THE EFFECT OF TIME AND DEPTH OF CULTIVATING A WHEAT SEED-BED UPON BACTERIAL ACTIVITY IN THE SOIL[1]

By

P. L. GAINEY, *Soil Bacteriologist, Kansas Agricultural Experiment Station*

Before the American Society of Agronomy 1914, L. E. Call (1) of the Kansas Agricultural Experiment Station, presented a very interesting paper concerning the effects that different methods of preparing a seed-bed for wheat have upon yield, soil moisture, and nitrates.

The principal fact with which we are concerned, brought out in this paper, is the very marked effects that the various experimental methods have upon the accumulation of nitrates. So striking and consistent have the results been for the past several years that a careful study of the problem was deemed advisable. A study of the bacteriological factors involved was given the writer.

The project, as conducted by the Department of Agronomy, consists in comparing eleven different methods of preparing seed beds. A detailed description of the various methods, with results secured, etc., is given in the above mentioned paper. The major points of comparison have been time of preparation and the depth of culture. The dates of preparation vary from July 15, to seeding time, about October 1. The depth of culture varies from discing to plowing 7 inches deep. In general, the effect upon nitrate accumulation has been that early and deep preparations give materially higher nitrate contents at seeding time than do late and shallow. A few specific examples from Call's paper will suffice to show the influence of these two factors.

Plot No. 1	disced	Oct. 1,	22.43 lbs.	NO_3 per acre.
Plot No. 14	plowed 3 in. deep	Sept. 15,	57.30 lbs.	NO_3 per acre
Plot No. 15	plowed 3 in. deep	July 15,	517.01 lbs.	NO_3 per acre.
Plot No. 13	plowed 7 in. deep	Sept. 15,	76.83 lbs.	NO_3 per acre.
Plot No. 10	**plowed 7 in deep**	**Aug. 15,**	**255.76 lbs.**	**NO_3 per acre.**
Plot No. 9	plowed 7 in. deep	July 15,	407.94 lbs.	NO_3 per acre.

[1] Contribution from the Research Laboratory in Soil Biology, Kansas Agricultural Experiment Station.

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From these data it is evident that some very important factors must be influencing the nitrate accumulation in the various plots.

For the bacteriological investigations it was deemed advisable to restrict the studies to a very limited number of plots. If the factors operating in the plots studied could be ascertained an application of similar methods to the whole series would be undertaken, provided such seemed advisable.

As representative of the extremes, both in methods of preparation and in results secured, so far as yield and nitrates are concerned, we chose as plots upon which to work Nos. 1 and 9. The treatment of No. 1 has been thorough discing at seeding time (about Oct. 1). The treatment of No. 9 has been plowing 7 inches deep July 15, and cultivating as often thereafter as necessary to insure a good seed-bed. For the four seasons '11, '12, '13 and '15, No. 1 has produced an average of 6.9 bushels of wheat per acre, while No. 9 has produced 27.59 bushels. The nitrate content for the four preceding seasons at the last analysis was for No. 1, 42.95 pounds of NO_3 per acre, and for No. 9, 318.69 pounds. From these data it is evident either that there has been much more NO_3 formed in No. 9, or that much has disappeared from No. 1. The only evident source of loss existing in No. 1 and not present in No. 9, is in the growth of weeds taking place prior to cultivation. Analysis has shown that in extreme cases this can account for only a small portion of the observed differences. It would seem then that those plots showing high NO_3 content actually form larger quantities. We have, therefore, for two seasons been directing our attention toward the nitrate forming power of soil from the two plots under study.

Experimental Data

The experiments given below include only a very few of those that have been carried out. An effort has been made to select representative experiments. At a later date a more complete report will be published.

Ammonia Forming Power

The relative ability of soil, from the two plots under study, to liberate ammonia from organic nitrogenous compounds is shown in Table I. Soil for the experiments here given was collected by means of a 2-inch soil auger. Twelve cores to a depth of 12 inches were drawn from each plot. Care was used to prevent, as far as possible, outside contamination.

The soil was brought to the laboratory, passed through a 3-mm. sieve, and thoroughly mixed. The moisture content and water holding capacity were determined. Four samples, the equivalent of 100 gm. dry soil, were then weighed. To two each of these cottonseed meal and dried-blood containing 60 mg. nitrogen were added, thoroughly mixed, and the whole placed in a 500-c.c. wide mouth bottle. The water content was made up

to optimum (2/3 saturation), the bottle plugged loosely with cotton, and incubated at room temperature 7 days. The ammonia was determined by direct distillation in presence of magnesium oxide. If the ammonia recovered by such means is any indication of the relative ammonifying power of the respective floras, it is evident that the difference in nitrate accumulation cannot be ascribed to a difference in this phenomenon. Further, repeated measurements of the ammonia content of the soils as they came from the field, revealed no appreciable difference. This was true whether such determinations were made by direct distillation or by the aeration method of Potter and Snyder (4).

TABLE I

THE PRODUCTION OF AMMONIA IN SOIL FROM PLOTS NO. 1 AND NO. 9: MG. NITROGEN RECOVERED AS AMMONIA PER 100 GM. OF SOIL AFTER ONE WEEK INCUBATION

Date	60 mg. Nitrogen as C. S. M.				60 mg. Nitrogen as D. B.	
	Soil Plot No. 1		Soil Plot No. 9		Soil Plot No. 1	Soil Plot No. 9
Oct. 23, '14.....	26.43 mg.		29.01 mg.		15.30 mg.	14.76 mg.
Nov. 7, '14.....	28.07 mg.		30.69 mg.		7.14 mg.	6.36 mg.
	A	B	A	B		
Nov. 21, '14.....	26.15 mg.	29.82 mg.	26.82 mg.	30.06 mg.		

C. S. M.—Cottonseed Meal. D. B.—Dried Blood.
A. Surface three inches. B. 5th to 7th inches inclusive.

Nitrate Forming Power

Experiments similar to those described above have been conducted to ascertain the nitrate forming power. Table II contains a description of treatment, etc., with the averages of three such determinations. The soil for these experiments was collected October 7, October 23, and November 7, 1914. Samples were incubated for four weeks, the water lost by evaporation being replaced from time to time. Nitrates were then determined by the phenol-di-sulphonic acid method as modified by Lipman and Sharp (2).

The results here presented indicate that in the absence of any addition of nitrogen, either with or without an addition of calcium carbonate, Plot No. 1 exhibits a materially higher nitrate forming power. On the other hand plot No. 9 exhibits a materially higher nitrate forming power when nitrogen as ammonium sulphate was added with calcium carbonate, and when nitrogen in form of cottonseed meal and dried blood was added in absence of calcium carbonate. In all other instances the differences are insignificant. A careful study of the data from which this table was constructed reveals, however, consistent differences in only the first three instances mentioned, namely, when no nitrogen was added either in the

presence or absence of calcium carbonate, and when ammonium sulphate was added in the presence of calcium carbonate. In all other comparisons sometimes Plot No. 1 and sometimes Plot No. 9 gave higher results.

The data contained in Table II indicate very strongly that, if the nitrate nitrogen produced under the experimental conditions here used gives any measure of the relative nitrate producing power, Plot No. 1 certainly contains as active a flora as No. 9. It is only just to add that in a number of experiments carried out in a similar manner, except that the nitrate content was measured at varying intervals, Plot No. 9 gave usually a somewhat more rapid accumulation during the early stages of incubation. However, such large quantities were in all cases formed in soil from Plot No. 1, as to show conclusively that the low accumulation under field conditions could not be attributed to a potentially weak flora. In addition there is absolutely no evidence to indicate a less active flora in the soil of Plot No. 1 when no nitrogen was added. Further, as pointed out above we have never been able to detect, either under field or laboratory conditions, a greater accumulation of ammonia in the soil of Plot No. 1 than in that of Plot No. 9.

TABLE II

THE PRODUCTION OF NITRATES IN SOIL FROM PLOTS NO. 1 AND NO. 9, INCUBATION FOUR WEEKS: AVERAGES OF DETERMINATIONS MADE ON DUPLICATE SAMPLES OCT. 7, OCT. 23, AND NOV. 7, 1914

Treatment		Mg. NO_3 per 100 gm. Soil	
60 mg. Nitrogen as	$CaCO_3$ gm.	Soil Plot No. 1 mg.	Soil Plot No. 9 mg.
0	0	2.93	2.26
0	1	8.43	6.85
Cottonseed Meal	0	67.02	76.59
Cottonseed Meal	1	153.89	154.45
Dried Blood	0	29.96	36.36
Dried Blood	1	102.33	103.31
Ammonium Sulphate	0	21.11	21.83
Ammonium Sulphate	1	155.23	190.51

The nitrate forming power in solution, as so vigorously recommended by Löhnis and Green (3), has also been tested. Data secured according to this method, for Sept. 14, 1915, are given below. The figures represent milligrams nitrogen converted into NO_3.

Incubation Period	10 days	28 days
Inoculum Soil of Plot No. 1	0.95 mg.	8.41 mg.
Inoculum Soil of Plot No. 9	1.36 mg.	8.47 mg.

Soil from Plot No. 1 has also been inoculated with soil from Plot No. 9 to see if the introduction of organisms from No. 9 would have any influence on the accumulation of nitrates. The results secured Sept. 14, 1915 are given in Table III. In both these last two mentioned experiments additional evidence is furnished to show that there is but slight

difference in the nitrifying floras of the two plots under study. Similar studies, giving similar results, have been made on other plots in the series.

TABLE III

THE EFFECT OF INOCULATING SOIL FROM PLOT NO. 1 WITH SOIL FROM PLOT NO. 9: MG. NO_3 RECOVERED PER 100 GM. SOIL

Soil	Inoculum	60 mg. Nitrogen as	$CaCO_3$ gm.	After 4 weeks mg.	After 10 weeks mg.
1	0	0	0	3.32	8.20
1	1	0	0	4.00	7.50
1	9	0	0	4.00	8.20
1	0	$(NH_4)_2SO_4$	1	.....	225.00
1	1	$(NH_4)_2SO_4$	1	133.30	200.00
1	9	$(NH_4)_2SO_4$	1	150.00	200.00

While no differences in the activity of the organisms could be detected in the above experiments, it was thought that possibly by sampling the soil at different depths and making similar studies, differences might be noticed. Accordingly, samples have been taken at varying depths down to 12 inches. Under our conditions very small quantities of nitrates are formed at lower depths. The results of one such analysis are given in Table IV. Soil for these experiments was collected August 20, 1915. A

TABLE IV

SHOWING NITRATE FORMATION IN SOIL FROM DIFFERENT DEPTHS OF PLOTS NO. 1 AND NO 9: Mg. NO_3 RECOVERED PER 100 GM. SOIL

Weeks Incubated	Treatment		Soil Plot No. 1				Soil Plot No. 9			
	60 mg. Nitrogen as	$CaCO_3$ gm.	A	B	C	D	A	B	C	D
0	0	1	1.42	1.24	1.24	1.24	2.80	2.34	2.40	1.60
2	0	1	5.40	3.00	2.25	1.50	9.00	9.60	7.80	2.49
4	0	1	18.00	2.64	2.49	1.29	13.80	8.59	9.60	...20
8	0	1	30.00	13.86	5.28	3.00	11.40	13.80	15.00	6.42
16	0	1	40.00	19.40	15.00	9.00	16.30	20.00	20.00	7.70
1	Cottonseed Meal	1	4.00	2.00	1.60	1.00	2.73	2.73	2.10	0.84
2	Cottonseed Meal	1	19.50	8.16	4.17	1.50	16.00	18.50	13.20	3.60
4	Cottonseed Meal	1	128.40	114.00	120.00	114.00	116.40	112.80	118.00	118.00
8	Cottonseed Meal	1	138.00	120.00	120.00	128.40	120.00	123.60	128.40	123.40
1	Ammonium Sulphate	1	6.40	2.66	2.00	1.42	7.20	6.90	5.20	2.00
2	Ammonium Sulphate	1	15.30	7.20	4.50	2.82	15.80	18.70	13.00	5.60
4	Ammonium Sulphate	1	189.60	114.00	133.30	60.00	180.00	180.00	180.00	75.00
8	Ammonium Sulphate	1	200.00	180.00	180.00	180.00	180.00	200.00	200.00	180.00

cylinder 5 inches in diameter was driven into the soil 12 inches deep, then was dug out and the core of soil divided into four equal parts. Five such cores were taken from each plot and the corresponding sections thoroughly mixed. Otherwise, the experiments were carried out as described earlier in the paper.

Several very interesting facts are to be noted in the data contained in Table IV. One of the most striking is the very rapid accumulation of nitrates in the surface soil of Plot No. 1, when no nitrogen was added. Below the surface section the accumulation fell off rather rapidly. On the other hand, the second and third layers of Plot No. 9 show just as rapid accumulation as the surface layer. Not until the fourth layer is reached do we note a decrease in nitrate accumulation. The results of this particular experiment are not nearly so marked in this respect as many others that have been carried out. It is reported only because it covers a wider range than the other experiments covering the same field. The results of a similar experiment, which was continued for a year, are graphically shown in figure 1. Soil for this experiment was collected Nov. 21, 1914. Here the second layers of soil differ from those in the experiment reported above in that they include the soil from the fifth to the seventh inches inclusive, instead of that from the fourth to the sixth. We shall take up a discussion of this particular phenomenon later.

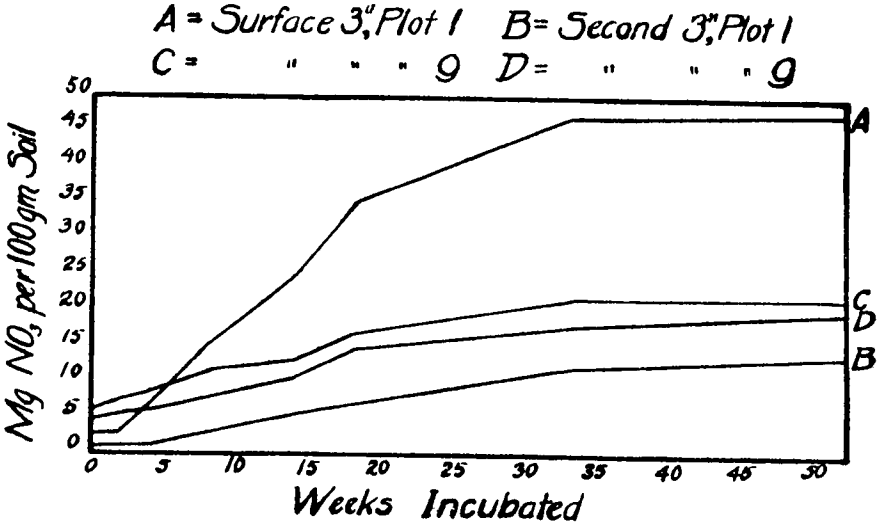

Fig. 1.—Diagram showing NO_3 accumulation in soil from different depths.

Returning to Table IV, we note that where nitrogen was added, somewhat similar results were obtained after one and after two weeks' incubation. That is, the NO_3 accumulation fell off rather rapidly immediately below the stirred area. Such differences, however, were in almost all instances eliminated by a longer incubation period. The question naturally arises as to whether this difference is due to some physical, chemical, or biological difference brought about by cultivation. We are at present in-

terested only in biological factors. If the differences are due to variations in the nitrifying floras, there are two ways in which we might detect the same. Either by introducing the respective floras into a standard solution and measuring their activity, or by cross inoculating experiments. Experiments have been carried out by both methods and yielded alike negative results, so far as exhibiting differences between the two plots is concerned.

In solution the following quantities of nitrogen, expressed as milligrams converted into NO_3 during four weeks' incubation, were found:

	Plot No. 1	Plot No. 9
1st 3 inches	3.69 mg.	4.42 mg.
2nd 3 inches	3.07 mg.	3.30 mg.
3rd 3 inches	2.31 mg.	2.29 mg.
4th 3 inches	4.24 mg.	4.11 mg.
Average	3.33 mg.	3.53 mg.

These results, together with others secured under similar conditions, give no indication either of a material difference in the nitrifying floras of the different plots, or of the different layers of an individual plot.

An extensive series of cross inoculation experiments was not conducted at this particular date. The results reported below were secured from soil collected May 3, 1915. Duplicate samples from each layer of both plots were inoculated from every other layer of both plots, giving a total of 72 samples. Half the samples were incubated 4 weeks, the other half, 12 weeks. Only the average nitrates, expressed in milligrams NO_3 recovered from all samples inoculated from the same source, are given.

	Plot No. 1			Plot No. 9		
Inoculum	A	B	C	A	B	C
Mg. NO_3	10.3	10.3	9.9	10.4	10.4	10.4

(A—1 to 3 inches inclusive; B—5 to 7 inches inclusive; C—8 to 12 inches inclusive). When only one experiment is considered, the differences here shown are well within the experimental error.

We have, therefore, been unable to detect any consistent differences in the nitrifying power of the respective floras which could, in our opinion, explain the large differences observed in nitrate accumulation under field conditions. This being true, the controlling factors must be other than biological. We have undertaken some experimental work studying the physical and chemical factors that might be influencing the activity of the nitrifying organisms. Work along these lines is by no means complete; still, certain facts brought out may throw some light on the general problem.

From the evidence in hand, and in part presented above, two points of importance seem to be clearly demonstrated. First, transferring the soil from field to experimental conditions eliminates the differences normally

existant. Second, the surface soil of Plot No. 1 possesses the ability to transform into nitrate nitrogen much more of its native nitrogen than does any other layer of either plot, or at least at a much more rapid rate. As accurately as we, by our present methods, are able to detect, these facts cannot be explained solely upon a difference in the respective floras either under their natural or under laboratory conditions.

The most evident changes brought about in transferring the soil from field to experimental conditions are: stirring, aerating, changing the temperature, and changing the moisture content. It is a common observation that stirring soils, apparently aside from all other influences, materially accelerates bacterial activity, and no doubt, has an influence on the phenomon under consideration. If this were the major factor, however, those layers of soil normally remaining unstirred i.e.—4 to 12 inches inclusive of No. 1, and 8 to 12 inches inclusive of No. 9, should show a greater response under the new environment. The evidence (see Table IV) does not bear this out. What has been said with regard to stirring also applies to aerating. It is hardly conceivable, though, that the surface soil of Plot No. 1 lacks aeration, yet this is the soil which gives the greatest response under laboratory conditions. In fact, Table IV shows that in all cases those soils receiving better aeration under field conditions give higher nitrate formation under laboratory conditions.

We believe temperature changes are a negative factor, since under field conditions the differences could be only very slight and in laboratory all were subjected to the same temperature.

As stated above, the soils in all experiments herein reported were made up to optimum moisture conditions, i.e. two-thirds saturated. In this respect they were materially changed from field conditions. Figure 2 illustrates the effect upon nitrate accumulation produced by varying the moisture content of soil from the experimental field. From this it can be seen that an increase of 1 per cent moisture, at or near the minimum for nitrification, may cause an increase of 100 per cent in nitrate production. If moisture is an influencing factor, however, it necessarily implies a variation of moisture content under field conditions. We have collected but little data on the moisture content of the soils under field conditions, but, through the courtesy of the Department of Agronomy, the data collected by them during the past seven years have been made available for study. They have sampled the various plots monthly in foot sections and made moisture determinations. Since the variations in nitrate content are largely brought about during August and September and almost entirely in the first foot of soil, we have confined our studies to the data collected during those two months, and for the first foot sections. It could not be expected that such meagre data would give us a clear insight into the relation existing between moisture content and nitrate pro-

duction. However, a remarkably close agreement can be traced for some seasons. Figure 3 illustrates graphically the correlation between the two factors for the season 1911. Similar relations, though not so close, can be traced for all other years for which data are available, except 1913.

It will be remembered that 1913 was omitted from the averages of yields and nitrates present in plots No. 1 and No. 9 given earlier in the paper. This was done because of the uniform results secured for that season. The yield was on Plot No. 1, 21.83 bushels, and on No. 9, 25.83 bushels of wheat per acre, a difference of only 4 bushels, while checks showed showed a variation of 6.25 bushels. The difference in nitrate content was at seeding time only 3 pounds per acre in favor of No. 9. The greatest variation in NO_3 content in the whole series was between

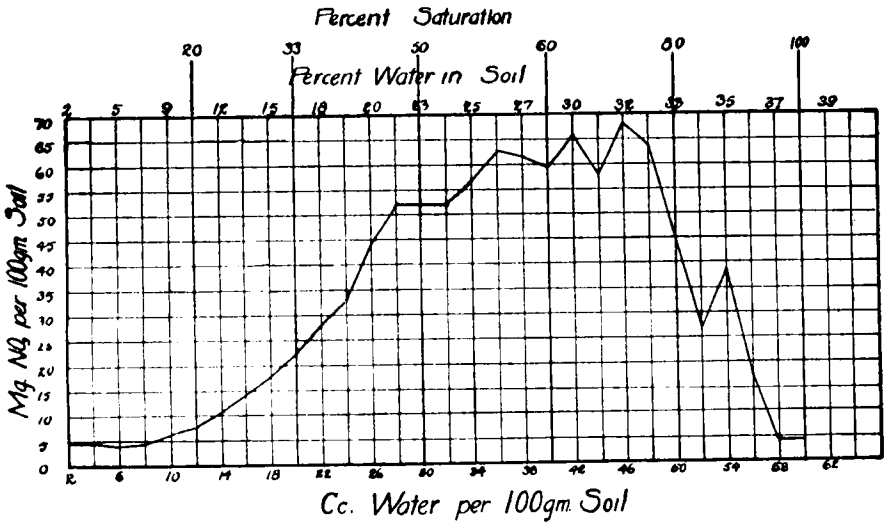

Fig. 2.—Diagram showing the effect of varying water content upon NO_3 formation in soil from experiment field.

checks. The same is true regarding moisture content. In other words, the whole series gave negative results for this season. A glance at figure 4, illustrating graphically moisture and nitrate content for 1913, shows absolutely no correlation between moisture content during August and September and nitrate content. In figures 3 and 4 the moisture lines give the average per cent of moisture actually available for nitrifying organisms when the August and September analyses were made. The nitrate lines represent pounds of NO_3 per acre three feet at the October analysis. We do not wish to be understood as saying that the large differences observed under field conditions can be entireely explained upon a moisture content basis. We do believe, however, that the few facts presented above are of considerable significance.

Turning our attention for a moment to the differences exhibited under laboratory conditions between the various layers of soil as illustrated in figure 1, it may be asked, how can these be explained? No evidence indicating a lack of active organisms in the lower layer soil of Plot No. 1 has been secured. The differences then must be due either to an inhibiting agent in the lower layer or to a difference, either in quantity or quality, of nitrogen present in the two layers. The fact that added nitrogen in any readily available form has been rapidly nitrified in the lower layer would seem to preclude the first possibility. As to the second possibility, it was found that the surface layer contained approximately 100 parts per million, or actually 100 pounds per acre, more nitrogen than the sec-

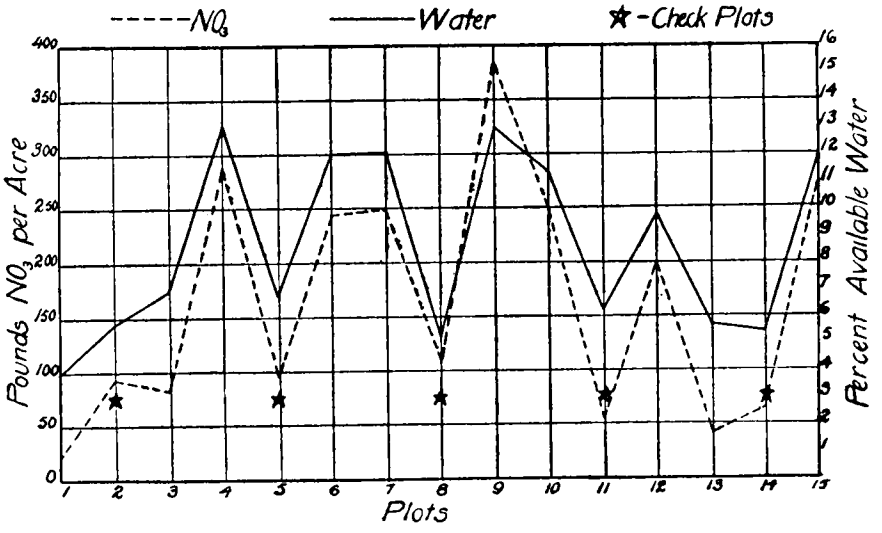

Fig. 3.—Diagram showing the relation between available water and NO_3 content under field conditions, 1911.

ond. There are, however, approximately 1500 parts per million nitrogen in the second layer, and unless this 100 parts per million exists in a different form or condition it would appear relatively insignificant. Besides, the second layer soil of Plot No. 1 contained slightly more nitrogen than either layer of Plot No. 9, yet, it failed to produce as much nitrates. As pointed out earlier, no difference could be detected in the ammonia content. This is rather significant since ammonia is the immediate forerunner of nitrates. Observations indicated a much larger quantity of undecomposed organic matter in surface soil from Plot No. 1 than in any of the other layers under study. By an arbitrary specific gravity method of separating this organic matter it was found in the following quantities in the four soils:

Surface layer No. 1 13,500 parts per million
Surface layer No. 9 3,400 parts per million
Second layer No. 1 2,200 parts per million
Second layer No. 9 3,700 parts per million

The nitrogen contents of this organic matter were in practically the same proportions. The excess of undecomposed organic matter in the surface layer of Plot No. 1, therefore, contained approximately 100 pounds of nitrogen, a figure just equal to the difference in total nitrogen between the two layers. A glance at figure 2 will show that when the NO_3 content of the surface soil of Plot No. 1 exceeds the second layer by approximately 100 pounds of nitrogen, the production in the two layers runs almost parallel (32nd to 52nd week). In other words, it appears

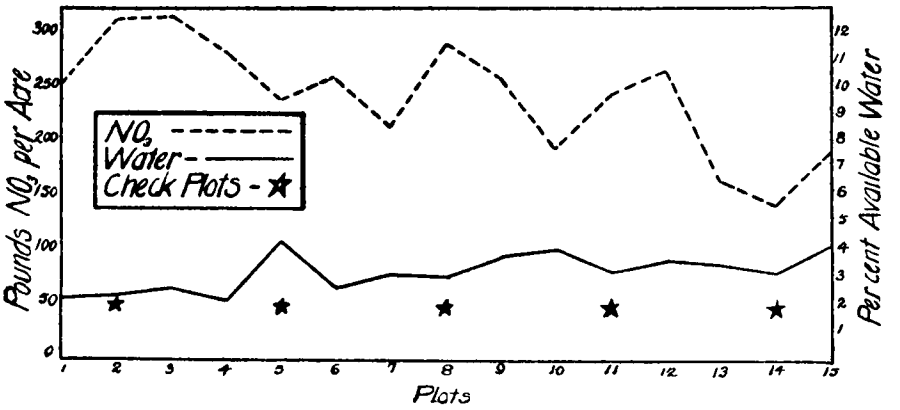

Fig. 4.—Diagram showing the relation between available water and NO_3 content under field conditions, 1913.

that something has prevented the decomposition of organic matter in the surface soil of Plot No. 1 under field conditions. This is, no doubt, due to the fact that the cultural method followed on this plot, i. e. discing, leaves the organic matter on or near the surface where, because of the normally dry conditions during the summer and fall months, decomposition is impossible. On the other hand, deep plowing incorporates the organic matter throughout the first 7 inches. More moisture being present, both because of depth and early culture, decomposition is made possible.

SUMMARY

As a summary we may state that the evidence here submitted indicates that:

1. The differences in nitrate content reported by Call cannot be attributed to a difference in the bacterial content.

2. Some non-biological condition existing in certain plots, under field conditions, prevents the normal activity of the bacterial flora.

3. Among the factors controlling bacterial activity the available moisture probably plays a paramount rôle.

ACKNOWLEDGEMENT

The writer wishes to express his appreciation of the valuable help rendered by the Department of Agronomy, through Prof. L. E. Call, in furnishing such data as have been used relative to yields, and moisture and nitrate content under field conditions.

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