XVII.—*On the Distribution of the Nitrifying Organism in the Soil.*

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IN a previous communication to the Society (Trans., 1884, 645), an account was given of some experiments made at Rothamsted in 1883 and 1884, to ascertain to what depth the presence of the nitrifying organism could be detected in the soil. The trials were confined to one field on the farm (Agdell), having a stiff clay subsoil. Portions of the soil were removed, with suitable precautions, from the side of a pit, and placed in sterilised solutions of diluted urine (4 c.c. per litre), containing a little calcium carbonate. The subsequent occurrence of nitrification in a solution was taken as proof that the soil introduced contained the nitrifying organism. Working in this way, it was concluded from three series of experiments, embracing 28 trials, "that in our clay soils the nitrifying organism is not uniformly distributed much below 9 inches from the surface. On much slighter grounds, it may perhaps be assumed that the organism is sparsely distributed down to 18 inches, or possibly somewhat further. At depths from 2 feet to 8 feet, there is no trustworthy evidence to show that the clay contains the nitrifying organism. It is however probable that the organism may occur in the natural channels which penetrate the subsoil at a greater depth than in the solid clay. In the case of sandy soils, we may probably assume that the organism will be found at a lower depth than in clay."

A further investigation of the subject has now been made. The new trials were carried out in the years 1885 and 1886. Besides the soil of the field with a clay subsoil, already experimented on, the soil of another field (Great Hoos), having a loamy subsoil, was also investigated. In all, six series of experiments, including 69 trials, have been made. The method employed was the same as that made use of in the earlier experiments, and described in the former communication, with the exception that *now* more soil (about 0·2 gram) was introduced into the trial solutions, and these solutions contained gypsum (0·1 gram per litre), in addition to the urine and calcium carbonate previously employed. The gypsum was added in consequence of the results obtained in another investigation (Trans., 1885, 758), showing that the presence of gypsum greatly facilitates the process of nitrification in the case of urine solutions. The solutions to which soil had been added were kept in a dark cupboard, the temperature being that of the air.

The first experiments to be noticed were made with soils obtained from Great Hoos Field, in which pits were dug for soil-sampling in July and August, 1885. In this field there is no sharp boundary between the surface soil and subsoil; the latter is loamy, and occasionally sandy. The previous summer had been dry; the soils were therefore dry, and towards the surface were found to be considerably disintegrated.

Fallow Soil, Hoos Field, sampled August 1, 1885.—This soil belonged to a plot of alternate wheat and fallow; it was in 1885 without a crop. The depths from which the soil was taken are shown in a subsequent table. Worm-holes were found to extend 2 feet from the surface. The subsoil at 2 and 3 feet was clayey, but that from 4, 6, and 8 feet was sandy.

White Clover Soil, Hoos Field, sampled August 4, 1885.—This plot had grown white clover, manured with ash constituents only, for several years. The roots of the clover were found to extend 3 feet from the surface. The soil near the surface was very dry. The subsoil consisted of clay and sand very irregularly distributed.

Bokhara Clover Soil, Hoos Field, sampled August 4, 1885.—On this plot, Bokhara clover had been grown for several years, and manured as the preceding plot. Only two samples of soil were taken; both were from an invasion of hard chalk, interspersed with a little brown loam, from a depth of about 7 feet from the surface.

Lucerne Soil, Hoos Field, sampled August 14, 1885.—This plot had a similar history to that of the two preceding. The soil was very dry, and towards the surface much disintegrated; the surface soil was full of cavities, and that at 16 inches very crumbly and porous. Worm-holes containing living worms extended 4 feet from the surface.

The roots of the lucerne were seen at 6 feet from the surface, but they did not penetrate the chalk. Chalk occurred at 6 feet from the surface. Both the samples of soil at 8 feet from the surface were from chalk. All the samples taken, down to the chalk, were selected from places in the immediate neighbourhood of lucerne roots.

It is to be noted that a strong breeze was blowing on the day when these samples were taken; and the surface soil being very dry, there was danger of soil-dust entering the sterilised solutions when the bottles containing them were opened for the introduction of the soil. A screen was erected to afford shelter from the wind; but it is impossible to say that the protection from dust was complete.

The urine solutions to which the samples of soil had been added were examined from time to time with diphenylamine, to ascertain if nitrification had commenced: the results of the examinations are shown in the following tables. Solutions in which the nitric acid found was "considerable," were not generally further examined. Samples of soil marked "C" were chalk.

Nitric or Nitrous Acid produced in Dilute Urine with Gypsum, seeded with Soil (Hoos Field, Fallow Plot) from various depths on August 1st, 1885.

Depth from which soil was taken.	August 24th (23 days).	September 22nd (52 days).	March 29th (240 days)
9 inches	distinct	large	—
16 " (a)	none	considerable	—
" (b)	"	considerable	—
2 feet (a)	"	considerable	—
" (b)	"	considerable	—
3 feet (a)	"	none	large
" (b)	"	"	none
4 feet (a)	"	"	"
" (b)	"	considerable	—
6 feet (a)	"	none	none
" (b)	"	"	"
8 feet (a)	"	"	"
" (b)	"	"	"

The results yielded by these experiments were in several respects distinctly different from those formerly obtained. The process of nitrification was far more speedy in most of the cases in which it occurred. Soil obtained from very considerable depths was also in many instances found capable of starting nitrification. Soil from the fallow plot was effective without exception down to 2 feet from the surface, and one sample from 3 feet, and one from 4 feet also produced

Nitric or Nitrous Acid produced in Dilute Urine with Gypsum, seeded with Soil (Hoos Field, White Clover Plot) from various depths, August 4th, 1885.

Depth from which soil was taken.	August 24th (20 days).	September 22nd (49 days).	March 29th (237 days).
9 inches	moderate.	—	—
16 " (a)	none	considerable	—
" (b)	"	considerable	—
2 feet (a)	"	considerable	—
" (b)	"	trace	large
3 feet (a)	"	considerable	—
" (b)	"	none	large
4 feet (a)	"	"	distinct
" (b)	"	"	none
6 feet (a)	"	"	"
" (b)	"	considerable	—
8 feet (a)	"	none	none
" (b)	"	"	"

Nitric or Nitrous Acid produced in Dilute Urine with Gypsum, seeded with Soil (Hoos Field, Bokhara Clover Plot), August 4th 1885.

Depth from which soil was taken.	September 23rd (50 days).	March 29th (237 days).
7 feet (a) C	none	none
" (b) C	"	"

nitrification. Soil from the white clover plot was effective without exception down to 3 feet, and one sample from 4 feet, and one from 6 feet also proved effective. Soil from the lucerne plot (Table, p. 122) was still more efficacious, all samples down to 4 feet producing nitrification, and one at 6 feet exhibiting the same property.

It seemed doubtful whether the unexpected exhibition of nitrifying power by these subsoils was due to the more favourable character of the solutions in which they were placed, or whether the results were simply the result of contamination of the solutions by soil-dust when the bottles were opened in the field, the surrounding soil having been throughout the taking of the samples unusually dry, and a brisk breeze at times blowing. It was resolved therefore to repeat the experiments under more favourable conditions of weather.

In the spring of 1886, further trials were made, both with the soil of Agdell field, and with that of Great Hoos field. The solutions into

Nitric or Nitrous Acid produced in Dilute Urine with Gypsum, seeded with Soil (Hoos Field, Lucerne Plot) from various depths, August 14th, 1885.

Depth from which soil was taken.	September 22nd (39 days).	March 25th (223 days).
9 inches	considerable	—
16 „ (a)	none	large
„ (b)	considerable	—
„ (c)	distinct	large
2 feet (a)	none	large
„ (b)	„	large
„ (c)	considerable	—
3 feet (a)	considerable	—
„ (b)	none	large
„ (c)	„	large
4 feet (a)	„	large
„ (b)	„	large
„ (c)	„	large
6 feet (a)	considerable	—
„ (b) C	none	none
8 feet (a) C	„	„
„ (b) C	„	„

which the soil was introduced consisted, as in the experiments of the preceding year, of dilute urine with gypsum.

Red Clover Soil, Agdell Field, sampled April 16, 1886.—The pit was dug in the unexperimental portion at the south end* of the field. Red clover had been sown the previous year, and had not yet borne a crop; there was a fair plant. Roots were not observed below 27 inches. The surface soil was 6 to 8 inches deep, and was sharply divided from the subsoil. This subsoil consisted generally of a rather loamy clay. The samples were taken in the intervals of a showery day.

Fallow Soil, Hoos Field, sampled May 7, 1886.—This soil was from an unexperimental portion of the same plot which had been examined in 1885. The subsoil down to 2 feet consisted of a friable loam, below this depth it became stiffer. At one side of the pit an invasion of chalk occurred. The weather was fine and calm during the taking of the samples.

The results of the examination of the solutions were as follow:—

* In the former paper (Trans., 1884, 648), this part of the field is by a mistake called the north end.

Nitric or Nitrous Acid produced in Dilute Urine with Gypsum, seeded with Soil (Agdell Field, cropped with Red Clover) from various depths, April 16th, 1886.

Depth from which soil taken.	May 28th (42 days).	June 28th (73 days).	August 3rd (109 days).	Nov. 20th (218 days).
6 inches.....	considerable	large	—	—
12 „ (a)	distinct	large	—	—
18 „ (b)	none	considerable	large	—
27 inches (a)	distinct	large	—	—
„ (b)	trace	large	—	—
3 feet (a)	trace	large	—	—
„ (b)	none	considerable	large	—
4 feet (a)	„	considerable	large	—
„ (b)	„	none	distinct	considerable
6 feet (a)	„	distinct	considerable	large
„ (b)	„	none	distinct	large
„ (b)	„	„	none	none

Nitric or Nitrous Acid produced in Dilute Urine with Gypsum, seeded with Soil (Hoos Field, Fallow Plot) from various depths, May 7th, 1886.

Depth from which soil taken.	June 11th (35 days).	July 14th (68 days).	August 16th (101 days).	Nov. 20th (197 days).
9 inches.....	large	—	—	—
16 „ (a)	distinct	large	—	—
„ (b)	trace	large	—	—
2 feet (a)	considerable	large	—	—
„ (b)	large	—	—	—
3 feet (a)	considerable	large	—	—
„ (b)	none	none	considerable	large
4 feet (a)	„	„	none	none
„ (b)	„	„	„	„
5 feet (a) C ..	„	„	„	„
„ (b)	„	„	distinct	large
6 feet	(not tested)	large	—	—

It is evident that these new trials, made under more favourable conditions as to exclusion of soil-dust, confirm the results of the previous year, and afford proof of the distribution of the nitrifying organism to a considerable depth in the subsoil. Taking the whole of the 69 trials, and classifying them according to the depth of the soil experimented on, we obtain the following results. Soil from the depth of 27 inches has been reckoned as 2 feet.

We have thus no failure to produce nitrification in samples of soil down to, and including, a depth of 2 feet from the surface. At 3 feet,

Depth of soil.	Number of trials.	Number of solutions nitrifying.	Number nitrifying out of 10 trials.	
			Including chalk.	Excluding chalk.
Less than 2 feet.	17	17	10·0	10·0
2 feet.....	11	11	10·0	10·0
3 „	11	10	9·1	9·1
4 „	11	7	6·4	6·4
5 „	2	1	5·0	10·0*
6 „	9	4	4·0	5·0
7 „	2	0	0·0	—
8 „	6	0	0·0	0·0

* This result represents a single experiment only.

only one sample out of 11 fails. Below 3 feet, failures become far more numerous, and increase in frequency as the depth increases. At 6 feet, if we exclude the experiment with chalk, one half of the trials resulted in nitrification. At 7 and 8 feet, the soil failed entirely to produce nitrification. The trials at 7 feet were, however, limited to two experiments with chalk; those at 8 feet included four experiments with clay. None of the six trials with chalk resulted in nitrification.

To what cause are we to attribute the production of nitrification by subsoils in these experiments, while subsoils from the same depth, and in one case from the same field, failed entirely to produce nitrification in the earlier experiments. The only apparent explanation is the presence of gypsum in the urine solutions employed in the later experiments. This explanation is probably sufficient. It has been abundantly proved by other experiments that nitrification will not commence if the alkalinity of the ammoniacal solution exceeds 400 parts of nitrogen per million, and that an alkalinity far below this point greatly retards the commencement of nitrification, and may altogether prevent it if a large quantity of the nitrifying organism is not present. It is quite true that the amount of ammonium carbonate produced in a 4 per 1000 urine solution would be very small, and quite insufficient to prevent nitrification if seeding were made with a vigorous nitrifying organism. We may naturally assume, however, that the organism present in a clay subsoil, several feet from the surface, is neither abundant nor vigorous in habit; it may consequently be unable to develop in a decidedly alkaline urine solution. The addition of gypsum to the solution, by neutralising all excess of alkali, removes any hindrance to the development of a feeble organism.

That the nitrifying organism occurring in subsoils is of comparatively feeble power is shown by the greater length of time which a subsoil takes to start nitrification, as compared with a similar quantity of surface soil. It is not possible from the results of these experiments to give the average time required to start nitrification by soils from various depths, the conditions as to temperature, and the frequency of observation, having been very different in the different series of experiments; but it is quite possible to deduce from the results an average order of priority, if we give numerical values to the amounts of nitric acid found at the different dates of the commencement of nitrification. Thus giving the value 1 to a "considerable" quantity of nitric acid found at the *first* examination of the solution, a value 2 for a "distinct" quantity, and a value 3 for a "trace." And again a value of 4 for a "considerable" quantity marking a commencement of nitrification at the *second* examination, a value 5 for a "distinct" quantity, and a value 6 for a "trace." And proceeding in the same order with commencements of nitrification occurring at a *third* examination, we are able finally to obtain the average order of priority in commencement of nitrification for each depth of soil.*

Order of Priority in commencement of Nitrification in Solutions seeded with Soil from different depths.

Depth of soil	9 ins.	16 ins.	2 ft.	3 ft.	4 ft.	5 and 6 ft.
Number of solutions nitrifying ...	6	11	11	10	7	5
Average order of priority in nitrification	1·33	3·36	3·73	4·90	6·57	5·00

We observe at once a great difference between the activity of soil 9 inches, and soil 16 inches in depth; and below this depth the activity of the soil continues gradually to diminish. The figures do not, however, afford a perfectly smooth order, those belonging to a depth of 4 feet being apparently rather high, and those for 5 and 6 feet rather low.

The general result of the experiments is thus to indicate that although the nitrifying organism may occur at a considerable depth in a subsoil of loam or clay, it occurs there in small quantity and in feeble condition.

Are we to conclude that nitrification occurs in all depths of the subsoil at which the nitrifying agent exists? I am strongly of

* In taking the averages, soils 6 and 12 inches deep have been reckoned as 9 inches; soils 18 inches as 16 inches; and 27 inches as 2 feet.

opinion that nitrification is practically confined to the surface soil, and occurs rarely, and to a very small extent, in a clay subsoil removed 2 or 3 feet from the surface. All the conditions for nitrification are present to a far greater extent in the surface soil than in the subsoil. The surface soil is in the large majority of cases far richer in nitrogenous matter suitable for nitrification than the subsoil. At the surface also, the amount of oxygen available is immeasurably greater than it is below. The principal hindrance to nitrification in a clay subsoil is in fact the impossibility of obtaining a sufficient circulation of air at considerable depths.

The amounts of nitric acid annually produced by the three masses of soil forming the drain gauges at Rothamsted are not in favour of any important portion of this product being generated in the subsoil. These masses of soil were originally portions of an arable field; they were isolated without being disturbed. They are of the same area, but of different depths. The quantity of nitric nitrogen annually found in the drainage waters from these soils has, on an average of nine years, been as follows:—

Soil 20 inches deep, 40·2 lbs. per acre.

„ 40 „ 35·0 „ „

„ 60 „ 38·8 „ „

There is here no evidence of any greater production of nitrates where the subsoil is included in the experiment.

The evidence obtained by analysing fallow soils at different depths points to the same conclusion. If after a cereal crop, which has removed the nitrates from the soil, the land be cultivated during the next summer as a bare fallow, the nitrates formed during this season of rest and tillage will be found chiefly in the surface soil, unless heavy rain has occurred to wash them below. Thus a fallow soil in Agdell Field at Rothamsted, sampled on September 8, 1882, yielded the following quantities of nitrogen as nitrates:—

First 9 inches, 40·1 lbs. per acre.

Second 9 inches, 14·3 lbs. per acre.

Third 9 inches, 5·5 lbs. per acre.

There can be no doubt that a considerable part of the small quantity of nitrates found in the subsoil had not been formed *in situ*, but had been carried to this depth by drainage and diffusion.

The discovery of large quantities of nitrates in a subsoil is no proof that they have been produced there; their occurrence at this depth is a natural result of drainage and diffusion in the absence of a growing crop capable of removing them from the soil. An accumula-

tion of nitrates in the subsoil below the ordinary depth to which the roots of crops extend is quite possible, and such accumulations may remain little altered in quantity from year to year, the annual additions balancing the annual waste by drainage.

The conditions which favour nitrification in the subsoil are such as enable air to penetrate it. Artificial drainage; a dry season; or the growth of a luxuriant crop, causing much evaporation of the water of the soil: such conditions, by removing the water that fills the pores of the subsoil, will cause air to penetrate more or less deeply, and render nitrification possible. A small amount of subsoil nitrification may thus take place in the drier periods of the year.

The nitrogenous matter existing in a clay subsoil is not only small in quantity, but also different in composition from that found in the surface soil. At Rothamsted, the ratio of nitrogen to carbon in the surface soil of the arable fields is about 1 : 10, while at 54 inches from the surface the proportion is only 1 : 6. The nitrogenous matter existing in the subsoil is apparently susceptible of nitrification, if placed under perfectly suitable conditions.

Experiments on the nitrifiability of soils and subsoils from Manitoba have been already published by Messrs. Lawes and Gilbert (*Trans.*, 1885, 410). The subsoils, freely exposed to air in a covered basin, were found to yield nitric acid, especially during the last period of the examination, when they had been supplied with nitrifying organisms from a fresh surface soil.

Some trials of the nitrifiability of clay subsoils were made in 1884. The surface soil (1st 9 inches), and a subsoil (3rd 9 inches) from two arable fields at Rothamsted were the subject of experiment. 100 grams

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of dry powdered soil were mixed in each case with the same weight of coarsely powdered recently ignited flint, and the mixture placed in cylindrical funnels, consisting of narrow-necked pint bottles having their bottoms removed. Each funnel was connected by a cork and tube with a bottle, into which any liquid draining from the funnel could pass. The funnel was closed at top by a tubulated caoutchouc cap. The tubulure contained cotton-wool moistened with weak sulphuric acid. The bottle receiving the drain water was also closed with a cork, and provided with a tubulure containing acidified cotton-wool. The passage of air through the apparatus was thus permitted, but any ammonia the air might contain was removed before entrance. As all the soils had been dried (at a temperature of about 56°), and any nitrifying organism they contained probably killed, they were each seeded by mixing with them 0.10 gram of fresh surface soil. In each case, the subsoil was experimented on in duplicate, one lot receiving 0.5 gram potassium phosphate, 0.5 gram magnesium sulphate, and 5.0 grams of calcium carbonate, and the other lot being without this addition.

The soil mixtures when placed in the funnels were treated with successive quantities of ammonia-free water till all ready formed nitrates were removed; the soils were then placed in the dark to nitrify. From time to time, the soils were extracted with ammonia-free water to remove the nitrates which had been produced; the extraction was performed without removing the soil from the funnel. The quantities of nitrates found in the successive watery extracts are shown in the following tables:—

Nitric Nitrogen obtained from 100 grams of Soil and Subsoil from Plot 10A, Broadbalk Wheat Field.

	Surface soil (1st 9 inches).	Subsoil (3rd 9 inches).	
		Alone.	With cinereal plant food.
	gram.	gram.	gram.
Jan. 24th—Feb. 9th (16 days).....	0.00067	trace	trace
Feb. 9th—Mar. 1st (21 days)	0.00093	0.00002	0.00022
Mar. 1st—Mar. 28th (27 days)	0.00053	0.00004	0.00061
Mar. 28th—May 9th (42 days)	0.00037	0.00050	0.00027
May 9th—Dec. 12th (217 days)	0.00135	0.00073	0.00104
Total nitric nitrogen obtained	0.00385	0.00129	0.00214
Nitric nitrogen per 100 of original nitrogen	3.8	2.4	4.0

Nitric Nitrogen obtained from 100 grams of Soil and Subsoil from Plot O2, Hoos Barley Field.

	Surface soil (1st 9 inches).	Subsoil (3rd 9 inches).	
		Alone.	With cinereal plant food.
	gram.	gram.	gram.
Mar. 18th—April 17th (30 days) ...	0·00023	0·00004	0·00015
April 17th—May 20th (33 days) ...	0·00060	0·00062	0·00064
May 20th—Dec. 23rd (216 days) ...	0·00086	0·00052	0·00063
Total nitric nitrogen obtained.....	0·00169	0·00118	0·00142
Nitric nitrogen per 100 of original nitrogen	1·9	3·0	3·6

The amounts of nitrate obtained from the experimental soils and subsoils at different periods are far from regular; we need not here, however, discuss the causes of this irregularity, our object being simply to show that the nitrogenous matter of a clay subsoil is nitrifiable, and of this fact the figures leave no doubt. The quantity of nitrates produced in the subsoil is smaller in every case than in the surface soils, but the proportion of the original nitrogen of the soil which was nitrified is on the whole quite as large in the case of the subsoils as in that of the surface soils.