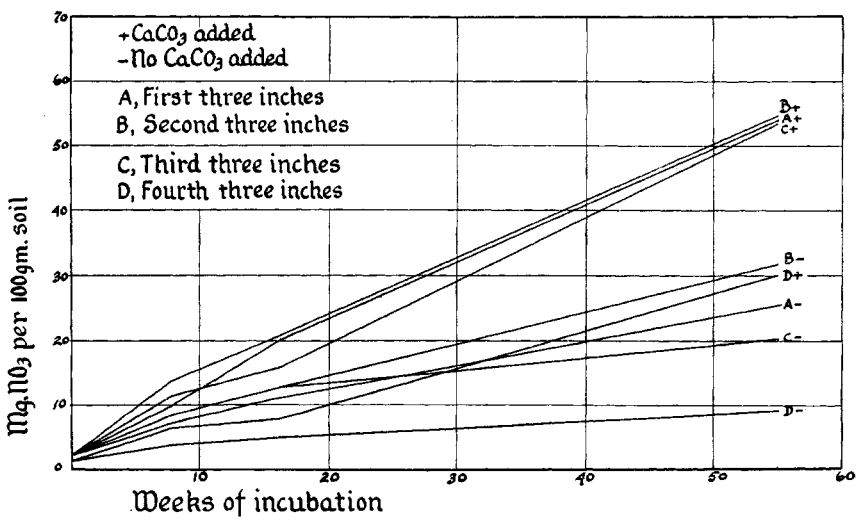

FIG. 9.—Graphs showing accumulation of NO_3 in soil from plot 9. (Soil collected August 20, 1915)

The major points brought out by the data here presented are as follows :

Soil from the surface three inches of plot 1, and to a less extent from plot 15, both shallow cultivated soils, showed very much greater nitrate gains than did soil from any other layers of any plot studied. This increased gain was strikingly evident only during the first few months of incubation after which the increases in all layers of all plots studied tended to run more or less parallel.

The increase in nitrate content in soil from the different cultivated layers in the deep cultivated plots, 9 and 13, were similar and approximately only one-half as great as the gain in the cultivated layer of the shallow cultivated plot 1.

Nitrate accumulations in soil below the cultivated layer, in either shallow or deep cultivated plots, were much less marked and decreased as the depth increased. Graphs of such gains tend to become a straight line from the beginning.

An addition of calcium carbonate materially increased the nitrate accumulation in all soils, but had little effect on the differences exhibited by the various soils.

The variations in nitrate accumulation were not so marked where the moisture content remained unchanged from field conditions as where it was corrected to two-thirds saturation.

The gains in nitrate nitrogen during the first few weeks were sometimes quite irregular, due perhaps to experimental error. There is the possibility, however, of a quantitative difference in that portion of the soil nitrogen capable of being very rapidly converted into nitrate nitrogen.

In all soils except where the moisture content was quite low, qualitative and quantitative determinations of ammonia nitrogen showed that there was no accumulation of nitrogen in this form. This would indicate that the nitrifying organisms in all layers of all soils studied were sufficiently active to oxidize ammonia nitrogen as rapidly as it could be formed from the native organic nitrogen. Ammonia nitrogen accumulated to a slight extent in some instances where the moisture was quite low, indicating that there are some ammonia forming organisms the minimum moisture requirement of which is lower than that of the nitrifying organisms.

CROSS INOCULATION EXPERIMENTS (NO NITROGEN ADDED)

The results of the cross inoculation experiments to which no nitrogen was added are reported in Tables XXI and XXII. In these, as in other cross inoculation experiments, the inoculum consisted of the supernatent liquid prepared by shaking one part of soil with two parts of water and allowing to stand a few minutes. Sufficiently large quantities of the suspension were added to insure its distribution throughout the soil.

TABLE XXI.—CROSS INOCULATION EXPERIMENTS WITH SOIL FROM DIFFERENT DEPTHS

(Mg. NO_3 per 100 gm. soil)

Inoculum (*a*)	1 A	9 A	1 E	9 E	Average
C. c. water per 100 gm. soil	40	39	40	39	
Soil 1 A	28.25	13.40	10.70	12.50	16.21
Soil 9 A	28.65	12.70	9.50	12.20	15.76
Soil 1 E	28.25	14.90	9.90	16.50	16.66
Soil 9 E	27.85	12.70	9.80	12.80	15.79
Average	28.35	13.42	9.97	12.75	16.12
Inoculum (*a*)	13 A	15 A	13 E	15 E	Average
C.c. water per 100 gm. soil	37	34	34	37	
Soil 13 A	9.74	10.78	12.30	4.89	9.36
Soil 15 A	8.78	10.78	12.30	6.22	9.52
Soil 13 E	9.42	9.38	11.34	5.72	9.96
Soil 15 E	7.42	9.38	11.82	6.22	8.71
Average	8.84	10.08	11.94	5.76	9.15

(*a*) Soil collected April 17 and 21, 1915. Incubation eight weeks.

TABLE XXII.—CROSS INOCULATION EXPERIMENTS WITH SOIL FROM DIFFERENT DEPTHS

(Mg. NO_3 per 100 gm. soil)

Inoculum (*a*)	1 A	9 A	1 E	9 E	1 F	9 F	Average
C. c. water per 100 gm. soil	40	32	35	31	40	42	
Soil 1 A (*b*)	11.92	4.52	4.31	5.80	2.54	5.44	5.75
Soil 9 A (*b*)	13.75	4.52	3.91	5.54	1.86	5.44	5.83
Soil 1 E (*b*)	13.38	4.52	3.81	5.14	2.18	5.44	5.75
Soil 9 E (*b*)	13.22	4.96	3.61	5.18	2.12	4.88	5.66
Soil 1 F (*b*)	11.92	4.00	3.31	4.47	2.08	5.44	5.20
Soil 9 F (*b*)	13.22	4.96	3.31	4.47	2.40	5.44	5.63
Average	12.67	4.58	3.71	5.10	2.20	5.35	5.62
Soil 1 A (*c*)	28.58	10.80	7.63	13.54	5.08	5.70	11.89
Soil 9 A (*c*)	27.98	10.80	8.61	13.84	4.84	6.00	12.01
Soil 1 E (*c*)	28.58	10.08	7.81	14.14	5.08	5.50	11.87
Soil 9 E (*c*)	28.58	9.36	7.81	15.44	4.84	6.30	12.05
Soil 1 F (*c*)	28.58	10.08	6.83	12.44	5.20	6.00	11.52
Soil 9 F (*c*)	30.38	10.80	7.81	11.94	6 16	6.00	12.18
Average	28.91	10.34	7.75	13.76	5.20	5.92	11.93

(*a*) Soil collected May 4, 1915. (*b*) Incubation four weeks. (*c*) Incubation twenty weeks.

The results here presented show that in the absence of an addition of nitrogen, despite the rather wide range in nitrate-accumulating power exhibited by the different layers of soil, the addition of the flora of those soils showing a high nitrate accumulation to those showing a low accumulation, was without effect. This would indicate that the low accumulations were due to an inherent quality of the soil rather than to its microorganic life.

AMMONIA IN SOIL UNDER FIELD CONDITIONS

In the data thus far presented there is little if any evidence to indicate the inability of the nitrifying organisms in all plots to oxidize ammonia nitrogen as rapidly as it is formed from the native nitrogen. This is evidenced by the failure of ammonia nitrogen to accumulate under experimental conditions. Such a failure might be due, not only to nitrification, but to the disappearance of ammonia nitrogen through other processes. Since the nitrifying organisms are more susceptible to the action of certain antiseptics than many of the ammonifying organisms it is possible to check the action of the former and still permit the latter to proceed in normal or perhaps slightly accelerated activity. We have carried out experiments of this nature using toluol as an antiseptic and it has been found in all cases that where the processes of nitrification are thus checked ammonia nitrogen will accumulate. Ammonia will continue to accumulate as long as the processes of nitrification are held in check. Such evidence tends to show that the failure to observe accumulations of ammonia in laboratory experiments has been due to the conversion of ammonia nitrogen into nitrate nitrogen as rapidly as it was formed, rather than to any inability of the soil to form or retain ammonia nitrogen as such. The writer has called attention (1917) to the probability that the failure to observe accumulations of ammonia nitrogen under field conditions is also due largely to the processes of nitrification. In this paper it was shown that checking the processes of nitrification under field conditions resulted in ammonia nitrogen accumulating.

Applying these principles to the problem under consideration, one would expect to find larger quantities of ammonia nitrogen under field conditions in those soils showing low nitrate nitrogen content, than in those showing high nitrate nitrogen

TABLE XXIII.—AMMONIA NITROGEN IN SOIL

(Mg. nitrogen as NH_3 per 100 gm. soil)

Method of determination	Plot No.	Date sampled											Average
		7-24-'15	7-27-'15	9-14-'15	10-30-'15	11-1-'15	11-4-'15	11-9-'15	3-22-'16	3-31-'16	4-5-'16	4-12-'16	5-10-'16	
Aeration	1	1.80	.....	1.35	0.94	.....	.....	1.23	1.33	.....	0.84	.....	1.42	1.27
Aeration	9	2.30	.....	1.45	1.28	.....	.....	1.99	1.15	.....	1.13	.....	1.65	1.52
Distillation	1	.....	.....	.....	1.92	.....	.....	1.83	1.21	.....	.....	.....	1.87	1.71
Distillation	9	.....	.....	.....	1.91	.....	.....	1.90	1.07	.....	.....	.....	1.89	1.69
Aeration	13	.....	2.80	.....	.....	1.81	1.64	.....	.....	0.69	.....	0.88	.....	1.56
Aeration	15	.....	1.38	.....	.....	1.21	1.16	.....	.....	.84	.....	1.15	.....	1.15
Distillation	13	.....	.....	.....	.....	1.20	2.33	.....	.....	1.28	.....	1.06	.....	1.47
Distillation	15	.....	.....	.....	.....	1.05	2.00	.....	.....	1.17	.....	1.17	.....	1.35

content, provided ammonia nitrogen were being formed with equal rapidity under the two conditions. In order to ascertain if such a condition existed a number of quantitative determinations of the ammonia nitrogen content of soil as it came from the field were made. The results are presented in Table XXIII. Ammonia determinations were made both by the magnesium oxide distillation method and by the aeration method (Potter and Snyder, 1914), the results secured from the two methods being reported separately. It will be observed that plots 1 and 9 were sampled on the same dates and that 13 and 15 were likewise sampled on the same day. The only accurate comparisons one is justified in making from these data are, therefore, between plots 1 and 9 and between 13 and 15. This suggestion is made because, as the data show, the ammonia nitrogen content of any individual plot is subject to variations from time to time. When the number of determinations are no larger than here recorded such variations might influence materially the averages.

The data secured by the distillation method show but slight difference between the ammonia nitrogen content of soil from plots showing high and those showing low nitrate accumulation under field conditions. The aeration method data, however, show more ammonia nitrogen liberated from the soil of plots 9 and 13 than from the soil of plots 1 and 15. Both 9 and 13 are deep cultivated plots, the former an early and the latter a late plowed one. If only the data accumulated during the seedbed preparation season and fall months, the time when the largest differences exist in the nitrate nitrogen content are considered, the differences in ammonia content by the aeration method are more marked.

There is much experimental data showing that when magnesium oxide is used as the alkali, and distillation carried out at normal atmospheric pressure, nitrogen in the form of ammonia will be liberated from many of the decomposition products of the protein molecule. This may account for the differences observed in the quantity of ammonia nitrogen as determined by the two methods. Such an explanation involves the existence in soil from plots 1 and 15 of larger quantities of such compounds. An effort to detect differences in the quantity of nitrogen present in the soil, soluble in dilute alkali and capable of being liberated as free nitrogen by nitrous acid, as

in the Van Slyke method for amino nitrogen, gave negative results. This would indicate that the increased quantity of ammonia distilled from soil of plots 1 and 15 did not come from amino acids. Furthermore, Jodidi (1912) has shown that such compounds as result from the more complete hydrolysis of proteins are, as a rule, readily further hydrolyzed or oxidized by microorganisms with the liberation of ammonia. This being true we should not expect an accumulation of such compounds. All these facts would indicate that the earlier stages of decomposition are probably lacking. On the other hand, the shallow cultivated soils do contain more easily decomposable organic nitrogen, otherwise magnesium oxide should not have liberated a larger quantity of ammonia nitrogen than was secured by the aeration method.

DISCUSSION OF THE VARIOUS FACTORS INFLUENCING AMMONIA AND NITRATE FORMATION UNDER FIELD AND LABORATORY CONDITIONS

GENERAL CONSIDERATIONS

The data thus far presented are conclusive on at least one very important point; namely, that transferring the soil of those plots which under field conditions show a very low nitrate accumulating power to laboratory conditions completely eliminates or overshadows the factor or factors responsible for low accumulation under natural conditions. It is also evident that the rate of nitrate accumulation is limited by the rate of ammonia formation.

The ability of the soil organisms to produce ammonia under laboratory conditions has been found to be equally as great in soil from plots showing a low nitrate accumulation under field conditions as it is in soils showing a high nitrate accumulation. The same is true regarding the various layers of soil from high and low nitrate producing plots. However, in the surface few inches of plot 1, late shallow cultivated, the formation of ammonia and subsequent accumulation of nitrates from the native nitrogen is very much more rapid than from any layer of plot 9, a deep early cultivated plot. On the other hand, any layer of plot 9 included within the stirred area will show a more rapid accumulation of nitrate nitrogen than any layer of plot 1, below the surface three inches. The formation of

ammonia and accumulation of nitrates in the layers below the cultivated soil in both plots differ but little.

The difference in ammonia production and subsequent nitrate accumulation in different layers of the various plots does not appear to be due to the inability of the organisms to liberate ammonia or to any physical or chemical condition which prevents their activity. When nitrogen in the form of cottonseed meal is added, as rapid ammonia production will take place in soil from one plot or layer as from another. Yet when the native nitrogen only is present large differences are exhibited in nitrate accumulation. Apparently the changed environmental conditions eliminate or render inactive an agent inhibiting ammonia production in certain soils, or there is a difference either qualitative or quantitative, or both, in the nitrogen content of the various soils. Perhaps a more specific consideration of the important factors influencing nitrate accumulation, which undergo rather marked changes in transferring the soil from field to laboratory conditions, will give valuable information. Some of the important factors influencing nitrate accumulation, which are effected by the change to which the soil is subjected, are (1) Distribution of organic nitrogen; (2) character of the nitrogen; (3) cultivation and aeration; (4) reaction; (5) influence of the addition of a new flora; (6) temperature; and (7) moisture.

DISTRIBUTION OF ORGANIC NITROGEN

Upon purely logical grounds a difference in the nitrogen content of the various plots or layers of the same plot might be expected. Where large wheat yields have been produced and entirely removed, save stubble, there have certainly been large quantities of nitrogen removed in the crop. No artificial return of nitrogen in any form has been made to any plot. Unless there is some natural compensating agent of which nothing is known one should expect to find a difference in the total nitrogen content of different plots equivalent to the differences in quantities removed. These plots have not been under the present treatment for many years and hence the difference in nitrogen content due to the removal of nitrogen in the crop could not be very large. However, if the present system is continued indefinitely, without compensating for such losses, the high yielding plots will certainly be the first to become depleted of nitrogen.

Again the major portion of the remains of a wheat crop and subsequent growth of weeds is found either on the surface or in the first few inches of soil. Where deep cultivation is practiced this accumulation of organic debris will become more or less thoroughly incorporated throughout the cultivated soil. Where cultivation is shallow, it will remain on the surface or in the first few inches.

A physical examination of soil from the different layers of the plots under study has revealed the effects of the operation of some such factors as those mentioned above. No differences can be observed in soil from the various layers of plot 9, down to the depth to which cultivation has taken place, i. e., seven inches. Soil from the surface three inches of plot 1 (shallow cultivated), however, appears to be thoroughly impregnated with undecomposed plant residue. Below this layer the soil of plot 1 resembles very much the soil below the cultivated area of plot 9.

Quantitative determinations of the nitrogen content of soil from the surface three-inch layer and from the layer lying between four and seven inches deep of plots 1 and 9 gave the following results:

Surface three inches, plot 1	0.1761	percent
" " " " 9	.1486	"
Fourth- to seventh-inch layer, plot 1	.1669	"
" " " " " " 9	.1501	"

It is not believed that all the differences observed between the nitrogen content of plots 1 and 9 have been brought about during the course of these experiments. Plot 1 lies somewhat lower and the soil is somewhat heavier, factors which would tend toward a collection and retention of organic matter. The differences between soil from the two layers of plot 1 have, however, probably been brought about during the course of the experiment.

An effort was made to measure that portion of the organic matter of the four layers of soil under consideration which has undergone little or no decomposition. Such a determination is rather difficult and can of course be only comparative. A purely arbitrary method based largely upon specific gravity was employed. The results are given in Table XXIV. Duplicate determinations agreed well and it is impossible that the major difference in figures given could have been due to accident. The organic matter separated consisted largely of plant tissue which had undergone little decomposition, the physical characteristics being preserved to a large extent.

TABLE XXIV.—ORGANIC MATTER IN DIFFERENT LAYERS OF SOIL

Source of soil	Lbs. of organic matter per 1,000,000 lbs. of soil	Percent of nitrogen in organic matter	Lbs. of nitrogen contained in the organic matter
0″ to 3″, plot 1	13,500	1.00	135.5
0″ to 3″, plot 9	3,380	1.03	34.9
4″ to 7″, plot 1	2,180	1.17	25.5
4″ to 7″, plot 9	3,740	0.90	33.6

The normal decomposition which organic matter of the soil undergoes results in an increased nitrogen content of the residue. The difference in the nitrogen content of the organic matter from the surface three inches and the four- to seven-inch layer of plot 1 would indicate that decomposition had proceeded further in the soil from the lower layer. It is believed that the high nitrogen content of organic matter from the second layer of plot 1 together with the low total quantity of organic matter are very significant facts.

According to these figures there is 10,000 pounds more undecomposed organic matter, containing 100 pounds nitrogen per 1,000,000 pounds soil, in the surface of plot 1 than in the surface of plot 9 or in the four- to seven-inch layer of either plot. The difference in the nitrogen content of the two layers of plot 1 can be accounted for in this manner. These experimental data are in perfect agreement with the theoretical conclusions previously arrived at and undoubtedly offer a possible explanation for a part of the differences in nitrate accumulation observed in soil from various layers under laboratory conditions. It will be noted from figure 5 that when the nitrate content of the surface soil of plot 1 exceeded that of the soil from other layers by approximately 100 pounds nitrogen per 1,000,000 pounds soil, succeeding accumulations of nitrate nitrogen ran more or less parallel.

CHARACTER OF THE ORGANIC NITROGEN

If the slight difference in nitrogen content, just noted, is accepted as an explanation of the difference in nitrate accumulation in soil from the various layers, it is necessary to make another assumption; namely, that this nitrogen is radically different in nature, physically or chemically, from the other 1,500 pounds present. According to Hilgard's (1914) view, one which has dominated soil science for many years, only that organic nitrogen present in soils in the form of "humus" is

capable of being rapidly transformed into nitrate nitrogen. The differences here reported appear to be in the form of undecomposed organic matter. There are certain facts, however, that have come to light since Hilgard formulated his views.

When organic matter with a relatively high nitrogen content, cottonseed meal, dried blood, etc., is added to soil there is a very rapid transformation of organic nitrogen into ammoniacal nitrogen during the first few weeks. This change involves only a fairly constant percent of the contained nitrogen, depending upon the material. After the expiration of this period of rather rapid transformation, conversions of the remainder of the nitrogen into ammonia take place very slowly. Rarely ever is it possible to recover, as ammonia, the total quantity added. Apparently there is a certain proportion of the organic nitrogen content of different materials that is capable of being rapidly transformed into ammonia and another portion which is incapable of being rapidly liberated as ammonia. It is believed that similar relations hold true regarding all organic plant and animal residue containing nitrogen and from which all available nitrogen must come. A certain proportion of the added organic nitrogen, or that which annually accumulates in the soil, is, under favorable conditions, rapidly changed into ammonia and thence to nitrate nitrogen. Another portion, constituting a varying percent of the organic nitrogen, is transformed into ammonia nitrogen with great difficulty and comparatively slowly under its normal conditions. This latter form has gradually accumulated and constitutes the major portion of the organic nitrogen of the soil. If the biological equilibrium of a soil is upset by some radical physical or chemical change a portion of the organic nitrogen which has been undergoing very slow change is, under the new environment, much more easily affected by the microorganisms. Such conditions are brought about by plowing virgin soil or by the addition of lime to a soil deficient in lime. Successful agriculture is dependent upon keeping up the supply of fresh organic matter, not because there is an insufficient supply of nitrogen in the soil to produce high yields but because it does not become available rapidly enough. A heavy demand for nitrogen can only be supplied from nitrogen capable of becoming readily available.

The Rothamsted Station has shown that after nearly three quarters of a century of continuous wheat culture upon the

same soil with no artificial addition of nitrogen, save that in the seeds, the soil still contains approximately 2,500 pounds of nitrogen in the first nine inches. Yet because of the lack of available nitrogen this plot (Broadbalk wheat No. 3) is incapable of producing more than approximately 12 bushels of wheat per acre. One is justified in saying that this low yield is largely due to lack of available nitrogen because another plot in the same series receiving complete mineral manure and containing approximately the same quantity of nitrogen produces practically the same yield, while still other plots receiving, in addition, nitrogenous fertilizers produce good yields.

During the first few years the yield on plot 3 declined rather rapidly. During the last half century the yield has remained practically constant. Since under these experimental conditions the yield of wheat is to a large extent proportional to the available nitrogen, it may be said that during the first few years there was a rapid decline in the nitrogen annually rendered available, while during the last half century the nitrogen annually rendered available has remained practically constant.

The conditions at Rothamsted Station on the Broadbalk wheat plots are such that drainage takes place readily. Any nitrogen rendered available under such conditions and not utilized by growth taking place on or in the soil will be removed in drainage water. The total loss of nitrogen from these soils should, therefore, give a fair conception of the rapidity with which the soil's original store of nitrogen is rendered available. Fortunately data are available covering a long period of years as to the quantitative changes taking place in the soil nitrogen of a number of the Rothamsted plots, including plot 3 mentioned above.

The first accurate analysis of the nitrogen content of these plots was made in 1865. From numerous analyses of adjacent soil, however, Gilbert (1895) estimated the nitrogen content of the first nine inches to be 0.125 percent when the present system of treatment was begun in 1843. According to Russell (1912) the first nine inches of soil weighed approximately 2,600,000 pounds, The nitrogen content of the first nine inches of plot 3 in 1843 was, therefore, according to the available evidence, 3,250 pounds. Up until 1865 this soil had lost, according to above estimate, 24 pounds of nitrogen per annum. From 1865 to 1912 the annual loss was only approximately 4.5

pounds per year. Expressed otherwise this soil during 47 years, 1865 to 1912, was able to render available annually 0.16 of 1 percent of its original store of organic nitrogen.

In this same series of plots there is one which has received an annual application of 200 pounds organic nitrogen in the form of stable manure. Calculating the total quantity of fresh organic nitrogen added from 1843 to 1865 together with that present in 1843 and subtracting that present in 1865, there is an annual loss of 150 pounds per acre. Similar calculations from 1865 to 1912 show an annual loss of 170 pounds per acre. The decomposition of fresh organic nitrogen probably, to a slight extent, prevents the decomposition of the original store of nitrogen, but assuming that the loss in the original store of nitrogen in plot 2 was the same as in plot 3 and subtracting this 4.5 pounds from the 170 pounds we have an annual net loss from the 200 pounds organic nitrogen added in manure of 165.5 pounds, or more than 80 percent of that annually added. Expressing these figures in comparative terms it is found, per unit of organic nitrogen, that contained in the manure is becoming available 500 times as rapidly as is the soil's original store of organic nitrogen. How can such a wide difference in the rate at which the organic nitrogen is being rendered available or soluble under similar soil and climatic conditions be explained?

Fortunately Dyer (1902) has reported so-called "humus" determinations on soil from these plots as well as from a number of others. The following results are copied from Dyer's table:

	Plot 2	Plot 3
Percent soluble organic matter	1.7930	0.8200
Percent total nitrogen	.2170	.0966
Percent soluble nitrogen	.1140	.0500
Percent nitrogen in soluble organic matter	6.3800	6.1300
Percent total nitrogen soluble	52.5300	51.7600

It will be observed that while the percent of total and of soluble nitrogen in plot 2 is more than twice as great as in plot 3, the percent of nitrogen in soluble organic matter and the percent of total nitrogen soluble are practically the same. If, as Hilgard contended, the nitrogen rendered available in a soil is dependent upon the "humus nitrogen" content, the quantity of nitrogen rendered available per unit of total nitrogen in these two soils should be practically the same and neither should exhibit nitrogen hunger, yet plot 3 is the poorest yield-

ing in the wheat series, the low yield being due almost entirely to a deficiency in available nitrogen, while plot 2 is the most fertile plot in the entire series. (The addition of a complete mineral fertilizer to plot 5 increased the yield only approximately two bushels per acre.) As mentioned earlier, the nitrogen in plot 2 is being rendered available per unit of nitrogen, 21 times as rapidly as is that in plot 3, while that annually added to plot 2 as organic nitrogen in barnyard manure is being rendered available 500 times as rapidly as is the soil's original store of nitrogen.

The total nitrogen content of all the Rothamsted soils examined by Dyer varied from 0.0966 to 0.5876 percent, while the total nitrogen soluble varied only from 41.24 to 66.8 percent.

Dyer says: "It would seem, therefore, that although the ratio on which Professor Hilgard lays so much stress (percent nitrogen in soluble organic matter) may serve as an index to initial nitrogenous fertility in soils comparatively newly brought under cultivation, it does not, afford a means of differentiating between a starved and a fertile soil in the case of long-cultivated land such as we have at Rothamsted." And further — "that as far as the Broadbalk wheat field is concerned the determination of soluble nitrogen in the manner described does not give us any more information as to potential fertility than would be derived merely from the determination of the total nitrogen."

As further evidence of the comparative narrow limits of fluctuation in the percent of the nitrogen of soils that would be classed as "humus nitrogen'' as compared with the wide variations in total nitrogen the following work is cited: Gortner (1916) has reported what is believed to be the most carefully executed study of this problem as yet carried out. Data are given secured from a number of soils in which the percent nitrogen varied from 0.128 to 1.34. The percent of the total nitrogen that would be classed as "humus nitrogen" only varied from 61.05 to 74.49. Expressing these variations in the percent that the maximum in each instance varied from the minimum we have 946 percent in the case of total nitrogen and 22 percent in humus nitrogen, giving a ratio of 43 to 1. Similar calculations based upon a large number of soils covering a wide range in total nitrogen, "humus nitrogen," and pro-

ductivity, reported by Fulmer (1896) give a ratio of 23 to 1. So close a relation did Fulmer find between the total nitrogen and the "humus nitrogen" in normal soils that he evolved a formula whereby given the percent of nitrogen in humus and the percent of humus, he could predict with a fair degree of accuracy, the percent of total nitrogen in the soil. Gortner has also shown that the percent of total nitrogen in "unhumified" vegetable matter (68.45) that would be classed as "humus nitrogen" does not vary materially from the percent of the total nitrogen in soils (68.53) that'would be classed as "humus nitrogen." These various facts mean that regardless of the total nitrogen present in a soil the percent of the total nitrogen existing in the "humus" condition does not vary widely. Therefore, if the nitrogen rendered available in a soil is dependent upon the "humus nitrogen" content, it should not vary widely per unit total nitrogen.

Fraps and Hammer (1910) and Gortner (1916) have also shown that the addition of organic materials (Fraps and Hammer used stable manure) to a soil does not increase the "humus" content, provided the "humus" content of the "unhumified" organic materials is taken into consideration. If the above assumptions are justified the wide relative difference in the rate at which the two classes of organic nitrogen are rendered available under Rothamsted conditions certainly cannot be explained upon a "humus" basis.

It is believed the explanation offered 35 years ago by Laws, Gilbert, and Warrington (1883) accounts for the differences cited above more satisfactorily than any succeeding substitute. Quoting verbatim: "Of the quantity of nitrates produced solely from the oxidation of nitrogenous humic matter, including crop residue, . , . we have examples in the case of plots 3, , . . In plots 2, . . . we have examples of the influence of barnyard manure . . , in increasing the amount of nitrates produced in the soil.

"Soil contains nitrogenous matter which nitrifies with different degrees of facility. The bulk of the nitrogenous matter of the soil is only capable of very slow oxidation, but a smaller proportion is far more readily converted into nitric acid. In thoroughly exhausted land the easily nitrifiable matter has to a large extent disappeared; in the soil in good agricultural condition it is being continually renewed by fresh crop-residues,

or by the application of organic manures. This easily nitrifiable matter constitutes a chief part of the floating capital of the soil, on which its immediate productiveness depends. The larger quantity of more inert nitrogenous matter constitutes the sunk capital which only very slowly becomes available."

Weir (1915) of Rothamsted Station carried out a series of experiments to ascertain whether or not active nitrogen ($NH_3 + NO_3$) was derived largely from that portion of the soil nitrogen soluble in weak alkali. After treating soils with N/5 HCl they were extracted with 2 percent NaOH for five days, the solution being renewed daily. The soils were then freed of NaOH, rendered alkaline with $CaCO_3$ and inoculated. During the first few weeks (up to 16) of incubation such treated soils, in every comparison except two, showed a higher active nitrogen content than unextracted soils. This was true in spite of the fact that the number of microorganisms in treated soils was two to ten times as great as in untreated soils and they had of course consumed two to ten times as much active nitrogen.

In the light of present experimental data it would seem to be much more rational to assume just the reverse of Hilgard's hypothesis; namely, that so-called "humus" is that portion of the organic matter which remains after active bacterial action. It is, therefore, necessarily more resistant to such action; in fact, is the most difficult portion of the soil nitrogen for the normal soil flora to render available for plant metabolism.

It is not wished to minimize the value of keeping up the supply of "humus" in agricultural soils. No rational system of soil fertility can long eliminate the addition to the soil of organic matter. "Humus" is the residue left from the decomposition of organic matter. If a sufficient quantity of easily decomposable organic matter is supplied, the "humus" supply will likewise be kept up; or if the up-keep of "humus" is maintained it necessarily follows that the supply of easily decomposable organic matter will be maintained.[1]

It is believed then, that the differences in the rate of nitrate accumulation in soil of the various plots, or layers of the individual plots under laboratory conditions, can be attributed to the quantity of nitrogen capable of being rapidly trans-

[1] "Humus" is a very intangible term. It has been used here in the sense that is in common use in agricultural literature. All the nitrogen extracted from soil by dilute alkali will not fall within such a classification, for it is well known that a part of the nitrogen in any organic substance can be and will be extracted by such solvents regardless of whether it has undergone the so-called process of "humification."

formed into ammonia. Also, that differences in nitrate accumulation under field conditions are in part due to differences in the rate of ammonia formation, which in turn is due to differences in the decomposition of organic matter. In no case, however, is the difference in rate of decomposition due to any lack of organisms capable of becoming active, but to some factor or factors inhibitory to their activity.

CULTIVATION AND AERATION

The above facts do not offer an explanation for the differences in the activity of the nitrifying flora in the stirred and unstirred soils of the various plots. Similar phenomena have frequently been observed and a number of explanations offered. Greaves (1914), Reed and Williams (1915), and others have called attention to greater activity being exhibited by the flora of soils which have been in cultivation than by those of adjacent virgin soils. Likewise, King and Whitson (1901) and a number of others have observed nitrifying activity to vary with variations in depth and frequency of cultivation. Such differences are more often attributed to aeration than to any other factor. Gainey and Metzler (1917) have recently submitted evidence tending to show that such an explanation, as far as local soils are concerned, is untenable.

Laboratory Experiments.—To ascertain the effect of aeration on ammonia and nitrate formation, the author conducted a number of experiments, among which are the following: Columns of soil seven inches long and five inches in diameter were taken from plot 1. These were brought to the laboratory and incubated intact. Other columns collected at the same time within a few inches of the same place were thoroughly cultivated by passing through a three-millimeter sieve and incubated. In order to prevent loss of moisture the columns were incubated in anaerobic jars through which a current of moist air was drawn. The results are given in Table XXV.

TABLE XXV.—ACCUMULATION OF NITRATES IN SOIL FROM PLOT 1

Sample No.	Condition of soil	Weeks of incubation	Moisture content in c. c. per 100 gm. soil	Gain mg. NO_3 per 100 gm. soil
1 (*a*)	Column undisturbed	7	25	9.60
2 (*a*)	Sieved soil	7	25	6.50
3 (*b*)	Column undisturbed	4	24	0.32
4 (*b*)	Sieved soil	4	24	0.07
5 (*b*)	Column undisturbed	12	24	2.50
6 (*b*)	Sieved soil	12	24	1.60

(*a*) Soil collected July 22, 1915. (*b*) Soil collected Sept. 22, 1915.

From the data given in this table it appears that this soil is capable of producing just as large quantities of nitrate nitrogen without being subjected to cultivation as when thoroughly stirred.

In addition to the above experiments similar columns were taken and subjected to the following conditions: One column seven inches long was well moistened over the bottom and lower sides with a solution of ammonium sulphate and dusted with calcium carbonate. It was then placed in an anaerobic jar and all space between column and jar on both bottom and sides was filled by pouring melted paraffin around the column. The paraffin ran into all cracks and holes made in transferring the soil to the jar. In this way all aeration was forced to take place through the normal surface. No method was available for regulating the concentration of ammonia and getting the calcium carbonate into the column. Furthermore, it has since been ascertained by the writer (1917) that paraffin in contact with soil will decrease the nitrate accumulation enormously. Nevertheless, the soil of this column showed an increase of 14.5 mg. NO_3 per 100 gm. soil. In the bottom of another jar was placed two inches of loose soil containing ammonium sulphate and calcium carbonate. On top of this was placed a five-inch unbroken column and all the space surrounding it was filled with paraffin as above. All oxygen reaching the loose soil was thus forced to pass through the column. In this experiment all the ammonia nitrogen was converted into nitrate, the loose soil below showing a net gain of 39.8 mg. NO_3 per 100 gm. soil.

In order to show that the loose soil in the above experiment did not contain sufficient oxygen to bring about the oxidation of the added nitrogen a bottle was filled with loose soil and sealed. This soil would be similar to the loose soil below a solid column provided aeration did not take place through the column. Soil in the sealed bottle not only failed to show an increase in nitrate content but showed an actual loss of 0.9 mg. NO_3 per 100 gm. soil, or one-half of that initially present. Loose soil containing the same quantity of ammonia and calcium carbonate, and incubated with free access to the air showed a net gain of 30 mg. NO_3 per 100 gm. soil. In no instance in these experiments was there any indication of a lack of sufficient aeration in the soil, approximating as near as possible uncultivated field conditions. In fact, when aeration was

forced to take place through the normal channels the supply of oxygen was sufficient for the nitrification of many times the quantity of ammonia normally available. This soil had not been cultivated for several years. The difference in nitrate accumulation in the seven-inch column and in the loose soil below the five-inch column was probably due to a difference in the concentration and distribution of ammonium sulphate and calcium carbonate rather than to aeration relations.

Field Experiments.—In addition to the above laboratory experiments, air was forced daily into perforated tubes placed underneath both cultivated and uncultivated plots during two seedbed preparation seasons without producing appreciable differences in nitrate accumulation.

Still another method was used in an effort to ascertain whether or not aeration, or oxygen, is the factor limiting nitrate accumulation in the soil of plot 1 under field conditions. This was to actually analyze the soil atmosphere. If oxygen is the factor limiting nitrate accumulation it would appear that an analysis of the soil atmosphere of plot 1 would reveal a deficiency of oxygen; or that the percent of oxygen in the soil atmosphere of plot 1 would be materially less than that of plot 9. On the other hand, if aeration is rapid and abundant in both plots, the exchange of gases between the soil and air above should be sufficiently rapid to maintain approximately the same composition in the two.

In a recent publication Gainey and Metzler (1917) called attention to the fact that previous analyses of soil atmosphere have usually shown only a slight decrease in the percent of oxygen while the CO_2 content has usually been very much higher than normal air, though never, as Plummer (1916) has shown, sufficiently high to interfere with nitrification. Attention was called to the work of Schloesing (1873), in which it was shown that the oxygen content of air available to nitrifying organisms could be reduced to one-half that of normal atmosphere without having an appreciable effect upon nitrate accumulation in soil.

In collecting samples of soil atmosphere for the analyses here reported use was made of a tube similar to the one described by Russell and Appleyard (1915). This was driven nine inches into the soil and by the use of mercury a 50 c.c. sample of gas was drawn into a pipette. Previous to collecting

the 50 c.c. sample, 30 c.c. were drawn and discarded. This quantity was sufficient to fill all the space in the soil tube and connections twice and was believed to be sufficient to free the apparatus of approximately all its original air content. At the same time it was not believed to be a sufficiently large quantity to cause an appreciable replacement of soil air by normal air thus causing the 50 c.c. sample taken for analyses to be composed of air just drawn into the soil. As a further precaution against securing air from above the soil, rather than from in the soil, an area of several inches surrounding the tube was removed, leaving a slight depression into which sufficient water was poured to form an effective seal. This prevented drawing air into the soil through any opening formed by the tube or any that might have existed in the immediate vicinity of the tube.

The samples collected as above, six in number, were brought to the laboratory and subjected to duplicate analyses for CO_2 and oxygen. The modified Haldane apparatus recommended by the Bureau of Mines (1913), was used and gave very satisfactory duplicate analyses. In Table XXVI is given a summary of the results. It is worthy of note that the variations in the composition of samples collected from various points on individual plots were very much greater than the variations in the average of the two plots.

Analyses were also made of the soil atmosphere from the corresponding plots of two other series, one of which had been running for four and the other for two seasons. These results varied but slightly from those just given.

It is evident, therefore, that if the analysis of the soil atmosphere gives any indication of actual conditions within the soil, the atmosphere of plot 1 varies but slightly from that of plot 9 and that in both plots the exchange of gases with the atmosphere above is sufficiently rapid to maintain approximately the same composition of oxygen in the soil air as in the air above the surface. The CO_2 content is very much higher than that of normal air, but it is not sufficiently high to exert any influence upon nitrification.

Experimental data presented in this bulletin show that cultivation affects beneficially the nitrifying organisms, but it is not believed that such beneficial effects are due to increased aeration. Furthermore, it is believed that the organisms in

TABLE XXVI.—COMPOSITION OF THE SOIL ATMOSPHERE

Date	July 18		Aug. 30		Sept. 7		Sept. 15		Sept. 25		Average	
Plot No.	Percent CO_2	Percent oxygen	Percent CO_2	Percent oxygen	Percent CO_2	Percent oxygen	Percent CO_2	Percent oxygen	Percent CO_2	Percent oxygen	Percent CO_2	Percent oxygen
1	0.263	20.68	0.311	20.91	0.404	20.10	0.242	20.38	0.233	19.95	0.291	20.40
9	.272	20.56	.315	20.54	.194	20.28	.800	20.25	.172	20.11	.227	20.35

the shallow uncultivated soils with which we have been working, have sufficient available oxygen to oxidize ammonia nitrogen as rapidly as it is formed. Cultivation facilitates the decomposition of organic matter. This necessarily supplies more ammonia and hence a larger source of energy to the nitrifying organisms. At the same time cultivation brings about a redistribution of the organisms breaking up and scattering groups which might be formed, thus bringing them into more intimate contact with the supplies of food and into better environmental conditions. Such factors would tend to keep up the number and vigor of the flora. Any factor that might tend to limit the growth and activity of organisms in uncultivated soils would tend to lower the total efficiency, but would not necessarily reduce it below the efficiency required to oxidize the ammonia normally available. If the organisms are supplied with an excess ammonia, as in nitrification experiments, those flora normally most efficient would oxidize the larger quantities.

REACTION

The increased accumulation of nitrates due to the addition of calcium carbonate is nothing out of the ordinary. Such a phenomenon is almost always observed in soils in which calcium carbonate is not present in large quantities. This soil had a lime requirement of 250 pounds per acre for the first seven inches, according to the Department of Agronomy.

The addition of calcium carbonate is no more essential for a rapid accumulation of nitrate nitrogen in soil of plot 1than in the soil of plot 9 when subjected to experimental conditions. Furthermore, calcium carbonate exerts just as great a stimulation on nitrate accumulation in the soil from plot 9 as in the soil from plot 1.

INFLUENCE OF THE ADDITION OF A NEW FLORA

The addition of the flora of soil from plot 9 to the soil of plot 1 was without effect, showing that the essential organisms are present in abundance in soil from those plots which under field conditions exhibit a low nitrate-accumulating power.

TEMPERATURE

There is no doubt that under laboratory conditions the temperature is more favorable than under field conditions. It is almost inconceivable, however, that the very slight differ-

ence in temperature between cultivated and non-cultivated soils (which Bouyoucos (1913) has shown to be only a fraction of a degree for this particular time of the year) could bring about an appreciable difference in nitrate accumulation under field conditions.

MOISTURE

No attempt has been made by the author to accumulate data as to the moisture relations of soil of the various plots under field conditions. This has been done by the Department of Agronomy and will appear in a forthcoming bulletin.

Under semi-arid conditions available moisture is perhaps more often than any other the limiting factor in microorganic

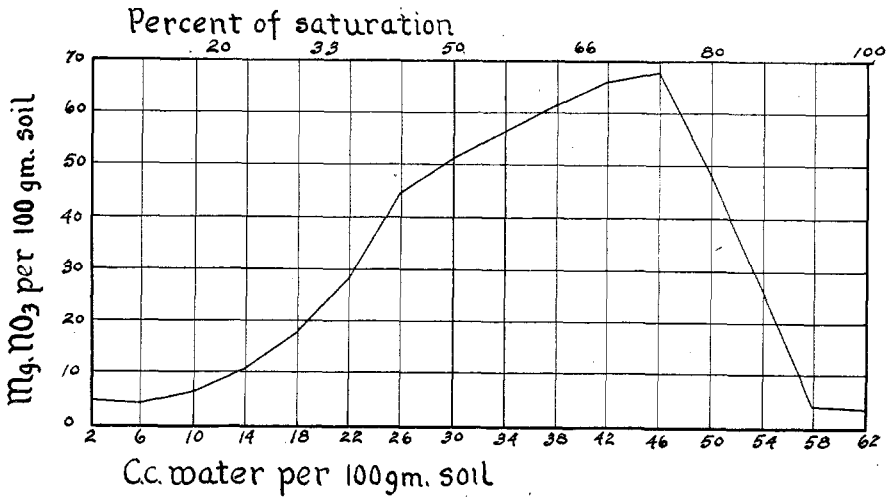

FIG. 10.—The effect of moisture upon NO_3 accumulation

activity under natural soil conditions. In figure 10 is shown the enormous effect of variations in moisture content upon nitrate accumulation. The lower the moisture content the greater the effect of slight variations. A difference of .1 percent in moisture content may have a possible effect of 100 percent in the accumulation of nitrate nitrogen when moisture content is near the minimum for the processes of nitrification. The moisture content of soils, especially the uncultivated ones, for this section is usually very low during the season of the year under consideration. Therefore, slight variations in the moisture content of the various plots might have a very marked effect upon the accumulation of nitrate nitrogen.

Under laboratory conditions we have usually adjusted the moisture relations to the optimum. It has been shown that in order to produce optimum moisture conditions, the soil of plot 1 requires a slightly higher moisture content than does the soil of plot 9. Any difference in moisture content of soil under field conditions favoring plot 9, as compared with plot 1, would therefore tend to exaggerate the more favorable conditions for nitrate accumulation, provided the moisture content did not exceed two-thirds saturation.

The data published by Call (1915) tend to show but slight differences in moisture content. It should be remembered, however, that these data are for the first four feet of soil. The data here presented indicate that the major decomposition of fresh organic matter must take place in the cultivated portion of the soil, i. e., in the first two or three inches of plot 1, and in the first seven inches of plot 9. In an effort to correlate moisture content and nitrate accumulation, one should look for differences in moisture content within these areas. There is no question but that quite marked differences exist in the moisture content of the stirred soil of these plots during seedbed preparation. In an earlier paper the writer (1916) has pointed out that these differences may extend to the depth of a foot, and that for some seasons, at least, they can be correlated with nitrate accumulation.

The differences in moisture content of the cultivated layers of soil are no doubt much greater than for the first foot, taken as a whole. In fact the surface three inches of soil from plot 1, with the rank growth of weeds present during the late summer months, rarely ever contains a sufficient quantity of moisture for any appreciable length of time, to permit active bacterial metabolism. It is believed that in the moisture relations may be found a sufficient explanation to account for all the differences in the rate of nitrate accumulations under field conditions that are not otherwise accounted for in this publication.

DISAPPEARANCE OF NITRATES FROM THESOIL

Attention was called early in this bulletin to the fact that the differences in nitrate accumulation might be due to differences in the rate at which nitrate nitrogen disappeared from the different soils rather than to differences in formation. Thus

far only nitrate formation has been considered. There are two general principles which might be involved in the disappearance of nitrate nitrogen as such from soils. It is well known that many organisms have the ability to bring about a reduction of the highly oxidized nitrate nitrogen. This process is assumed to be brought about in an effort on the part of organisms to supply themselves with oxygen and hence occurs only where the supply of this element in the free state is limited. Again all organisms require nitrogen in their metabolism. For most higher plants, and many lower, the highly oxidized nitrate nitrogen has been shown to be more readily assimilated than other forms. The growth of any such forms of plant life in the soil would, of course, be expected to utilize nitrate nitrogen in preference to all other forms present as long as the supply lasted.

DENITRIFICATION

It is extremely difficult to ascertain whether the processes of reduction, commonly called denitrification, actually take place under field conditions. There is no question but that organisms capable of bringing about such changes are almost universally distributed in soils. As mentioned above such processes are supposed to take place to an appreciable extent only under limited aeration and in presence of the active decomposition of organic matter. It has been shown that the decomposition of organic matter is less rapid in the soil of plot 1, for example, than in the soil of plot 9. It has also been shown that under laboratory conditions the undisturbed soil of plot 1, with a moisture content as high as 24 c.c. per 100 gm. soil, will permit aeration far in excess of that required to maintain aerobic conditions. This is true even when activity is far in excess of that under field conditions. The pore space of this soil undisturbed is approximately 42 percent, and 24 c.c. of water per 100 gm. of soil is much in excess of the average for the seedbed preparation season. Furthermore, the moisture content of the soil of plot 9 is nearly always in excess of that of plot 1. The water holding capacity of the soil of plot 1 exceeds that of plot 9. All these facts taken together certainly do not point to more favorable conditions for the reduction of nitrates in the soil of plot 1 than in the soil of plot 9.

NITRATE ASSIMILATION BY MICROORGANISMS

As to the utilization of nitrates for food by microorganisms, the factors pointed out above indicate lower consumption in soil of plot 1, than in soil of plot 9. Assimilation takes place only where active metabolism or growth is taking place. All the evidence accumulated indicates that the active metabolism, under field conditions, is certainly as great in the soil of plot 9 as in the soil of plot 1.

NITRATE ASSIMILATION BY HIGHER PLANTS

Regarding the utilization of nitrogen by higher plants much more definite data can be secured. A marked difference occurs in the amount of growth of higher plants on the various plots during seedbed preparation. For the seasons of 1915 and 1916 a comparison of such growth on plots 1 and 9 was made. The method followed was to select a number of representative areas scattered uniformly over the plot and then remove, by pulling, all growth thereon that had occurred since harvest. This, of course, did not remove all organic matter formed, since very few roots were removed by such a procedure. In most instances only that portion of the weed above the ground, and the rootstock was removed. Such determinations were necessary only on plot 1, for plot 9 was kept cultivated to prevent weed growth. After such material was gathered it was brought to the laboratory, dirt washed from the roots, dried, weighed and subjected to a quantitative analysis for total nitrogen. The results secured for two seasons are shown in Table XXVII.

TABLE XXVII.—ASSIMILATION OF NITRATES BY WEEDS

Year	Weight of weeds per acre	N content of weeds per acre	Equivalent NO_3
	Lbs.	*Lbs.*	*Lbs.*
1915	3,850	34.07	150
1916	5,360	49.44	210

From these results it is evident that a large amount of nitrogen has been annually consumed by the growth of weeds on those plots receiving cultivation late. The growth on plot 1 has been greater than on any other because it is the last to receive cultivation. The growth on plot 1, also varies from season to season but was not excessively heavy for 1915 and 1916.

Assuming that all the nitrogen consumed by this weed growth in its metabolism was assimilated as nitrate nitrogen, it represents for 1915, 150 pounds NO_3 and for 1916, 210 pounds NO_3. These two determinations give an average of 180 pounds NO_3. The average NO_3 content of plot 1 for the seasons of 1909 to 1917 was 74 pounds and for plot 9, 274 pounds, a difference of only 200 pounds. Assuming the growth of 1915 and 1916 to be average, a difference of 180 pounds NO_3 has been accounted for, leaving only 20 pounds unaccounted for. In this one factor alone then the difference in nitrate accumulation between plots 1 and 9 has been accounted for almost entirely. If the years 1915 and 1916 be considered, those for which data are available, the results given in Table XXVIII are to be noted.

TABLE XXVIII.—COMPARISON BETWEEN NITRATE ACCUMULATION IN SOIL AND NITROGEN ASSIMILATED BY WEED GROWTH

Year	Nitrate accumulation in pounds NO_3		Difference in favor of Plot No. 9	Pounds of nitrogen calculated as NO_3 in weeds Plot No. 1
	Plot No. 9	Plot No. 1		
1915	136	30	106	150
1916	362	96	266	210

In other words for 1915 the nitrates formed in plot 1 were 44 pounds greater than in plot 9. In 1916 the nitrates formed in plot 1 were only 56 pounds less than in plot 9. If the nitrogen consumed by the weed growth was in the form of NO_3, nitrification was actually more active in soil of plot 1 for 1915 and only slightly less active for 1916 than in soil of plot 9. In these calculations it should be remembered the nitrogen content of the major portion of the roots has not been taken into consideration. One is perhaps not justified in assuming that all the nitrogen assimilated was in the form of NO_3, nevertheless this is the form regarded as being most readily available and analyses show that there is always more or less present in this form in the soil of plot 1 throughout the seedbed preparation season. Whether or not the nitrogen assimilated by the growth of weeds be regarded as coming from the nitrate supply, it certainly, as far as is now known, came from some of the more simple decomposition products of organic nitrogen. This being true it would necessarily limit the quantity of nitro-

gen actually available for nitrification. As far as affecting the accumulation of nitrate nitrogen it would have the same ultimate effect.

ANTAGONISTIC EFFECT OF HIGHER PLANTS

There is still another factor worthy of consideration but upon which no data have been accumulated. It has been fairly well established that the growth of certain higher plants in a soil has a marked effect upon the activity of certain microorganisms growing in the same soil. May not the growth of certain weeds that are found in abundance on plot 1, have a harmful influence upon the metabolism of certain individual species or groups of organisms which are endeavoring to grow in the same pabulum? There would certainly be a struggle for existence and necessarily a limiting of available food.

CONCLUSIONS

It has been the purpose of this investigation to try to offer a satisfactory biological explanation for the differences in the rate of accumulation of nitrate nitrogen following various methods of seedbed preparation. The study has been confined to four, and primarily two, of the methods of preparation which have, as an average, given the widest variations in nitrate nitrogen accumulation. The study has brought out the following facts :

1. Under laboratory conditions no appreciable differences could be detected in the relative efficiency of the various soil flora in forming ammonia or in bringing about the accumulation of nitrate nitrogen.

2. No indications of differences in the potential possibilities of the different flora as they exist under field conditions could be detected.

3. No indications of subnormal activity on the part of the nitrifying organisms under field conditions could be detected in the soil of any plots studied.

4. There are differences in the rate at which organic decomposition takes place in soil of deep- and shallow-cultivated plots under field conditions. The difference in the decomposition of organic matter results in the formation of larger quantities of ammonia in deep-cultivated soil and hence renders possible a greater formation of nitrates. This difference is

due to the environmental conditions of the flora rather than to any difference in the various flora.

5. The principal environmental factors responsible for variations in organic decomposition are the distribution of organic matter and available moisture.

6. The major difference in the accumulation of nitrate nitrogen observed to occur between early and late cultivation can be explained upon a basis of the utilization of nitrate nitrogen by the growth of weeds on the late-cultivated plots.

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