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BACTERIAL ACTIVITIES IN FROZEN SOILS

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AGRICULTURAL EXPERIMENT STATION
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AGRONOMY SECTION
SOIL BACTERIOLOGY

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SUMMARY.

1. By means of the "modified synthetic" agar plate method bacteria are shown to be present in large numbers in a typical Wisconsin drift soil when it is completely frozen and the temperature is below zero degrees Centigrade; furthermore, increases and decreases in numbers of organisms occur during this period and large numbers are found after the soil has been frozen for a considerable period than before it begins to freeze.

2. During the fall season, the number of bacteria present in the soil diminishes gradually with the lowering of the temperature.

3. Frozen soils possess a much greater ammonifying power than non-frozen soils whether they are tested by the peptone solution method or by the dried blood or cottonseed meal method.

4. During the fall season, the ammonifying power of the soil increases until the temperature of the soil almost reaches zero, when a decrease occurs, and this is followed by a gradual increase and the ammonifying power of the soil reaches a maximum at the end of the frozen period.

5. The nitrifying power of frozen soils is weak and shows no tendency to increase with extension of the frozen period.

6. Frozen soils possess a decided denitrifying power which seems to diminish with the continuance of the frozen period.

7. During the fall season, the denitrifying power of the soil increases until the soil freezes, after which a decrease occurs.

8. Frozen soils possess a nitrogen-fixing power which increases with the continuance of the frozen period, being independent of moderate changes in the moisture conditions but restricted by large decreases in moisture.

9. In the fall, the nitrogen-fixing power of the soil increases until the soil becomes frozen, when it almost ceases, after which a smaller nitrogen-fixing power is established.

10. These results confirm Conn's conclusion that bacteria are alive and multiply in frozen soils. The results of the physiological determinations lend support to his theory of the existence of specific groups of bacteria in the winter which are adapted to growth at low temperatures.

11. The theory is advanced that because of the surface tension exerted by the soil particles on the films of water, the presence of salts in this water, and the concentration in salts which may occur in it when the main body of soil water begins to freeze, it seems justifiable to assume that under average winter conditions, when the soil temperature is not depressed far below zero, the hygroscopic water in soils remains uncongealed and consequently bacteria may live in it and multiply sometimes to a comparatively large extent.

BACTERIAL ACTIVITIES IN FROZEN SOILS.

BY

PERCY EDGAR BROWN AND ROY EUGENE SMITH.*

INTRODUCTION.

It is a matter of common knowledge that changes in temperature have an important influence on the multiplication of bacteria and consequently, on their activities. Every organism has what is known as an optimum temperature at which point it is the most vigorous. Each also has a maximum and a minimum temperature at which its characteristic activities cease. The temperatures involved are as varied as the organisms and the optimum for one organism may prove the maximum or the minimum temperature for another.

These facts apply directly to the myriads of bacteria of varying characters and functions now known to inhabit the soil, and variations in the optimum, maximum, and minimum temperatures for the growth of the different species are clearly recognized. Under normal seasonal and climatic conditions, changes in temperature, and also in moisture conditions in the soil, are constantly occurring. Many other factors, such as aeration, reaction and food supply, have an important influence on bacterial activities in the soil and the combined action of these various factors determines largely the character and extent of bacterial changes in the organic and inorganic food constituents in the soil, and consequently determines the crop producing power of the soil, or its fertility.

It is evident, therefore, that the soil, under natural climatic conditions, may be the seat of important activities from the fertility standpoint, whose extent and far-reaching results are determined largely by the temperature or other climatic conditions.

Some experiments have been conducted to determine the effects of seasonal and climatic conditions on the bacterial flora of soils, but practically all the investigations have been confined to the growing season of crops. It has been deemed entirely unnecessary to carry on the work through the winter months, the assumption being that bacteria remain dormant when the soil is frozen, and that their activities, therefore, are of no importance in winter.

It may be asked of what interest it is to determine bacterial activities in the winter.

If the transformation of plant food in the soil proceeds to any great extent during the winter months, then the economic importance of such a transformation is evident. With no

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crops to utilize the food made available, an accumulation of soluble constituents might be produced and a loss of such constituents by early spring leaching would be the result naturally to be expected. It may be, however, that if increased production of soluble plant food is followed by increased bacterial development, the food is utilized by the organisms in their growth to such an extent that very little accumulation is possible. Furthermore, it is recognized that the complex plant food from bacterial bodies is more readily available than that from other sources, due largely to the better distribution through the soil, and consequently if this action of the organisms in transforming insoluble constituents to soluble is followed by increased assimilation by bacteria, it may actually be of economic advantage.

On the other hand, if bacterial activities are entirely suspended during the winter, why is it regarded profitable to plow under green manures in the fall, when such substances must be acted upon by bacteria to be of value to crops? Why is it that fall applications of such materials as ammonium sulfate are discouraged because of the danger of loss of nitrogen?

Other common agricultural practices might be cited to show that knowledge regarding bacterial activities in frozen soils is of importance, not only from the scientific but also from the practical standpoint.

HISTORICAL.

As has already been stated, previous investigation of bacterial activity in frozen soils has been very limited. While Remy[1], Fabricius and Von Feilitzen[2], Kruger and Heinze[3], and others studied the effects of seasonal conditions on the numbers of bacteria in soils, their experiments were all confined to the growing season and have no direct bearing on our problem. In the course of their investigation of the effects of treating a soil with carbon disulfide, Hiltner and Störmer[4] studied some samples taken during the winter months. Their results seem to indicate that the number of organisms in soils decreased with the temperature, being practically at a minimum when the soil was frozen. Conn, in his work which will be mentioned later, made a careful study of the above results in an effort to show that they do not disagree with his. After a thorough, critical discussion of the methods of Hiltner and Störmer and the conditions under which their experiment

1. Centbl. f. Bakt. (etc.) 2 Abt. 8 (1902) pp. 657, 699, 728, 761.
2. Centbl. f. Bakt. (etc.) 2 Abt. 14 (1905) p. 161.
3. Landw. Jahr. Bd. 36 (1907) p. 382.
4. Studien uber die Bakterienflora des Ackerbodens etc. Berlin 1903.

was carried out, he concludes that "considering the difference in the locality, in the weather, and in the media employed, and the probable differences in the soil, it is certainly remarkable that the two investigations can be shown to agree so well."

Hiltner and Störmer also found in their work that the number of bacteria was very closely related to the moisture content of the soil. In their opinion, the moisture conditions had more influence on bacterial numbers than temperature.

Engberding[1] carried on a series of experiments more recently in which he made comparative studies of bacterial activities in manured and unmanured plots, and also in fallow and cropped plots, but here again his work was done mainly during the growing season and only two samples were taken during the winter months. He concluded that raising and lowering the soil temperature exerted only a very slight action on the soil bacteria; that in the warm season, the numbers rose and fell with the water content of the soil regardless of temperature. He also found that while the numbers present in the soil in January were smaller than those found in September, they were larger than those obtained in the summer, so that while the number of samples taken were too few for the results to be conclusive, they seem to show the presence of bacteria in frozen soils in considerable numbers.

CONN'S INVESTIGATIONS.

Conn's experiments constitute practically the only previous attempt to make a careful study of the bacteria in frozen soils. His first work[2] extended through one year, 1909-1910, and twelve samples in all were taken, three of these being obtained when the soil was frozen. The conclusions which were drawn from his work were:—

1. "Quantitative determinations by means of the plate method have shown that bacteria may be present in large numbers during the winter in the soil * * * * there seems indeed, to be an unexpectedly rapid multiplication while the soil is completely frozen, the numbers becoming higher than any found during summer or fall." * * * *

2. "In general, the bacteria seem to increase and decrease nearly parallel to the moisture content of the soil: the rapid multiplication, however, during the winter was a striking exception to this" * * * *.

In a continuation of this work carried on through the succeeding year[1], the results of the previous investigation were largely confirmed. It was found that:—

1. "Quantitative determinations * * * * have shown

1. Centbl. f. Bakt. (etc.) 2 Abt. 23 (1909) p. 571.
2. Centbl. f. Bakt. (etc.) 2 Abt. 28 (1910) p. 422.

* * * * an increase in numbers of bacteria in frozen soils almost as great as that noticed the first winter.

2. "During the second winter, the numbers have increased while the soil has been well frozen, but have decreased whenever it thawed.

3. "Throughout most of the year, the numbers of bacteria have been nearly parallel with the moisture content; but these fluctuations in winter as the soil was frozen and thawed have shown no relation to the moisture.

4. "This increase during the winter seems to be due to an actual multiplication of the bacteria, rather than to a mere rise of the organisms from lower depths brought about by mechanical forces alone."

In this second work, Conn reports some very interesting and careful experiments in an attempt to classify soil organisms into groups according to the character of their growth on the soil extract gelatin medium, which he employs. His classification is essentially the same as that adopted by Hiltner and Störmer in their work, which has already been cited. They divided the organisms developing on gelatin plates into liquefiers, non-liquefiers, and Streptothrix species. Conn also made three divisions: rapid liquefiers, slow growers, and Actinomycetes. He states that, as all the colonies liquefy gelatin eventually, his slow growers undoubtedly correspond to Hiltner and Störmer's non-liquefiers and his Actinomycetes correspond to their Streptothrix. Considering then the soil organisms as falling into these three groups, Conn points out some interesting facts.

5. "The greatest increase during the winter occurs in a group of bacteria called here the slow growers, a group readily distinguished by its gelatin colonies from the two other large groups, rapid liquefiers and Actinomycetes.

6. "Qualitative work with pure cultures has shown that certain types of soil bacteria occur throughout the year; but that others apparently exist in the soil investigated for short periods only, and tend to recur at other times under similar weather conditions. The greatest variety of these types has been found in fall and winter.

CONN'S THEORY OF SUMMER AND WINTER BACTERIA.

7. "This seasonal variation suggests a possible explanation for the increase during the winter. It is probable that a different class of bacteria is in the ascendancy in winter from that which is benefited by the warm weather of summer: and it may be the hostile effect of summer organisms which prevents the other types from multifying rapidly in warm weather.

1. Centbl. f. Bakt. (etc.) 2 Abt. 32 (1911) p. 70.

In that case the increase in frozen soils is not due directly to the low temperatures, but to the depressing effect of the cold upon that group of bacteria which is able in summer to keep the winter bacteria in check."

These results and conclusions are somewhat surprising, to say the least, for the questions immediately arise:

How may bacteria multiply in frozen soils?

Where can they obtain food which must come to them in solution?

And finally the question upon whose solution that of all the others depends: When the soil is frozen is all the soil moisture congealed?

Conn's theory of the existence of specific groups of what might be called winter and summer bacteria seems plausible, but it fails to account for the multiplication of organisms in frozen soils. It fails to answer the above questions.

The results obtained in this work, as will be seen later, include no attempt to arrange the bacterial species into groups but they confirm Conn's conclusion that bacteria are alive and active in frozen soils, and they show also that the freezing of soils increases their ammonifying power, while reducing their denitrifying, nitrogen-fixing, and nitrifying powers. An attempt is therefore made to answer the question which, as has just been pointed out, immediately arises from such a conclusion: i. e., When the soil is frozen, does all the water congeal? The theory which is advanced later in this bulletin has been evolved from known physical facts, and as there is no experimental evidence to prove or disprove it, it is offered as a theory which is possible of experimental proof.

THE PURPOSE OF THE EXPERIMENTS.

The purpose of the experiments herein reported was to study the total numbers of bacteria in frozen soil; or, in other words, to study the effect of freezing on the total number of organisms in the soil and also its effect on the ammonifying, nitrifying, denitrifying, and nitrogen-fixing powers of the soil.

The number of organisms present in the soil at any time was determined by counting the colonies developing on plates of the "modified synthetic" agar proposed by Lipman and Brown[1], and the ammonifying, nitrifying, denitrifying, and nitrogen-fixing powers of the soil were tested at the various samplings by the beaker method. Determinations were made of the moisture conditions at each sampling, and the soil and air temperatures together with the general weather conditions were carefully observed and recorded. There was also in mind

an attempt to ascertain the relative influence of temperature and moisture conditions on the multiplication of bacteria and also on the various powers of the soil already mentioned.

THE PLOT EMPLOYED.

The plot employed in the experiment was carefully selected with the purpose of eliminating, as far as possible, disturbing factors. It is located on a tract of Wisconsin drift soil now used for experimental purposes by this department, and consists of a black, sandy loam, classed by the Bureau of Soils as Marshall Sandy loam. This surface soil is underlaid by a layer of clay which in turn rests on gravel, good drainage, and consequently undisturbed aeration, thus being insured. The plot is somewhat higher than its neighbors and is therefore protected from their wash. This fact, together with the excellent underdrainage, prevents the disturbance which constant, artificially induced changes in moisture relations would occasion. For the preceding five years the plot was in continuous meadow, receiving no cultivation and no treatment of any kind. Prior to that time it was under an ordinary four year rotation. Here again artificial conditions were very largely eliminated. The plot seemed, therefore, particularly well suited for the experiment as planned.

METHODS.

THE QUANTITATIVE METHODS.

After a careful consideration of all the various media which have been suggested from time to time as the bases for the quantitative estimation of soil bacteria, the synthetic medium already mentioned seemed the most satisfactory. Bouillon agar and gelatin have been shown to be of little value and various agars and gelatins made up from soil extracts are obviously open to objection because of the great differences in soil extracts depending on the character of the soil employed. Consequently, while it is manifestly impossible to formulate a medium which would permit of the development of all soil organisms, the "modified synthetic" agar gives the largest counts of any medium yet employed, and also eliminates some of the difficulties encountered in the case of some other media.

The plates were made by the usual dilution method. One hundred gram quantities of the soil prepared as described later were shaken for five minutes with 200 cc. portions of sterile water. 1 cc. portions of these infusions were transferred by means of sterile pipettes into 99 cc. portions of sterile water. From these suspensions dilutions were made in the order of 1-2,000, 1-20,000, and 1-200,000, care being taken that the con-

1. Centbl. f. Bakt. (etc.) 2 Abt. 25 (1910) p. 447.

tents of the flasks were thoroughly shaken before making the transfers, which in every case were performed with sterile pipettes. 1 cc. portions of the 1-20,000 and 1-200,000 dilutions were placed in sterile Petri dishes and the medium carefully mixed with them. The plates were then incubated for three days at room temperature, which was about 22° C. The results which are given are the average of the counts obtained on the two dilutions, and these counts agreed very closely in every case. No count was made of the molds, but they seemed to be slightly more numerous during the time that the soil was frozen.

PHYSIOLOGICAL METHODS.

The results of so many experiments have shown so irrefutably the unsatisfactory nature of the solution method for testing the physiological activities of soil bacteria that it was used in only one case in this experiment, and in that mainly for the purpose of comparison. In all cases the beaker method was employed, the soil itself being used as a medium. At the beginning of the experiment a large quantity of soil from the plot chosen was obtained, sieved, and thoroughly air-dried and stored for use. One hundred gram quantities of this soil were weighed off in tumblers for the various experiments, the proper materials added and stirred in thoroughly by means of a sterile spatula. The materials which were chosen to encourage the development of certain groups of organisms were:

Ammonification:—5 grams dried blood (D. B.)
5 grams cottonseed meal (C. S. M.)
Nitrification:—100 mgs. $(NH_4)_2SO_4$.
200 mgs. dried blood (D. B.)
Denitrification:—500 mgs. sodium nitrate.
Nitrogen Fixation:—1 gram mannite.

One hundred gram portions of the freshly sampled soil obtained as described later were shaken with 200 cc. of sterile water for five minutes and 10 cc. of this infusion (=5 grams of soil) were added to the medium in the tumblers. Sterile water was then added in order to offer optimum moisture conditions, 20% being the content determined for the soil.

Additional amounts of 12 cc. were added in the ammonification experiments where dried blood and cottonseed meal were employed to provide for optimum moisture conditions in the organic matter, (70%). The tumblers were then covered and incubated for varying lengths of time, the ammonification experiments, seven days; the nitrification experiments, twenty-seven days; the denitrification and the nitrogen-fixation experiments, ten days. In case of the nitrification experiments, the loss of moisture occasioned by evaporation was replaced every week by additions of sterile water to weight.

AMMONIA AND NITRATE DETERMINATIONS.

In the ammonification experiments the ammonia was determined by the usual method, i. e., the soil was transferred to copper flasks with 250 cc. of water, paraffine and heavy magnesium oxide were added, and the ammonia was then distilled off and collected in standard acid and titrated against standard alkali.

In the nitrification experiments the nitrates were leached out of the soil, aliquots evaporated to dryness and the determination made by the phenolsulfonic acid method.

In the denitrification experiments the nitrates were leached and then determined by the phenolsulfonic acid method and the residues were dried, ground, and analyzed for total nitrogen by the regular Kjeldahl method.

In the case of the nitrogen-fixation experiments, the soils were dried, ground, and analyzed for total nitrogen by the Kjeldahl method.

METHOD OF SAMPLING.

The samples of soil were drawn from the plot already described within an area about five feet square, in order to eliminate as far as possible local differences in the soil. They were taken to a depth of 20 cm. by means of a two and one-half inch auger except during the time the soil was frozen, when it became necessary to substitute a mattock or grub hoe for the auger. The samples were collected on a clean mixing cloth, and then placed in sterile glass jars and taken to the laboratory and the inoculations performed as quickly as possible. When the soil was not frozen, the preparation of the sample consisted merely in breaking up the lumps and mixing thoroughly, but when it was frozen further work was necessary. It has been suggested that frozen soils should be allowed to thaw and should then be stirred, after which the inoculations may be performed, but in this work it was not deemed advisable to permit such a multiplication of organisms to occur in the sample as would undoubtedly take place if it were allowed to stand long enough to thaw out completely. Consequently the frozen samples employed here were thoroughly comminuted by means of a sterile spatula, carefully mixed, and then subsampled for the inoculations. The maximum time required to prepare the sample in this way was ten minutes.

MOISTURE AND TEMPERATURE DETERMINATIONS.

Moisture determinations were made at each sampling, and the results are expressed on the air-dry basis. Careful note was made of the temperature and condition of the soil at the time of sampling, and the facts regarding the atmospheric tem-

perature and the rainfall for the entire period were obtained from the statistician located at the college.

THE QUANTITATIVE DETERMINATIONS.

Eight samples were drawn during the winter of 1910-1911, four of these being taken when the soil was frozen. Plates were made as already described and the counts obtained at the different dates are shown in Table I. which also shows the moisture determinations, and the soil and the air temperatures.

At the first sampling October 17, there were 10,858,000 bacteria per gram of air dry soil. The percent. of moisture in the soil at that date was 20.8, the soil temperature was 15.0°C. and the air temperature was 13.5°C. Twelve days later the temperature of the air had dropped to 3.0°C. and the soil temperature was lowered to 7.0°C. The moisture, however, had increased to 22.7%, but notwithstanding this increase in moisture the

TABLE I.

THE QUANTITATIVE DETERMINATIONS.

Date	Bacteria per gram air dry soil	Percent Moisture	Soil Temp. °C	Air Temp. °C
Oct. 17	10,858,000	20.8	15.0	13.5
Oct. 29	10,478,000	22.7	7.0	3.0
Nov. 15	8,252,000	17.3	6.0	—0.5
Dec. 3	5,200,000	20.4	1.0	—5.5
Jan. 11	4,821,000	21.6	—1.0*	—11.0
Jan. 26	7,723,000	24.9	—1.0*	—0.5
Feb. 11	4,744,000	15.7	—1.0*	—6.8
Mar. 1	16,870,000	26.5	—1.0*	—1.0

*Ground frozen and snow covered.

number of bacteria present was reduced to 10,478,000, a reduction of 380,000 per gram of soil. November 15, the number diminished to 8,252,000, the air temperature having dropped slightly below zero and the soil temperature being reduced to 6.0°C. In this case, the moisture content also diminished to 17.3%. Two weeks later, on December 3, a further reduction in numbers was noted, only 5,200,000 bacteria being present, a reduction of over three million bacteria per gram of soil from the previous date. The air temperature at this date had fallen to —5.5°C., while the soil temperature was only 1.0°C. Here again the moisture increased to 20.4%, but notwithstanding that increase the numbers of bacteria diminished with the air and soil temperatures. January 11, in spite of a gain in moisture to 21.6%, the numbers decreased to 4,821,000 bacteria, following again the air and soil temperatures which dropped to —11.0° and —1.0°, respectively, the soil then becoming frozen and snow covered.

ORGANISMS INCREASED AND DECREASED IN FROZEN SOIL.

Fifteen days later the number of organisms had increased to 7,723,000, this increase occurring while the soil was frozen and snow covered and at a temperature of —1.0°. At this date there was a rise in the temperature of the air to —0.5° and also an increase in moisture to 24.9%. February 11, there was a decrease in numbers to 4,744,000 bacteria, the soil still being frozen and at a temperature of —1.0°, but the air temperature had dropped to —6.8° and the moisture had diminshed to 15.7%. March 1, the soil was still frozen and at the same temperature, but the air temperature had increased to —1.0° and the moisture to 26.5% and a considerable increase in numbers was the result, 16,870,000 bacteria per gram of soil being found at that date.

Considering these results as a whole, many interesting facts are shown. In the first place it may be noted that there was a gradual decrease in the air temperature from October 17 to January 11, and this caused a drop in the soil temperature which was, however, more gradual and on the latter date went only to —1.0° then becoming frozen. Notwithstanding the fact that at succeeding dates the air temperature fluctuated, always remaining below zero, the soil temperature remained constant at —1.0° and the soil was frozen during the entire period from January 11 to March 1. The number of bacteria decreased gradually from October to January, following thus very closely the drop in soil temperature and also in air temperature. During this period the moisture conditions were exceedingly variable, rising and falling with the rainfall. This fluctuation in moisture conditions was apparently without influence on the numbers of bacteria in the soil, or at least had much less influence than the temperature conditions, for the numbers decreased with the temperature, the several increases in moisture recorded during the period proving ineffectual in checking this gradual decline.

In the work of Fabricius and von Felitzen, which has already been cited, it was found that "the bacterial content of the soil stands in direct relation to the temperature of the soil, rising and falling with it." As has been stated, their work was carried on during the growing season. Conn could not confirm their results and concluded that the moisture content of the soil had more influence on numbers than the temperature, during the time that the soil was not frozen. The work reported here seems to confirm the earlier experiments of Fabricius and von Feilitzen rather than those of Conn, for the numbers of bacteria recorded during the time that the soil was not frozen were influenced mainly by the temperature conditions, the effects of changes in moisture being non-apparent.

INFLUENCE OF MOISTURE ON BACTERIAL COUNT.

During the time that the soil was frozen, the numbers of organisms seemed to increase and decrease with the changes in moisture conditions, and at the last sampling such a large increase in numbers occurred coincident with a large increase in moisture that the count recorded at that date showed more organisms present than were found during the previous fall when the soil was not frozen. It might seem, therefore, from these results that during the time when the soil was frozen, moisture conditions governed the number of organisms present, but it will be shown later that another explanation of the increase in numbers may be offered according to the theory advanced by Conn.

Conn's conclusions regarding the presence and multiplication of bacteria in frozen soils are therefore confirmed, and furthermore the largest number of organisms was found in the soil when it was frozen, confirming thus his observation that maximum counts may be obtained in the winter. His statement, however, that bacteria seem to increase and decrease nearly parallel to the moisture content of the soil except during the winter is not borne out. These results show that the bacteria increase and decrease with the temperature of the soil during the fall until the soil becomes frozen, when they seem to follow the changes in moisture conditions.

EXPLANATION OF DIVERGENT RESULTS.

In connection with this divergence of results from those of Conn, several important facts should be noted. In the first place, Conn did not employ the same medium as was used here. He used a soil extract gelatin; the objections to which have been discussed in previous publications, and a synthetic medium was employed here which, while not free from criticism, has been shown to allow of the development of much larger numbers of organisms than any other medium. This difference in medium alone might account for the variation in the results obtained. Furthermore, the results secured with one soil are not necessarily applicable to other soils. They may or may not be confirmed by other experiments. The bacterial flora of different soils, and the varying mechanical and chemical conditions pertaining to them are exceedingly variable, while the latter conditions will affect the results obtained at different places when the same medium is used, the bacterial flora of the soil will affect the results when different media are employed, for species which will grow on one medium may refuse absolutely to grow on another, and vice-versa. Furthermore, different species are affected differently by varying moisture and temperature conditions, so that the effect of variations in

these conditions would depend largely on the character of the bacterial species present in the soil.

There is one thing further which may be mentioned in this connection. The statement which is frequently found in scientific articles that "numbers are parallel to moisture conditions," is evidently based on the relations shown by the curves. Now, parallelism should not be assumed between two curves in whose construction there are adopted arbitrary units of division on the ordinates and abscissae which are not the same for the two curves, as a change in any of the units would necessarily alter the relations between the curves. The only interpretation which should be put upon curves so constructed is that they proceed in the same or in opposite directions, and if they chance to be parallel, it is due to the accident of construction.

SUPPORT FOR CONN'S THEORY.

It should be noted here that these results may be interpreted to lend support to Conn's theory of the existence of different specific groups of predominating organisms in normal and in frozen soils. There was a gradual decrease in numbers from October 17 to January 11, when the soil became frozen, showing that the conditions were becoming less and less favorable for the growth of soil organisms. While the soil was frozen, however, with one exception, there was an increase in numbers and the maximum count was obtained on March 1, the last date of sampling. The exception to this increase, on February 11, occurred when a very low moisture content was found, so that probably in this case the drop in moisture was sufficient to cause the decrease in numbers from the previous date after the increase had begun which led eventually to the maximum count on March 1, but of course there is the possibility that some other unrecorded factor might have governed the numbers present at that time.

However this may be, it is interesting to note how closely Conn's theory fits these results. The conditions which caused the retardation in bacterial development as the soil cooled, might well explain the subsequent increasing development of bacteria after the soil became frozen, and the bacterial species in which this increase occurred probably were different from those originally present, and certainly were better adapted to growth at low tempreatures. It is perfectly possible, also, that these resistent species are present throughout the year but are held in check by the groups which are favored by the warmer temperatures.

Instead of concluding from these results, therefore, that when the soil is frozen moisture conditions govern the number of organisms present in it at any time, it may well be as-

sumed that after the soil is frozen there is increasing development of particular species favored by low temperatures, and that this increase ordinarily proceeds regardless of moisture, unless the depression in moisture content becomes very great, when its effect is felt even on these hardy varieties.

In general, therefore, it may be said that the conclusions from this work, in spite of the differences in the methods, in the soil, in the climatic conditions, etc., confirm Conn's conclusion that bacteria are active in frozen soils and also lend support to his theory of the existence of specific groups of winter and summer bacteria.

CONCLUSIONS FROM THE QUANTITATIVE DETERMINATIONS.

The conclusions which may be drawn from the quantitative determinations are, therefore:—

1. By means of the "modified synthetic" agar plate method, bacteria are shown to be present in large numbers in a typical Wisconsin drift soil when it is completely frozen and the temperature is below zero degrees Centigrade; furthermore, increases and decreases in number of organisms occur during this perid and larger numbers are found after the soil has been frozen for a considerable period than before it begins to freeze .

2. During the fall season, the number of bacteria present in the soil diminishes gradually with the lowering of the temperature irrespective of moisture conditions.

3. When the soil is frozen, an increase in numbers occurs. Two explanations may be offered for this increase. In the first place, it may be assumed that when the soil is frozen the number of organisms present depends on the moisture conditions. On the other hand, the results may be interpreted to confirm Conn's theory of the existence of a special group of organisms favored by low temperatures. If this latter explanation is accepted, an additional conclusion is brought out; i. e., while ordinarily when soils are frozen, the numbers of the particular species increase very rapidly and with no relation to moisture conditions, a depression in moisture content may be so great that it will check the development even in this hardy species.

PHYSIOLOGICAL DETERMINATIONS.

As has been stated already, it was intended to study the ammonifying, nitrifying, denitrifying, and nitrogen-fixing powers of the soil at the different dates of sampling. These were determined by the beaker method, and the ammonifying

power was also tested by the solution method for the purpose of comparison.

AMMONIFICATION IN SOLUTION.

The usual one percent peptone solution was employed, and the inoculations performed as described with 10 cc. (=5 grams soil) of a five minute infusion of a fresh sample of the soil. The results are given in Table II.

TABLE II.

AMMONIFICATION IN PEPTONE SOLUTIONS.

Date.	Lab. No.	Ammonia mgs. N.	Average mgs. N
Oct. 17	513	31.80	
	514	37.76	34.78
Dec. 3	523	70.34	
	524	72.81	71.57
Jan. 11	533	87.62	
	534	87.91	87.76
Jan. 26	543	53.94	
	544	55.49	54.71
Feb. 11	553	68.20	
	554	76.57	72.38
Mar. 1	563	94.86	
	564	96.17	95.61

Studying these results, we find that on October 17, 34.78 mgs. N. were produced as ammonia; December 3 that amount was more than doubled, 71.57 mgs. N. being found. This increase corresponded to a large decrease in numbers of organisms which grew on the modified synthetic agar plates, and also to a decrease in the soil and air temperatures, while the moisture conditions remained about constant. January 11, still more ammonia was produced, 87.76 mgs. N. being found, and this increase corresponded to a slight increase in moisture, the number of organisms being smaller and the soil temperature being lower than at the previous date. As the temperature of the soil was lowered from October 17 to January 11, there was a gradual increase in the ammonifying power of the soil, and this may be due to a gradual removal of conditions which had been limiting the ammonifying power. January 26, after the soil had been frozen for two weeks, a large decrease in ammonia production was observed, only 54.71 mgs. N. being formed, although the number of organisms had increased and the moisture conditions had increased. February 11, an increase to 72.38 mgs. N. was observed, corresponding to a decrease in numbers and moisture while the soil temperature remained constant. March 1, a large increase to 95.61 mgs. N. was occasioned, this gain coinciding with an increase in moisture and in numbers, the soil still being frozen and snow covered.

AMMONIFYING POWER GREATER IN FROZEN SOIL.

The results obtained by these tests in solution are very interesting. We note that there was a gradually increasing production of ammonia by the samples until the soil became frozen, when a drop occurred. During the time that the soil was frozen the ammonifying power gradually reasserted itself and at the last date of sampling, after the soil had been frozen for a considerable period, a greater ammonifying power was found than before the soil became frozen.

It is seen from these results, therefore, that the ammonifying power of the soil increased as the temperature was lowered, independently of the moisture conditions, so that from the fact that there was a gradual diminution in numbers during the time, it would seem that the lowering of temperature gradually removed conditions inimical to the ammonifying species. These conditions may have been chemical or bacterial in nature. When the soil became frozen, however, there was an abrupt termination of this state of affairs and the ammonifying power was reduced. There appeared then to be a gradual readjustment to the changed conditions and the ammonifying power began to increase. This increase corresponded to increased numbers, and if we accept Conn's theory, therefore, of the existence of specific winter species, we might assume that these specific bacteria possessed greater ammonifying power than the summer species; or, at any rate, the assumption seems warranted that the relationships of the species in the frozen soil were so altered that the ammonifying power increased beyond that which was observed when the soil was not frozen.

These results will be discussed further, comparisons made, and conclusions drawn, after the results of the ammonification tests in beakers have been studied.

AMMONIFICATION IN SOILS.

The results of the ammonification tests in beakers are given in Table III and the separate results for the ammonification of dried blood and cottonseed meal will be found in Table IV.

Considering first the results of the experiment with dried blood on October 17, 81.51 mgs. N. were found and on December 3, an increase to 103.87 mgs. N. was observed. This increase corresponded to a decrease in numbers, a decrease in the air and soil temperatures and to a slight decrease in moisture. January 26, a big increase in ammonia production was recorded, 120.30 mgs. N. being found and this corresponded to an increase in numbers and in moisture while the soil tem-

TABLE III.

AMMONIFICATION IN SOILS.

Date	Lab. No.	Addition mgs.	Ammonia mgs. N.	Average mgs. N.
Oct. 17	113	5 gms. D. B.	77.77	
	114	5 gms. D. B.	85.33	81.51
	115	5 gms. C. S. M.	79.18	
	116	5 gms. C. S. M.	84.74	81.86
Dec. 3	123	5 gms. D. B.	103.18	
	124	5 gms. D. B.	104.57	103.87
	125	5 gms. C. S. M.	48.53	
	126	5 gms. C. S. M.	55.94	52.23
Jan. 11	133	5 gms. D. B.	49.40	
	134	5 gms. D. B.	lost	49.40
	135	5 gms. C. S. M.	62.40	
	136	5 gms. C. S. M.	52.40	57.40
Jan. 26	143	5 gms. D. B.	122.50	
	144	5 gms. D. B.	118.10	120.30
	145	5 gms. C. S. M.	118.70	
	146	5 gms. C. S. M.	119.10	118.90
Feb. 11	153	5 gms. D. B.	138.51	
	154	5 gms. D. B.	142.68	140.59
	155	5 gms. C. S. M.	124.62	
	156	5 gms. C. S. M.	125.86	125.85
Mar. 1	163	5 gms. D. B.	157.79	
	164	5 gms. D. B.	148.49	153.14
	165	5 gms. C. S. M.	128.03	
	166	5 gms. C. S. M.	lost	128.03

TABLE IV.

THE AMMONIFICATION OF DRIED BLOOD AND COTTONSEED MEAL.

Date	Dried Blood mgs. N.	Cottonseed Meal mgs. N.
Oct. 17	81.51	81.86
Dec. 3	103.87	52.23
Jan. 11	49.40	57.40
Jan. 26	120.30	118.90
Feb. 11	140.59	125.85
March 1	153.14	128.03

perature remained constant. February 11, a further increase in ammonia production occurred, to 140.59 mgs. N. while the numbers diminished and the moisture decreased. March 1, an increase was observed, 153.14 mgs. N. being formed, and this again corresponded to an increase in numbers and in moisture.

At first glance these results would seem so irregular that no conclusions could be possible, but some facts may be noted from a careful study of the figures obtained, and a comparison with the temperature and moisture conditions. From October 17 to December 3, we note an increase in the ammonification of dried blood, notwithstanding the lowering of the soil temperature. It will be remembered that a similar increase was

found in the case of the ammonification in peptone solutions, and it may be attributed here as it was in that case to the removal of inimical conditions, chemical or bacterial in nature, by the changed temperature conditions. In this case, however, the minimum ammonifying power was reached at an earlier date, and on January 11, when the maximum ammonification was observed in the peptone solutions, meager ammonification was found, a considerable depression having occurred.

After the soil became frozen, however, just as in the solutions, a gradually increasing ammonifying power was observed, and the maximum power was noted at the end of the period during which the soil was frozen.

In the case of the cottonseed meal, on October 17, 81.86 mgs. N. were formed, and on December 3 there was a big decrease to 52.23 mgs. N., this corresponding to a decrease in temperature conditions and in numbers while moisture conditions remained about constant. January 11, 57.40 mgs. N. were found, a slight gain over that found on the previous date and corresponding to a slight increase in moisture, a decrease in temperature and a decrease in numbers. January 26, a large increase in ammonia production was observed, 118.90 mgs. N. being found, this coinciding with an increase in numbers and in moisture conditions, the soil temperature being constant. February 11, as in the case of the dried blood, a gain in ammonifying power occurred, 125.85 mgs. N. being formed while there was a decrease in moisture and in numbers and the soil temperature remained the same. March 1, again there occurred a gain, 128.03 mgs. N. being formed, corresponding to a gain in moisture and in numbers.

Here again, the irregularity of the results might seem so great that conclusions would be difficult, but some similarity and differences between these results and those obtained by the other methods should be noted. In the first place, instead of an increase in ammonifying power occurring as the soil cooled off in the fall, as was observed in the other two cases, we find here a decrease from October to December, indicating that instead of the removal of inimical conditions, chemical or bacterial in nature to which was attributed the increased ammonifying power as shown in peptone solutions and in dried blood in beakers, here the temperature merely caused a depression in the ammonifying power. As has been pointed out in previous publications the difference in the chemical composition of dried blood and of cottonseed meal is of considerable moment in a consideration of the results of tests of the ammonifying power of soils when they are employed. There is an indication in these results that the ammonification of dried blood and cottonseed meal does not always run parallel, and this difference is due in part at least to their different

carbon nitrogen ratio. After the soil became frozen, however, we find that there was increased ammonifying power observed, the maximum power being found at the end of the period when the soil was frozen. Here also, therefore, it is evident that the freezing of the soil brought about a greater ammonifying power than was previously observed.

GENERAL RESULTS OF AMMONIFICATION TESTS.

Considering the results of all the ammonification tests, we find that in the first place there seems to be no relation between the ammonifying power of the soil and the moisture or temperature conditions, either when the soil was not frozen or after it became frozen. In the case of the peptone solutions there was increasing ammonification until the soil became frozen, then a decrease which was followed by a larger increase; a maximum being reached at the end of the frozen period. In the case of the dried blood in beakers, there was increasing ammonification until the soil temperature reached 1.0° C. after which a decrease occurred and this was followed by a big increase. Where cottonseed meal was employed, however, the decrease in ammonification occurred before the soil temperature reached 1.0° C. Owing to lack of samples between October and December, we are unable to determine whether or not any increase in ammonification occurred between those dates, but from the results at hand it would seem that such was probably the case. When the soil was frozen, a big increase in ammonification such as was observed in the other cases also occurred here. As was mentioned under the discussion of the peptone solution results, as the soil cooled off there was a gradual removal of the conditions inimical to the ammonifying power of the soil and an increase in ammonification occurred until a certain temperature was reached after which a decrease occurred and this was followed by a large increase in the ammonifying power of the soil, it being greater after the soil was frozen for a considerable period than it had been before. The temperature at which the drop occurred seemed to depend on the material which was employed to test the ammonifying power of the soil.

In the case of the peptone, the decrease occurred after the temperature had gone below zero, with dried blood it occurred between 1.0° C. and —1.0°C., and with cottonseed meal it seemed to occur before 1.0° C. was reached, probably however being very close to that temperature. There is evidence here therefore that the ammonification of these materials proceeds slightly differently, and that the combined species action which produces the ammonifying power of the soil is not exactly the same on these three materials, when the soil is not frozen. After the soil becomes frozen, however, there is a big

increase in ammonifying power of the soil, no matter what material is employed.

Fitting these results to Conn's theory, we find that it is possible that different species are prevalent after the soil becomes frozen than predominate before. These species multiply to a great extent and, furthermore, they probably possess greater ammonifying power than the others. At any rate, the combined species present when the soil is frozen show greater ammonifying power than that noted previously. It will be seen, therefore, that the results obtained here agree very well with Conn's theory and suggest the additional possibility that freezing the soil removes or reduces species which restrict its ammonifying power, and consequently this power increases far beyond the point which it can attain when the soil is not frozen.

CONCLUSIONS FROM THE AMMONIFICATION TESTS.

The conclusions which may be drawn from these ammonification tests, therefore, are:—

1. Frozen soils possess a much greater ammonifying power than non-frozen soils, whether they are tested by the peptone solution method or by the dried blood or cottonseed meal beaker method.
2. When the soil is cooled gradually, e. g. during the fall, its ammonifying power increases until the temperature of the soil almost reaches zero when a decrease occurs, and
3. After the soil becomes frozen its ammonifying power begins to increase and reaches a maximum at the end of the frozen period.
4. These results lend support to Conn's theory of the existence of specific groups of winter bacteria and suggest also that either these particular groups possess greater ammonifying power or that there is a reduction in the growth of species which ordinarily limit the ammonifying power of the soil.

NITRIFICATION IN SOILS.

Nitrification tests were carried on in soils according to the usual methods and the results may be found in Table V.

October 17, 1.74 mgs. N. as nitrate were found, there was an increase from this amount to 7.76 mgs. N. on December 3, and here as in the ammonification experiments an increase in nitrifying power occurred notwithstanding the fact that there was a slight decrease in moisture and a decided drop in temperature. January 11, only 4.44 mgs. N. as nitrate were found, a decrease occurring here which corresponded to a drop in numbers, a drop in temperature and an increase in moisture. Jan-

uary 26, when the soil was frozen and snow covered and a gain in moisture and in numbers occurred there was a gain in nitrate production. February 11, the soil temperature was the same, but there was a drop in numbers and moisture and a gain in nitrate production to 5.22 mgs. N. occurred. This same gain was noted in the ammonification experiments. March 1, there was a decrease in nitrate production to 4.16 mgs. N. this coinciding with a gain in moisture and in numbers. At the last two dates of sampling a test was also conducted using 200 mgs.

TABLE V.

NITRIFICATION IN SOILS.

Date.	Lab. No.	Addition	Nitrates mgs. N.	Average mgs. N.
Oct. 17	213	100 mgs. $(NH_4)_2SO_4$	1.64	
	214	" " " "	1.84	1.74
Dec. 3	223	" " " "	7.24	
	224	" " " "	8.28	7.76
Jan. 11	233	" " " "	4.44	
	234	" " " "	lost	4.44
Jan. 26	243	" " " "	4.56	
	244	" " " "	4.94	4.75
Feb. 11	253	" " " "	5.68	
	254	" " " "	4.76	5.22
	255	200 mgs. D. B. "	9.48	
	256	" " " "	6.40	7.94
Mar. 1	263	100 mgs. $(NH_4)_2SO_4$	4.00	
	264	" " " "	4.32	4.16
	265	200 mgs. D. B.	10.64	
	266	" " " "	7.08	8.86

of dried blood instead of the ammonium sulfate. February 11, 7.94 mgs. N. were found and then there was a gain to 8.86 mgs. N. on March 1, so that by the use of dried blood, the indication is that moisture conditions governed nitrate production when the soil was frozen.

As a whole the results show that the nitrifying power of the soils was rather weak, very small amounts of nitrates being produced in practically every case. Consequently the differences which are apparent may not be considered as of great significance, for they are too small. In fact they are so small that any conclusions from them would be injustifiable. It may merely be pointed out that the indications are that the nitrifying power of the soil was restricted by the low temperature and that during the time that the soil was frozen it remained practically constant. It would seem therefore that the species which according to the other parts of this work are encouraged by the low temperatures which remove harmful competition, do not include nitrifying organisms.

THE DENITRIFICATION TESTS.

The results of the denitrification tests may be found in

Table VI. The denitrifying power of the soil at the different dates has been calculated in percent of the sodium nitrate denitrified, the loss from the sodium nitrate being first obtained by subtracting the loss in the checks from that in those receiving additions of sodium nitrate. There seems to be considerable variation in the amount of nitrogen lost from the untreated soils. October 17, 8.53 mgs. N. were lost; December 3, 10.33 mgs. N. and January 11, a gain of 16.11 mgs. N. was observed probably due to accidental contamination in the determination which unfortunately could not be checked owing to the loss of the leachings from the duplicate sample.

January 26, only 3.45 mgs. N. were lost while February 11, 62.40 mgs. N. were lost, a remarkably large loss occurring on this date when the smallest number of bacteria was recorded and the lowest moisture content observed.

March 1, again a slight gain was found of 1.04 mgs. N. The gain here occurred in one sample, due again undoubtedly to accidental contamination for in the duplicate a loss was observed.

Considering now the losses which were occasioned from the sodium nitrate by the different samples, we find that on October 17, 16.85% of the nitrogen added was lost. December 3, when there was a decrease in temperature and numbers, moisture conditions being constant, 28.13% of the nitrogen disappeared. Here the lowering of the temperature seemed to increase the denitrifying power of the soil. January 11, all of the nitrate nitrogen was apparently lost. This result is somewhat questionable, however, as on that date there occurred the gain in nitrogen in the untreated soil already mentioned, and probably the percentage of nitrogen lost from the sodium nitrate should be somewhat smaller.

However this may be, the loss was evidently greater than at the proceeding date and occurred coincident with a drop in temperature and in numbers and an increase in moisture.

January 26, only 69.20% of the nitrogen was lost, although there was an increase in moisture, the soil temperature remaining constant. February 11, a depression in moisture conditions occurred coincident with a slight increase in the nitrogen lost, and March 1, an increase in moisture was followed by a depression in denitrifying power.

Considering these results as a whole there seemed to be an increase in the denitrifying power of the soil until it became frozen, after which there was a depression which became greater at each sampling until the end of the frozen period was reached. The results show very little effect of changes in moisture conditions, and the temperature conditions seemed to govern the denitrifying power of the soil until it became frozen. The depression in numbers of bacteria which occurred

TABLE VI.

DENITRIFICATION IN SOILS.

Date	Lab. No.	N. added mgs.	Total initial mgs.	N. in residues mgs.	N. in leachings mgs.	Total final N. mgs.	Loss N. mgs.	Avr. loss mgs. N.	Loss $NaNO_3$ mgs. N.	Pct N. $NaNO_3$ Denitrified
Oct. 17	311	None	189.95	173.60	3.41	177.01	12.94			
	312	"	189.95	182.90	2.82	185.72	4.23	8.53		
	313	82.00	271.95	189.10	62.60	251.70	20.25			
	314	"	271.95	198.40	49.10	247.50	24.45	22.35	13.82	16.85
Dec. 3	321	None	189.95	173.60	6.12	179.72	10.23			
	322	"	189.95	173.60	5.92	179.52	10.43	10.33		
	323	82.00	271.95	158.10	80.00	238.10	33.85			
	324	"	271.95	155.00	84.00	239.00	32.95	33.40	23.07	28.13
Jan. 11	331	None	189.95	201.50	4.56	206.06	+16.11			
	332	"	189.95	189.10	lost	.....	....	+16.11		
	333	82.00	271.95	173.60	15.36	188.96	82.99			
	334	"	271.95	189.10	lost	.....	....	82.99	82.99	101.20
Jan. 26	341	None	189.95	179.80	4.00	183.80	6.15			
	342	"	189.95	186.00	3.20	189.20	0.75	3.45		
	343	82.00	271.95	186.00	28.40	214.40	57.55			
	344	"	271.95	189.10	20.00	209.10	62.85	60.20	56.75	69.20
Feb. 11	351	None	189.95	124.00	4.00	128.00	61.95			
	352	"	189.95	133.30	3.80	127.10	62.85	62.40		
	353	82.00	271.95	139.50	21.31	160.81	111.14			
	354	"	271.95	127.10	17.60	144.70	127.25	119.19	56.79	69.25
Mar. 1	361	None	189.95	195.30	5.00	200.30	+10.35			
	362	"	189.95	176.70	4.24	180.94	9.01	+1.04		
	363	82.00	271.95	204.60	26.40	231.00	40.95			
	364	"	271.95	198.40	17.60	216.00	55.95	48.45	48.45	59.08

while the temperature of the soil was dropping undoubtedly brought about indirectly the increased denitrifying power of the soil. After the soil was frozen there occurred a depression in its denitrifying power, due probably to the fact that the species which were beginning their big increase were unfavorable to the groups which determine the denitrifying power of the soil.

CONCLUSIONS FROM THE DENITRIFICATION TESTS.

From the results of the denitrification tests the following conclusions may be drawn:—

1. Frozen soils possess a decided denitrifying power which seems to diminish with the continuance of the frozen period.
2. During the fall, when the soil is gradually cooling, its denitrifying power increases until the soil becomes frozen, and this increase may be attributed to the restriction of the growth of species which limit denitrification.
3. The denitrifying power of frozen soils is less than that found just before the soil freezes, but greater than that observed when the temperature begins to decrease in the early fall.
4. The decrease in the denitrifying power of frozen soils may be attributed to the competition brought about by the large increases occurring in the particular groups of winter bacteria.

NITROGEN FIXATION IN SOILS.

Table VII. contains the results of the nitrogen-fixation experiments. October 17, 50.30 mgs. N. were fixed and on December 3, a gain to 67.35 mgs. N. was observed, although there was a slight decrease in moisture and a drop in temperature. January 11, the lowered temperature made its effect felt and only 0.70 mgs. N. was fixed, notwithstanding an increase in

TABLE VII.

NITROGEN FIXATION IN SOILS.

Date	Lab. No.	Initial N. mgs.	Nitrogen found. mgs.	Average mgs. N.	Nitrogen fixed. mgs.
Oct. 17	413	189.95	226.3		
	414	189.95	254.2	240.25	50.30
Dec. 3	423	189.95	291.4		
	424	189.95	223.2	257.30	67.35
Jan. 11	433	189.95	189.1		
	434	189.95	192.2	190.65	0.70
Jan. 26	443	189.95	207.3		
	444	189.95	208.1	207.70	17.75
Feb. 11	453	189.95	198.4		
	454	189.95	198.4	198.40	8.45
March 1	463	189.95	219.7		
	464	189.95	204.6	212.15	22.20

moisture. On January 26, there was an increase in the nitrogen-fixing power of the soil, 17.75 mgs. N. being fixed. This was followed by a decrease to 8.45 mgs. N. and subsequently by an increase to 22.20 mgs. N. fixed.

Taking these results in their entirety we find that as the soil cooled off in the fall there was an increase in its nitrogen-fixing power due probably to the same cause that was suggested for the increase in ammonifying power during that time, i. e., the removal of competition. When the soil became frozen, however, on January 11, there was almost a complete absence of nitrogen-fixing power. At the subsequent dates, however, there occurred an increase in the fixing power, but it never reached the original fixing power possessed before the soil froze. It seems probable from these results that lowering the temperature removed or restricted the growth of species which limit the nitrogen-fixing power of the soil, and consequently there was an increase in this power until the soil became frozen which abruptly terminated this state of affairs.

After this probably entirely different species relationships were established in the soil and these permitted of the development of a nitrogen-fixing power which, ordinarily independent of the moisture conditions gradually increased. In one case, however, a depression in moisture occurred which was sufficient to restrict the nitrogen-fixing power of the soil. It will be remembered that a depression in numbers also occurred at this time so that the possibility presents itself that perhaps some unknown factor may have entered here and caused the decrease. The ammonification experiments, however, gave no indication of the presence of any such disturbing factor, so it may have been some peculiar condition of affairs which affected the total numbers and the nitrogen-fixing power without having a noticeable effect on the ammonifying species, although it is possible, of course, that the moisture conditions may have had that peculiar effect.

CONCLUSIONS FROM THE NITROGEN FIXATION TESTS.

The conclusions which may be drawn from the tests of the nitrogen-fixing power of the soil at the different dates are:—

1. Frozen soils possess a nitrogen-fixing power which increases with the continuance of the frozen period, being independent of moderate changes in the moisture conditions, but restricted by large decreases in moisture.

2. In the fall when the soil is gradually cooling, its nitrogen-fixing power increases until the soil becomes frozen, when it almost ceases, after which a smaller nitrogen-fixing power is established.

3. This increase in nitrogen-fixing power in the soil as it cools may be due to the restriction of competition and the in-

crease after the soil is frozen may be due to the establishment of entirely new species relationships.

THEORETICAL.

Taking the results of this experiment as a whole, we find that they are in part in accord with the work of previous investigators already mentioned. That is, this work confirms the observation that bacteria are alive and may multiply rapidly in frozen soils.

This brings us back, therefore, to the questions asked earlier in this bulletin:

How may bacteria multiply in frozen soils?

Where can they obtain food?

It brings us also the question upon which it is deemed the others depend.

When the soil is frozen, is all the soil moisture congealed?

Only one answer to this latter question, only one explanation of the phenomenon of the existence and multiplication of bacteria in frozen soils, seems plausible, and that is that when the soil is frozen not all the soil water is congealed.

In other words, the theory which is advanced is that while the soil as a whole may be frozen and the temperature below the freezing point, that portion of the soil water known as the hygroscopic moisture may be in a liquid state. If this is the case, then in frozen soils there exists a state of affairs similar to that in some frozen ponds and streams, namely the occurrence of ice and water in juxtaposition. Now there is only one condition under which such an occurrence is possible, and that is that the freezing point of the water be lowered below the normal.

There are various conditions which bring about this lowering of the freezing point of water in ponds, streams, etc., and some of the same conditions and some additional ones peculiar to soils may bring about a lowering of the freezing point of the hygroscopic moisture to such an extent that it remains liquid while the main body of the soil water is congealed.

It is a well known fact that the hygroscopic moisture is held around the soil grains with great force and while this force has not been accurately determined, it has been estimated at from 6,000 to 25,000 atmospheres. This pressure is sufficient, therefore, to lower the freezing point of the hygroscopic moisture below zero degrees Centigrade.

There are other conditions, however, which may also exert a depression of the freezing point. All soil water contains salts in solution, the amount and character varying with the chemical character of the soil and also with physical conditions per-

taining to the soil. The amount of salts in soil water has been estimated at from five or six hundred parts to considerably less than one hundred parts per million. Now the presence of small amounts of salts in water has been shown to depress its freezing point, and consequently it is certain that the freezing point of hygroscopic soil water is below the normal. Furthermore, as hygroscopic water is known to contain normally more substances in solution than the main part of the soil water, due to absorption, and as a concentration of salts occurs in it as the capillary and gravitational water freezes, such an accumulation of salts takes place that the freezing point of the hygroscopic film may be considerably lowered.

Because of these three factors, therefore, which may cause a lowering of the freezing point of the hygroscopic moisture in soils, namely, the surface tension exerted by the soil particles on the films of water, the presence of salts in this water, and the concentration in salts which may occur in it when the main body of soil water begins to freeze, it seems justifiable to assume that under average winter conditions, where the soil temperature is not depressed far below zero, the hygroscopic water in soils remains uncongealed and consequently bacteria may live in it and multiply sometimes to a comparatively large extent.

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