NITRIFICATION AND NITRIFYING ORGANISMS

IN SOME QUEBEC SOILS

By

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I. INTRODUCTION

Nature of Nitrification

The formation of nitrates in soil, under favourable conditions as to moisture, temperature, and aeration is a reaction well known and frequently studied.

The bodies yielding nitric acid in the soil are, firstly, the various nitrogenous organic substances which arise from the decay of vegetable or animal matter; and secondly, ammonium salts, either produced in small quantity during the decay of organic matter, or carried to the soil by rain, or in some cases, applied as fertilizers. A further source of the nitrates contained in the soil is to be found in the free nitrogen of the atmosphere, but of any supply of this source, other than the ready-formed nitric acid contained in rain, and the nitrogen fixed by the nitrogen-fixing organisms, there is at present no substantial proof.

The production of nitric acid, both from ammonia and from the organic matter of arable soil is well illustrated

by the field experiments at Rothamsted as reported by Warington (77). The winter drainage-water from the various plots in the experimental wheat field was found to contain nitrates nearly in proportion to the quantity of ammonium salts applied the preceding autumn, while the drainage-water obtained at the drain-gauges from soil which had been unmanured for the previous eight years, clearly derived the greater part of its nitrogen from the organic matter of the soil.

Many investigations have been made as to the mode in which nitrification takes place.

Until the middle of the nineteenth century the phenomenon was thought to be produced by chemical processes. Some observers showed that ammonia may be oxidized into nitric acid by prolonged contact with ferric oxide at ordinary temperature. Ammonia is also oxidized to nitrite by ultra-violet radiation. Hydrogen peroxide and ammonia will react with each other giving rise to nitrous acid. The interaction between ozone and ammonia to give ammonium nitrate may also be mentioned. The quantities of nitrites and nitrates formed by chemical agencies are probably insignificant and of little importance in the soil.

In 1862, Pasteur, in view of the active oxidation induced by "mycoderms" in various kinds of organic matter, expressed the opinion that nitrification required to be studied over again from this point of view. Fifteen years

later, Schloesing and Müntz gave results proving that nitrification was due to the action of an organized ferment. The aim of their experiments was to ascertain if the presence of humic matter was essential to the purification of sewage by soil. The nitrification they obtained after 20 days in a glass tube, one meter in length, filled with mineral soil and allowed to filter the sewage, permitted the observers to ask why 20 days elapsed before nitrates appeared, if not because of the growth of some organism. After holding that nitrification during 4 months, vapours of chloroform took 10 days to stop the reaction which resumed in 8 days only after a new inoculation with fresh soil.

Successive researches by Schloesing and Műntz, (57), Schloesing (58), and Warington (77, 78, 79, 80) definitely established the biological oxidation of ammonia to nitrites and subsequently to nitrates in soils.

Winogradsky (85) succeeded in isolating the organisms responsible for that action and thus permitted the study of their physiological characteristics.

Ten years ago a new theory was put forward. Dhar and others, (6), have shown that in tropical countries 85.8% of an ammonium phosphate solution with sterilized soil was oxidized when exposed to sunlight in presence of air during 700 hours. Rao (54) reinforced that point of view by a long

series of experiments which brought him to conclude that nitrification in the soil is at least partly photochemical, taking place without the agency of bacteria under the action of sunlight at the surface of various soil photocatalysts like alumina and titania.

Singh and Nair (61), experimenting on the same subject, reached the conclusion that biological reactions are largely responsible for the processes of ammonification and nitrification in soil, though nitrite is formed also as a result of photochemical action.

On the other hand, Waksman and others (76), and Fraps and Sterges (10), while not excluding the possibility of photonitrification in soil, showed by elaborate experiments that biological oxidation is the all-important factor in nitrification in soils of temperate regions.

Significance of Nitrification in Soils

There is good evidence that nitrification is of more than passive significance for plants (66). Nitrates are absorbed and renewed in the soil many times throughout the growing season. No other strong acid (in the form of its salts), is so abundant in any but saline or alkali soils. No other anion (if absorption of nitrogen in the anion form is assumed) is needed in such large amounts for plant growth.

With nitrification eliminated there are no adequate means

for dissolving a sufficient quantity of the cations of the mineral soil and for getting sufficient of either nitrogen or the cations into the plant.

It seems that the crop yield is very closely correlated with the nitrifying power and the production of nitrates in the soil (7, 16). Whenever conditions are favourable for complete mineralization of the organic matter, there is also an abundance of available mineral nutrients important to plant growth.

Among essential soil processes, therefore, nitrification stands high in its importance to the nutrition and growth of higher plants.

In the province of Quebec, the high percentage of organic matter found in most of the soils seems to indicate a low activity of the soil microorganisms. This could be explained by the short growing season in which the soil biological processes may go on, and also by the low average temperature of that season.

Nitrification in some Quebec soils was found to proceed at low rates (41, 42, 21, 23), thus showing that this process follows the tendency of the biological activities of these soils.

The present work has been undertaken to ascertain if the low rates of nitrate production occur also in other representative soils of Quebec, which the author has selected after

having studied their distribution during the Quebec Soil Survey in 1942, and to attempt to relate nitrification with other biochemical factors.

II. LITERATURE REVIEWED

A. Nitrification in Soils

1. Soil type

It has been found that the texture of the soil will affect this process. Thus, Reed and Williams (55), have shown that the light open sandy soils had a strikingly low nitrifying power, while in the loams and clay loams it reached its maximum, and in the heavy clays there was again depression, yet not at low as in the extremely open soils. The high nitrifying power of certain cultivated soils irrespective of texture suggested, however, that this power might have been largely increased by proper cultivation. The deficiency in the light soils was probably due to a lack of organic matter whereas the depression in the clay soils could doubtless be ameliorated by aeration after tillage.

In Quebec soils it was found (42), that nitrification was highest in the clay soils and lowest in the lowland podsol soils. The sandy clay soils were intermediate between the heavy clays and the podsol soils.

2. Soil conditions

The beneficial effect of tillage is derived from the fundamental purposes for which cultivation is practiced (72):

1. To improve the physical condition of the soil.

2. To eliminate competing weed growth.

3. To prepare a suitable seedbed.

It is known that the best conditions of heat, moisture, and oxygen created by the tillage will encourage a better growth of the microorganisms, including the nitrifying bacteria.

The elimination of weed growth will keep the necessary moisture of the soil and moreover will hinder the utilization of the accumulated nitrates.

Naturally deriving from the two preceding factors, the suitable seedbed will have the essential stored up nitrates for the growth of the plants.

It is to be noted though that tillage of the right type must be done according to the conditions met with. Thus it has been reported (40) that inter-tillage did not promote nitrate accumulation in a friable soil while the same cultivation with a compact soil considerably increased the nitrates.

The tillage is regulated by the rotation established by the farmers. The succession of the crops grown must be arranged in such a manner as to restore the nitrate content of the soil after it has been exhausted by the crops removed. A well organized rotation has great advantages in maintaining the nitrogen balance that there existed in the soil before its agricultural use. Thus it has been shown (72) that the successive growth of wheat on the land for 12 years reduced

the nitrogen to about 26% of that originally found in the soil at the beginning of the test. The crops grown accounted for less than one fourth of this nitrogen showing that three fourths or more had been lost mainly through decay of the soil organic matter under this type of continuous farming.

3. Crops

Plants use nitrates for their nutrition, but they also secrete soluble organic matter (38) containing only a small proportion of nitrogen. It was suggested that this highly carbonaceous organic matter may act as a source of energy for soil organisms that assimilate the nitrates and cause their disappearance. This capacity of living plants to cause a transformation of nitrate-nitrogen into other forms is of some practical benefit. The nitrate-nitrogen that the crop does not use is taken up by the soil organisms and its loss by leaching prevented. On the other hand, there is the possibility that there will not be sufficient available nitrogen adequately to supply the crop.

Among the investigations done on the plants, one shows the effects of the different growth on the nitrate (47). The mean annual nitrogen absorption of hay crops (entire plant to plow depth) based on sods one and three years old was, in pounds per acre: alfalfa, 241.8; timothy, 152.2; western rye, 137.8 and brome, 154.2. For entire wheat plants following one-, three-, and five-year old sods of these hay crops for

six, four, and two successive years, respectively, the mean values were: 63.4, 58.6, 56.3, and 51.9. These figures are taken to indicate roughly the relative rates of soil nitrification under and after the crops in question, except under alfalfa. The roots and stubble of the hay plants contained about 71% of the dry matter and 68% of the nitrogen of these plants, whereas the wheat roots and stubble contained only 19% of the dry matter and 9% of the nitrogen of these plants.

Thus if the crop residues incorporated to the soil are taken under consideration it may be thought that they affect the soil nitrates differently. The percentage of total nitrogen in the incorporated material is an important factor because it usually changes the ratio of nitrogen to carbon in the soil. Ordinarily the ratio is narrower in the soil in which organic matter decomposition has reached an equilibrium than it is in plant tissues. That the nitrogen-carbon ratio in the incorporated plant tissue is, for all practical purposes, the main consideration in the assimilation of nitrates by microorganisms is emphasized by the following data (38) obtained from an experiment in which the roots of a number of hay crops were incorporated in the soil and allowed to decompose. At intervals the soil was leached and nitrates were determined in the leachings. It will be seen that the total quantity of nitrate-nitrogen in the leachings is in the same order as the nitrogen percentages in the materials added to the soil.

Roots of	Weight of roots	% Nitrogen	Total nitrogen in the leachings
Oats	153.3 mgs.	0.45	207.3 mgs.
Timothy	96.8 "	0.62	398.4 "
Clover	35.1 "	1.71	924.4 "

It is then obvious that the nature of the crop and its placement in a rotation may exert an important influence on the yield of the succeeding crop because of the carbon-nitrogen ratio of its residues and the effect on nitrate assimilation.

4. Seasonal changes

Nitrates of the soil fluctuate from season to season and differently in different soils.

Gowda observed (18) that in soils in Iowa there was a large accumulation of nitrates in June with a gradual decrease in July, and a rapid decrease in August and September when it reached the minimum. In October there was a slight increase.

Reuszer (56) observed that a bare soil exhibited such seasonal fluctuations while this was not the case for a soil under pasture. He also found evidence that part of the autumn rise in soil nitrate may be due to mineralization of microbial protein.

In Quebec soils such a seasonal fluctuation has been found (19), but it would seem merely that the maximum production comes in the later part of the season. Studies on three fallow soils, during two successive years showed that the mean highest nitrate accumulation occurred in late June or beginning of July; it was less in August and reached a minimum at the end of September.

5. Influences in soils

Before proceeding any further in the study of nitrification in soils, it is worth while to mention the care that has to be taken in the interpretation of solutions tests as compared to soils tests. The study of Stevens and others (68) establish that "tests in solutions are not adequate to indicate the nitrifying vigor of a soil" because, as they found, some nitrifying soils do not nitrify when placed in solutions even though a very large inoculum is used.

This seems to be confirmed in our experiments where soils No. 1 and 12, respectively cultivated and under forest, both showed about a three-fold increase in nitrate-nitrogen while incubated during some 200 days from sampling. They were, however, very slow to nitrify in solution and in fact never could survive a first transfer after the crude culture.

Outside of the fact that the solution provides all the necessary nutrients while the soil may not, it has been observed

frequently that in a saturated soil, which is the state of matter existing in the solutions, nitrification is nil or very slight.

Many factors certainly govern these differences and it seems that information is still required on this subject.

a. Physical.

If the effect of sunlight is first taken into consideration as it has been established that the photonitrification was of no great importance in the temperate regions, the germicidal action of that light will be the only possible effect one might expect. The experiments of Fraps and Sterges (10) are quite confirmatory. To determine how light affects nitrification, samples of soils exposed to sunlight for four weeks and stirred daily to present new surfaces to the light were compared with corresponding samples protected from the light. On an average of 72 comparisons conducted on six soils, each with four treatments and three different salts of ammonium, 5% of the ammonia was oxidized in sunlight and 41% in the dark. The decrease in the sunlight was slower with ammonium phosphate than with the ammonium chloride or the ammonium sulphate, though this might have been due only to differences of intensity of the light at time of exposure. Since only a small portion of the soil is exposed to direct sunlight, the destructive action of sunlight upon nitrifying organisms is not likely to be of agricultural importance.

In the first investigations on nitrification in soils and the organisms involved in the phenomenon, Schloesing and Müntz (57) noted the disadvantageous effect of desiccation and they also observed the increasing benefit of increasing amounts of moisture content up to a maximum which will not saturate the soil. Fred (12) observed no loss from denitrification provided the moisture content does not exceed one half total saturation capacity. This is generally accepted and is of current use when remoistening dried soils to bring them to conditions best suited to most biological activities.

The function of the soil moisture is that of a carrier of soluble gas and mineral or organic food to the plants and to the microorganisms, which gas and food have a direct effect on the growth and physiological activities of the microorganisms.

According with the physiological characteristics of the nitrifying organisms, the oxidation process will proceed only if oxygen is present. Plummer (51) has established the optimum concentration of oxygen in cultures to be around 40%.

Aeration in soil being dependent on the texture of the soil materials, it is evident that the more porous the soil will be, the best nitrification will it show. Fred (12) succeeded in obtaining increased nitrification with two clays which were diluted with sand.

The mention made (75) of the deeper distribution of the organisms with the more porous soils seems to find its explanation by the deeper penetration of oxygen through the soil mass.

The optimum conditions of temperature are around 25°C, in the temperate soils, while according to Tandon and Dhar (69), it would be 35°C in the tropical regions, for the nitrous organism. Waksman (75) gives 27.5°C as optimal for the process to go on. This was confirmed by Nelson (46). Warington (78) in his preliminary studies gave a temperature of 30°C as optimum and said that 40°C apparently killed the ferment. Later (79) he established that nitrification proceeded at temperature as low as 3.2°C. Schloesing and Müntz (57) gave the following range of temperature:

5°C or lower	: very weak action or nil.
12°C	: appreciable.
37°C	: maximum action. Ten times more than at 14°C.
45°C	: less than at 15°C.
50°C	: very little action.
55°C	: nil.

but it does not seem to be in accordance with Nelson's more recent studies (46).

Jensen (32) found that the effect of varied range of temperature in the soil have a decided indirect effect on nitrification, when he demonstrated with incubated soil, that, at 5°C, the numbers of bacteria and the density of fungi was

higher than at higher temperature. This, he suggested, would have meant that the lower the temperature of decomposition, the greater a proportion of nitrogen in the transformed organic matter was temporarily locked up as microbial substance before eventually appearing as ammonia and nitrate. At 5°C the numbers of bacteria were such as to account for approximately one-third of the nitrogen present in the added organic material.

b. Chemical

(i) Effects of mineral elements - Fraps and Sterges (11) state that the average order of effectiveness of phosphates (average of 14 soils) to promote nitrification, beginning with the most effective, is as follows: monopotassium phosphate, 20% superphosphate, dipotassium phosphate, monocalcium phosphate, tricalcium phosphate, dicalcium phosphate, disodium phosphate, rock phosphate with colloidal clay. These results are in the order one could expect from the knowledge of the availability of the phosphoric acid in the phosphates.

Hall and others (27) explained the nitrate accumulation in very acid soils as due to the fact that nitrate formation takes place in films surrounding the small isolated particles of calcium carbonate. The addition of calcium carbonate has, therefore, a decided effect on nitrate formation, particularly in acid soils. The opposite effect may be met with in alkali soils poor in organic matter, since it tends to

liberate free ammonia from ammonium salts and that at the expense of nitrification economy. Lime does not stimulate the activities of the nitrifying organisms; it serves as a base for neutralizing the acid formed from the oxidation of ammonia.

The addition of lime to acid peat and muck soils leads often to a rapid diminution of available nitrogen because of the organisms becoming very active.

The observations of Turk (71) on that matter are not confirmatory; he found that nitrate accumulation in all but one of the acid mucks studied was favorably affected by the addition of calcium carbonate when applied to neutralize acidity of the soil, while excess lime, particularly in the form of calcium hydroxide was in some cases detrimental to nitrate accumulation. He also noted a beneficial effect of addition of lime to acid muck on the carbon dioxide evolution. In alkaline or neutral mucks lime caused a decrease in the carbon dioxide evolution.

Fraps and Sterges (9), studying the causes of low nitrification capacity of certain soils, reached the conclusion that when field soils are compared for nitrifying power of ammonium sulphate the differences observed may be due chiefly to differences in the numbers of nitrifying bacteria at the time the experiment began and to the abundance or deficiency of calcium carbonate.

Fred (12) had already found that the applications of lime caused an enormous increase in the rate of nitrification. The total amount of nitrate formed during one year in soils treated with lime is more than is possible from the ammonium sulphate alone.

Field studies of the effect of lime on the biological activities of acid silty clay loam soil in Oregon (48) showed that larger quantities of nitrates were found in the limed plots, and laboratory tests showed that the nitrifying and nitrogen-fixing powers of the soils were stimulated.

Gray and Atkinson (22), have observed that the highest nitrate-nitrogen contents were obtained after applications of sodium carbonate with calcium carbonate to satisfy the lime requirement. They also noted that the sodium carbonate appeared to be more effective alone than with calcium carbonate.

Greaves (25) summarizes a review of investigations carried on by many workers and himself in asserting that many salts when applied to soil in small quantities increase the bacterial activities; production of ammonia, nitrates and soluble and organic phosphorus being particularly studied.

Usually, although not always, those salts which become toxic in the lowest concentrations are the greatest bacterial stimulants.

A close correlation has been observed between toxicity

of the various salts and the osmotic pressure produced in the soil, thus showing that toxicity is due in part to osmotic disturbances. Another factor of equal importance is the change in chemical composition of the protoplasm resulting from the formation of salts of the protein other than those normally occurring in the living protoplasm, thus incapacitating them for their normal functions.

Chlorides, nitrates, sulphates and carbonates of Na, K, Ca, Mg, Mn, and Fe exert a toxic effect upon nitrate formation in the soil according to the following order of the anions: $CO^3 > NO^3 > SO^4 > Cl$, while for the cations the order would be: K > Mg > Fe > Mn > Ca > Na.

The quantity of a salt which can be applied to a soil without decreasing the nitrate-nitrogen accumulation varies with the nature of the salt. Those compounds which become toxic in lower concentrations are not necessarily most toxic in higher concentrations, as the toxicity of some salts increases more rapidly than the toxicity of others.

It has been noted also in the numerous works on the subject that there exists an antagonism between salts, which means that a toxic salt may be rendered not toxic by the addition of another salt. For example, iron nitrate with magnesium chloride increased nitrification 420.7% over a soil treated with magnesium chloride alone.

Certain acid soils were reported by Whiting (81) to contain large amounts of soluble manganese and this element appears to support nitrification. Results with copper sulphate were not consistent, and for the most part the results showed a retarding rather than a stimulating effect. Sodium chloride with lime decreased the nitrate content causing an ammonia accumulation. Sodium chloride without lime caused increases in half of the samples. The treatment consisting of sodium chloride, copper sulphate and 0-8-24 fertilizer resulted in less nitrate accumulation than in the check. Increases in nitrates occurred where the 3-8-24 fertilizer was added with sodium chloride and copper sulphate. Manganese sulphate showed erratic results and no appreciable effect could be noted from the use of potassium iodide, barium chloride, aluminum sulphate, zinc sulphate, and boric acid.

Whiting also observed that the benefit obtained from ferrous sulphate on nitrification was apparently due to the iron rather than to the sulphate. Sulphur definitely stimulated nitrification in all the naturally alkaline mucks and also in these mucks that were heavily limed. The sulphur caused an increase in acidity, thereby reducing the toxicity of the alkali to the nitrifiers. No stimulation of nitrification was produced by the addition of sulphur to unlimed acid mucks but an excess of sulphur decreased nitrate accumulation and increased ammonia accumulation. That effect

had previously been observed, (48), and application of sulphur alone to soils had been found to depress the nitrate producing power of soils while sulphur with lime stimulated that power in clay loam. With sulphur alone the acidity was greatly increased.

Heavy metals inhibit nitrate formation (81) according to their protein-precipitating properties, mercury and silver salts being more injurious.

Carbon dioxide is also necessary but to a less extent, the amount of growth being limited. Larger concentrations of carbon dioxide seem to act merely as an inert gas. The investigations of Smith and others (63) of carbon dioxide effects on nitrification in soil are quite conclusive. They show that carbonic acid had a stimulating effect while treatment with carbon dioxide gas was without significance. They expressed the idea that the stimulation of nitrate production was undoubtedly related to the increased solubility of the mineral constituents required by the nitrifying organisms. The failure of carbon dioxide gas to involve any such increase indicated that carbon dioxide was not a limiting factor in nitrification.

(ii) Organic matter - Small amounts of soluble organic matter were found to retard the activities of nitrifying bacteria in pure cultures. In the soil, however, the organisms can stand high concentration of organic matter and furthermore

may benefit from it.

The nitrification process in the soil is immediately dependent on the quantity and the availability of the soil nitrogen and, therefore, a study of the forms of nitrogen and their nitrification rates is of main importance.

(a) Nitrification of soil nitrogen.

A very early investigation (77) established the fact that organic matter of the soil accounted for the high nitrate content of the drainage-water from a Rothamsted soil which had not been manured for 8 years.

Waksman and Diehm (74) have found that only the soluble nitrogen undergoes rapid nitrification in the soil. Both forms of organic nitrogen, namely, that of the organic matter and that synthesized by the microorganisms, become slowly available.

The soils of Quebec have been found to contain high percentages of carbon and nitrogen (41), but low nitrification always occurred, except when the soils were treated with combined sodium carbonate and calcium carbonate (24). The sodium carbonate was believed to have released more soluble nitrogenous material for the ammonifying bacteria, which would in turn supply more energy for nitrification, while the calcium carbonate very probably is limited in its effect, merely reducing the soil acidity.

(b) Added organic matter.

The soluble organic matter must be mineralized through the agency of ammonifiers and other groups of organisms before nitrate formation takes place, if no injurious effect is to occur.

In studying that mineralization, Batham (2), found that the nitrogen of the compound tryptophane containing both ring and chain nitrogen was more readily nitrifiable than that of compounds containing only chain nitrogen. The average of the results showed that the amino acids are nitrified at about the same rate. Tyrosine and cystine were less available than phenylalanine, leucine, alanine, and tryptophane. The presence of sulphur in cystine appeared to depress the nitrification of its nitrogen.

Under field conditions the oxidation processes can hardly be analysed so specifically. The analysis generally embraces many of these amino acids which are the bases of protein complex structures, and when mention is made of the soil nitrogen, it is termed the total nitrogen.

It is a current farming practice to add to the soil certain amounts of organic matter in the form of manures. These may be the farmyard, or green manures, or chemical compounds. Each kind of manure will affect nitrification according to its chemical composition.

Jensen (31) reports from a study of the effects of farmyard manure in various soils that at the early stage of decomposition there was observed a very high number of micro-organisms, especially when fresh straw was present in the manure. This increase was sooner or later followed by a rather sudden decrease which caused the numbers of bacteria to approach that of the control soils. The nitrification of the manure nitrogen became active at the period when the bacterial, or fungal, numbers were decreasing.

The C/N ratio of the manure exerted a great influence upon the degree and the rapidity of nitrification of the manure. The narrower the C/N ratio, the greater was the nitrification; this was suggested by a farmyard manure which had undergone intense composting, the Edelmist manure, and which showed a stronger nitrification than ordinary farmyard manure, though its nitrogen compounds did not seem more easily decomposable than those of ordinary manure.

The comparatively low fertilizing value of the organic nitrogen of the manure seemed to be dependent on the following phenomena: the organic matter of the farmyard manure is a mixture of compounds of a fairly wide C/N ratio which induced an increase growth of the various microorganisms causing at the same time a large part of the available nitrogen to be used up as nitrogenous food. When the supply of readily available energy is exhausted, i.e., with a narrow C/N ratio,

the bacterial numbers drop, and a production of mineral nitrogen begins but it never reaches the total of nitrogen in the manure. In this respect the farmyard manure resembles other organic manures, which generally yield only a fraction of their nitrogen as nitrate, but here the phenomena are somewhat more complicated owing to the presence of the resistant humus fraction in the manure which has been determined to consist largely of lignin, probably combined with some protein material.

The effect of the C/N ratio seems to be confirmed by a study of Brown (4). It was found that highest increases were registered with 8 and 12 tons of manure while the 16-ton application showed only a very slight increase and the 20-ton was depressive.

The effects of artificial manures in the soil (62) were the same as those from the farmyard manure as long as the straw and cellulose residues were allowed to compost properly.

The ploughing under of a crop is an additional source of organic matter and thereby of nitrogen. The order of effectiveness for a few crops to bring about greatest accumulation of nitrates was established by Lyon and Wilson (39) to be vetch, rye, field peas, oats and buckwheat.

Waksman (73) established that the behavior of the green manures in the soil follows their chemical composition.

Proteins and their derivatives are the carriers of the nitrogen in the plant and are readily subject to decomposition, but since celluloses and hemicelluloses are readily attacked by the soil microorganisms, the nitrogen that becomes liberated from the decomposition of the proteins is immediately assimilated and transformed into microbial cell substance.

According to Page (49) the situation would be the same as for farmyard manure with the exception that a deep-rooted green manure crop, by opening up the subsoil, will not only bring up, from the subsoil, mineral substances which on its decomposition will be added to the surface soil, but also, the ensuing main crop will itself have a better chance of penetrating into the subsoil with its roots, and tapping the mineral resources there. Furthermore, the nitrogen brought in the soil by the green manure is almost a net gain in that it represents either fixed nitrogen by nodule bacteria or by non-symbiotic nitrogen fixing bacteria, or absorbed nitrates from the soil which otherwise would have been leached out or partly removed in the digestive tracts of farm animals before being turned back to the soil as farmyard manure.

(iii) Correlation of biological activities with soil constituents - The rate of decomposition of organic matter has been shown (60) to be in proportion to the nitrogen content of the soil, and a high soil nitrogen content is also associated with a wide carbon-nitrogen ratio. It is concluded that the beneficial effects on the physical conditions of the soil, and

on productivity resulting from the incorporation of organic matter, have been found to be in direct proportion to the nitrogen content. The total nitrogen is believed to exist in two distinct forms in the soil (36), one of which, showing variations of the same order as the total nitrogen, would be the available form for the nitrifying organisms.

A relationship has in fact been found (20) to exist between the total nitrogen and nitrate-nitrogen in incubated samples of 38 Quebec soils of four different groups. The correlation was highly significant.

The results of Feher (8) have been submitted to statistical analysis (20). A close relationship was found to exist between the two variables nitrate-nitrogen and total nitrogen. It may be noted that the podsol soils form a group having lower mean values for these two factors than the remaining (brown earth) soils.

In regard to other factors that might be related to the activity of the nitrifying bacteria, Potter and Snyder (53) gave results for which the author has calculated the correlation to be highly significant. These are for nitrate-nitrogen in incubated samples and carbon dioxide evolved from soils which had been dried, remoistened and differently treated in the laboratory.

A close correlation has been found by the author between

the nitrates produced from the soil nitrogen after incubation (185 days) and the carbon dioxide evolution in fresh soils in the present work. This would suggest that there may be a close relationship between the rate of decomposition of organic matter and the nitrification process. That correlation may also be explained by the fact that a close relationship has been found between nitrate-nitrogen and phosphorus (19) and on the other hand by the method that Kalnins (33) recently introduced, which consists in determining the soils need of phosphorus and potassium by the growth of yeast in a glucose medium inoculated with soil.

It is a well known fact that in the process of decomposition of plant residues in the soil there is a tendency for the carbon-nitrogen ratio to be narrowed until it approaches that of the microorganisms responsible for the decomposition. This is the most pronounced where optimum conditions are provided for the decomposition of soil organic matter and where little or no provision is made to return plant residues. Where such provisions are made a noticeable depressing action has been observed on nitrifying processes. Fuller (15) noted that the dry weight of tomato plants was considerably reduced by the addition of cellulose to a soil low in nitrogen. In a soil high in nitrogen the addition of cellulose reduced the nitrate content, but not to a level that impoverished the soil for the requirement of the crop.

Studies (30) of the effect of straw applied alone and

in combination with other nitrogenous materials on the accumulation of nitrates in soils and on crop growth showed that when straw was applied with clovers, ammonium sulphate, or sodium nitrate, it does not retard plant growth.

Gowda (18) could establish a correlation between crop yield and the nitrate content and the nitrifying power of the soil. In the same series of experiments he showed that the application of manure up to 12 tons per acre caused the greatest increase in nitrate accumulation and in nitrifying power over the untreated soil. Sixteen- and twenty-ton applications caused smaller increases than those secured when twelve tons per acre were applied.

It seems, therefore, very probable that the widening of the carbon-nitrogen ratio is depressive on nitrifying power and resulting nitrate accumulation, in that the microorganisms active in organic matter decomposition drain larger amounts of the soil nitrogen to build their own protoplasm. This decrease would then be a temporary one and the results of Gowda (18) seem to confirm that opinion when he finds that the crop yield effected by these manure applications were in the following decreasing order: 20 tons > 16 tons > 12 tons > 8 tons.

(iv) Nature of soil constituents - A new line of investigation which still is obscure is the decomposition of forest litter composed of coniferous or deciduous residues. Gray and Taylor (23) have noted that no nitrates were formed in the A^1 and A^2 of a coniferous podsol soil, though

nitrogen in these horizons was not considerably less than in the corresponding horizons of a Laurentian podsol soil with a deciduous cover. Their analysis showed that horizon B of soil with a coniferous cover, in which nitrates were formed to a greater extent than in any other horizon except A^1 of the Laurentian soil, contained a high proportion of organic carbon as well as of nitrogen. From this they concluded that there was less of any inhibitory substances such as may have been present in the two superficial horizons, or that the nitrogen compounds present in the illuvial horizons were different from those in the eluvial horizons. A study of the production of ammonia from urea is interesting in that the illuval horizon of the coniferous soil produced more ammonia than the eluvial, suggesting that there was more available carbon for energy in the lower horizon.

Confirmation of this fact together with the author's last conclusion seem to be given in studies of the nitrogen forms (36) in different soils, which revealed fixed differences in the character of the forms of nitrogen occurring in podsol and chernozem soils. Those in the latter soil were more stable in character, less easily leached out, and tended to accumulate in difficult hydrolyzable forms. The nitrogen forms in the podsol were, on the contrary, more mobile, more easily hydrolyzed and more soluble in acids. Under aerobic conditions in the absence of nitrates, nitrate formation occurred mainly at the expense of acid-soluble forms of nitrogen.

Moreover, there also seems to be a biological factor in the nitrate deficiency of coniferous forest soil. Winogradsky (87) has called for more investigations on the probable occurrence of less active species of nitrifying organisms. This statement seems to agree with the experiments of Hale and Halversen (26) who established that although no nitrates were present in the majority of original samples of soils under white pine-forest growth in northern Idaho, ammonia-oxidizing bacteria were present. Under the usual conditions of 28-day incubation, however, serial dilutions of forest litter gave negative results; only after an incubation period of 90 days was a stabilized count possible.

c. Physico-chemical.

In the energy relationships of the nitrifying organisms the reaction of the medium shows a marked influence (43, 44). Optimum of pH 8.4 to 8.8 with limiting reactions of pH 7.6 to 9.3 have been given for nitrite-forming organisms while the optimum values at pH 8.3 to 9.3 and the limits at pH 5.6 and 10.3 were found for the respiration of the nitrate-forming bacteria.

This is true, however, only for the respiration of the organisms and not for their growth.

The limiting acid reaction for the growth of nitrifying bacteria in soil has been found to be at pH 3.9 to 4.5 depending on the origin of these bacteria and the reaction of they were obtained. The limiting alkali

reaction was found to be at pH 8.9 to 9.0.

Wilson (82), enumerating the ammonia-oxidizing bacteria, gave a very suggestive table of their number as compared to the pH.:

Soil reaction	Bacteria in 1 gm. of soil
6.2	1,000
6.4	3,500
6.6	6,280
6.8	25,000
7.0	35,000

Pohlman (52) found that in incubated fresh soils, incubated for 4 months, there was a relationship between the nitrate and calcium content of the soil extracts.

The continuous use of ammonium sulphate as a fertilizer without the addition of lime will lead to a gradual increase in soil acidity because of the additive effect of the nitric acid formed and the residual sulphuric acid, which are not neutralized in the absence of sufficient buffer or base. Nitrate accumulation will proceed until the reaction of the soil has reached a pH of about 4.0.

Another physico-chemical factor which has its importance is the osmotic pressure of the soil solution, of which mention

has already been made. Greaves (25) has found that the nitrifiers are more sensitive to osmotic changes than are the ammonifying organisms. The range at which the different salts become toxic, studied in relation with osmotic pressure, is very narrow and that is what called the attention of soil scientists to that subject.

d. Biological.

It is a well known fact that the oxidation of sulphur by autotrophic bacteria, increasing the acidity of the soil, may become a limiting condition for nitrification to proceed if no available base is present to neutralize the acid formed.

Mention has been made in the preceding pages that the number and strains of nitrifying organisms present in soil may prove to be limiting factors for nitrate accumulation in soils.

A new line of investigation has recently been set up in the biological interrelation of soil microorganisms. Pandalai (50), discussing the incompatibility, showed by the nitrifying bacteria as to their behavior towards organic matter in pure culture, studied the possible association of nitrifying organisms with the saprophytes of the soil. Studies were therefore carried out by that author with cultures of Nitrosomonas in an Omeliansky medium to which different forms of soil and other microflora were added, both by themselves and

in presence of various forms of organic matter. As a result of these, it was found that, although the organic matter tended to depress nitrification when Nitrosomonas was present by itself, the adverse effect was completely removed in presence of other organisms. In most cases there was also enhanced nitrification. Exactly similar results were obtained when these experiments were repeated in presence of soil.

These and other observations, he concluded, would show that by utilizing the interfering organic matter in some way, the associated saprophytes (Azotobacter and Bacillus mycoides) assist Nitrosomonas in its function. The exact mechanism by which nitrification is stimulated in some of these conditions is still obscure. Nevertheless, a correlation is possible between nitrification in pure culture and that in soil if we assume the occurrence of a regulated "chemomixotrophic" metabolism for these organisms.

An experiment in pure culture seems to strengthen that point of view. Nelson (46) outlined an experiment in which 0.25% peptone was added in the place of ammonium sulphate to a salt solution containing traces of manganese and ferrous sulphates, and the common nutritive and buffer salts. Combinations of Bacillus mycoides, Proteus vulgaris, Bacillus megatherium or Bacillus subtilis with Nitrosomonas and Nitrobacter were inoculated into this medium. Tests were made for ammonia, nitrites and nitrates after 40 days incubation.

be assumed that the organisms are sparsely distributed down to 18 inches, though possibly somewhat further in lighter soils through the agency of the natural channels which penetrate the subsoil at a greater depth than in solid clay.

Waksman (75) mentions that in humid soils the organisms are present in the few top inches while in the arid soils they can be found as low as a few feet from the surface.

Wilson (82) reported that the ammonia-oxidizing organisms varied in numbers from a few hundred to a million per gram of soil. These variations were related to the reaction of the soil. Soils whose reaction was around pH 4.5 supported fewer organisms than those whose reaction was pH 7.0. Soils whose reaction was more alkaline than pH 8.2 were not studied.

2. Types of nitrifying bacteria

The organisms composing that population and generally designated as the nitrifying bacteria are of two types:

a. Ammonia-oxidizers: which transform ammonia to nitrites.

The original organism isolated by Winogradsky (85) has been named Nitrosomonas, but, as that author stated, there might be some other species or at least strains according to the climate and the different soils. That statement was confirmed by Winogradsky himself (86) who found a smaller species of Nitrosomonas from a soil of Java, and a new genus he called

Nitrosococcus, isolated from a soil of Quito, South America, different from the Nitrosomonas in that it is non-motile and larger than the latter. Bonazzi (3) also isolated the Nitrosococcus from a soil of North America.

The existence of several forms of nitrite-forming organisms in the soils from different continents was explained by Winogradsky as due to the probability that local conditions favoured the adaptation of a particular variety. That would mean that very probably there will be some more organisms to introduce into that specific class of organisms.

Recently, Winogradsky and Winogradsky (87) suggested a classification of the nitrite-forming bacteria into three groups:

(i) Nitrosomonas - free, motile forms, rods and cocci, form of zero, obligately aerobic. Found in good cultivated soils.

(ii) Nitrosocystis - cocci united in rounded masses and surrounded by a membrane forming the zooglea. These organisms are the forest ammonia-oxidizers which are much slower than the Nitrosomonas in their action.

(iii) Nitrosospira - Spiral shaped forms. They were found in uncultivated soils. Their physiological functions are very slow.

b. Nitrite-oxidizers: which transform nitrite into nitrate.

Only one form of active nitrate-forming bacteria has been yet isolated and it was Winogradsky (86) that succeeded in this discovery. In a recent work, the same author (87) opened a new field of investigation when he stated that he isolated a species

resembling Nitrosocystis but which he lost before testing its oxidation properties. Other forms of very slowly active bacteria have also been found and observed in that study, the organisms being isolated from what the authors (Winogradsky and Winogradsky (87)) named "les voiles", kinds of veils that tardively invaded the nitrobacter cultures. The situation may be thus summarized:

(1) Nitrobacter - Non-motile, rod-shaped bacterium, obligate aerobic, non-spore forming. Found in all kinds of soils. It is to be noted that motile Nitrobacter have been found.

(2) "Les Voiles" - Which group includes as yet found four types of bacteria which all nitrified to some extent, but in general so little that their role is still doubtful as nitrifiers.

They are:

Bactoderma alba.
Bactoderma rosea.
Microderma minutissima.
Microderma vacuolata.

3. Energy relationships of the nitrifying bacteria

The most striking characteristic of living organisms is the continuous change which they exhibit. These changes are of two distinct types: a building up of matter and a breaking down of matter. In order to effect changes, as is stated by elementary mechanics, work must be done and that capacity to do work is due to the possession of something that is called energy. The great physiologist Claude Bernard, quoted by Steel (65), says that "all the manifestations of life are composed of phenomena borrowed from the outside cosmic world, so far as

their nature is concerned, possessing, however, a special morphology, in the sense that they are manifested under characteristic forms and by the aid of special physiological instruments".

Viewed, therefore, from the physico-chemical point of view, the living cell is a peculiarly constructed energy machine or energy transformer, through which energy continuously flows, and the entire life of the cell is an expression of variations and alterations in the rates of flow of energy, and changes in equilibrium or balance between the various types of energy.

Before the discovery of microorganisms, more exactly before the studies of their physiological behavior, the physiologists divided living organisms in two groups because of the opposed vital processes they showed: the higher animals, which degrade the complex organic structures, and the higher plants which had built these structures.

Further advances in the study of the lower forms of life, namely, the filamentous fungi, protozoa, and most of the bacteria, enabled scientists to divide living microorganisms in two distinct groups: the Heterotrophs: deriving the energy for their metabolic processes as well as the carbon used for synthesizing their cells from complex organic compounds such as carbohydrates, fats, proteins, degradation products of these, or other compounds of the aliphatic and cyclic series.

A typical reaction by which such organisms meet their energy requirements may be represented as follows: (64).

$C^6H^{12}O^6$ (solid) + 6 O^2 (1 atmos.) = 6 CO^2 (1 atmos.) + 6 H^2O (liquid)

ΔF_{298} = -689,000 calories.

ΔF_{298} being the total energy decrease at 25°C. or 298 absolute. The Autotrophs: organisms effecting the synthesis of their own organic compounds from inorganic substances.

Up to the last twenty years of the nineteenth century, the higher plants and the smaller algae were the only known representatives of the autotrophs. These build up the organic matter within their cells by photosynthesis, which is the elaboration of organic compounds from carbon dioxide and water to formaldehyde and by subsequent polymerisation or condensation, to more complex structures under the energy of sunlight acting on chlorophyll.

The energy requirements for these would be: (5)

H^2CO^3 = HCHO + O^2 - 118.6 Calories.

6 HCHO = $C^6H^{12}O^6$ + 47.6 Calories.

But by 1887 (83), 1888 (84) and 1890 (85), Winogradsky's observations showed a new and very distinctive group of the autotrophants when he claimed that the sulphur-oxidizing bacteria, as well as the ammonia-oxidizing bacteria could synthesize organic compounds in the absence of light.

These autotrophic bacteria are distinguished from the heterotrophic forms in that they have the specific ability of obtaining energy for their metabolism by the oxidation of certain inorganic substances. Autotrophic bacteria differ from the autorophic plants in that this energy, derived from the oxidation of inorganic materials is utilized for the reduction of carbon dioxide to organic compounds.

It was subsequently found that this group of autotrophic bacteria could be divided into strictly and facultative organisms, the latter being able either to derive their energy from the oxidation of inorganic substances and reduce carbon dioxide for synthesizing their organic structures, or to derive their energy, like the heterotrophs, from purely organic substances. Furthermore, in the sulphur bacteria there are found representatives of forms which appear to be intermediate between the autotrophic bacteria and the chlorophyllous plants. These are the purple bacteria which require both hydrogen sulphide and light for their development. It would appear that their nutrition is dependent upon both a photosynthetic reaction and an oxidation of an inorganic substance.

Obligate autotrophic bacteria and facultative forms existing under autotrophic conditions show certain distinctive physiological characteristics:

1. They thrive on strongly elective, purely mineral media containing the specific inorganic oxidizable substances.

2. Their existence is dependent upon these substances which are oxidized in the performance of the life processes of the organisms.

3. These oxidation processes furnish the only source of energy for the organisms.

4. They require no organic nutrients as sources of energy.

5. They use carbon dioxide (dissolved) as their exclusive source of carbon. The carbon dioxide is reduced by means of the energy obtained from the oxidation of the inorganic foods.

Before discussing the particular energy relations of nitrifying group, it would seem that the classification given by Starkey (64) should be introduced here to clarify the physiological as well as the morphological characteristics of the autotrophants.

A. Bacteria which oxidize compounds of nitrogen.

a) Oxidize ammonia to nitrite. (Nitrosomonas, Nitrosococcus).
b) Oxidize nitrite to nitrate. (Nitrobacter).

B. Bacteria which oxidize sulphur or compounds of sulphur.

a) Simple bacteria (genus Thiobacillus).

1. Strictly autotrophic.

(a) Aerobic.

(1) Develop at reactions close to neutrality. (species Th.thioparus Beijerinck.)
(2) Develop under very acid conditions. (species Th.thiooxidans Waksman and Joffe.).

(b) Anaerobic. (species Th.denitrificans Beijerinck).

2. Facultative autotrophic.

(a) Facultative anaerobic (species of Trautwein).

b) Higher bacteria (complex in morphology).

1. Colorless (includes the genera Beggiatoa, Thiothrix, Thioploca, Achromatium, Thiophysa, Thiovulum, and Thiospira).

2. Pigmented-red or purple bacteria (includes the genera Thiocystis, Thiocapsa, Thiosarcina, Lamprocystis, Thiopedia, Amoebobacter, Thiothece, Thiodictyon, Thiopolycoccus, Chromatium, Rhabdochromatium, Thiospirillum, Rhodocapsa, Rhodothece).

C. Bacteria which oxidize ferrous or manganous compounds.

a) Simple bacteria.

1. Long excretion filaments. (genus Gallionella).

2. Coccoid or oval shapes in masses (genera Siderocapsa and Sideromonas).

b) Filamentous bacteria (genera Leptothrix and Crenothrix).

D. Bacteria which oxidize hydrogen.

The present thesis is concerned only with bacteria that oxidize compounds of nitrogen.

The discovery and isolation of the nitrifying bacteria by Winogradsky was the climax to a long series of investigations aiming to explain the mechanism of nitrate formation in soils. In his work of isolating the organisms, Winogradsky found that these bacteria grew only on culture media from which organic matter was absent. After experimenting with a culture media completely free of organic material and obtaining an accumulation of organic matter as the result of the growth of the

bacteria as shown in the following table, he could claim that the "ferment nitrique" was an organism oxidizing ammonia to nitrite to obtain the necessary energy for the reduction of carbon dioxide, the sole source of carbon.

Winogradsky's (85) results in Organic Carbon from Cultures of Nitrosomonas

Culture No.	Carbon dioxide in milligrams					Organic Carbon Net Gain
	Sediment	Liquid	Total	Control	Remainder	
11	59.5	26.0	85.5	13.1	72.4	19.7
12	49.6	16.0	65.6	9.8	55.8	51.2
26	87.0	26.0	113.0	16.0	97.0	26.4
30	70.8	21.2	92.0	10.0	82.0	22.4

Further investigations (86) showed to Winogradsky that the oxidation of ammonia to nitrates was performed by two different organisms:

1) Oxidation of ammonia to nitrite. (Nitrosomonas and Nitrosococcus).
2) Oxidation of nitrite to nitrate. (Nitrobacter).

The energy equation has been established for the nitrite producer (Nitrosomonas), as follows:

$$NH^3 + 30 = HNO^2 + H_2O + 79{,}000 \text{ calories.}$$

whilst the nitrite oxidizer (Nitrobacter) depends on the reaction:

$$HNO^2 + 0 = HNO^3 + 21{,}600 \text{ calories.}$$

The ratio of nitrogen oxidized to carbon entering into synthesis in the two cases has been determined by Winogradsky (85, 86) and later, more exactly by Meyerhof (44) for the Nitrobacter. The values obtained were:

Nitrosomonas (Winogradsky).	N/C = 35
Nitrobacter (Winogradsky).	N/C = 135
Nitrobacter (Meyerhof).	N/C = 101

As expected these values are roughly inversely proportional to the amount of heat liberated by the oxidation of 1 g. of nitrogen in the two cases:

$$35/101 = 0.346; \quad 21,600/79,000 = 0.276.$$

From these ratios, the energy efficiency can be calculated. The calculations though will only be approximate because, in the absence of data on the heat of combustion of the organisms, the ratio heat of combustion of cells/heat lost in medium in the production of cells cannot be given. The amounts of carbon synthesized, however, having been determined, this was assumed for purposes of calculation to possess the same heat of combustion as glucose, i.e., 113,000 calories per g. atom.

Meyerhof (44) found that the oxidation of 1 g. mol. KnO^2 results in the assimilation of $\frac{14}{12 \times 101}$ g. atom of carbon, of which the heat of combustion $\frac{14 \times 113,000}{12 \times 101} = 980$ calories.

but since 1 g. mol of KNO^2 on oxidation to KNO^3 liberates 21,600 calories, the total energy efficiency of the process will be:

$$\frac{980 \times 100}{21,600} \% = 4.53\%$$

From the standpoint of free energy instead of from heats of combustion the relations become: (1)

$$NH^{4+} + 1\tfrac{1}{2}\ O^2 = NO^{2-} + H^2O + 2H^+(10^{-8})$$

$$\Delta F_{298} = -66,500 \text{ calories},$$

$$NO^{2-} + 1\tfrac{1}{2}\ O^2 = NO^{3-}$$

$$\Delta F_{298} = -17,500 \text{ calories}.$$

The changes are calculated from concentrations of ammonium and nitrite shown to be optimal for the organisms, viz.:

$$(NH^{4+}) = 0.005M. : H^+ = 10^{-8} : (NO^{2-}) = 0.03M.$$

The reduction of 1 g. mol CO^2 to glucose requires 118,000 calories free energy:

for Nitrosomonas to free energy efficiency will then be:

$$\frac{14 \times 118,000 \times 100}{12 \times 35 \times 66,500} \% = 5.9\%$$

for Nitrobacter: $\frac{14 \times 118,000 \times 100}{12 \times 101 \times 17,500} \% = 7.8\%$

From these figures one would expect about 95% of the energy liberated by the oxidation to nitrate to appear as heat.

The efficient free energy is used in the building processes which are still obscure. Klein and Svolba (35) have claimed to have shown that formaldehyde is an intermediate product in the reduction process, an observation which would bring nitrifying organisms into relation with the green plants. From the N/C ratio of 35 for Nitrosomonas it was calculated that if all the carbon assimilated passed through the stage of formaldehyde, 19.5 mg. of the latter would be formed during the oxidation of 2 g. of ammonium sulphate. Using sulphite and dimedon as fixatives they demonstrated that formaldehyde was actually formed, but only half the amount theoretically possible was actually obtained in the most favourable experiments.

The same authors claimed to have obtained, by the same fixation method, acetaldehyde, which they regarded as representing the respiration or degradation processes.

With regard to the actual process of oxidation, no experimental data exist. Suggestions about the intermediate products as hydroxylamine and hyponitrous acid are as yet unsupported by experimental evidence (67).

4. Factors influencing the growth of nitrifying organisms

Winogradsky (85) first established the conditions governing an optimum growth of the autorophic ammonia-oxidizers, such as the necessity for carbonates of calcium or magnesium, the inhibitive effect of many organic compounds, the concen-

tration of ammonium salts, and free access of oxygen.

Meyerhof (43, 44, 45) in later and more detailed studies of both types of bacteria, considered that the action of carbonates was due to their buffering effect, and demonstrated their necessity by showing the extreme sensitiveness of the organisms to hydrogen-ion concentration. The optimum pH for the oxidation of ammonia is between 8.5 to 8.8, that for nitrite oxidation between 8.3 to 9.3. The pH of a 0.2 molar solution of sodium carbonate and free access of carbon dioxide is seen therefore to provide the conditions best suited to maintain a reaction favourable to the development of these bacteria.

It is important from the standpoint of soil economy to note that organisms isolated from peat soils (pH 4.6) display a greater tolerance for acid, nitrification continuing as low as pH 4.1. Experiments of our own agree fully with that point of view. Out of fourteen different soils in Quebec, one, developed on St. Lawrence River alluvial blue mud (pH 4.6), showed an outstanding behavior for inoculum from 1 g. of soil subsequently transferred (two drops) to fresh media up to the 5th generation as well for nitrosation as for nitratation. The strains were the most active on artificial media and also in fresh soil samples kept in a cool store room; the increase in NO^3-N of these samples, determined at 6 or 7 weeks intervals, was at the level of other soils of higher pH values.

The early investigators on nitrifying organisms showed that organic matter was an inhibiting factor on the growth of these organisms. The situation is not very well defined. Fred and Davenport (13) could subcultivate the organisms after two to six weeks on 1% solution of gelatine, peptone, casein, milk, and yeast water; 0.1% concentrations of peptone, skimmed milk, beef extract and beef infusion, when added to a nitrite media favoured growth; on the other hand the same concentrations of gelatin, urea and asparagin retarded it.

The recent work of Kingma Boltjes (34) showed that pure cultures of both types of nitrifiers develop large colonies if 0.7% "Nährstoff-Heyden" is added to the inorganic medium. This preparation appears to be an incomplete acid digest of egg albumin; in liquid medium it also acts favourably but does not replace any constituent of the inorganic medium, the organisms continuing their autotrophic mode of life. In fact the only evidence that the substance is used by the organisms is that the colonies are larger in its presence than its absence, and the possibility is not excluded that its action is physical rather than chemical.

Growth of both nitrifiers also occurred in media containing a 4% concentration of glucose, which itself was not attacked.

Observations made by Meyerhof (44) led him to the conclusion that many of the remarkable properties of the nitrifying

organisms were to be ascribed to their very high permeability by lipoid-soluble substances; thus, the lipoid-soluble ammonia is an inhibitor of oxidation, whilst the lipoid-insoluble ammonium salts are comparatively inactive; furthermore, only those amines which are lipoid-soluble resembled ammonia in their inhibitive action; among many inorganic anions borates were found to be the most strongly inhibitive in agreement with the lipoid-solubility of boric acid; finally, the lipoid-soluble mercuric chloride was found to be more strongly inhibitory than the lipoid-insoluble mercuric nitrate.

Aeration has been shown of importance by Winogradsky (85, 86) who was not able to cultivate any of the nitrifiers under anaerobic conditions. Gowda (17) obtained very high daily oxidation by the nitrifiers with a process of aeration which consisted of a long tube containing limestone of the size of split peas on the surface of which a flora of nitrite-formers had been established and a current of air was drawn through the mass when a nutrient solution of ammonium sulphate was allowed to drip into the top of the tube. The rate of oxidation of the ammonium sulphate reached the maximum of 318 mg. per day to be compared with the daily oxidation of 7.7 mg. first obtained by Winogradsky.

III. EXPERIMENTAL

A. Soils

1. Source

The soils were selected in such a manner as to cover some of the most representative soils of Quebec. Their origin, field characteristics, and management are fully described in Table I.

2. Sampling and subsequent treatment

Each soil was sampled as follows: a composite sample of five six-inch deep holes, taken at 50 feet distance, was thoroughly mixed. Two tin cans were filled of that well-mixed sample, free of rough organic debris, and brought to the laboratory.

Two weeks after sampling, the soils were finally stored in glass containers in case the metal of the tin cans would affect their nature. They were stored in a dark cupboard in which the temperature varied from 14° to 18°C. The covers of the containers were loosened so as to allow a certain amount of aeration.

3. Analyses

The moisture analyses, made by the author, were the total moisture content of fresh soil to oven-dried, the

hygroscopic moisture, and the water-holding capacity. The results are given in Table II.

Total moisture: About 10 grams of fresh soil were weighed in an aluminum soil moisture dish, and this was put to dry in an oven at 105°C for 24 hours. The dry soil was weighed after it had cooled in a desiccator. Calculations were based on the differences from weights of moist soil to dry soil.

Hygroscopic moisture: The same operation as the preceding but with air-dried soil. The moist soil was allowed to dry for three weeks after which the determinations were done.

Water-holding capacity: The method employed was that described by McKibbin and Gray (42). Air-dried soil, passing through a 1-mm mesh sieve, was ground in a porcelain mortar; a 10 gm. portion was placed in a small funnel, having at the neck a small cone of filter paper (one inch in diameter), saturated with distilled water. The soil was made to settle by tapping the funnel, and distilled water run in slowly from a burette until the first drop in excess of water-holding capacity exuded from below the cone. The value was taken from the mean of two determinations.

The physical analyses on the soil texture were carried out by "Le Laboratoire Provincial des Sols" of Ste Anne de la Pocatiere, Que., and the methods followed were those of Bouyoucos (See Table II). The same applies to the chemical analyses (Table III) by methods described in A.O.A.C.

The samples so analyzed were taken a few feet distant from the author's samples for soils Nos. 1, 2, 3, 4, 6, 7, 8, and 9, while the analyses of samples 10, 11, 13, and 14 refer to the analyses of a soil which was the most typical of each particular type of soil, the characteristics of which can be obtained in the reports published by the Quebec Department of Agriculture (59, 70).

The pH was determined, by the author, by means of a Beckman glass electrode meter (Industrial model M.).

B. Nitrifying Capacity of the Soils

The fresh soils stored in glass containers, in a cool and dark cupboard, were allowed to nitrify their own nitrogen for periods varying from 6 to 7 weeks before the nitrate-nitrogen was determined.

Each soil was taken out of the container on a paper, which was changed at each sampling. The soil was thoroughly mixed by means of a spatula and 50 grams (25 if muck) were weighed, small portions being taken at random in the mass of the sample.

Harper's (28) modification of the phenoldisulphonic acid method was used to determine nitrates, which are expressed in parts per million on a basis of oven-dry soil (Table IV).

C. Carbon Dioxide Evolution

1. Fresh soil

Three hundred grams of the mineral soils (150 for the muck) were placed in large suction flasks of 1 l. capacity (Fig. 1, c.), and air, purified by means of passage through a column of "carbosorb" (Fig. 1, a.) and by passage through a barium hydroxide solution (b), was passed over the soil at a slow rate and then allowed to bubble in three successive tubes (d), each containing 33.3 ml. of 0.1N $Ba(OH)^2$. At the end a trap (e) hindered the water of the suction pump from turning back to the tubes if a back pressure established.

Residual barium hydroxide was titrated against N/5 oxalic acid in the proximal tubes at intervals and in the three tubes at the end of a period of 14 days.

Three successive 14-day periods were recorded with the same soil undisturbed. In the intervals of a few days between each period, all connections were tightened on the flask containing the soil and the carbon dioxide was allowed to accumulate.

The results are reported in Table V as milligrams of carbon dioxide per hundred grams of oven-dried soil.

2. Air-dried soil

a. With addition of water only.

Soils were spread to dry for two weeks at room temperature.

100 gm. of the sample were remoistened to 60% of water-holding capacity and placed in a flask for aeration as for fresh soil.

b. Air-dried soil with water and glucose.

0.3 gm. of glucose was added to 100 gm. of air-dried soil, in the remoistening water.

In view of the fact that hygroscopic moisture differed in the different soils, and that the results are calculated on oven-dried soil, the amount of glucose available to the organisms in equal masses of oven-dried soil varied from 0.302 to 0.312 gm. in the mineral soils; the equivalent amount in soil No. 6 was 0.346 gm.

The carbon dioxide of the two series was then collected during one 14-day period and the results are reported in Table V as milligrams of carbon dioxide evolved from 100 gm. of oven-dried soil.

D. Cultural Studies

1. Nitrite organisms

a. Oxidation and transfers

In order to have a good idea of the activities of the nitrifying bacteria, and also as a preparation for the isolation of the specific organisms, a series of elective cultures was set up for each soil.

One gram of fresh soil was inoculated into 50 ml. of the

following sterile solution in Erlenmeyer flasks of 500 ml. capacity.

Ammonium Sulphate Solution (Fred and Waksman (14))

$(NH^4)^2 SO^4$	1.0 gm.
$K^2 HPO^4$	1.0 "
NaCl	2.0 "
$MgSO^4 .7H^2O$	0.5 "
$FeSO^4 .7H^2O$	trace
Water, distilled	1,000 ml.

The medium was sterilized by steaming for 45 minutes. After sterilization, 1% sterile $MgCO^3$ was added.

The cultures were incubated at a temperature varying from 26° to 28°C.

The oxidation of ammonia to nitrites was tested by means of the Trommsdorf reagent, the preparation of which is given by Fred and Waksman (14).

At the time of the earliest appearance of a strong dark-blue coloration, two drops of the crude cultures were transferred to fresh 50 ml. portions of the medium, and so on up to the fifth transfer when it was thought that the major part of the contaminating heterotrophic bacteria would have been killed by so long a starvation.

The time each generation took to carry on a strong nitrification was recorded and is reported in Table VI below.

b. Isolation.

It was planned to isolate the organisms responsible for the nitritation, but unfortunately a first series of transfers in elective culture media failed to carry on further than the crude culture except for three soils of which only two proceeded up to the fifth transfer. This failure was attributed to a too high concentration of free ammonia secured from a 10% stock solution of ammonium sulphate. A new series was set up but up to now it has not gone further than the second transfer.

However, an attempt to isolate such bacteria responsible for nitritation has been carried on. A silica jelly medium is recognized as the only actually sure method of isolating the nitrous organisms. As, however, the present work was planned more to study the nitrifying capacity of the soils and the activities of the nitrifying organisms than to obtain knowledge of their specific characteristics, and also due to lack of time, the preparation of the silica jelly was considered to be less important and it was decided to choose a less complicated culture medium, namely, Heinemann's (29) ammonium sulphate agar, No. 1420 in Levine and Schoenlein (37), the composition of which is as follows:

Distilled water	1,000 ml.
Fe SO^4	0.4 gm.
Mg SO^4	0.5 "
K^2 HPO^4	1.0 "
NaCl	2.0 "
$(NH^4)^2$ SO^4	1.0 "
Agar	20.0 "
Ca CO^3	10.0 "

This agar was plated and allowed to harden; it was then inoculated with the most advanced elective cultures. The inoculation was done by spreading a loopful of a suspension (one drop in 2 ml. sterile water), in a criss-cross manner on the surface of the agar. The plates were incubated for a week at 28°C.

Where attempts have been made for the isolation of the organisms that grew on the agar, the procedure was as follows: The colonies were selected by means of the low-power lens and some bacteria were extracted with the aid of a needle, mounted in a diamond-objective piece, and centered so as to come in contact with the selected colony. The needle so charged with bacteria was washed with a drop of sterile water held in a wire-loop. That drop of water was then either put to dry on a slide, to check the morphology of the organisms, or transferred to 25 ml. of the ammonium sulphate solution in an Erlenmeyer flask of 125 ml. capacity.

These inoculated solutions were incubated at 28°C. and tested daily with Trommsdorf's reagent for the nitrite reaction.

2. Nitrate organisms

a. Oxidation and transfers

The method followed here was the same as that described under the nitrite organisms with a difference only in the culture media for the elective cultures, which was the same throughout the work with nitric organisms, as well for oxidation as for isolation. It will be referred to as the sodium nitrite solution, and was composed as follows:

Sodium Nitrite Solution (Fred and Waksman (14))

$NaNO^2$	1.0 gm.
Na^2CO^3	1.0 "
K^2HPO^4	0.5 "
NaCl	0.5 "
$MgSO^4$.$7H^2O$	0.3 "
$FeSO^4$.$2H^2O$	trace
Distilled water	1,000 ml.

The oxidation was tested first by the Trommsdorf reagent and when no nitrite reaction was observed the presence of nitrates was confirmed by a test with the diphenylamine reagent, the preparation of which is given by Fred and Waksman (14).

The time taken for each soil to bring about the complete oxidation of nitrites to nitrates is recorded in Table VII.

b. Isolation

The manipulations were the same as for the isolation of nitrous organisms. Heinemann's sodium nitrite agar (Levine and Schoenlein (37) No. 1427) was used for plating.

Distilled water	1,000 ml.
$MgSO^4$	0.3 gm.
$FeSO^4$	0.4 "
NaCl	0.5 "
K^2HPO^4	0.5 "
Na^2CO^3 (fused)	1.0 "
$NaNO^2$	1.0 "
Agar	20.0 "

The solution used for the culture of the isolated bacteria was the sodium nitrite solution in 25 ml. quantities. The course of oxidation was daily followed with the Trommsdorf reagent and two confirmatory tests for nitrates have been secured by the use of the diphenylamine with parts of the agar which was nitrified three weeks after inoculation.

IV. RESULTS

1. Nitrifying capacity

The aim of the present study was to obtain more information on the rates of nitrification in few representative soils of Quebec.

The figures obtained (Table IV) from the nitrification of the soil nitrogen after incubation show that, although there are large variations from one soil to another, the nitrifying capacity of all the soils, except one, is low.

The exception is given by an organic soil, the St.Edouard muck, which could not be compared in any respect with the mineral soils, according to its organisation, i.e., high content of well decomposed organic matter, high content of calcium and available phosphoric acid as well as total nitrogen and available nitrogen (Lamotte tests), kept in good tilth by a short rotation, and supplied with 4-8-10 fertilizer and manure.

If the mineral soils are studied in their decreasing order of nitrifying power, some information may be secured to explain the possible reasons of their different behavior. Some of the factors that may influence the nitrifying power are discussed below.

Soil No. 2 - Heavy loam. Nitrifying power: 80.7.

This soil presents a very good structure under field conditions although the texture is heavy. The chemical analysis revealed a high content of available phosphorus, which is probably held at a good level by the application of 2-12-6 fertilizer once every four or five years. The rotation is organized so as to permit the soil to rest and recover through one or two years of sod.

Yearly application of farmyard manure replaces the loss of organic matter.

Soil No. 5 - Clay loam. Nitrifying power: 74.2.

The annual additions of organic matter to this virgin soil is composed of mixed debris of deciduous and coniferous trees. The texture is somewhat lighter than that of the preceeding soil, and, although no analysis was secured, the calcium content may be assumed to be high because of its calciferous nature. It may be assumed, too, that the soil nitrogen was less available since there was no rapid formation of nitrates during the first days of incubation; the increase throughout the incubation period was regular.

Soil No. 4 - Clay loam. Nitrifying power: 72.4.

This cultivated soil, though well supplied with fertilizers, (4-8-10 and 2-12-6), presents a lower nitrifying power than the same type of soil under forest (No. 5). The factor here would seem to be the depletion of organic matter; farmyard

manure was applied only once every five years. The calcareous nature and the alfalfa culture probably furnish the reaction and the nitrogen necessary for its comparatively high nitrifying capacity. The phosphorus content, both total and available, is high.

Soil No. 14 - Clay loam. Nitrifying power: 69.0.

This soil presents very special characteristics and, like the muck soil, has not been considered with the other mineral soils when correlations have been calculated between nitrifying capacity and the factors believed to affect it.

It is a marsh soil, deposited by sea-water and occasionally flooded by the annual high-seas not so long ago. The saline vegetation first established gave place to farm plants when the soil came under agricultural operations.

As it is shown by the analysis, the top horizon is very rich in organic matter and in total nitrogen but due to some factors all entangled together, very little of this total nitrogen is available. The analysis has shown a high percentage of exchangeable magnesium and by difference we could expect a high percentage of sodium as well. The structure is not good, probably caused by a high sodium concentration giving a peculiar flocculation of the clay. Furthermore, a very slow drainage prevents the soil from attaining the temperature which other soils of the area will afford for the growth and activities of the microorganisms.

The calcium content is exceptionally low and consequently

the hydrogen ion concentration high, the pH being 4.6 for the sample studied and ranging from 3.7 to 5.6 in the samples collected by the Soil Survey in 1940. The available phosphorus also is very low.

Soil No. 7.- Clay. Nitrifying power: 66.1.

It seems that the activity of the microorganisms is high in this forest soil, according to its narrow C/N ratio, but, although it is well supplied with carbon and total nitrogen, the available nitrogen is very low and the phosphorus content, total and available, is also too low to secure a good nitrifying capacity. It may be that the organic residues, derived from deciduous trees for the most part, are rapidly turned into microbial substance and a resistant humus complex. It may also be assumed, from its high acidity, pH 5.3, that the calcium content is low in the surface horizon. The Soil Survey classed that type of soil as calcareous because of effervescence usually found at depth of 4 to 5 feet.

Soil No. 9 - Clay. Nitrifying power: 65.7.

The situation met with here resembles much to that mentioned above with soils Nos. 4 and 5. Under forest the soil had a slower start for nitrification but it surpassed the cultivated soil of the same type which showed a greater activity at the start. It is to be said though, that the differences are very small and probably not significant. If the present cultivated soil be compared to the preceding one (No.7) is shows the same content of organic matter, and a highest content of phosphorus, both

total and available. The higher content of available nitrogen does not appear to be significant. The percentage of calcium is low and there is a decrease in the total nitrogen content. The wider C/N ratio would indicate a somewhat slower activity of the microorganisms.

The type of farming very probably affected the nitrifying power in that it depleted the soil of its nitrogen without making any provision for its return as manure, fertilizer or leguminous crop.

Soil No. 3 - Sandy loam. Nitrifying power: 65.1.

This soil with its very low content in calcium and phosphorus, total and available, and available nitrogen, has a high carbon content and a total nitrogen which ranks about the mean of all the other mineral soils. This would be indicative of relatively slow microbiological activities and this expectancy is met with by the wide C/N ratio.

It is believed that the management given to the soil some ten years ago has been highly depressive on the organic matter content which caused the available nutrients to be washed down by leaching at the time the soil was turned to sod without addition of fertilizers. Now the organic matter has been increased by continuous sodding for seven years, but the nutrients are still lacking and the low nitrifying capacity appears to be due to this lack.

Soil No. 8 - Sandy clay loam. Nitrifying power: 61.5.

This is calcareous soil which is low in all the elements

that would make for a high nitrifying capacity. The total nitrogen ranks in the average of all the other mineral soils, but the carbon is low, indicating that the organic matter has been depleted probably by continuous cropping without any return except the impoverished roots and stubble of grasses turned under after the sod.

Soil No. 1 - Sandy clay loam. Nitrifying power: 59.2.

The field conditions are the same for this soil as for the preceding. Unfortunately, it has not been possible to secure the total analysis. The available phosphorus is high and it may be assumed also that the calcium content is high; the pH value is near to neutrality. The soil is also calcareous.

The content of available nitrogen is low and may act as the limiting factor. The management may be assumed to be depressive on organic matter and essential nutrients, though a longer sodding period probably restored part of the organic matter content.

As will be seen below there probably exists a biological factor to explain the low nitrifying power.

Soil No. 12 - Clay loam. Nitrifying power: 58.9.

It is believed that this forest soil presents two factors which could cause a low nitrifying capacity. Firstly, its chemical nature, namely, organic matter resulting from a coniferous forest litter, and the assumed low calcium content giving rise to a high hydrogen-ion concentration as expressed by a pH value of 5.3.

Secondly, a biological factor. The nitrifying organisms, as it will be seen below, presented a very slow action and they never produced the oxidation in pure elective culture, showing that they are either in very low numbers or that they have very slow oxidative activity.

Soil No. 13 - Clay. Nitrifying power: 58.6.

This soil contains a low percentage of total and available phosphorus, and of available nitrogen. The calcium, total nitrogen and carbon content stand at the level of all other mineral soils.

A factor to cause a low nitrification is probably the low moisture, either total or hygroscopic.

Soil No. 10 - Sand. Nitrifying power: 51.9.

The low nitrifying capacity of this podsol sand is undoubtedly dependent on the abnormally low content of total nitrogen and phosphorus. The total carbon is also indicative of a very low content of organic matter which in turn makes the moisture content far too low to be in good condition for the normal activities of the nitrifying organisms.

Soil No. 11 - Clay loam. Nitrifying power: 47.9.

The limiting factors of this soil may be assumed to be the low calcium and phosphorus content.

The narrow C/N ratio indicates that probably the microbial activities have reached an equilibrium and that the organic matter as well as the nitrogen content of the soil are stored

up in the resistant humus fraction, bearing very little available nitrogen, as is shown by the Lamotte tests. A factor which very probably indirectly influenced the nitrifying power is the excessive drainage which seems to have leached out the essential nutrients. The rocks from which the soil originated are not particularly rich in lime, and drainage probably continued washing out what was left after the soil had been formed by glacial drift deposits.

The relatively high content of organic matter is probably due to the soil being under permanent pasture, though this cannot supplement the lack of phosphorus and lime.

2. Factors related to nitrification

The only data with which it was possible to establish correlations with the nitrifying capacity were the field data taken at time of sampling, the analyses, chemical and physical, the evolution of carbon dioxide, and the activities of the nitrifying organisms in culture.

The assumed influences due to field characteristics have been considered above with general considerations on the chemical and physical state of the soils, while the nitrifying organisms will be discussed in the following section.

The coefficient of correlation has been calculated for each of the different mineral elements given by the chemical analysis as compared to the nitrification of the fresh soil

nitrogen. No significant value could be found but it cannot be said that none exists. This is probably due to the fact that no experiment could be carried on to study the influence of a selected factor only, all others being equal. It so happened that the factor correlated was the limiting one in some samples whilst in others, although the factor studied was in condition to produce high activities, another factor was in such state as to be limiting.

If the whole of the results are taken under consideration and compared on a basis of the mean phosphorus content of 0.18% and the mean nitrogen content of 0.27% as established in Quebec soils (41, 42), it may be claimed that the phosphorus content more than the lime content and, to a less extent, than the nitrogen content, exerts an influence on the nitrifying capacity of these soils. The moisture, either total or hygroscopic, shows some relationship. No definite correlations, as those already found (20), could be established between nitrification and the elements of the soil, probably because the analyses relate to the type of soil instead of to the sample incubated for its nitrifying capacity.

It is not indeed advisable to draw sharp conclusions out of such a small number of samples taken only once in the growing season.

The only other analyses made from the same samples were the carbon dioxide evolution with fresh and air-dried

soils, and there only could a significant correlation be found. In fact, there is a high probability that there exists a relationship between the carbon dioxide evolution of untreated fresh soil and the nitrifying capacity. (See Fig.2).

The author has calculated the correlation coefficient from Potter and Snyder's data (53) and finds them to be highly significant between the two variables, nitrates and carbon dioxide. These authors made no mention of such correlation; it is shown here, Fig. 3. From these correlations it may be assumed that the highest nitrification occurred where the highest respiration processes went on.

The fact that a close relationship was found to exist between the carbon dioxide evolution from the remoistened air-dried soils, and that of the same soils to which glucose was added, while no such correlation could be established between the carbon dioxide evolution of the untreated fresh soils and that of the remoistened air-dried soils with or without addition of glucose, suggests that a different flora developed after the drying and remoistening; and that the flora that developed after the addition of glucose was the same as that which developed without it.

This seems to be confirmed also by the fact that no correlations could be found between the nitrifying capacity of the fresh soils and the carbon dioxide evolution from remoistened air-dried soils either with or without addition

It seems that more information is required, not only in laboratory, but also in field experiments, to ascertain if such a close association between these two dynamic systems will be found in a larger number of soils.

3. Nitrifying organisms

a. Oxidation

(i) Ammonia-oxidizers - The soils differed greatly in their behavior when their nitrifying organisms were required to oxidize in elective medium. There again no correlation could be established between the nitrifying capacity and the different activities of the organisms.

It has already been mentioned that the major part of a first series failed to carry on any further than the crude culture probably because of a too high ammonia concentration, but it may be worth while mentioning too that under these conditions the organisms of soils Nos. 3, 6, 10 and 14 succeeded in growing and oxidizing for few transfers; soils Nos. 10 and 14 alone went as far as the fifth transfer.

The soil complex factors being removed from that experiment, it seems that only biological factors could be responsible for the differences noted here. Of these it is believed that the resistance to acid conditions played no part because of the similitude of results obtained from soils Nos. 1 and 12 which show a significant difference in their pH value. For these two soils also it is doubtful that the numbers had to

be considered because an inoculum with 5 gm. of soil brought no increase of activity over that of one gram.

It is to be noted that the only soils which yielded organisms that could oxidize after a few transfers were the fastest oxidizers. This may indicate that certain soils contain strains of nitrifiers that are more active. And if the soils are divided on that basis, we see that the major part of the soils have a mean oxidation-time of 20 days while few soils with highly active organisms will take only an average of 15 days or less to complete the process. Two forest soils showed delayed oxidation after over 30 days.

From the work of Nelson (46), and Pandalai (50), it may be assumed that the fastest oxidizing organisms may have benefited from the action of saprophyte contaminants which resisted as far as the fifth transfer. As a matter of fact the few soils that were plated for isolation of the nitrifiers all showed a dense growth of contaminants, but it may be that there existed a specific one, helpful for the nitrifiers, which was present in certain soils. Due to lack of time, no study on that subject was carried on.

The suggestion made by Winogradsky and Winogradsky (87), namely, that there exist types of organisms varying in activity may be applied here also.

(ii) Nitrite oxidizers - The nitrate forming bacteria proved to be present in all soils when cultivated in elective

culture medium, and in all but three they succeeded in oxidizing up to the fifth generation. One soil, No. 8, went as far as the 4th transfer, but as for the fifth, two different inoculations failed to bring about the oxidation. The two other exceptions were soils Nos. 1 and 12. These two never succeeded the oxidation of a first transfer after the crude culture. The situation was exactly the same as for the ammonia oxidizers and yet these soils certainly contained the nitrifying organisms as it was shown by their increased nitrate content after incubation of the fresh soil. Again here a test was made with 5 gm. inoculum of soil in the elective culture medium and no increased activity was observed. This is suggestive that the numbers were not in cause and it may be assumed that the type of organisms differed from that of other soils, or that the state of matter that existed in the solutions was affecting the growth of the nitrifiers of these two soils.

Among the soils that proceeded normally, a variation in the speed of oxidation is also observed. The question of more active strains again is suggested as a probable explanation of this fact, but it may be invalidated if we consider that the most rapid nitrate-oxidizers correspond to the fastest ammonia-oxidizers. This difference of oxidation-time between the organisms of different soils may be attributed to disadvantageous conditions that caused the organisms to alter their usual behavior, and it is assumed that only five generations is not long enough to permit such ill-treated organisms to recover

;e of activity.

This proposition of possible ill-treatment, seems to receive support by the fact that the fastest oxidizing organisms were not necessarily found in the soils according to their order of nitrifying capacity but rather distributed here and there.

b. Isolation

(i) Ammonia-oxidizers - The few tests carried on in the isolation of these organisms proved to be inadequate. It is assumed that the agar selected for that operation is not suitable at all.

The more advanced soils (Nos. 6, 10 and 14) of the first series (not tabulated) of elective cultures were plated out with a few of the more active of the second series, (Nos. 3, 10 and 14).

A first result that may be pointed out is that after five successive transfers in pure elective medium, starvation was inoperative for few contaminants that grew plentifully on the elective Heinemann's ammonium sulphate agar. Of these contaminants two were more often met with, one, forming yellow colonies of nearly one millimeter of diameter, proved to be a medium long fat rod, with oblong spores like those of the "clostridium" cells; the other with its red-brown colonies of 500-800 microns in diameter was first thought to be the "licht rood-bruine" colonies mentioned by Kingma Boltjes (34) on the "Nährstoff-Heyden", but a microscopical examination

showed that it was a medium long fat rod which probably presents a vacuole at each end. Gray's spore stain (20a) did not stain those vacuoles, but a very faint wall could be seen around the highly refractile terminal body. The stain with carbol-fuchsin presents this cell as a rectangle with each end concave and each corner acuminate.

One colony, colorless and crateriform, gave under microscopical examination an organism resembling Nitrosomonas. It was isolated and transferred to 25 ml. of ammonium sulphate solution. After three weeks incubation at 28°C., it had not yet oxidized. Three weeks after plating, the agar still gave no reaction with the Trommsdorf reagent, showing that the nitrifying organisms had not developed on the culture medium.

It was impossible to proceed any further because of the time at our disposal.

(ii) Nitrite oxidizers - In spite of the fact that more work has been done in attempts to isolate the nitrite oxidizers, none of the organisms isolated has proved to be a nitrifier.

Soils Nos. 2, 3, 4, 7, 9, 10, 11, 13, and 14 have been plated in duplicates and the growth has been thoroughly followed by microscopic examination. They all presented the same four kinds of colonies. The organisms of these four colonies taken from soil 13, were transferred by the needle process to 25 ml. of the sodium nitrite solution and after one month none had oxidized the nitrite to nitrate while the

same amount of the same solution was oxidized in 24 days by inoculation with a drop of the fifth transfer of the same soil, No. 13.

A preliminary test carried on with soils Nos. 6 and 14 plated on the Heinemann's nitrite agar, but with washed agar instead of regular agar as used throughout the isolation work, gave rise to doubts whether the nitrifying organisms ever grew on the unwashed agar.

As a matter of fact, after 5 weeks of incubation, the nitrite agar still gave a nitrite reaction with the Trommsdorf reagent, while when the nitrite washed agar of the preliminary tests was tested with the same reagent 3 weeks after inoculation it gave a negative reaction and the presence of nitrates was confirmed by a positive diphenylamine test.

Unfortunately, when that fact was established, all the inoculations were done with the unwashed agar and no time was available to start the experimentation all over again.

Thus no further information as to the nitrifying capacity of the soils studied has been secured by the isolation of the organisms, but it may be assumed that Heinemann's agars, both ammonium and nitrite, as given by Levine and Schoenlein (37) are not suitable for the isolation of the nitrifying organisms from these soils.

V. CONCLUSIONS

The studies undertaken in the present work have shown that there exist wide variations in the nitrifying capacity of certain Quebec soils.

If the muck soil is excepted because of its five-fold nitrate content over the average of the mineral soils, it may be said that generally the process of nitrification is low in the soils studied. That confirms the findings of McKibbin and Gray (42) on soils of Quebec.

Although the author could find no correlation between the nitrifying capacity of these soils and their content in essential elements, it is not concluded that none really exists. The conditions under which the present study was carried on might have been the only cause for which the correlations already found (20) could not be confirmed.

However, a general tendency seems to establish that the total phosphorus content of the soils was the factor influencing the most the process of nitrification. Nitrogen content, lime content and, to a less extent, moisture conditions have been shown to be the probable influencing factor in some soils.

The carbon dioxide evolution from the untreated fresh soil incubated in the laboratory proved to run parallel to the nitrifying power of the soils.

When these soils were air-dried and remoistened, with or without addition of glucose, they showed no correlation with the nitrifying capacity. No correlation could be established between the carbon dioxide evolution of such treated soils and the carbon dioxide produced by the fresh untreated soils, while a close correlation exists between the carbon dioxide evolved from the remoistened air-dried soils alone and with addition of glucose. This suggests that in the treated soils, a microflora developed after drying which was different from that of the natural soils and which was the same in the treated soils either with or without addition of glucose.

The organisms themselves were studied in elective media, and the results point to the fact that there may exist at least few strains, if not species, that are less active than others. Again, after Stevens and others (68), doubts are expressed as to the validity of studying nitrification in solutions when two soils which nitrified in the fresh state could not proceed normally in the solution.

The strains which were the fastest in solution, were derived from the soils irrespective of the order of nitrifying power. This seems to be another line of evidence that the soils constituents were in most samples the factors causing a low nitrifying power.

Attempts were made to isolate the two types of nitrifiers on specific Heinemann's agar. The ammonium and nitrite

of the agars were not oxidized to nitrite and nitrates respectively after incubation for one month. It is believed that this agar which composition and preparation is given by Levine and Schoenlein (37) is not suitable for the isolation of the nitrifying organisms and that probably the washing of the agar may prove to make it fit for such isolation.

An observation was made of the contaminants found in the nitrifying elective cultures after five transfers for the nitrite-oxidizers and it is seen that the same forms are met with in all the soils.

The author expresses the wish that more investigations of this kind be linked up with field fertility studies in Quebec soils. It seems that an inventory of the total chemical content of a soil by no means can be interpreted as indicative of a high or low level of fertility if such studies as the biological activities and fertility analyses are not available for such discussions.

VI. SUMMARY

It was planned to establish the rate of nitrification in some of the most representative Quebec soils.

A thorough review of the literature on the factors influencing the nitrifying power of the soils has been given.

The soils under study, when permitted to nitrify their own nitrogen content in the fresh state and after 185 days incubation, have been shown to possess a low nitrifying capacity, if a muck is excepted.

The principal causal factor seems to be the lack of phosphorus while in some instances influences have been attributed to low content in nitrogen or lime or to a bad moisture distribution.

A correlation has been found to occur between the nitrifying capacity and the carbon dioxide evolved from the untreated fresh soil in the laboratory, which very probably indicates that the nitrification proceeds parallel to the activity of the soil organisms composing the normal soil flora.

Drying and subsequent remoistening of the soils is thought to have permitted to a new flora to develop, which flora, it is believed, is different from that of the soils under natural conditions but may prove to be the same one

either the remoistened soils received additions of glucose or not.

Cultural studies have been also carried on and they tend to show that there may exist in certain soils more or less active strains or species which may have influenced the nitrifying capacity of the soils. The numbers, for two soils, were not found to bring any increase in the oxidation processes.

The isolation of the organisms had to be cut short from lack of time. After five weeks of selection in the isolation investigations no nitrifiers had been yet isolated. It has established that the Heinemann's ammonium or nitrite agars do not seem to be suitable for such work if the agar is not previously washed.

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IX. FIGURES

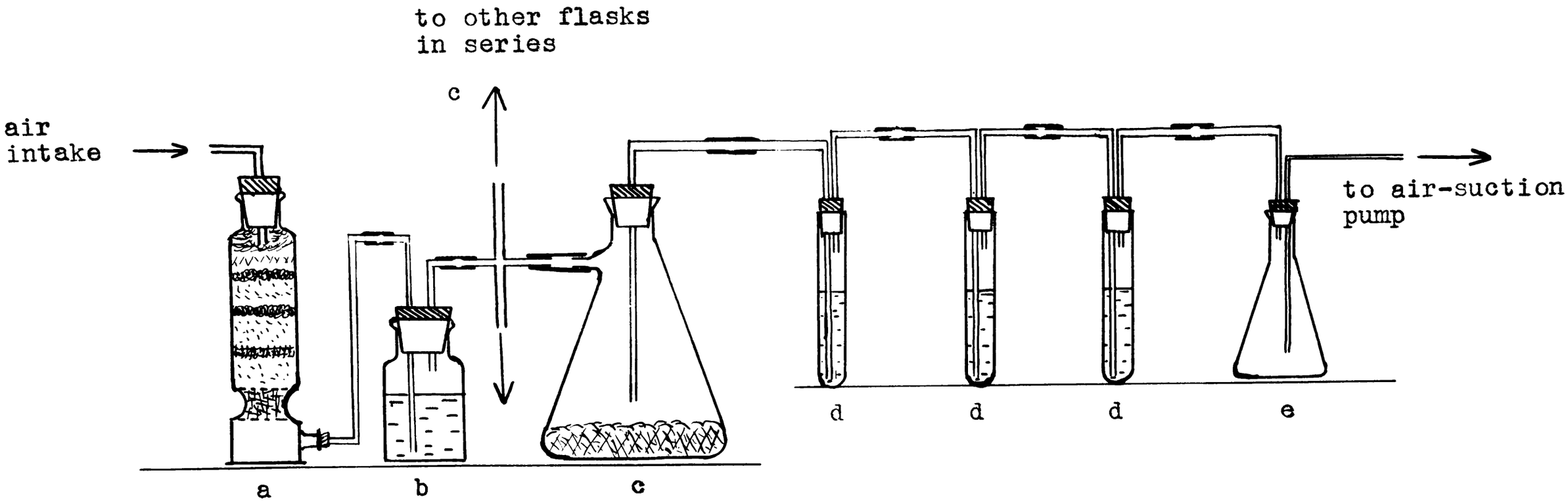

Fig. 1 - Apparatus for the determination of carbon dioxide.

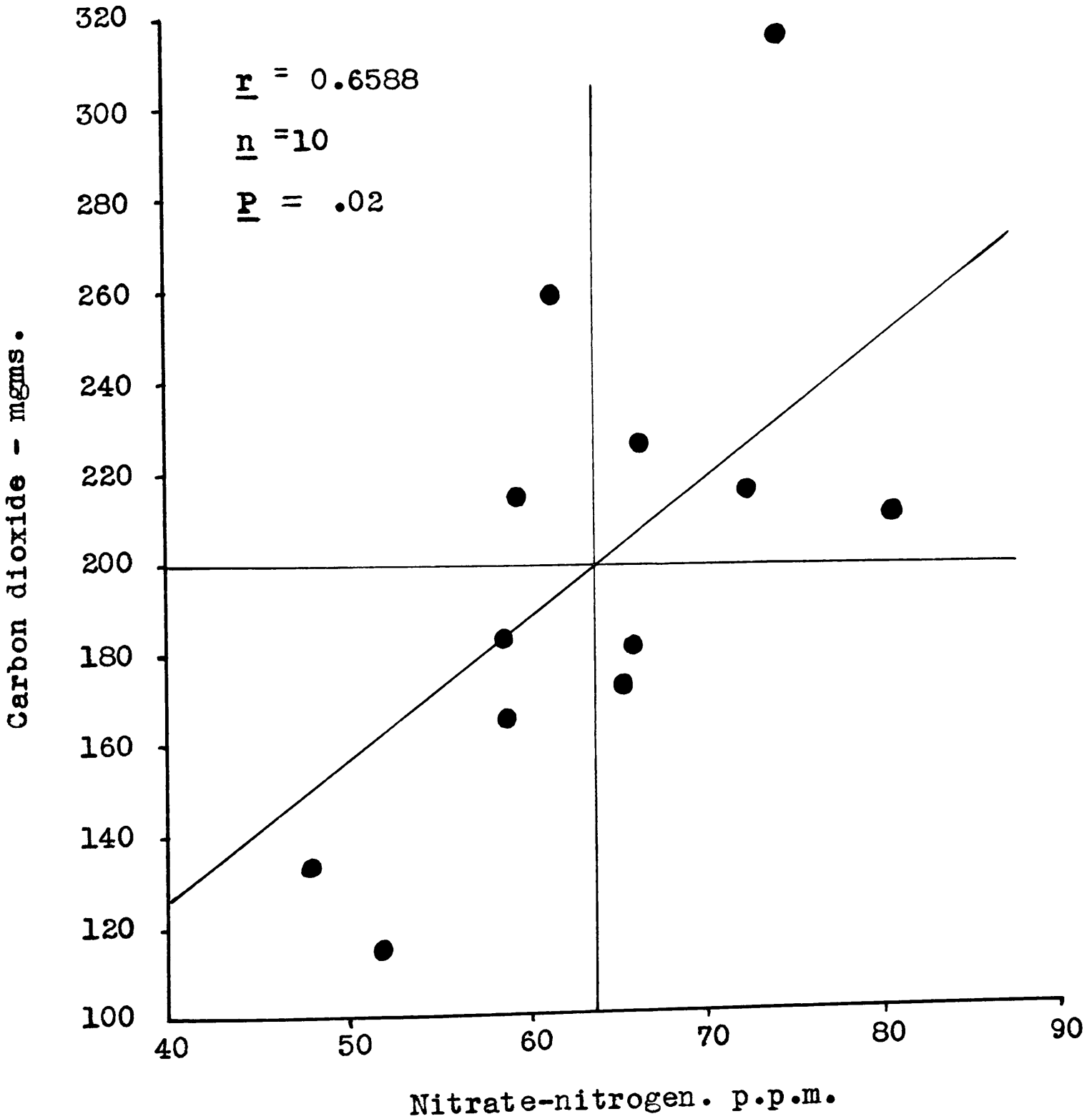

Fig. 2 - Correlation between nitrate-nitrogen (p.p.m.) after 185 days incubation and the total carbon dioxide evolution (mgms. per 100 gms. of oven-dried soil) of three successive 14-day periods from fresh soil.

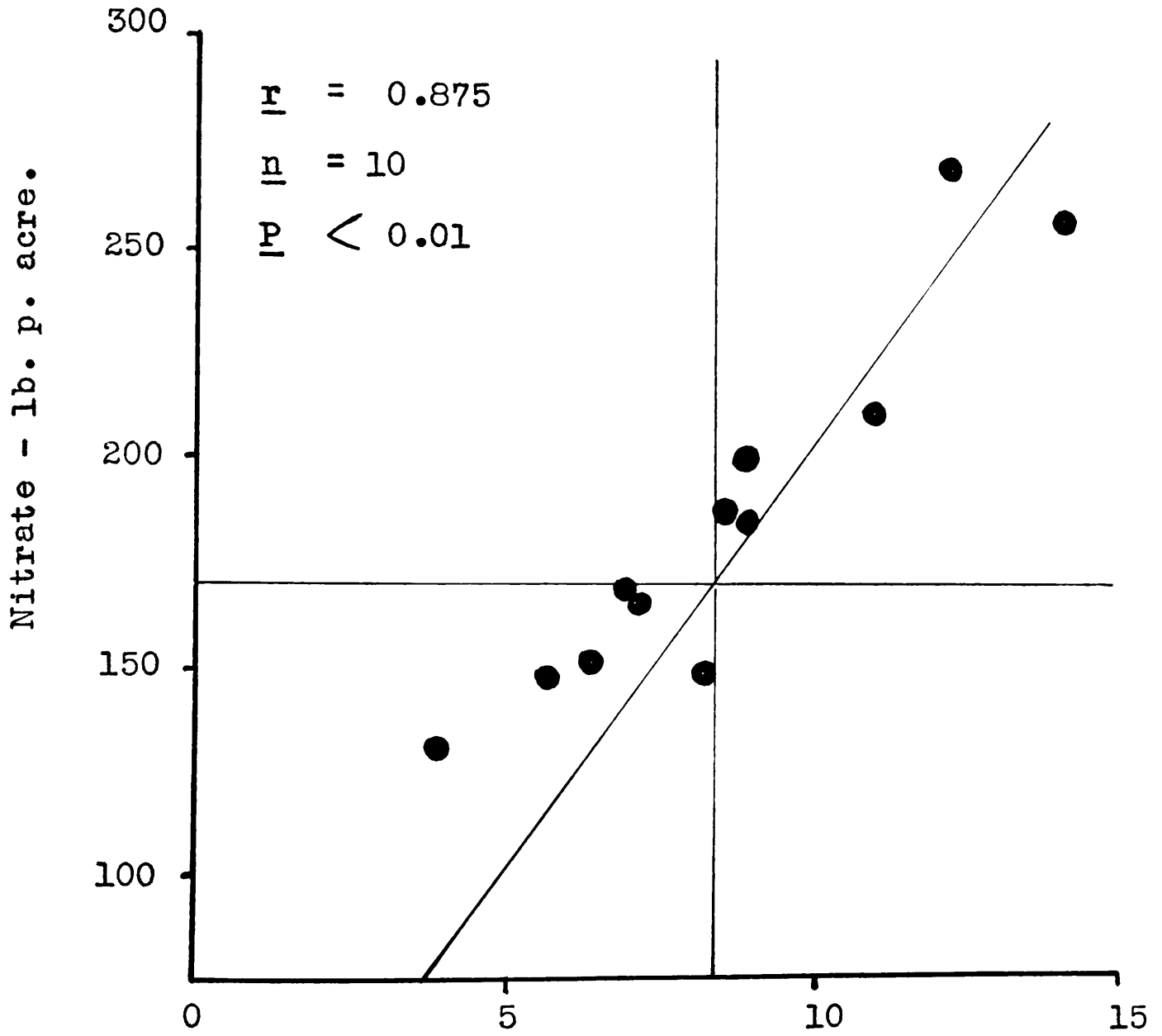

Fig. 3 - Correlation between nitrate-nitrogen and carbon dioxide from soils variously treated. Data from Potter and Snyder (53).

X. TABLES

TABLE I - FIELD DATA

Sample No. and Date of Sampling	Locality and nature of profile	Ecology	Previous treatment Rotation..Treatment
1 September 23rd 1942	Napierville Co. Flat plain. Depth of 6 in. Earthworms. Dark brown. Efferverscence with HCl 1:5 at 18 inches. Slow drainage. Loam	Elm, maple Red, yellow and white wild clover. Grasses. Chicory, green foxtail. Small ragweed.	Oats. 1 year. Hay. 2-3 years. Sod. 3-4 years. Fall ploughing. No fertilizer. No manure. Sampled on last year of sod.
2 September 23rd 1942	Napierville Co. Flat plain Depth of 6 in. Earthworms. Good drainage. Heavy loam.	Elm. Red, yellow and white wild clover. Green foxtail Common plantain. Grasses.	Corn or peas. 1 year. 2-12-6 + manure. Oats or barley. 1 year. Manure. Hay and Sod. 2-3 years. Manured each year. Sampled on last year of sod.
3 September 23rd 1942	Napierville Co. Gently rolling. Depth of 6 in. No earthworms. Very good drainage. Hardpan in B. horizon. Podsolized sand.	Birch, spruce and pine. Red and white wild clover. Grasses. Small ragweed.	It was formerly organized with tomatoes, oats and hay with manure occasionally. Now in sod since 6 or 7 years.
4 September 23rd 1942	Napierville Co. Rolling. Depth of 6 in. Earthworms. Effervescence not found here but usually this is a characteristic of the soil type. Very good drainage. Calciferous loam.	Elms. Red, white and white wild clover. Alfalfa. Cow vetch. Grasses.	Potatoes. 1 year. 1000 lbs. 4-8-10 + manure. Peas. 1 year. 90-100 lbs. 2-12-6. Barley. 1 year. 90-100 lbs. 2-12-6. Sod. 2 years. (Sown with hay and alfalfa). Sampled on 2nd year of sod.

5 September 23rd. 1942	Napierville Co. Rolling. Depth of 6 in. No earthworms Same as No.4 for effervescence. Very good drainage. Surface organic debris were taken off to sample only the mineral top layer of the soil. Calciferous loam.	Elm, maple oak. Fir, spruce and pine. Very little white wild clover. Goldenrod. Strawberry. Aster. Raspberry. Hawkweed.	Virgin soil. Under forest.
6 September 23rd. 1942	Napierville Co. Flat plain. Depth of 6 in. Earthworms. Slow drainage. Not burnt since. very long. When potatoes are grown two subsequent years scab appears on the second year. About 6 feet deep on clay. Well decomposed muck.	Elm, birch, ash-leaved maple, (Box Elder). Grasses Small and great ragweed. Mint.	Potatoes. 1 year. 4-8-10. Oats or barley. 1 year. Manure. Hay. 1 year. Sampled after the hay.
7 September 24th. 1942.	Chateauguay Co. Flat plain. Depth of 6 in. No earthworms. Surface organic debris were taken off. Effervescence not found there but usually a characteristic of the soil type - at 4-5 feet. Good drainage. Heavy rain 3 hours before sampling. Clay.	Birch, oak, maple, elm. Grasses. Cow vetch. Aster. Goldenrod. Small ragweed.	Virgin soil. Under forest

8 September. 24th. 1942.	Chateauguay Co. Rolling. Depth of 6 in. No earthworms. Effervescence at 15 inches. Slow drainage. Heavy rain 4 hours before sampling. At a depth of 15" the soil is very compact. Calciferous loam	Oak, elm, maple. Grasses, red and white wild clover. Goldenrod. Green foxtail.	Oats or barley. 1 year. Hay. 1 year. Sod. 2 years. Usually not manured or very little. No fertilizers. Sampled on 2nd year of sod.
9 September. 25th. 1942.	Laprairie Co. Flat plain. Depth of 6 in. Earthworms. Drainage medium. Heavy rain the day before. Clay.	Elm. Red and white wild clover. Grasses, aster. Small ragweed. Chicory.	Oats. 1 year. Buckwheat. 1 year. Hay and sod. 2-3 years. No fertilizer. No manure. Sod ploughed under at the end of the cycle. Sampled after the hay.
10 September. 25th. 1942.	L'Assomption Co. Undulating. Depth of 6 in. No earthworms Drainage very good. Podsolized sand of the wind-blown type.	Birch, poplar, maple, vinegar tree. Grasses. White wild clover. Common Yarrow. Goldenrod. Ox-eye Daisy. Great Mullein	Rotation and treatments unknown. Sampled on sod.
11 September. 29th. 1942.	Kamouraska Co. Undulating. Depth of 6 in. No earthworms. Drainage excessive. Clay loam.	Maple, birch, fir, spruce. Common juniper. Fescue-grass. Hawkweed. Strawberry. Mosses. Raspberry.	Permanent pasture.

12 September 29th 1942.	Same as preceding.	Under forest	
13 September 29th 1942.	Kamouraska Co. Flat plain. Depth of 6 in. No earthworms. Drainage good. Clay	Fir, spruce, poplar, birch. Couch-grass. Strawberry. Hawkweed. Grasses.	Rotation and treatments unknown. Sampled on old sod
14 September 29th 1942.	Kamouraska Co. Flat plain. Depth of 6 in. No earthworms. Slow drainage. Soil developed on alluvial blue mud of the St. Lawrence River. Silty loam.	Fir, spruce, poplar, willows Couch-grass. Dock.	Permanent pasture.

TABLE II - PHYSICAL ANALYSIS

Soil	Texture				Moisture			
	Gravel %	Sand %	Clay %	Silt %	Total oven-dried %	Hygroscopic moisture %	Water-holding capacity %	Moisture in fresh soil as % of water-hold capacity
1	1.5	53.6	21.8	24.6	19.3	1.6	44.2	43.7
2	0.3	26.0	28.0	46.0	25.6	2.8	63.8	40.2
3	0	63.2	14.0	22.8	19.7	2.0	40.5	48.6
4	5.0	50.0	23.8	26.2	20.7	2.4	48.5	42.7
5	-	-	-	-	27.4	3.5	69.5	39.5
6	-	-	-	-	69.4	13.4	274.0	25.3
7	0	22.4	47.2	30.4	26.9	4.1	65.5	41.1
8	9.5	57.2	20.8	22.0	21.3	2.6	50.5	42.2
9	0	22.0	58.0	20.0	27.4	4.1	62.0	44.2
10	0.6	90.7	6.0	3.3	10.3	0.9	29.5	34.9
11	7.5	36.9	20.9	42.2	15.7	2.1	40.5	38.8
12	-	-	-	-	19.5	2.3	47.5	41.1
13	0.5	7.4	58.6	34.0	16.8	1.6	44.5	37.8
14	0	36.2	24.9	38.9	31.9	4.0	85.0	37.5

TABLE IV - NITRIFICATION IN THE DIFFERENT SOILS

(Nitrate-Nitrogen, parts per million, oven-dried soils)

Soil	Periods of incubation (days)			
	31	82	139	185
1	12.6	28.4	54.2	59.2
2	22.0	49.5	74.4	80.7
3	9.9	30.5	61.0	65.1
4	16.4	32.3	67.8	72.4
5	13.1	31.1	69.8	74.2
6	61.1	154.9	335.1	342.9
7	7.4	28.1	54.3	66.1
8	24.8	40.1	50.4	61.5
9	12.3	24.2	44.4	65.7
10	3.6	16.1	43.7	51.9
11	3.2	18.7	46.1	47.9
12	8.2	29.2	52.7	58.9
13	6.7	25.5	52.2	58.6
14	13.2	37.0	57.6	69.0

TABLE V - CARBON DIOXIDE EVOLUTION FROM THE DIFFERENT SOILS

(Total mg. per 100 gm. per 14-day period;oven-dried soil)

Soil	Fresh Soil				Dried Soil (one period)	
	Three successive periods				Remoistened	Remoistened + glucose
	1st	2nd	3rd	Total		
1	118.4	58.3	37.8	214.5	112.9	412.2
2	115.7	55.3	40.1	211.1	153.3	422.6
3	86.4	49.6	37.6	173.6	88.8	359.8
4	115.1	56.8	44.6	216.5	67.4	390.4
5	133.1	100.5	82.4	316.0	249.2	523.5
6	486.5	346.7	271.0	1104.2	294.6	621.5
7	107.1	67.6	51.8	226.5	309.1	562.2
8	139:1	69.2	51.6	259.9	152.8	454.0
9	69.1	55.2	56.7	181.0	139.2	437.9
10	42.4	35.7	36.3	114.4	82.4	345.5
11	48.5	44.0	41.1	133.6	97.2	382.9
12	61.6	48.6	55.8	166.0	157.5	420.0
13	65.8	54.9	63.1	183.8	167.8	416.1
14	62.5	49.5	57.2	169.2	191.0	489.0

TABLE VI - CULTURAL STUDIES OF THE NITRITE-FORMING BACTERIA IN AMMONIUM SULPHATE SOLUTION

(Number of days for a strong nitrite reaction)

Soil	Crude Culture	Transfers 1st	2nd
1	21	-	-
2	21	-	-
3	14	12	13
4	18	-	-
5	18	21	-
6	14	16	14
7	39	-	-
8	16	19	14
9	21	-	-
10	18	16	13
11	21	19	14
12	35	-	-
13	21	-	-
14	21	7	16

TABLE VII - CULTURAL STUDIES OF THE NITRATE-FORMING BACTERIA IN SODIUM NITRITE SOLUTION

(Number of days for a strong nitrate reaction)

Soil	Crude Culture	Transfers				
		1st	2nd	3rd	4th	5th
1	27	(1) -	-	-	-	-
2	23	18	29	19	17	20
3	16	10	11	11	13	11
4	20	13	12	12	11	13
5	23	24	17	24	16	17
6	16	10	11	12	11	12
7	27	14	14	15	27	47
8	23	21	17	15	17	-(2)
9	23	15	15	13	11	14
10	20	13	12	10	11	11
11	20	13	12	12	11	16
12	44	(1) -	-	-	-	-
13	23	21	12	14	16	31
14	16	14	11	11	11	8

(1) For soils No. 1 and 12 - the first transfers failed to oxidize. Soil No. 1 was transferred anew but failed again.

(2) The 5th transfer had not oxidized after 98 days incubation.