SOME ASPECTS OF THE NITROGEN CYCLE IN SOIL OF THE DOUGLAS-FIR FOREST

by

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ABSTRACT

Studies were carried out on nitrifying capacities of duff mull and raw humus soils of the Douglas-fir forest. For this purpose, perfusion apparatuses were set up according to the technique described by I.J. Audus (8) with certain modifications which seemed to improve considerably the application of this apparatus for soil studies. Certain changes were introduced into the method as given by H. Lees and J.H. Quastell (54). The quantitative estimations of ammonia and nitrates were made by colorimetric method using phenoldisulphonic and NaOH-EDTA solution for nitrates and Nessler's reagent for ammonium determinations. Soils used for this experiment were tested for total organic matter and total nitrogen.

From the results obtained, the most striking difference between the raw humus (mor) and duff mull was observed in the total absence of nitrification in all samples of mor and the comparatively vigorous nitrification in all samples of mull. There was further confirmation that the acidic condition of mor humus alone is not the limiting factor in nitrification in such forest soils. In duff mull, nitrification occurred over a wide range of pH. In raw humus, after adjusting pH to 6.5 and 7.0 and inoculating with actively nitrifying garden soil, no nitrification was observed. It was of interest to note that nitrification

occurred only when mor soil was subjected to complete drying or steam sterilization before being inoculated. This phenomenon might indicate the presence of some inhibitory action against nitrifying organisms. This inhibitory effect is typical of raw humus (mor) but lacking when it is sterilized by steam. In all leachates of raw humus some ammonium was always detected. As far as could be determined, nitrifying duff mull soils have failed to show any significant seasonal variation. It was difficult to establish any correlation between total nitrogen of soils, their carbon/nitrogen ratio, and their capacity for nitrification.

It is understood that nitrates are not the only source of nitrogen for the metabolism of forest trees; nevertheless, nitrates should be regarded as an important ecological factor in the evaluation of forest sites.

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I. INTRODUCTION

Of fifteen elements known to be required for the nutrition of plants, nitrogen is regarded as one of the most important of the macroelement group. Very often it becomes a limiting factor. For these reasons the nitrogen cycle in nature and its economy has been the subject of thorough studies especially in agricultural soils, in which the constant removal of crops reduces the nitrogen level of these soils and their productivity.

In virgin soils under the forests, a state of dynamic equilibrium has been established over time. Any natural changes in this equilibrium are minor and occur only after relatively long periods of time by very slow changes in climatic, geological, and biotic factors. On this assumption, therefore, it was presumed that there is no immediate necessity for a strict control or possible corrections of the nitrogen level in these soils. Recently, in countries where the forest is a major asset in the national economy, it became obvious that the soil under the forest should not be considered of less importance than the trees growing on it. With the removal of trees by logging or the destruction of most of the organic matter by fire or any other drastic changes in the environmental conditions, it is logical to assume that nature's long established nitrogen equilibrium in these soils will be

altered. To continue, therefore, an expensive and laborious program of reforestation without regard to the productive capacity of the soil might lead to unpredictable and disappointing results.

II. LITERATURE REVIEW

A. Nitrogen in Agricultural Soils

Most of our knowledge of the interchangeability of various forms of nitrogen in nature is derived from studies of the nitrogen cycle in agricultural soils.

a. Total Nitrogen in Agricultural Soils

Total nitrogen in soils depends mainly upon the amount of organic matter in the soil and bears only a slight relationship to the soil nitrogen available for the growth of plants. In general, the organic fraction of soil nitrogen is very resistant to decomposition and therefore, relatively unavailable to the growing vegetation (96). According to Bremner (16), only 1% - 3% of organic nitrogen is mineralized during the growing season. Recent work (24,33,76,106) performed by a number of investigators at the Iowa Agricultural Experiment Station suggests nitrates rather than total nitrogen as a relative measure of the potential nitrogen supplying power of agricultural soils. For data showing the average values of total nitrogen in certain agricultural soils see Appendix (Table I).

b. Nitrogen of Organic Origin in Agricultural Soils

Nitrogen in the organic fraction consistutes about 97 to 98 percent of total nitrogen (96). Considerable variation in the nitrogen content of humus and of different

organic residues renders difficult the establishment of an accurate quantitative relationship between total nitrogen and total organic material in soil (114). There is some evidence to show that the carbon/nitrogen ratio decreases with depth in the soil and with the degree of humus decomposition (96,114). It might be expected, therefore, that the value of this ratio would fluctuate considerably with different soils, but according to Russell (96), in most agricultural soils the carbon/nitrogen ratio "... is surprisingly constant and surprisingly independent of soil treatment." For the variations of percentage nitrogen in humus in different soils and some data regarding carbon-nitrogen ratios, see Appendix (Tables II, III).

In general, a high value of carbon/nitrogen ratio implies an undecomposed status of a humus. A carbon/nitrogen ratio of 10:1 or less points rather to an advanced humus degradation and the likelihood of decreased microbial action (114). The chemical nature of the various forms of nitrogenous compounds in soil organic matter is not yet well understood (117). Kojima (16) and Bremner (16) have shown that the protein fraction of amino-acid polymers can account for not more than 40% of total nitrogen in soil. Waksman (117) analyzing some prairie soils in Alberta, Saskatchewan, and Manitoba found average protein values to lie within 33.3 to 37.4 percent of total organic matter. Clark (16) stated that the nucleic

acid portion of soil nitrogen is negligible. The presence of amino sugars was verified by some workers and stated by Bremner (16) to account for 5 to 10 percent of soil nitrogen. As no other nitrogen compounds have, as yet, been isolated in reasonably substantial quantities, the identity of approximately half of the total organic fraction of soil nitrogen remains obscure. Work of Rod (16) with tropical soils suggests that some of this unidentified nitrogen may be fixed ammonia in close association with clay minerals. Other investigators (16), postulate an interaction between ammonia and oxidized lignins or amino groups and quinone rings of some humic substances (16).

c. Nitrogen of the Inorganic Fraction

The inorganic nitrogen in the form of ammonium (NH_4^+), ammonia (NH_3), nitrites (NO_2^-), and nitrates (NO_3^-) constitutes approximately 2 to 3 percent of the total nitrogen in soils (114). The amounts of mineralized nitrogen and the proportions of different forms varies considerably depending upon changes in the process of formation and removal (16). Ammonium is usually present in only relatively small quantities of a few parts per million (97). It is interesting to note that its level in arable soils is fairly low but constant (96). In grasslands, ammonium comprises the main portion of inorganic nitrogen and its concentration is unaffected by artificial additions

(96). Whereas nitrates are totally dissolved in soil water, ammonium is held on the exchange surfaces of both mineral and organic soil fractions. There are differences of opinion among some investigators regarding the importance of ammonium absorption for nitrification and the availability of absorbed ammonium ions to oxidizing organisms and growing plants (2,4,32).

Relative concentrations of nitrites are usually negligible as their rate of formation is less than their rate of oxidation. Russell (96) states that in some desert soils the accumulation of nitrites might reach levels detrimental to the growing crop. It has been reported (96) that soils with low phosphate content and high amount of calcium carbonate are able to accumulate nitrites. Sterilization of a soil or treatment with chlorides will prevent nitrite oxidation with the consequent accumulation of nitrites. Generally, the presence of nitrites in soils is regarded as harmful to plants though some investigation (97) points to a possible utilization by plants. The approximate quantities of nitrate in arable soils were estimated to be from 2 to 60 mgm/Kg of soil (96). The concentration of nitrates according to Russell (96), varies considerable with fallow, season, type of growth, amount and nature of decomposed material, percent moisture, temperature, and fertilization practices. Clark (105) concludes that for these reasons the relative concentration of nitrate in a given soil sample should not be used as an index of soil fertility. More

recent investigators (129) claim that nitrate production occurs over a wide range of soil moisture with an optimum temperature of 35°C ± 1°C. Iowa workers (33,76,106,129) regard these variations as negligible and state that nitrate production, together with other information, will provide a reliable indication of the nitrogen-supplying power of the soil, which, they claim, remains unchanged for a period of years.

B. Nitrogen Intake in Soils in General

The fixation of elemental nitrogen by either biotic or nonbiotic factors is indispensable to the nitrogen economy of agricultural soils. The gaseous inert form of nitrogen that is part of our atmosphere can be utilized by plants only in a combined form. Two general groups of nicroorganisms participate in the fixation of atmospheric nitrogen namely the symbiotic organisms and the so-called free-living nitrogen fixers.

a. Fixation of Nitrogen by Symbiotic Bacteria

In agricultural soils the best known and the most important examples of this group are the bacteria of the genus Rhizobium associated with some higher plants of the family Leguminosae. The bacteria infecting these plants stimulate the growth of cells scattered throughout the root tissue of the host, resulting in the formation of characteristic nodules. Most of the species of the

family Leguminosae have not developed this habit of harbouring nodular bacteria (79). Among the identifiable bacteria found to colonize the roots of the few leguminous species that are susceptible, certain elements of specificity have developed. According to Waksman and Starkey (115), at least twelve different strains or species of legume bacteria have been established. Each strain is able to invade only one or a few leguminous plants.

The fixation of atmospheric nitrogen occurs only in the union of a healthy vigorously growing plant-host and a specific bacterial symbiont. Neither the plant nor the bacterium alone can fix nitrogen. Various claims for the fixation of nitrogen by excised nodules have been criticised (111), and even if accepted as true, the amounts fixed are small and almost insignificant (82).

The quantities of nitrogen fixed depend upon many factors, among them the presence of molybdenum (66) and the sufficiency of the sulphur supply. In fact, any nutritional deficiency that lowers the vitality of the host plant will hamper nitrogen fixation. Soils in which leguminous plants are to be grown require an ample supply of calcium, potassium, sulphate, and phosphate (72). The presence of readily available sources of inorganic nitrogen, especially nitrates, in such soils, tends to decrease nodulation since the plants take up nitrates and ammonium preferentially (79). Leguminous bacteria, especially Rhizobium trifolii,

are present in almost all agricultural soils and the most favorable pH range for these organisms is in region of neutrality (86,115). Furthermore, they are cold tolerant, even being able to withstand prolonged freezing (61).

Plants in symbiotic consortium with such Rhizobium species are capable of utilizing the bacterially fixed nitrogen as is the bacterial associate itself. Remaining quantities of nitrogen compounds are excreted into the soil. Sloughing off and decay of leguminous root tissue is regarded as a more important source of nitrogen than such root excretions (22). It should be understood that the presence of legumes does not necessarily imply an increase in soil nitrogen, and this is particularly true in the case of soils already high in nitrogen or those from which a crop has been recently harvested. In addition, the species of host plant and the age of the stand of which it is a part will affect the relative amount of fixed nitrogen (70).

b. Fixation of Nitrogen by Nonsymbiotic Bacteria

Soil may acquire a part of its nitrogen as a result of the activities of free-living bacteria like Clostridium, Azotobacter and some photosynthetic and chemosynthetic organisms.

The organisms from the above groups are present in varying combinations and amount. This may be partly due to the methods of isolation (61). Azotobacter, known to be

of world-wide distribution (absent only from the arctic soils), is generally considered to be less abundant than the anaerobic Clostridium species (26,96). Lochhead (61) estimated that there were from several hundred to a thousand Azotobacter cells per gram of soil, whereas the same amount of the same soil might contain as many as one hundred million bacteria of other types. Waksman (117) states that in general, the number of anaerobic Clostridium species found in one gram of soil is greater than 100,000; he concludes that any quantitative estimates of anaerobic nitrogen-fixing population can only be regarded as approximate. That this is so is due largely to the fact that only a few organisms of this group can develop on artificial media.

Nitrogen fixed by Azotobacter is mostly incorporated as cell material and the amount of nitrogen fixed can be regarded as an index of cell growth and increase in cell material (115); this nitrogen can be utilized by plants only after the death of such bacteria. The process of decay of microbial protein is fairly slow.

Fixation of nitrogen by Azotobacter species is influenced strongly by factors such as the presence of phosphate, molybdenum, readily available carbohydrates, suitable pH, and the absence of inorganic nitrogen. Jensen (43) states that molybdenum is essential only to some species of Azotobacter and then only in certain conditions. The optimal pH range, with but few exceptions,

(Azotobacter indicum) is 7.5 - 6.8 (43,117). Calcium is regarded (66) only as a neutralizing agent and not as an essential element. Ammonium immediately inhibits nitrogen fixation. Nitrites and nitrates have a similar effect but, compared to that of ammonium, it is somewhat delayed (43).

In the case of Clostridium species, Waksman (117) states that they are more abundant in acid soils. Bogell (117) reports, that heavy rains favor anaerobic nitrogen fixation by Clostridia species. Waksman (117) finds Clostridium butylicum to be present everywhere. In conclusion, Lochhead (61) says that one is entitled to doubt the importance of Azotobacter as an important source of fixed nitrogen in nature. Waksman (117) and Jensen (43) observed a stimulation in the growth and nitrogen-fixing capacity of Azotobacter when grown in association with other organisms. Somewhat later, Jensen (43) concluded that the fixation of nitrogen by species of Azotobacter in natural environmental conditions is not well understood. (See Appendix, Table V, for data of average values of nitrogen fixed by nonsymbiotic bacteria).

c. Fixation of Nitrogen by Other Organisms

It is now a well established fact that many species of blue-green algae belonging to the genera of Nostoc, Anabaena, Aulosira and Cylindrospermum have the ability to fix atmospheric nitrogen (96). Fogg (25) states

that, of forty algal species tested, only about half have been found to be capable of fixation. According to the same author (25), this process is considerably slower with algae than with bacteria since it usually takes several weeks to accumulate 20-30 ug. N/ml.

According to Russell (96), fixation of nitrogen by algae does not depend on light, since if supplied with a source of carbohydrates, they are functional in this regard even in the dark. In nature algae may live in some type of symbiosis with liverworts, fungi, and cycads. Scott (99) has demonstrated fixation of nitrogen by a species of Nostoc living in symbiosis with Peltigera praetextata; he did not find any fixation with Cladonia impexa.

In general, nitrogen fixation among blue-green algae becomes more important when we consider that these completely autotrophic organisms are universally distributed and are able to adapt themselves to a great variety of environmental conditions (96). Algae are regarded as "invasive colonizers", actively responsible for colonization of barren mineral soils, burnt-over areas or saline-lake bed soils. Their ability to act as pioneer organisms is the result of this unique ability to synthesis their own nitrogen. The presence of nitrates, nitrites, or ammonia inhibits fixation by these organisms; molybdenum seems to be required (96).

The fixation of nitrogen by Phoma species, usually in association with roots, has been reported in many instances though without absolute certainty. Foster (28) regards soil fungi as very active agents in nitrogen fixation, though of lesser importance, in this regard, than bacteria. A fungus associated with the root system of the grass Lolium temulentum, has been reported to possess a fixative ability with regard to nitrogen. Scott-Wilson H.W. (98) quotes Lipman as having found some pseudo-yeast Tulare No46b exhibiting this ability. Handley (35) concludes that the use of isotopic nitrogen in recent investigations has made it even more certain that some fungi are able to fix atmospheric nitrogen.

d. Fixation of Nitrogen by Nonbiological Factors

Apart from the biological fixation of atmospheric nitrogen, there are some factors which contribute rather small but constant supplies of nitrogen to growing vegetation. There are some forms of fixed nitrogen in our atmosphere which originated either from electrostatic discharges or from industrial pollution and volatilization of ammonia from certain soils. Usually two main forms of nitrogen are recognized in our atmosphere as being brought back to the soil by means of snow and rain. These are nitrates and ammonium. The amount of nitrogen-containing compounds occurring in snow and rain fluctuates with the season of the year, geographical location, and the

the amount of precipitation (see Appendix, Table VI). From some of the data available, it seems safe to suggest that under a humid temperate climate, the average annual amount of 5 lb. of nitrogen (in form of ammonium and nitrates) is brought by precipitation to every acre of land. This amount, according to Lyon (70) is equivalent to about 31 lb. of commercial nitrate of soda, artificially applied.

Reports of some investigators on the fixation of atmospheric nitrogen by purely physico-chemical or photo-chemical reactions occurring in soil itself still remains unconfirmed. Dhar, through his work, claimed that a fairly large percentage of gaseous nitrogen could be fixed by soils. Fairly recent work in this line seems to confirm earlier claims that light supplies energy for the fixation of atmospheric nitrogen by clay particles and that this process is catalyzed by glucose.

C. Nitrogen Transformation in Soils

a. Ammonification and Denitrification

A very large number of organisms among bacteria, fungi, and actinomycetes are able to produce ammonium from different nitrogenous compounds and under different environmental conditions. The amount of ammonium produced varies and depends on: (a) the nature of material being attacked; (b) the organism involved in the decomposition; and (c) the environment. The great majority of soil bacteria

that develop on petri plates is able to form ammonium from proteins of animal or plant origin (117). Gelatin-liquefying organisms, able to induce greater protein decomposition, constitute 15 percent or more of the total number of soil bacteria. According to Waksman (117), proteins make up 1 to 20 percent of all plant residue. Plant residues that are low in nitrogen form little ammonium on decomposition, and then only after prolonged periods of bacterial activity. Young plants decompose more readily with a higher production of ammonium than older (115). The degradation of plant proteins is carried out in different stages by different organisms with a variety of end products (117). The final amount of ammonium liberated may be as much as 50 to 80 percent of total protein nitrogen (117). There is no case known in which the total organic nitrogen present can be changed into ammonium (115).

Nitrates can be reduced to ammonium by organisms such as Clostridium welchii and Escherichia coli (117). In soils saturated with water and containing large amounts of organic matter, nitrates are reduced either to nitrites or ammonium, and these soils become more alkaline as a consequence (117). Ammonification by bacteria occurs in a relatively narrow range of pH whereas fungi may be active under both neutral and acidic conditions. In acid forest

soils fungi are the organisms mainly responsible for the degradation and breakdown of plant proteins. The rate of fungal growth and ammonia formation, according to Waksman (117) depends upon the nature of the proteinaceous material, its nitrogen content, the species of fungi participating in decomposition and the nature of the carbon compounds or protein carbon. In the absence of carbon sources, a fungus such as Aspergillus niger utilizes proteins for its carbon and nitrogen requirements (117).

Actinomycetes, as a rule, prefer proteins to carbohydrates as a source of carbon and, according to Waksman (116), in the presence of both will attack preferentially proteins with considerable liberation of nitrogen in form of ammonium. Waksman (117), comparing the decomposition of proteins by pure cultures of bacteria, fungi, and actinomycetes, states that the relative amount of liberated ammonium is highest in the case of bacteria because they themselves synthesize less. In addition, Waksman states that the presence of an excessive amount of carbohydrates has a depressing effect on the liberation of ammonium from any soil.

Quantitatively, ammonia or ammonium present in soils is usually in the range of few parts per million (97). Arable soils have a fairly low but constant amount of ammonium. In the case of grassland, the main portion of inorganic nitrogen is in the form of ammonium and nitrates (96).

The function of ammoniacal nitrogen in soils varies as follows:

a. it is used by many soil organisms and the ammonifiers themselves;

b. it can be actively absorbed by most higher plants, especially those that are in the early stages of growth (1).

c. a considerable amount is used in nitrification.

d. portions may be adsorbed on soil particles or lost to the atmosphere.

Waksman and Wilson (122) state that ammonium is the simplest nitrogen source for many bacteria, yeasts, and moulds. Autotrophic Nitrosomonas can oxidize ammonium to nitrate and utilize the obtained energy for the assimilation of carbon dioxide (82). Many heterotrophic organisms will grow on ammonium salts as the sole source of energy (82). Perhman (83) states that many actinomycetes are able to utilize ammonium and nitrates for their nitrogen requirements.

In the case of higher plants, ammonium is readily absorbed from soil by root system of the plants, especially in moderately aerated soils with a sufficient supply of carbohydrates (72). This absorption may be direct or mostly through the mycorrhizal fungi (Hymenomycetes) (70). It is a well accepted fact that plants can utilize ammonium and, in some cases, prefer

this source of nitrogen to nitrates (70). Krajina (lecture notes) finds that hemlock fares best with ammonium as a nitrogen source whereas Douglas-fir grows best in an ample supply of nitrates.

b. Nitrification

The literature on this subject is too voluminous for a comprehensive representation. Most of the research on nitrification has been done with agricultural soils. Work with forest soils is comparatively scanty and some of the results cannot be used now to any advantage as they contain no reference to the ecological conditions of the soils studied.

Nitrification until very recent times was regarded as the most important link in the nitrogen cycle because of the value of nitrates to the nutrition of plants. Recently, however, some authorities (70) have come to regard nitrification as an overemphasized phase of bacterial physiology and a very weak link in the nitrogen cycle due to the fact that the nitrifying bacteria are extremely sensitive to environmental changes.

Nitrification appears to be a two-stage process being characterized by a gradual disappearance of ammonium with a concurrent formation of nitrites. This is followed by further oxidation of nitrites and an accumulation of nitrates. Both stages are affected by only two groups of highly specific, strictly autotrophic soil

bacteria. Good aeration, an adequate amount of moisture, the presence of buffering substances, an absence of large quantities of soluble organic matter, a suitable pH range (pH 70-90) and fairly high temperature are among the more important requirements of these organisms (15,112,114). The sixth edition of Bergey's manual (15) lists five genera of bacteria capable of oxidizing ammonia to nitrite and two genera that oxidise nitrites to nitrates. The oxidation of ammonia to nitrite and atmospheric carbon dioxide are the respective energy and carbon sources fundamental to these organisms.

Recent investigations suggest certain heterotrophic bacteria and other organisms to be actively engaged in the process of transformation of ammonia nitrogen into nitrates. Kalinenko (45) reports that many heterotrophic bacteria isolated from water, sewage, and soil are capable, under certain conditions, of nitrifying inorganic nitrogen. Quastel (90) isolated three species of soil bacteria oxidizing oximes of pyruvic acid or certain other alpha-keto acids to nitrites. Jensen (94) reports isolation of some actinomycetes species (Nocardia corallina) producing nitrites from pyruvic acid oxime. Isenberg (42) states that a certain organism of the genus Streptomyces oxidises ammonium to nitrites.

Fisher (23) and Hutton (39) found various unidentified heterotrophic and methane-oxidizing soil

bacteria capable of ammonium to nitrite transformations. Schmidt (100) succeeded in isolating a fungus (Aspergillus flavus) capable of carrying the nitrification reaction to completion with the formation of nitrate.

The difficulty experienced by a number of investigators (78) in isolating nitrifyers by conventional selective culture techniques lies in suppressing heterotrophic organisms in primary enrichment or isolation. This pointed to the possibility of nitrification as a symbiotic phenomenon (40,74).

A change in the method of study of nitrification, from pot culture methods (30) to soil-perfusion techniques (8,19,54,55,58,59,94), has provided much fundamental information on soil nitrification. The study of soil metabolism by perfusion techniques is advantageous in that the many uncontrollable variables, by being included in each test sample, are eliminated from comparative conclusions.

Twenty to fifty grams of soil are placed in a glass tube in such a way that a liquid can be passed through the tube from the top, collected in a reservoir at the bottom and automatically returned through the soil tube again. The device is fairly simple and permits of excellent control of aeration and the rate of perfusion (8). From studies such as these, nitrification appears to occur wholly at the soil colloid surface where ammonium is adsorbed and the bacteria adhere (54,55,56). The addition

of calcium ions diminishes the rate of nitrification due to the displacement of ammonium from exchange surfaces by the divalent base. The curve of the rate of nitrification with fresh soil samples is sigmoid and implies that nitrification is effected by actively multiplying cells.

The importance of the base exchange phenomena in nitrification gave rise to certain conflicting views. Lees and Quastel (54,55,56) held that ammonium has to be adsorbed by soil particles for it to be available to nitrifying bacteria. Bower (14) Allison (2,4) and Goldberg indicated a possible detrimental effect of surface phenomena on the process of nitrification. According to Goldberg and Gainey (32) absorption of ammonium from the soil solution may reduce the rate of nitrate accumulation as much as 70%; it would seem therefore, that Quastel's (89) claim to the almost complete recovery of nitrogen as nitrate from soils containing ammonium salts during continuous perfusion, is doubtful.

D. Forest Soils

a. Mull and Mor General Characteristics

Many systems have been proposed for the classification of forest soils, based usually on different soil properties such as soil maturity (profile morphology), or soil forming processes. At one time, it was not uncommon for pedologists to disregard the surface humus as part of the soil. Süchting, for instance, stated emphatically that

humus was an insignificant and unimportant factor in soil analysis. In 1879 and 1884, Müller (77) divided the forest soils of beech woods and oak forests of Denmark into two general, biologically distinctive groups underlying two different types of humus, mull and mor. He also recognized certain transitional phases. Waksman (114) stated that the humus layer is the important factor in forest soil development and concluded that Müller's concept, with some modifications, fulfilled present needs in forest soil classification. Wilde (120) recognized three types of forest humus:

i. Earth mull: (a) mixture of amorphous organic matter and mineral soil (A.).
(b) pH range 5.0 - 8.0.
(c) organic matter rarely exceeding 10 percent.
(d) predominantly occurring under hardwood stands.

ii. Duff mull: (a) friable organic remains of Ao and A_1 horizons of high biological activity and abundant supply of readily available nutrients.
(b) pH range 5.0 - 6.5.
(c) occurring mostly under mixed hardwood-coniferous stands.

iii. Mor or raw humus: (a) thick organic layer comprised of tenacious horizon of Ao interwoven with mycelia of fungi overlying leached mineral soil.

(b) pH range 3.0 - 5.0.

(c) usually supporting dense stands of northern conifers.

Handley (35) utilized this classification extensively in his characterization of forest soils. Handley (35), studying factors involved in the formation of mull and mor conditions, regarded these two types as the extremes of the various biological systems occurring in forest soils in general. From a review of past literature, Handley concluded that there was insufficient evidence for assuming that any single factor was causal in the formation of mull or mor humus. Krajina (lecture notes), in his ecological studies of hemlock and Douglas-fir forests in British Columbia, recognizes two main types of humus condition, namely raw humus (mor) and duff mull. The "earth mull" condition, developed on some alluvial soils, results from frequent flooding and mixing of organic matter with subsoil. According to Krajina, raw humus conditions are most likely to develop in areas with a short vegetative period, low temperature, high precipitation, and under heavy forest cover. Raw humus accumulates over longer periods of time than duff mull and may reach a thickness of up to 1 foot.

It is composed mainly of free organic remains interwoven with fungal hyphae and is compressed into a matted Ao horizon overlaying a leached mineral soil which, only in rare instances, is infiltrated by humates. Its usual pH range is 3.5 - 5.0.

Duff mull in British Columbia coastal areas, develops either on lime-stone substrata or igneous rocks of a basic character. Its development is favored by base-saturated seepage water. It is formed where the precipitation-evaporation ratio is small. In general, it is characterized by friable organic remains intergrading into a more or less developed layer with incorporated humus. The actual humus layer is not very thick (1 to 3 inches). Underneath duff mull there is always a melanized layer. Podsolization in duff-mull soils is negligible. The pH range is from 5.0 - 7.2. Krajina concludes that in the coastal forests of British Columbia, raw humus, as it occurs in salal associations, is the climatic climax condition. Duff mull, on the other hand, is more restricted in occurrence and is an edaphic climax. He further regards raw humus as being advantageous to hemlock but less so for western red cedar or Douglas-fir. The most excellent sites for Douglas-fir are those in which duff mull conditions prevail.

b. Microbial Studies of Forest Soils

Research in the field of forest soil bacteriology, in comparison with the amount of work of similar

nature on agricultural soils, is of very meagre proportions. This state of affairs can, in part, be explained by difficulties experienced in forest soil classification, indiscriminate terminology regarding humus types, and the mistaken viewpoint that there is no pressing and no practical value in a more thorough study of forest soils. Pfeil (84), for instance, a German authority on forest management, has introduced the paradox: "The only general rule in forestry is that there are no general rules." Süchting, too, has stated emphatically: "only that forest humus is best, which is never formed." The majority of papers dealing with forest soil microbiology cannot be used now to any advantage as they contain no references to the ecological conditions surrounding the soil they seek to describe. Even today, it is a common occurrence to refer to the soil under study as "Forest soil", "Forest soil under hardwood" without any reference to humus conditions, climate, topography, history of the site, and characteristic plant associations inhabiting these soils.

Müller (77) was one of the first to distinguish between mor and mull largely on the basis of biological differences. He particularly stressed the striking differences in bacterial and animal life abundantly present in mull and absent in mor. Romell (92), from fairly extensive studies, came to the conclusion again that mull humus is definitely characterized by a more active fauna

than mor. Plice (85), in his study of forest soils, states that, though bacteria and fungi inhabit both humus forms, bacteria predominate in mull, and fungi in mor humus. Handley (35), reviewing the subject of microbial population of mull and mor, concludes that there is insufficient information to enable one to say that bacteria are restricted to mull and fungi to mor. Works of Vandecaveye (123, 124, 125), Mitrofanova (75), and Ambroz (5,6) have accumulated a fairly large amount of evidence pointing to the possibility that each soil process has its own characteristic microbial population. Mishustin (74), in his work, even goes as far as to say that there is a clear correlation between the direction of the soil forming process and the composition of the soil microflora. He states, with emphasis, that the microbial population of different soils is no less specific than the natural vegetation of higher plants. He implies, therefore, that the soil condition can be determined from a study of the soil micro-populations of different groups of microorganisms present and the number of their component species.

c. Nitrogen in Forest Soils

Most of the nitrogen in forest soils is present in organic form. Waksman (114) states that there is no correlation between quantity of nitrogen and the amount of organic matter; but a definite relation has been demonstrated between nitrogen content of humus and its reaction. Alkalinity favors humus decomposition and a consequent

reduction of the carbon/nitrogen ratio. In general, the carbon/nitrogen ratio of forest soils is wider than in agricultural soils but it becomes narrower with increasing depth (see Appendix, Table VII). The amount of humus in forest soils varies considerably and, according to Muller (quoted by Waksman, 114), true mull contains less than 10 percent, mull-like 30-60 percent and raw humus around 60 percent organic matter. This organic part of forest soil develops from plant debris that varies in nitrogen content (see Appendix, Table VIII). Handley (35) reviewing the topic of the nitrogen content of forest litter, stated that percent nitrogen in plant residues varies with species and it is difficult to relate this variation to the type of soil on which plants grow. This conclusion leads Handley to the qualitative rather than quantitive consideration of nitrogen in forest organic debris. Further on, he hypothesizes that nitrogenous material in mor is characteristically resistant to breakdown due to the presence of tannin-like materials that are responsible for the stabilization of leaf proteins. This fixation of plant protein in mor humus lowers the level of available nitrogen for microorganisms.

The inorganic forms of nitrogen are considerably less abundant in forest soils than in agricultural soils. Waksman (114) states that humus at pH 3.5 - 5.0 contains mostly ammonium as the principal form of

inorganic nitrogen. Little or no nitrates are found at a pH below 3.9. In raw humus, nitrogen transformations culminate in the production of ammonium.

As ammonium is practically independent of organic matter accumulation, nitrates are totally absent when the organic content of a soil exceeds 70 percent. For the surface eight inches of virgin forest soil, Wilde (120) puts the maximal levels of nitrates and ammonium at 25 and 70 parts per million respectively. Russell (96) states that mor soils, because of their acidity, contain no nitrates, whereas mulls have a higher concentration of both ammonium and nitrates. This particular characteristic is regarded by many workers as the most consistent and striking difference between raw-humus and duff-mull forest soils.

Fixation of atmospheric nitrogen in forest soils by both biotic and nonbiotic factors has not yet been investigated sufficiently. The presence of Azotobacter in raw humus is unlikely because of the low pH range common to such material. Optimal pH conditions for Azotobacter are in the 6.8 - 7.5 range. The lack of proteolytic enzymes and a very feeble deaminating ability will exclude Azotobacter from highly organic surface layers of any forest soils. Furthermore, the presence of ammonium in raw humus will preferentially inhibit nitrogen fixation by these organisms, as it does in cultures (43,86). The same

assumptions might apply to the anaerobic Clostridium species which are inhibited by the presence of fixed nitrogen compounds (86). The very extensive root systems in forest soils will undoubtedly create unfavorable conditions for the establishment of Azotobacter, as has been shown to be the case with other plants (48,63).

The role of free-living nitrogen-fixers other than Azotobacter and Clostridium is not known. Nitrogen fixation by Pseudomonas-like soil bacteria (7), several strains of Azotobacter aerogenus (34), many photosynthetic bacteria, purple and green sulphur bacteria, and autotrophic anaerobic sulphate-reducing bacteria (66), point only to the possibility that nitrogen fixation, in general, might be a very common and widespread phenomenon. Fixation of atmospheric nitrogen by fungi, algae, and symbiosis of non-leguminous plants with their respective microorganisms (79,115) substantiates the previous statement and makes us realize that this might be a missing link in the nitrogen cycle of forest soils.

III. EXPERIMENTAL PART

A. Characterisation of Habitats Under Investigation

a. Ecological Analysis of Sites

Soils used for the experimental part of this work were collected from two very well defined forest associations:

1. *Pseudotsuga menziesii* - *Thuja plicata* - *Polystichum munitum* association with characteristic duff mull conditions of this forest soil.
2. *Pseudotsuga menziesii* - *Tsuga heterophylla* - *Gaultheria shallon* association with characteristic raw humus conditions of this forest soil.

   Both sites are located on the University of British Columbia Endowment Lands.

   Plot size used for floristic analysis of the two associations was 1/5 of an acre.

   The relative abundance and dominance of the plant species present was evaluated.

   (Ecological analysis of both sites was done with the help and under the supervision of Dr. V.J. Krajina.)

TABLE OF SCALES ADAPTED FOR ECOLOGICAL DESCRIPTION OF SITES

I. Wind exposure scale:

strong exposure to wind:	!!
moderately strong exposure:	!
intermediate exposure:	+ +
slight exposure:	+
well sheltered from wind:	0

II. Scale for the estimation of total abundance and dominance:

+	quite solitary, dominance very small
1	seldom
2	very scattered, dominance small
3	scattered
4	often dominating 1/20 - 1/10 of area
5	often dominating 1/10 - 1/5 of area
6	dominating 1/5 - 1/3 of area
7	- " - 1/3 - 1/2 - " -
8	- " - 1/2 - 3/4 - " -
9	- " - over 3/4 - " -
10	- " - 100%.

III. Vigor is expressed by figures, attached as exponents to the figures expressing the total estimate of abundance and dominance. The following is the scale used for vigor:

3	most vigorous growth
2	growth moderately vigorous
1	poor vigor, but the plant may reach a considerable age
0	vigor non, plant may vegetate only for a short time

ECOLOGICAL DESCRIPTION OF STUDIED FOREST ASSOCIATIONS

I. *Pseudotsuga menziesii* - *Thuja plicata* - *Polystichum munitum* association

General ecological characterization:

1. Date: September 18, 1956.
2. Place: U.B.C. Endowment Lands, SW of the proposed site for the Forest Product Laboratory.
3. Altitude: 260 feet above sea level.
4. Exposure: south, in the center of a col.
5. Sloping: 20 - 30 degrees.
6. Wind exposure: at the ground: + (in the proximity of the sea level).
   at the top of the tree layer: !
7. Snow cover period: usually 2, weeks, occasionally 5 - 6 weeks.
8. Depth of fine weathered soil: over 1 meter.
9. Soil: gley of alluvial material.
10. Plot size: 1/5 acre.
11. Total cover of vegetational layers:
    - A: 20-25% (dominant & codominant trees)
    - A: 75-80% (intermediate & suppressed trees)
    - B: 60% (tall shrubs & small trees, 2-20 meters).
    - B: 25% (shrubs 20-200 cm.)
    - C: 65-70% (herbs)
    - D: 2% (mosses)
12. List of plants:
    - A: *Pseudotsuga menziesii* 6^{2-3}
      Thuja plicata 1^{2}
      Abies grandis $+^{2}$

A: Acer macrophyllum 7 $^{2(-3)}$

Thuja plicata 3 ^{2}

Alnus rubra 2 $^{2-0}$

Abies grandis + ^{2}

B: Sambucus pubens 7 $^{2(-3)}$

Rubus spectabilis 3-4 ^{2}

Acer macrophyllum 3 $^{1-0}$

Tsuga heterophylla (on decaying wood) 1-2 ^{1}

Thuja plicata 1-2 $^{1-2}$

Abies grandis + $^{1(-0)}$

B: Sambucus pubens 4 $^{2(-3)}$

Rubus parviflorus 4 $^{2(-0)}$

Rubus spectabilis 4 ^{2}

Ribes lacustre + ^{2}

Symphoricarpos rivularis + ^{2}

Ilex aquifolium + ^{1}

C: Polystichum munitum 7-8 $^{2-3}$

Tiarella trifoliata 4-5 $^{2-3}$

Rubus vitifolius 3-4 $^{2-0}$

Dryopteris austriaca 3 ^{2}

Bromus vulgaris 3 $^{2-3}$

Sambucus pubens 2-3 $^{1-2}$

Rubus parviflorus 2-3 $^{2-0}$

Rubus spectabilis 2 ^{2}

Claytonia sibirica 2 $^{2(-3)}$

Tellima grandiflora 2 $^{2-0}$

Athyrium filix-femina 1-2 $^{2-1}$

Galium triflorum 1 + 2-1

Trientalis latifolia + 1-2

Equisetum telmateia + 2

Geum macrophyllum + 1-2

Carex leptopoda + 2-1

Acer macrophyllum + 1-0

Dicentra formosa + 1-2

Cinna latifolia + 1-2

Vicia americana + 1-2

Sorbus sitchensis + 0

Polypodium vulgare var. occidentale (on decaying wood & bark) + 2

Adenocaulon bicolor + 2-3

Ranunculus bongardii + 2-3

Osmorhiza chilensis + 2-3

Stellaria crispa + 2

C: Eurhynchium stokesii 2 2-3

Mnium insigne 1-2 2

Brachythecium asperrimum + 1-2

II. *Pseudotsuga menziesii* - *Tsuga heterophylla* - *Caultheria shallon* association

General ecological characterization:

1. Date: September 18, 1956.
2. Place: U.B.C. Endowment Lands, Secondary forest on the plateau south of 16th Ave.
3. Altitude: 300 feet above sea level.
4. Exposure: N 75 E.
5. Sloping: 0-5 degrees.
6. Wind exposure: at the ground, 0
   at the tops of the tree layer, ++ .
7. Snow cover period: 3 (-4) weeks; occasionally 6-7 weeks.
8. Depth of the fine soil: over 1 meter.
9. Podzol (A_2 Layer rather thin, $\frac{1}{2}$-3 cm. thick).
10. Plot size: 1/5 acre.
11. Total cover of vegetational layers:
    - A 95-100% (dominant & codominant trees)
    - A (intermediate & suppressed trees)
    - B. 10% (tall shrubs & small trees, 2-20 meters).
    - B. 85-90% (shrubs 20-200 cm.)
    - C. 10 - 15% (herbs)
    - D. 10% (mosses)
12. List of plants:
    - A. *Pseudotsuga menziesii* 3^{1-2}
      Tsuga heterophylla 9^{2}

Thuja plicata 1^{1}

Cornus nuttallii $+^{2-1}$

B. Tsuga heterophylla 2^{1}

Thuja plicata $2\text{-}3^{1(-0)}$

Cornus nuttallii $+^{1}$

Sorbus sitchensis $+^{1}$

Malus diversifolia $+^{1-10}$

Prunus emarginate $+^{1}$

B. Tsuga heterophylla $4\text{-}5^{1-2}$

Gaultheria shallon $9\text{-}10^{2}$

Vaccinium parvifolium $5^{2(-3)}$

Thuja plicata $1\text{-}2^{1}$

Mahonia nervosa $+^{2}$

Rosa gymnocarpa $+^{2-3}$

Vaccinium ovalifolium $+^{2-1}$

Abies grandis $+^{1-0}$

C. Mahonia nervosa $1\text{-}2^{2}$

Pteridium aquifolium 3^{2}

Rubus vitifolius 3^{1-2}

Tsuga heterophylla 3^{1}

Vaccinium parvifolium $2\text{-}3^{1-2}$

Gaultheria shallon $^{2-3}$

Thuja plicata $+^{1}$

Moneses uniflora $+^{1-2}$

Acer circinatum $+^{0}$

Blechnum spicant $+^{1}$

Dryopteris austriaca +[1]

Polystichum munitum +[1]

D. Plagiothecium undulatum 4[2]

Lepidozia reptans (on decaying wood) 1[2]

Dicranum scoparium 1[2]

Scapania bolanderi (on decaying wood) +[2]

Hypnum circinale +[2]

b. ANALYSIS OF SOME PLANTS FOR THE ABILITY TO ACCUMULATE INORGANIC NITROGEN

1. Determination of the Presence of Nitrates in Plant Tissue by Diphenylamine Reagent

(1) Experimental part

Reagents:

(1) Diphenylamine Reagent for Nitrates. Dissolve 0.7 grams of diphenylamine in a mixture of 60 cc. of concentrated H_2SO_4 and 28.8 cc. of distilled (nitrate free) water. After cooling the mixture, 11.3 cc. of concentrated HCL is added and the reagent is left overnight.

(2) Trommsdorf's Reagent for Nitrites. Add slowly a boiling solution of 20 grams of zinc chloride in 100 cc. of distilled water to a mixture of 4 grams of starch in water, and continue to heat until the solution is nearly clear.

Then 2 grams of zinc iodide are added and the mixture is diluted with distilled water to the final volume of 1 liter. After filtering, the reagent should be stored in a well-stoppered, dark bottle.

(3) N-Phenylanthranilic Acid Reagent. Prepare 0-1% solution of N-Phenylanthranilic acid in hot methanol in order to insure complete dissolution. In testing for nitrites and nitrates, concentrated H_2SO_4 should be used. Positive test is indicated by pink-violet coloration. Substitution of concentrated HCl for concentrated H_2SO_4 gives a positive test for nitrites in presence or absence of nitrates.

Procedure:

Analyses were carried out on a fairly large number of plants taken from dull mull and raw humus habitats. Parts of a leaf or stem of the plant to be tested were slightly crushed or broken and then placed into the porcelain spot plate. Several drops of the diphenylamine reagent plus a fixed number of drops of concentrated sulphuric acid was used. A blue colour was taken as indicative of the presence of nitrates. To ascertain that a positive reaction was not due to the possible presence of nitrites, the Trommsdorf's reagent was used, following the same technique.

Realizing that a diphenylamine reagent will give a coloured reaction with a variety of compounds, the same test was repeated using N-phenylanthranilic acid (Chem. Abstracts 15630 f., 1955).

(ii) Results:

Scale used:

- NO_3^- absent	0
- NO_3^- trace	1
+ NO_3^- in slight conc.	2
- NO_3^- in medium conc.	3
- NO_3^- in large conc.	4-5

(Exactly the same scale is used for nitrites concentrations.)

N-PHENYLANTHRANILIC ACID REAGENT TEST FOR NITRATES IN LEAVES AND STEMS OF PLANTS

Results of the test for some plants taken from both duff mull and raw humus sites

Plants tested	Site	N-Phenanthranilic test for NO_2^-	N-Phenanthranilic test for NO_3^-	Diphenylamine test for NO_3^-
Alnus rubra	duff mull	0	0	0
Polystichum munitum	raw humus	0	0	0
Mnium insigne	duff mull	0	0	0
Sambucus pubens (leaves)	"	0	2	4
Rubus spectabilis	"	0	2	3
Tiarella trifoliata	"	0	2	3
Galium triflorum	"	0	1	2
Rubus parviflorus (leaves)	"	0	2	4
Gaultheria shallon	Raw humus	0	0	0
Pteridium aquilinum	"	0	0	0

DIPHENYLAMINE SPOT TEST FOR THE PRESENCE OF NITRATES IN LEAVES AND STEMS OF PLANTS

Scale used:

$-NO_3^-$	absent	0
$-NO_3^-$	trace	1
$-NO_3^-$	in slight conc.	2
$-NO_3^-$	in medium "	3
$-NO_3^-$	in large "	4-5

1. Results of the Test for Some Plants Collected from the Duff Mull Site

Plant	Test result
Alnus rubra	0
Acer macrophyllum	0
Pseudotsuga menziesii	0
Abies grandis	0
Thuja plicata	0
Tsuga heterophylla	0
Polystichum munitum	0
Dryopteris austriaca	0
Symphoricarpos rivularis	0
Ilex aquifolium	0
Equisetum telmateia	0
Ranunculus bongardii	0
Vicia americana	0

Mnium insigne	0
Eurhynchium stokesii	0
Brachythecium asperulum	0
Sambucus pubens (seedling)	5
" " (leaves adult plant)	5
" " (stem young plant)	1-2
Claytonia sibirica	2-4
Geum macrophyllum	3
Tiarella trifoliata	4-5
Rubus spectabilis	3-4
Dicentra formosa	4
Cinna latifolia	4
Carex leptopoda	4-5
Ribes lacustre	2-3
Bromus vulgaris	4
Athyrium filix-femina	2-3
Tellima grandiflora	4-5
Galium triflorum	3-4
Rubus parviflorus (leaves)	4-5
" " (stem)	1-2
Rubus vitifolius	3-4
Adenocaulon bicolor	4-5
Osmorhiza chilensis	4-5
Stellaria crispa	3

2. Results of the Test for Some Plants Collected from the Raw Humus Site

Pseudotsuga menziesii	0
Thuja plicata	0
Tsuga heterophylla	0
Abies grandis	0
Cornus nuttallii	0
Sorbus sitchensis	0
Malus diversifolia	0
Prunus emarginatus	0
Vaccinium parvifolium	0
Vaccinium ovalifolium	0
Gaultheria shallon	0
Rosa gymnocarpa	0
" " (fruits)	0
Pteridium aquilinum	0
Polystichum munitum	0
Rubus vitifolius	0
Moneses uniflora	0
Lepidozia reptans	0
Dryopteris austriaca	0
Dicranum scoparium	0
Blechum spicant	0
Plagiothecium undulatum	0

(iii) Discussion

Accepting the tests as reliable, the following conclusions can be drawn:

1. Some plants are able to accumulate a certain amount of inorganic nitrogen in the form of nitrates, mostly in the leaves.
2. This accumulation occurs only (as far as it was possible to detect) in plants growing on duff mull soils.
3. It appears that only certain plants are able to accumulate nitrates in their tissue.

It is interesting to note that the quantity of nitrates, detected in plant tissue by the methods described, seems to vary considerably during the year. This was ascertained from a prolonged series of tests conducted throughout the year by the author and Dr. V.J. Krajina.

In view of the fact that nitrates were detected from the distilled water leachates of duff mull and "A" soils (see experiments on nitrification), one can detect, therefore, a certain relationship between the presence or absence of soil nitrification and the ability of some plants to accumulate nitrates in their tissues.

c. DETERMINATION OF ORGANIC MATTER AND TOTAL NIGROGEN OF SOILS

1. Determination of Total Organic Matter

(i) Experimental part (modification of Walkley-Black method)

References: "Methods of Soil Analysis for Soil-fertility Investigations" by Peech, Micheal, L.T. Alexander, L.A. Dean, and J. Fielding Reed. U.S.D.A. Circular No. 757, 1952.

Reagents:

(1) N- Potassium dichromate.
Dissolve 98.06 gm. of $K_2Cr_2O_7$ in water and dilute to 2 liters with water.

(2) Sulfuric acid, con. (not less than 96%).

(3) 0.5N - Ferrous sulfate.
Dissolve 278 gm. $FeSO_4 \cdot 7H_2O$ in water, add 80 ml. conc. H_2SO_4, cool, and dilute to 2 liters.

(4) Ortho - Phenanthroline Ferrous Sulphate indicator.

Procedure:

Dried soils were sieved through a 20-mesh screen. Three 50 mgm. portions were used for highly organic soils such as raw humus and duff mull, and three 100 mgm. portions for subsoils and garden soil. Soil samples were placed in 250 ml. Erlenmeyer flasks and 5 ml. of N dichromate and 10 ml. of concentrated sulfuric acid were added. Flasks were shaken for about 15 seconds and then left standing for 30 minutes. Then 30 ml. of water and 2 drops of (Ortho)-Phenanthroline Ferrous Sulphate indicator were added.

Titration with standard Ferrous Sulphate was continued until there was a change of colour from green to brownish gray. As the potassium dichromate is a reasonably stable solution, it was taken as the standard. The ferrous sulfate is subject to oxidation, hence, it was standardized against the dichromate each time the solutions were used. Standardization was as follows:

5 ml. of the standard dichromate solution in a 250 ml. flask was mixed with 10 ml. of concentrated sulfuric acid. Then 30 ml. of water and 2 drops of the (Ortho)-Phenanthroline Ferrous Sulphate indicator were added. Titration with standard ferrous sulphate was continued until the brownish gray endpoint was reached. Assuming the dichromate to be correct, the normality factor for the ferrous sulphate was calculated.

Calculations:

The mean recovery of carbon by this method has been found to be 75%, hence, the correction factor, $\frac{100}{75} = 1.33$, should be applied.

% organic matter in soil sample =

$$\frac{(\text{ml. N dichromate reduced}) \times 0.003 \times 1.33 \times 1.724 \times 100}{(\text{Wt. of sample (gms.)})} =$$

$$\frac{(\text{ml. N dichromate reduced}) \times 0.69}{(\text{Wt. of sample in grams})}$$

Notes:

Chlorides interfere by reducing the dichromate; carbonates up to at least 50% of the soil do not interfere; MnO_2 does not interfere.

(ii) Results of the Organic Matter Estimation in Soils

(Soil Samples Collected from Duff Mull and Raw Humus Sites of University Area Forest, Vancouver, B.C.)

Soils	Amount of soil used for test	% organic matter for Test II soil (average)	% organic matter for Test III soil (average)	Mean values %
Garden	50 mg.	18.90	17.25	18.07
Duff humus	50 mg.	23.70	18.35	21.02
A_1	50 mg.	11.10	11.34	11.22
Raw humus	50 mg.	67.00	62.37	64.68
B	100 mg.	4.07	4.62	4.34

(All estimates were made on dry weight bases)

Note: Test II and test III soils were used for nitrification experiment; see page 65.

2. DETERMINATION OF TOTAL NITROGEN

(i) Experimental part:

Reference: (27)

Reagents:

(1) Digestion mixture:

16 gm. sodium selenate

503.0 gm. Na_2SO_4 . 10 H_2O

17.4 gm. Cu SO_4 . H_2O

667 ml. conc. H_2 SO_4

(made up to two liters with distilled water)

(2) Indicator:

33 mg. Brom-cresol green

66 mg. Methyl red

100 ml. 95% ethyl alcohol

(3) Absorption solution:

Saturated (4%) boric acid solution

(4) Sodium hydroxide (1 lb. of Na OH per liter of water)

(5) Standard hydrochloric acid (0.1N).

Procedure:

With humus samples and with subsoils, 2 grams and 10 grams respectively, of oven dried samples were used. Sixty ml. of the digestion mixture in an 800 ml. Kjeldahl flask, plus a given weight of soil sample, was heated gradually for 3 hours. After cooling, the mixture was diluted with 100 ml. of distilled water and 100 ml. of

Na OH. (Before NaOH is added several pieces of mozzy zinc had been placed into the flask.) After mixing the contents, the liquid in the flasks was distilled over the saturated boric solution, to which 1 ml. of the indicator solution had been added. The ammonia collected was titrated with 0.1 N HCL acid. From the fact that the alkaline solution of this mixture is green and the acid red, the end point is easily observed.

Calculation:

$$\text{Percentage nitrogen} + \frac{(T-B)\ N \times 1.4}{W}$$

T = volume of acid used with soil

B = " " " " " blank

N = normality of acid

W = weight of dry soil used

(ii) Results of the total nitrogen estimations

1. Values for soils collected from duff mull and raw humus sites. (University area, Vancouver.)

Soils	% -N test II soils (average)	% - N test III soils (average)	Mean values
Garden soil	0.604	0.687	0.645
Duff mull	0.567	0.598	0.583
A_1	0.292	0.281	0.286
Raw humus	1.211	1.165	1.188
B	0.078	0.091	0.084

Note: Regarding test II and test III soils, see page 65.

2. Value for soils collected from University Forest in Haney, B.C.

(Given as additional, comparative values only)

Soils	% - N (average)
Raw humus	1.165
Hardwood mull	1.018
Black muck	1.425
Lake peat	2.418

Table of Carbon/Nitrogen ratio for soil samples from University Area, Vancouver

Soils	Percent carbon soils test II	Percent carbon soils test III	C/N ratio soil test II	C/N ratio soil test III	Average C/N ratio
Garden soil	11.0	10.0	18.2	14.5	16.3
Duff-humus	13.7	10.6	24.2	17.7	20.9
A_1	6.4	6.6	21.4	23.5	22.4
Raw humus	38.9	36.2	32.2	31.1	31.6
B	2.3	2.7	29.5	29.7	29.6

(Percent carbon was estimated on the assumption that soil organic matter contains 58% of carbon.) Ref.: (21).

Note: Regarding test II and test III soils, see page 65.

(iii) Discussion

Soil collected for these determinations were from the following depths:

a. duff mull - usual depth from 0.5 to 1.0 inch.

b. A_1 - from 0.5 up to 7.0 inches.

c. raw humus - up to 30 inches.

d. B - up to 11.0 inches.

Before taking small samples for both total organic or total nitrogen determinations, soils were thoroughly mixed to insure the uniformity of samples. The calculated carbon/nitrogen ratios point to the fact that there is considerable variation between raw humus and duff mull soils in this regard. It would be difficult to predict the fertility of the soils from these two sites, taking into consideration only total nitrogen or total organic matter present.

Destruction of the humus layer, by fire or some other agency, in raw humus sites has a more detrimental effect on growth than similar destruction in soils overlain by duff mull. The former soil type has a B horizon characteristically low in nitrogen.

The distribution of total nitrogen in duff mull soils is more uniform. Considerably lower carbon/nitrogen ratios are indicative of a more advanced type of humus degradation and likewise a more intensive type of microbial action.

d. pH MEASUREMENTS OF SOILS

(i) Experimental part

The pH of soil samples was determined immediately after their collection using approximately 25 cc. of each soil in a 50 ml. beaker, and adding enough distilled water to each to produce a thick paste. The beaker and its contents were allowed to stand for 30 minutes, prior to any measurement, to permit the soil and water to come to equilibrium.

A portable Beckman Model N pH-meter was used.

(ii) Results:

Soils	18th of February	11th of May	26th of May	15th of June	26th of June	12th of July	Average values
Garden soil	6.10	7.10	7.20	7.35	6.95	7.22	6.98
Duff mull	4.25	5.63	4.45	4.26	4.40	5.30	4.71
A_1	6.25	6.05	6.43	5.85	6.43	5.43	6.07
Raw humus	3.60	4.20	3.70	3.50	3.65	4.34	3.83
B	5.25	4.88	5.08	4.60	5.08	4.45	4.89
Dist. water	5.80	5.85	-	5.68	5.86	5.72	-

(The values quoted are the average values of two sample measurements per soil per horizon.)

e. SOIL MOISTURE

(i) Procedure:

Soils to be analysed were collected in sealed glass jars and weighed to the nearest milligram; then dried in an oven at 105°C. to a constant weight, for nearly 48 hours. The dried samples were re-weighed and the percentage of moisture was calculated as follows:

$$\% \text{ moisture} = \frac{(\text{wt. lost}) \times (100)}{\text{Dry weight.}}$$

(ii) Results:

Percent Moisture Values

(Soils collected from University Area, Vancouver, B.C.)

Soil	3rd of Dec.	15th of Jan.	18th of Mar.	11th of May	15th of June	12th of July	Average values
Garden	40.32	41.61	42.52	48.20	38.50	35.80	41.16
Duff mull	39.15	41.30	45.60	38.30	40.72	39.41	40.75
A_1	40.20	40.61	40.31	38.30	32.10	39.41	38.49
Raw humus	71.31	68.05	69.50	61.20	66.52	61.28	66.31
B	14.24	11.40	12.33	13.72	9.36	8.95	11.66

(All figures are the average values of two measurements per sample of soil.)

(iii) Discussion:

The highest amount of total moisture was found in raw humus. This is evidence of the fact that organic matter is capable of retaining a considerable amount of water, and that soils rich in humus are likely to be less subject to drought than humus deficient soils. In the case of a duff-mull soil, moisture is retained by both the organic matter and the not inconsiderable colloidal fraction of the underlying mineral material.

In order to be able to obtain a more exact understanding of soil moisture and soil-water relationships in forest soils from different sites, five principal forms of soil water should be studied, namely:- gravitational, capillary, hygroscopic, water vapor and ground water. Determination of percent moisture, as given in this work, serves only as an additional, and very general factor in the characterization of the soils studied.

F. Quantitative Studies of Soil Microorganisms in Forest Soils

(i) Experimental Part

The quantitative estimation of aerobic populations in duff mull and raw humus soils was made according to the method of A.G. Lochhead and R.H. Thexton (1951),

with some modifications. It was found that the soil extract agar, as described by Lochhead, gave smaller bacterial counts than soil infusion agar.

The soil infusion agar plates were prepared as follows:

250 grams of soil was left in 250 ml. of distilled water for 24 hours at 37C.; then, before filtration, the temperature was raised to just below boiling point, and the infusion was filtered while hot; the "Case" nutrient agar was dissolved in the above soil extract without any pH adjustments being made. As a consequence, a separate infusion agar medium was used with the soil samples. There was no attempt made to control any possible occurrence of "spreaders," but relatively few plates were discarded on this account. In order to group the microorganisms according to their morphology, 50 colonies were selected at random from acceptable plates and a microscopic examination made of the gram-stained preparations. (Table B.)

(ii) Results

See Table A and Table B.

TABLE A.

Results of Plate Counts of Microorganisms by Soil Horizons in Forest Soils

(Soils collected from the University Area Forest, Vancouver, B.C.)

(Numbers given are in hundred thousands per gram, oven-dry soil)

Soils	pH	% moisture	Bacteria	Actinomyc. and fungi
Garden soil 0-15cm.	7.20	41.20	190.40	31.80
Duff mull 0-10cm.	4.45	39.10	121.83	41.21
A 10-25cm.	6.43	38.70	126.33	38.20
Raw humus 0-15cm.	3.70	63.40	13.81	97.43 mostly fungi
B 15-25 cm.	5.08	14.10	7.26	40.38 mostly fungi

TABLE B.

Morphological Groups Present in Each Soil Horizon

(Soils collected from the University Area Forest, Vancouver, B. C.)

	Gram positive cocci forms	Gram positive spore-forming bacilli	Gram negative Bacilli	Spore formers	Pleomorphic organisms	Others: Actino and Fungi
Garden soil	18	31	1	23	10	17
Duff mull	6	38	4	32	10	10
A_1 horizon	4	32	3	37	14	10
Raw humus	-	5	2	32	40	21
B horizon	1	4	-	12	52	31

(Figures given are percent values calculated from each plate by picking up 50 colonies at random.)

(iii) Discussion

From the above results, it is obvious there is a relatively rich population of bacteria in garden soil, duff mull, and A_1-horizon soils. Conversely, few bacteria inhabit raw humus and its immediate subsoil.

The fungal population, on the other hand, seems to be at a considerably higher level in raw humus.

Plating methods, as a means of obtaining a quantitative representation of soil microflora, are admittedly of dubious worth. Nevertheless, the above conclusions are in accord with those of others (77,85).

B. PRELIMINARY STUDIES OF NITRIFICATION BY PERFUSION TECHNIQUES

(i) Experimental Part

Preliminary studies were carried out on duff mull and raw humus (mor) soils of the Douglas-fir forest comparing their relative nitrifying and denitrifying capacity. For this purpose, perfusion apparatuses were set up according to the technique described by I.J. Audus (8) with certain modifications that improved considerably the application of this apparatus for soil studies. The change made was the use of a "by-pass tube" that obviated any possibility of overflowing or plugging,

though still insuring adequate soil aeration (see Appendix, Fig. I and Fig. II). This apparatus was in continuous operation for more than thirty days without any technical difficulties.

The soils were collected from two different localities in sterile glass jars. They were not dried or sieved, as was suggested by H. Lees and J.H. Quastell (54), but rather used in their natural state. Each soil sample was leached completely with a sufficient amount of distilled water to insure the removal of any detectable traces of ammonium, nitrite or nitrate; then the leachates were replaced by 200 cc. of N/50 $(NH_4)_2SO_4$. The perfusion with the above solution was continued for 24 days with a regular daily check for the presence of ammonium, nitrates and nitrites. Adjustments for evaporation were made one hour before removing 0.5 cc. of leachate for analysis.

The detection and approximate quantitive estimations of ammonium, nitrites, and nitrates were made as follows:

a. For determination of ammonium: one drop of Nessler's solution and one drop of the solution to be tested were used in a spot plate.

b. For determination of nitrites: Trommsdorf's reagent was used.

c. For determination of nitrates: one drop of diphenylamine reagent was added to one drop of leachate and two drops of concentrated sulphuric acid.

All reagents used and the method followed were according to E.B. Fred and S.A. Waksman (30). An additional test was run on soil samples of raw humus and its subsoil treated with 0.5 percent calcium hydroxide ($Ca(OH)_2$) to raise their pH. (from 3.85 to 6.85 in the case of raw humus and from 4.20 to 7.05 in the case of the "B" horizon. The intention was to determine whether any relationship exists between the low pH values characteristic of such media and the absence of nitrification as a process in them.

RESULTS OF AMMONIUM OXIDATION

(Soil samples collected from University Area Forest, Vancouver, B.C.)

No. of days	Duff mull			A_1			Raw humus			B			Garden soil		
	NH_4^+	NO_2^-	NO_3^-	NH_4^+	NO_2^-	NO_3^-	NH_4^+	NO_2^-	NO_3^-	NH_4^+	NO_2^-	NO_3^-	NH_4^+	NO_2^-	NO_3^-
1	4x	-	-	4x	-	x	4x	-	-	4x	-	-	4x	-	x
3	3x	-	x	3x	-	1x	4x	-	-	4x	-	-	3x	x	2x
5	3x	-	1x	2x	-	2x	4x	-	-	4x	-	-	1x	-	3x
7	2z	-	2x	2x	x	3x	4x	-	-	4x	-	-	x	-	4x
9	2x	-	2x	1x	x	3x	4x	-	-	4x	-	-	x	x	4x
11	1x	-	2x	x	-	3x	4x	-	-	4x	-	-	-	x	4x
13	x	-	3x	-	-	3x	4x	-	-	4x	-	-	-	x	4x
15	x	-	3x	-	-	3x	4x	-	-	4x	-	-	-	-	4x
17	x	-	3x	-	x	3x	4x	-	-	4x	-	-	-	-	4x
19	-	-	3x	-	-	3x	4x	-	-	4x	-	-	-	-	4x
21	-	-	3x	-	-	3x	4x	-	-	4x	-	-	-	x	4x
23	-	-	3x	-	-	3x	4x	-	-	4x	-	-	-	-	4x

Scale used:

4x - maximum colour intensity of positive reaction.

3x)
2x) - gradually decreasing intensity of colour.
1x)

x - slightly detectable colour.

x - uncertain.

RESULTS OF AMMONIUM OXIDATION

(Soil samples collected from Nanaimo River Valley, Vancouver Island, B.C.)

No. of days	Duff-mull			A_1			Raw- humus			B			Garden soil		
	NH_4^+	NO_2^-	NO_3^-	NH_4^+	NO_2^-	NO_3^-	NH_4^+	NO_2^-	NO_3^-	NH_4^+	NO_2^-	NO_3^-	NH_4^+	NO_2^-	NO_3^-
1	4x	-	-	4x	-	-	4x	-	-	4x	-	-	4x	-	-
3	4x	-	x	4x	-	x	4x	-	-	4x	-	-	4x	x	x
5	4x	-	x	4x	-	x	4x	-	-	4x	-	-	3x	x	1x
7	3x	-	1x	4x	-	x	4x	-	-	4x	-	-	2x	x	2x
9	3x	-	1x	3x	-	1x	4x	x	-	4x	-	-	2x	X	2x
11	3x	-	1x	3x	-	2x	4x	-	-	4x	-	-	2x	-	3x
13	3x	-	2x	2x	x	3x	4x	-	-	4x	-	-	2x	x	3x
15	2x	-	2x	2x	-	3x	4x	x	-	4x	x	-	1x	x	3x
17	2x	-	2x	2x	-	3x	4x	-	-	4x	-	-	1x	x	4x
19	2x	-	2x	1x	x	3x	4x	x	-	4x	x	-	x	x	4x
21	1x	-	2x	1x	-	3x	4x	-	-	4x	-	-	x	x	4x
23	1x	-	3x	x	x	3x	4x	-	-	4x	-	-	x	x	4x
39	-	-	2x	-	-	2x	4x	-	-	4x	-	-	-	-	4x

To simplify the data, readings, originally taken daily, are given above for every second day.

Scale used: 4x - maximum colour intensity of positive reaction.

3x)
2x) - gradually decreasing intensity of colour.
1x)

x - slightly detectable presence.

x - uncertain.

RESULTS OF AMMONIUM OXIDATION FOR DEACIDIFIED SOILS

(Soil samples collected from University Area Forest, Vancouver, B.C.)

No. of days	Raw humus pH	NH_4^+	NO_2^-	NO_3^-	B pH	NH_4^+	NO_2^-	NO_3^-	Garden soil pH	NH_4^+	NO_2^-	NO_3^-
1	6.85	4x	-	-	7.05	4x	-	-	6.90	3x	-	x
2	4.20	4x	-	-	6.80	4x	-	-	6.75	2x	-	2x
3	4.35	4x	-	-	7.15	4x	-	-	7.05	x	-	3x
4	4.65	4x	-	-	7.05	4x	-	-	7.00	x	-	3x
5	5.05	4x	-	-	7.00	4x	-	-	7.00	-	x	3x
6	5.85	4x	-	-	6.95	4x	-	-	7.05	-	-	2x
7	5.70	4x	-	-	7.00	4x	-	-	7.10	-	-	3x
8	5.60	4x	-	-	7.00	4x	-	-	7.00	-	x	4x
9	5.60	4x	-	-	7.00	4x	-	-	6.95	-	-	4x
10	5.65	4x	-	-	-	4x	-	-	6.90	-	-	4x

(iii) Discussion

It is appreciated that the above analyses were only rough, qualitative estimates. To obtain more reliable data, quantitative microchemical analysis should be used. Even with this reservation, it appears evident from the present data that the nitrifying capacity of the duff mull and A_1 (melanized) horizons tested is considerable and is comparable with that of the garden soil. No ammonium oxidation was observed in any instances with raw humus or its B (subsoil) horizon, even if the usual acidity of these soils was artificially corrected.

It is of further interest to note that raw humus was unable to build up any ability to oxidise ammonium even when left for a period of more than forty days. There is a strong likelihood that nitrifying bacteria are totally absent from raw humus.

An analysis of the leachates collected directly from the fresh soils showed a complete absence of any detectable traces of nitrite and nitrate in raw humus and its B horizon. The duff mull, A_1-horizon and garden soil leachates had a fair amount of nitrates, the highest concentration being in the garden soil, the lowest in the A_1 horizon of forest soil. The leachates from the raw humus, however, gave a positive test for ammonium.

C. NITRIFICATION IN FOREST SOILS AS INVESTIGATED BY THE PERFUSION TECHNIQUE

(1) Experimental part:

Soils used for these experiments were collected in sterile glass jars and transferred immediately to the laboratory. Samples of soils for the various tests were collected as follows:

Soils for test I April 10th.
" " " II June 15th.
" " " III July 12th.
" " " IV January 24.

Small amounts of the soil samples were weighed for moisture and pH determinations.

Remaining portions of soils were air-dried for 24 hours, then mixed thoroughly and passed through an 8-mesh sieve. 35 gram samples were used for garden and B-horizon soils and 25 gram samples were used for duff mull, A_1 and raw humus soils.

140 cc. of N/50 $(NH_4)_2 SO_4$ was added to garden and B-horizon soils, and 100 cc. of N/50 $(NH_4)_2 SO_4$ to the other samples in order to give approximately the same concentration of $(NH_4)_2 SO_4$ per each gram of soil.

Before perfusion was carried out each apparatus containing soil samples and perfusate was weighed exactly to within 0.1 gram. (See Appendix.)

A continual temperature check was maintained throughout experiment.

Every day, one hour before taking the samples for determination of ammonium and nitrates, each of the sets was adjusted for evaporation with sterile distilled water.

1. Determination of Nitrates

Reagents:

(1) Sodium hydroxide, 1 N solution

(2) Phenoldisulphonic acid

150 ml. of concentrated H_2SO_4 in flask to which 25 gm. of pure white phenol and 75 ml. of fuming H_2SO_4 is added. Mixture is heated at 100°C for 2 hours, cooled and stored.

(3) Standard nitrate solution containing 10 g NO_3^--Nitrogen/ml.

(4) I 0 N NaOH - EDTA solution

Dissolve 15 gm. of disodium EDTA in 800 ml. of water. Add 400 gm. of NaOH, cool and make to 1 liter.

Procedure:

1 cc. of leachate was pipetted into a 50 ml. beaker to which 1 ml. of I N NaOH was added, the contents were evaporated to dryness at low temperature and then 2 ml. of phenoldisulphonic reagent was introduced in such a way as

to cover all the residue. Then 20 ml. of H_2O and 10 ml. of NaOH - EDTA solution was added and the mixture cooled.

Colour was read on an AC Model Fisher Electrophotometer at 425 millimicrons (filter 425-B).

2. Determination of Ammonium

Reagents:

(1) Sodium tartrate-10%

(2) Nessler's reagent: 45.5 g. of mercuric iodide. 35.0 g. of potassium iodide are dissolved in a 1000 ml. flask in as little water as possible. Then 112 g. of KOH is added, mixed, cooled and diluted to volume. Reagent should be allowed to stand a few days before using.

(3) Gum Acacia solution
10 g. of gum acacia is dissolved in 195 ml. of distilled H_2O and 3 ml. of Nessler's solution is added.

(4) Standard solution of ammonium having 10 g. NH_4 - nitrogen/ml.

Procedure:

1 cc. of leachate was pipetted into a test tube into which the following amounts of different solutions were added:

20 cc. of distilled H_2O

1 cc. of Sodium tartrate

1.5 cc. of Nessler's reagent

0.5 cc. of gum acacia

giving a total volume of 24.00 cc. . After allowing 15 minutes for full colour development, colour was read on AC Model Fisher Electrophotometer at 425 millimicrons (filter 425-B).

(iii) Results:

All values plotted on the attached graphs are amounts of ammonium and nitrate nitrogen in g/ml. of leachage. Graphed values were obtained from standard curves for ammonium and nitrates of known concentrations. All plotted values are mean values of two simultaneous runs and four estimates (two estimates per soil sample).

(ii) Results:

Amounts of Nitrates and Ammonium Present in Distilled Water Leachates of Duff mull and Raw humus Soils

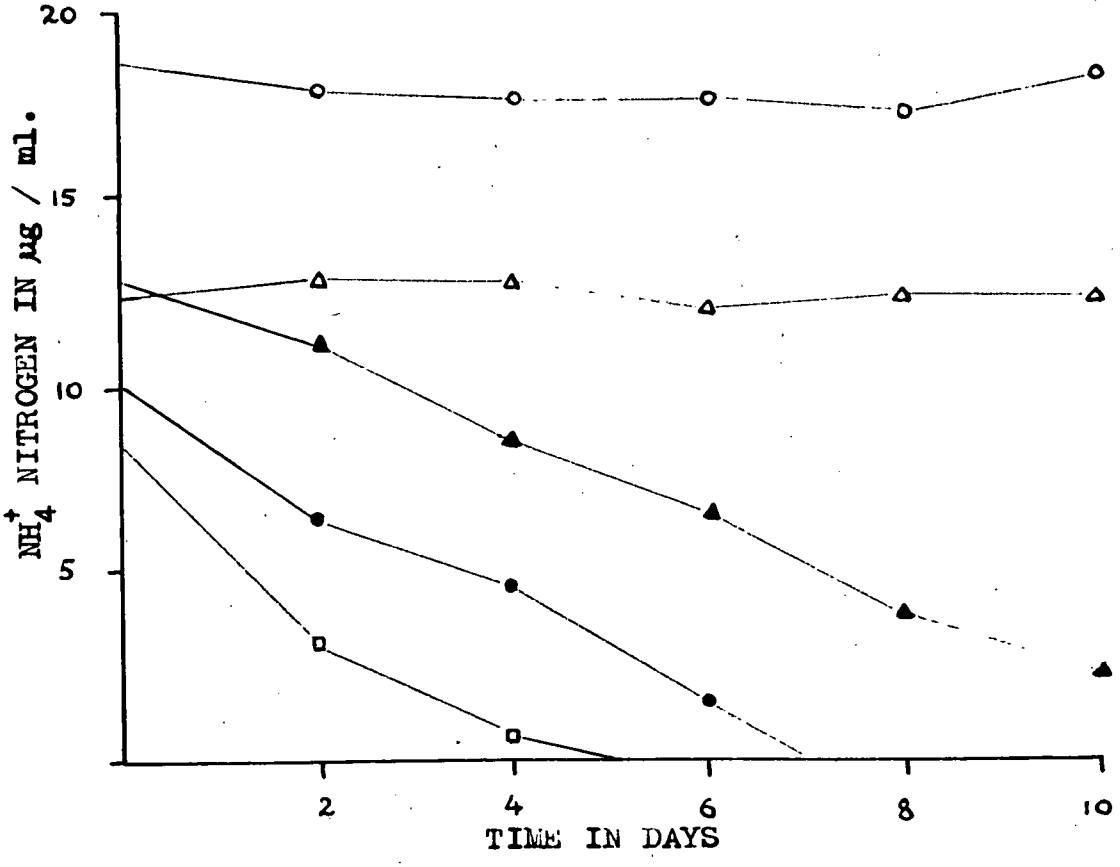
Date	Duff mull		Raw humus		A_1		B	
	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+
April 10	1.0	-	-	1.0	1.0	-	-	-
June 15	1.5	-	-	1.0	1.0	-	-	-
June 16	1.5	-	-	1.0	2.5	-	-	-
July 24	-	-	-	1.5	1.5	-	-	-
January 18	-	-	-	2.0	1.5	-	-	-
February 6	0.5	-	-	1.5	2.0	-	-	-

(All values given in ug/ml. of leachate, using 1 cc. of distilled water per 1 gram of soil)

to follow page 69

TEST No I.

CURVES SHOWING THE DISAPPEARANCE OF AMMONIUM.



□ - GARDEN SOIL

▲ - DUFF MULL

● - "A"

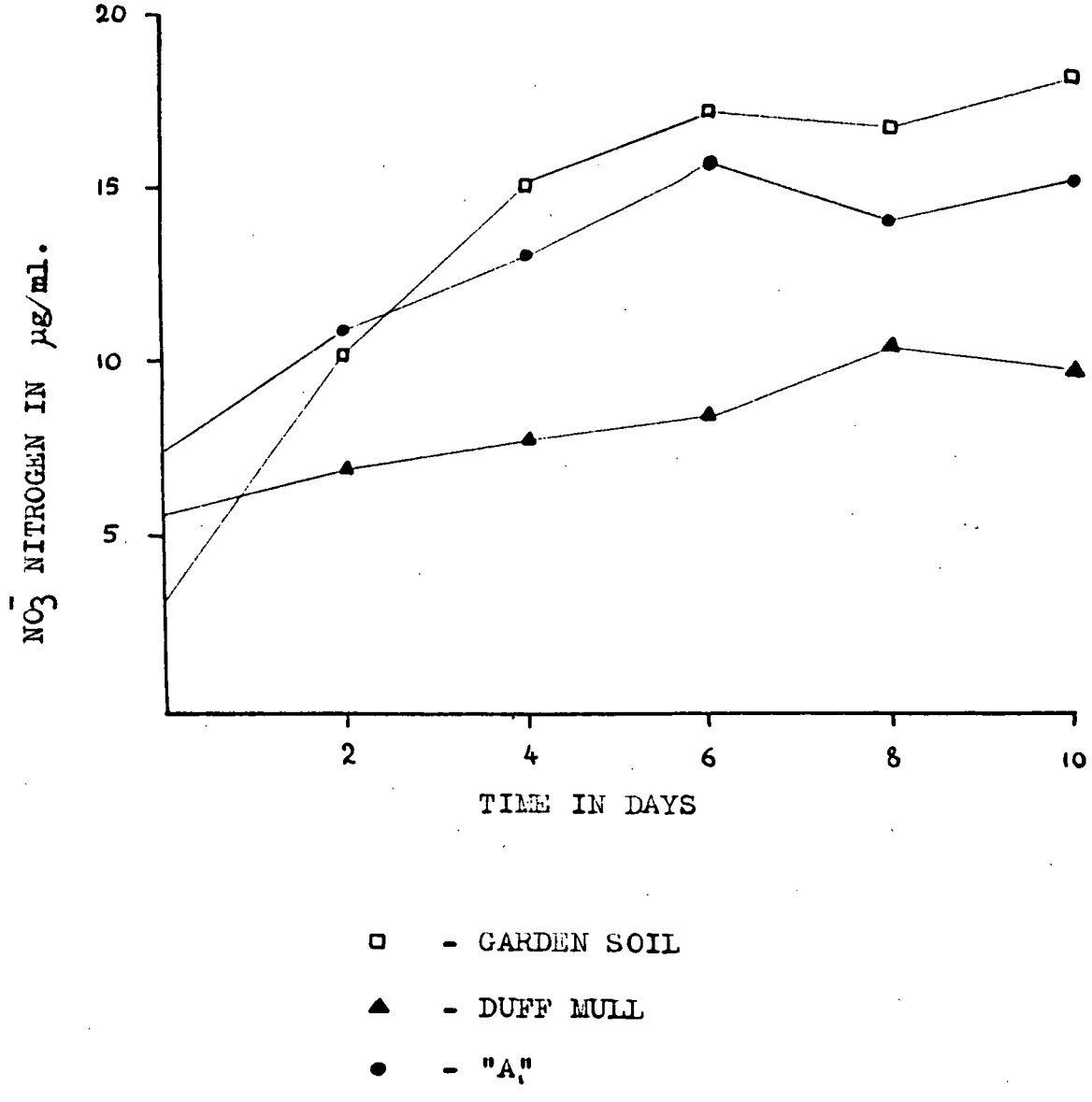
△ - RAW HUMUS

○ - "B"

to follow page 69

TEST No I

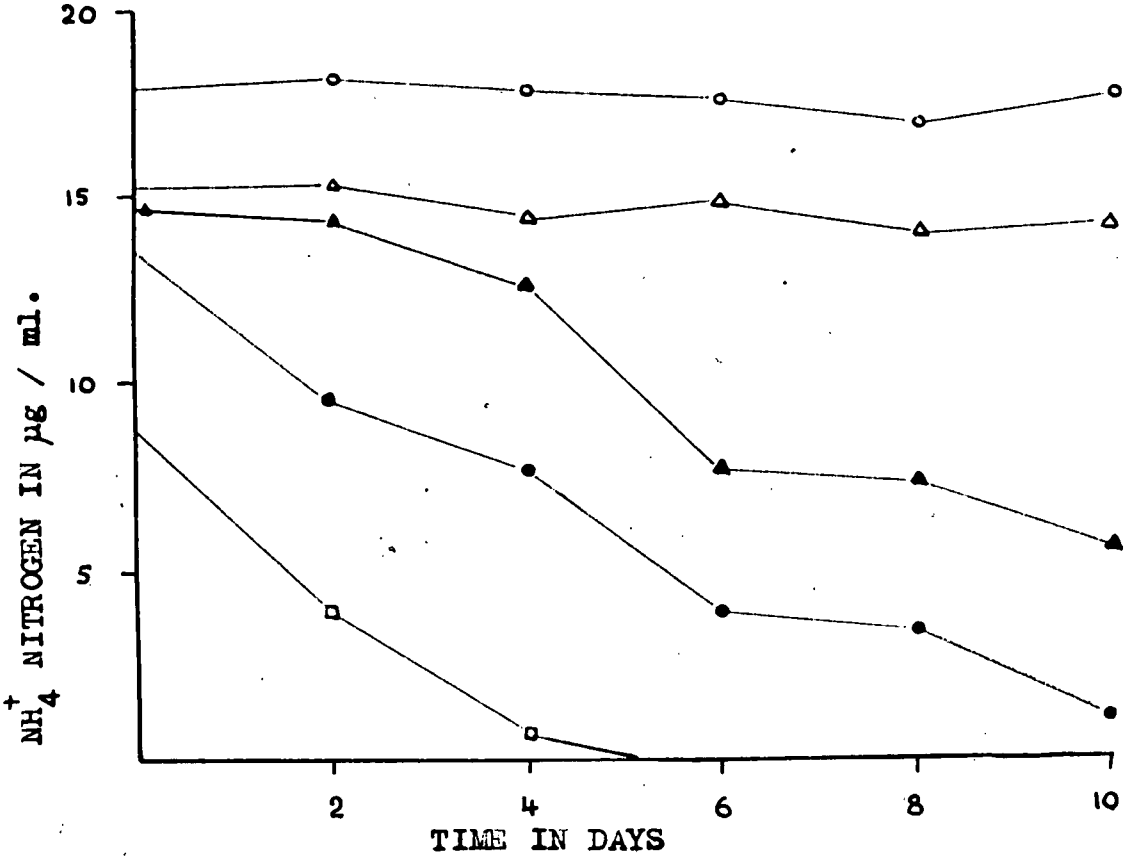
CURVES SHOWING THE ACCUMULATION OF NITRATES.



to follow page 69

TEST No II

CURVES SHOWING THE DISAPPEARANCE OF AMMONIUM.



□ - GARDEN SOIL

▲ - DUFF MULL

● - "A"

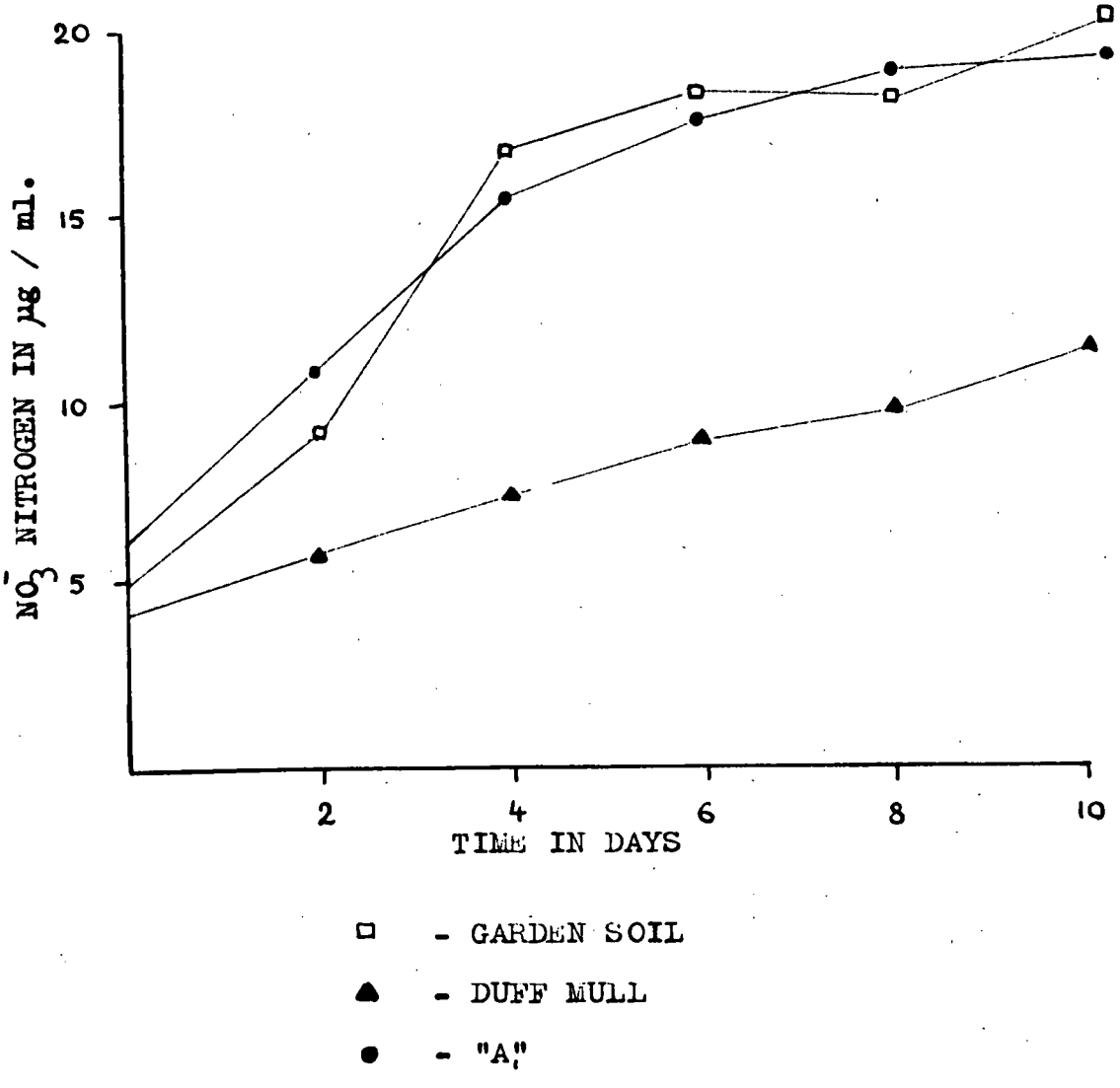
△ - RAW HUMUS

o - "B"

to follow page 69

TEST No II

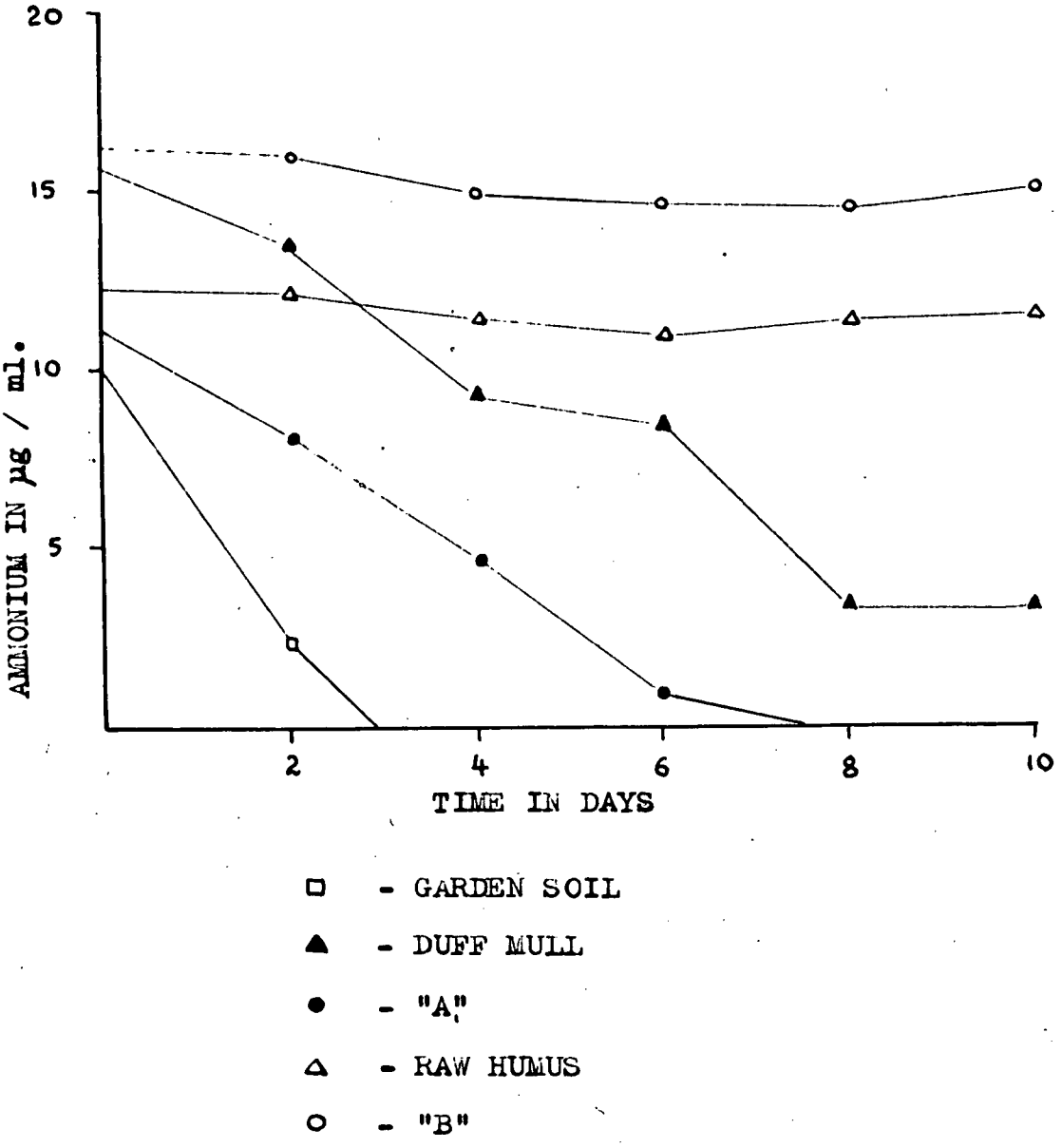
CURVES SHOWING THE ACCUMULATION OF NITRATES.



to follow page 69

TEST No III

CURVES SHOWING THE DISAPPEARANCE OF AMMONIUM.



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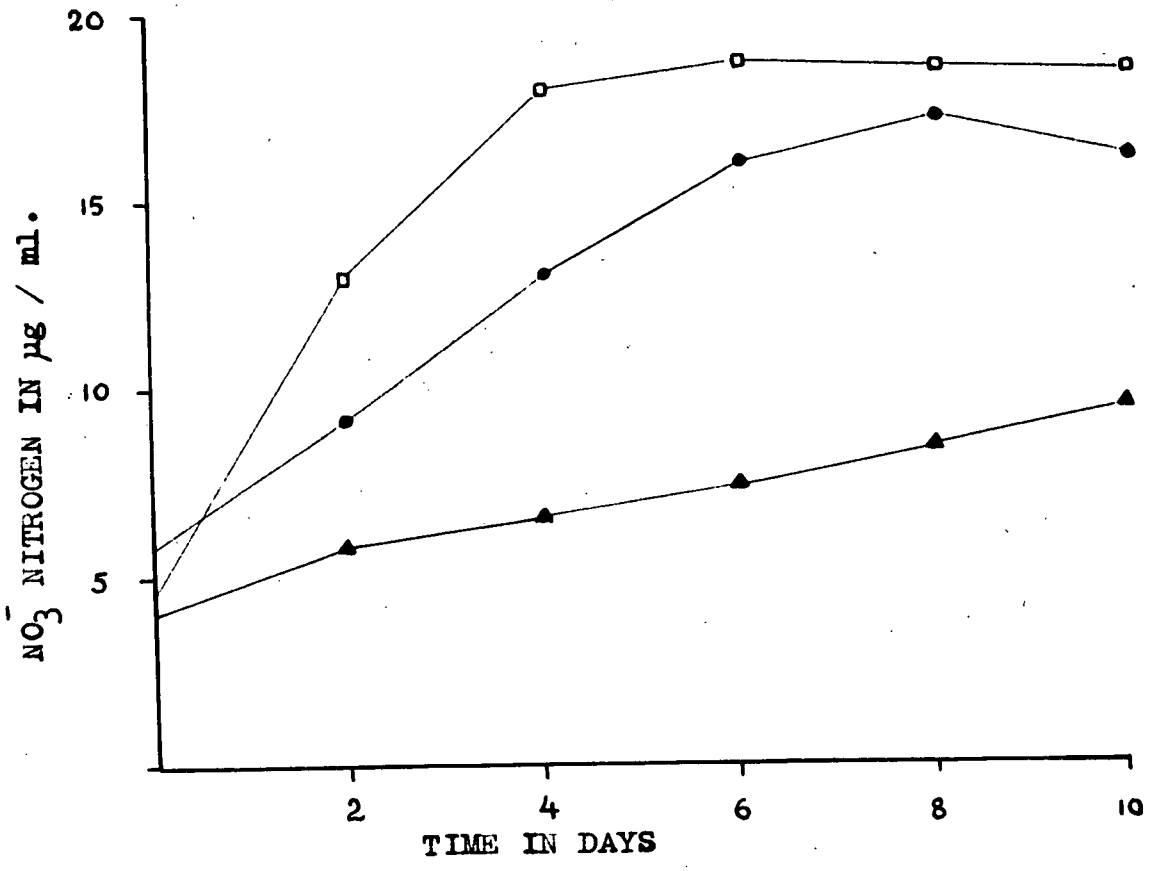
TEST No III

CURVES SHOWING THE ACCUMULATION OF NITRATES.



□ - GARDEN SOIL

▲ - DUFF MULL

● - "A"

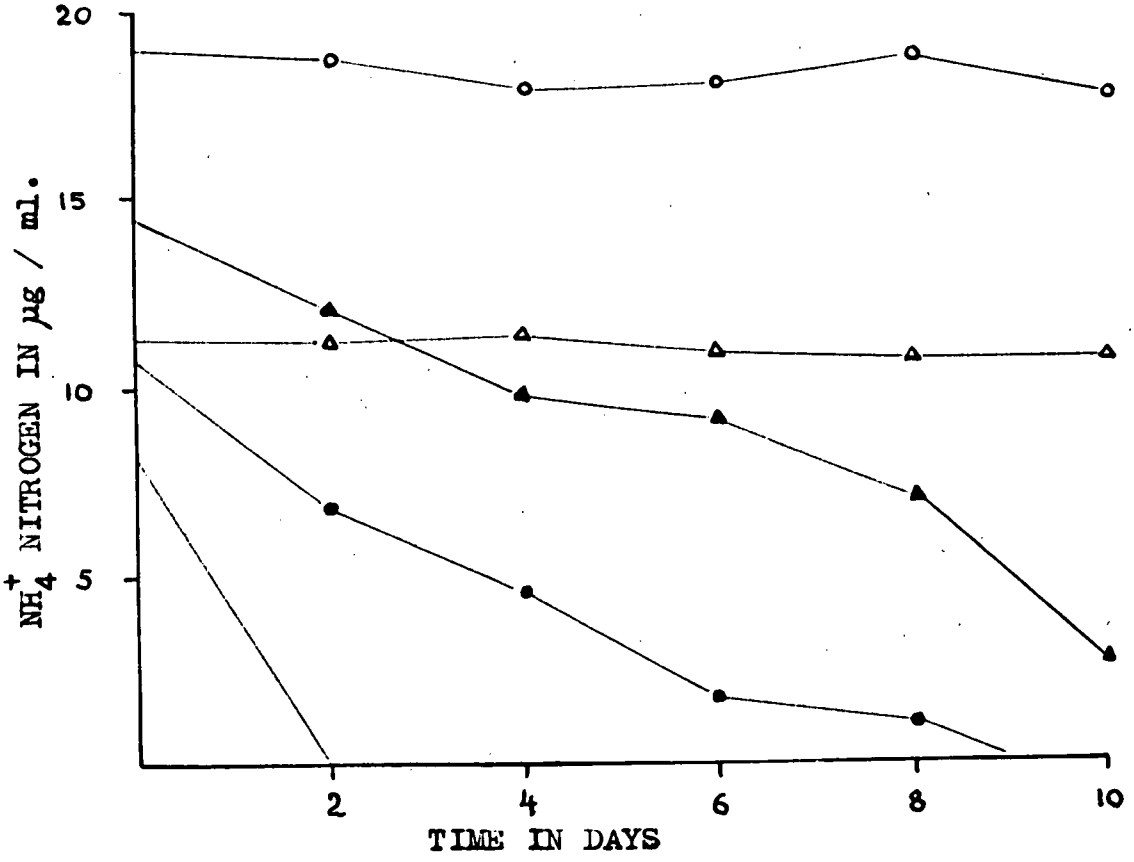
△ - RAW HUMUS

○ - "B"

to follow page 69

TEST No IV

CURVES SHOWING THE DISAPPEARANCE OF AMMONIUM.



□ - GARDEN SOIL

▲ - DUFF MULL

● - "A"

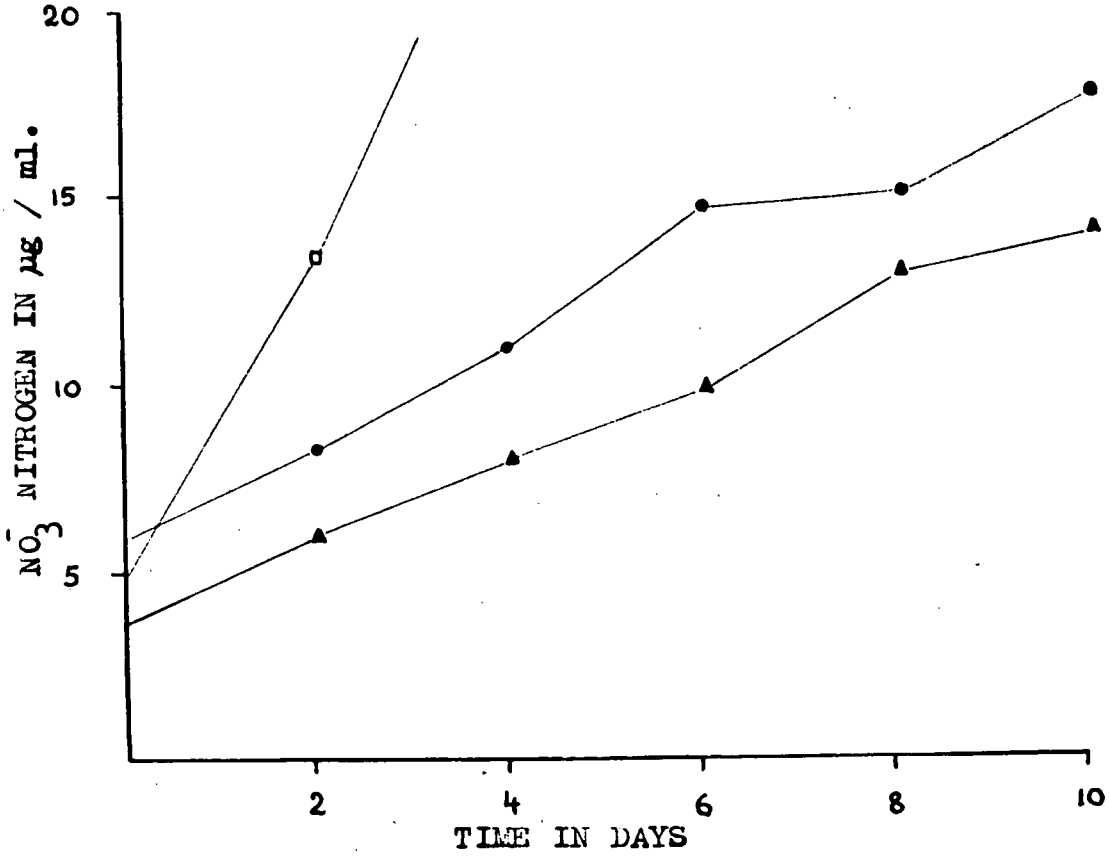
△ - RAW HUMUS

○ - "B"

to follow page 69

TEST No IV.

CURVES SHOWING THE ACCUMULATION OF NITRATES.



□ - GARDEN SOIL

▲ - DUFF MULL

● - "A"

(iii) Discussion:

These four consecutive tests show a complete absence of nitrification in raw humus soils and their subsoils eg. B-horizon. This absence of nitrification in raw humus and the underlying soil horizonz cannot be explained by the unfavorable pH conditions.

Soils that lack nitrifying ability will not acquire it when their acidity is corrected.

All samples of duff mull and A_1 horizon soils show fairly active nitrification.

From the graphs, one can conclude that rates of nitrate accumulation and ammonium disappearance vary little with season.

Tests of distilled water leachates of freshly collected soils have shown constantly detectable amounts of nitrates in duff mull and A_1 - horizon soils. Only raw humus has shown easily detectable quantities of ammonium in distilled water leachates.

CONCLUSIONS

1. A study of nitrification in the two humus horizons, duff mull and raw humus, has indicated a close dependence of the process on the ecological nature of the site. The results lend weight to attempts by ecologists and others to amplify and enlarge classical soil descriptions by an account of the biocoenotic environment that has given rise to them.

2. The two distinctive humus types, and the equally distinctive ecotypes from which they originate, have their own characteristic microflora and they, in turn, have definite counterparts in the floristic structure of the higher plants.

3. The respective microfloral populations, typical of the two distinctive and most widely occurring humus types, duff mull and raw humus, vary in their ability to oxidize ammonium. The resultant forms of nitrogen are specifically suited to the growth of certain species, whether tree, shrub, or herb.

4. In the raw humus type there are no plants storing nitrates in their leaves, whereas in the duff mull type some plants store rather large quantities of nitrates in their leaves. Among plants that store nitrates, there are some (nitrophilous plants) that may grow with

lower vigor in other habitats and not store nitrates. Others, mainly those that store relatively large quantities of nitrates, are incapable of migration to other habitats (obligatory nitrophytes).

5. Bacteria are present in greater quantity in duff mull than in raw humus, which has a markedly higher number of fungi per unit weight.

6. Studies of nitrification can be considered as an index of soil fertility and the suitability of a soil for tree growth. It is known, for instance, that Douglas-fir prefers nitrates rather than ammonium salts as a source of nitrogen supply, whereas western hemlock exhibits no such preference.

7. As most transformations of nitrogen into the forms required by plants take place in the organic increment of any soil, any drastic changes in the nature of the humus (by fire or other means of destruction) will alter the long established equilibrium in the natural nitrogen cycle of these forest ecosystems.

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APPENDIX

LIST OF TABLES ATTACHED

TABLE I.

NITROGEN IN CERTAIN AGRICULTURAL SOILS

	Soils	% Nitrog.	Ref.
Marbut (1929)	Prairies: Surface up to 6 inch.	0.05-0.15	(71)
	Depth up to 40 "	0.12 averag.	
	Chernozem: Surface up to 6 inch.	0.15-0.30	(71)
	Depth up to 40 "	0.12 averag	
Rost (1943)	Prairie soils (Minnesota)	0.17-0.35	(95)
	Average value	0.266	
Dep. of Agric. Canada (1949)	Majority of agricultural soils in Canada	0.10-0.50	(21)
	Peats and mucks	1.00-2.00	

TABLE II.

NITROGEN CONTENT OF SOIL HUMUS

	Soils	% Nitrogen in humus	Ref.
Zacharow	Russian soils (given as total range)	4.48-8.49	(114)
Waksman	American soils(given as total range)	2.57-8.03	(113)
Allison	Average value for agric. soils	5.5	(3)
	Undecomposed crop residue, (wheat and corn straw)	0.50-1.00	(3)

TABLE III.

C/N RATIO OF SOILS AND CERTAIN ORGANIC RESIDUES

		C/N Ratio	Ref.
Lyttleton Lyon, Buckman and Brady, (1952)	Organic matter of the furrow-slice of arable soils.	10-12/1	(70)
	Plant material (Legumes)	20-30/1	
	Farm manure	up to 90/1	
	Microorganisms (it is narrower for bacteria and wider for fungi)	4-9/1	
Waksman (1949)	Fungi (average)	10/1	(115)
	Bacteria (average)	5/1	
	Actinomycetes (average)	6/1	
Deherain (1902)	Cultivated soils of France	9.5/1	(114)
Stewart (1910)	Brown silt loam soils of Illinois:		
	- surface	12.1/1	
	- subsoil	8.9/1	
McLean (1930)	Average for fifty British soils	10.0/1	
Leighty (1930)	Most frequent range for sixty-three American soils:		
	- surface	8.5-11.4/1	(96)
	- subsoil	5.5- 8.4/1	
Waksman (1952)	Average for sixteen chernozem soils (Alberta)	10/1	(117)
	Average for twenty-one chernozem soils (Manitoba)	11/1	
	Average for eighteen brown soils (Saskatchewan)	11/1	

TABLE IV.

QUANTITIES OF NITROGEN FIXED BY LEGUMINOSAE AND RHIZOBIA

		N in lb/acre	Ref.
Lyttleton Lyon, Buckman, and Brady (1952)	Average crop of alfalfa	200-250	(70)
	" " " red clover	100-150	
Mayer (1955)	Good crop of alfalfa	400	(72)
Nicol (1955)	Good crop of lucerne	100	(79)
	Legume nodule bacteria	40-50	

TABLE V.

QUANTITIES OF NITROGEN FIXED BY NON-SYMBIOTIC BACTERIA

		Amount fixed	Ref.
Wilson (1951)	Azotobacter in optimal conditions:	100-150 mg/ml in 24 hours.	(122)
		15-20 mg/per 1 gram carbohydrates.	
	Clostridium spp.	10-12 mg/per 1 gram carbohydrates.	
Waksman (1952)	Azotobacter	10 mg/per 1 gram carbohydrates	(117)
Jensen (1954)	Azotobacter	15-16 mg/per 1 gram carbohydrates	(43)
Waksman (1952)	Butyric bacteria in pure cultures:	2-3 mg/per 1 gram carbohydrates	(117)
	Bact. asterosporus (facultative anaerobe):	1-3 mg/per 1 gram carbohydrates	
	(Clostridium spp. when freshly isolated fix more than Azotobacter)		
Lochhead	Cl. pasterianum approaches Azotobacter in some cases.		(66)

TABLE VI.

QUANTITIES OF NITROGEN IN PRECIPITATION

		Nitrogeh lb/acre/year	Ref.
Shutt (1917)	Average for 10 years for neighborhood of Ottawa, for 23.39 inch/year	6.583	(41)
Miller (1949)	Rain contains NH_3, NO_3	4.00	(95)
Leland (1949)	Average for 1931-49 for Ithaca N.Y., for 35.55 inch/year $-NH_3$	4.02	(53)
	$-NO_3$	1.25	
Meyer and Anderson (1955)	Quotes data taken for 5 years in Rothamsted Exper. Stat.	4.4	(72)
Lyttleton Lyon, Buckman, and Brady	Quoting a number of data gives an average	5.0	(70)
Fogg (1955)	Rain may supply in average	1.0	(26)
Nicol (1955)	In temperate climates the rain contributes about	4.0	(79)

TABLE VII

C/N RATIO OF FOREST SOILS

		C/N Ratio	Ref.
Waksman	Minnesota forest	19/1	(117)
	Alpine soils	9.7-34/1	
Isaac and Hopkins	Douglas Fir Region (Pacific Northwest): - average for duff (cut-over area)	57/1	(41)
	- mineral soils at depth of: 0 - 3 inches	27/1	
	3 - 6 "	24/1	
	6 - 12 "	22/1	
	12 - 30 "	21/1	
	- adjoining old-growth timber area: for duff mull	52/1	
	for rotten log	171/1	
	(common C/N ratio for agricultural soils)	10/1	

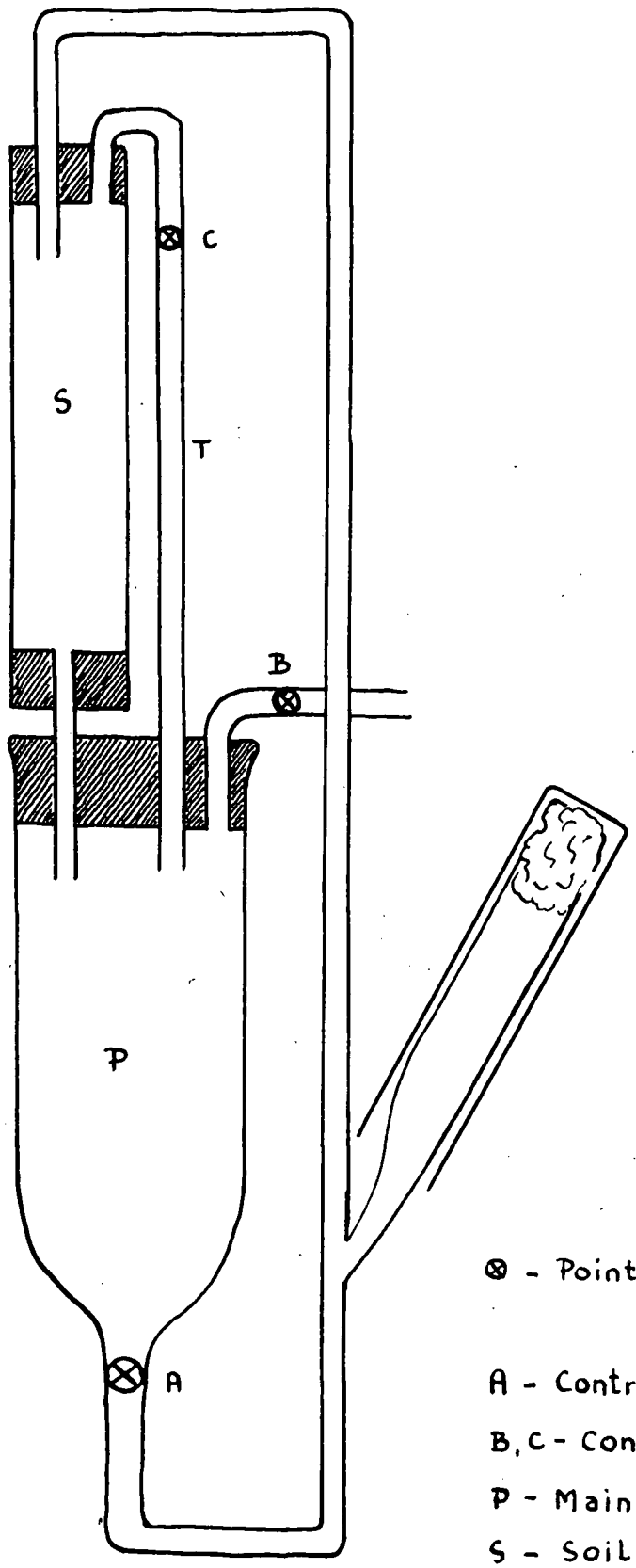
TABLE VIII

NITROGEN CONTENT OF FOREST LITTER

		Nitrogen % ppm.	Ref.
Isaac and Hopkins	Douglas Fir Region (Pacific Northwest) - average for duff (cut-over area)	0.92%	(41)
	- average for old growth timber area	0.87%	
Wilde	Surface 8-inch layer of virgin forest soil	0.1-0.3%	(119)
	(content of nitrates seldom exceeds 20 ppm.		
	(content of ammonia may accumulate up to 70 ppm.		

LIST OF FIGURES ATTACHED

Fig. I



⊗ - Points of control and adjustment.

A - Control of perfusate.

B, C - Control of air flow.

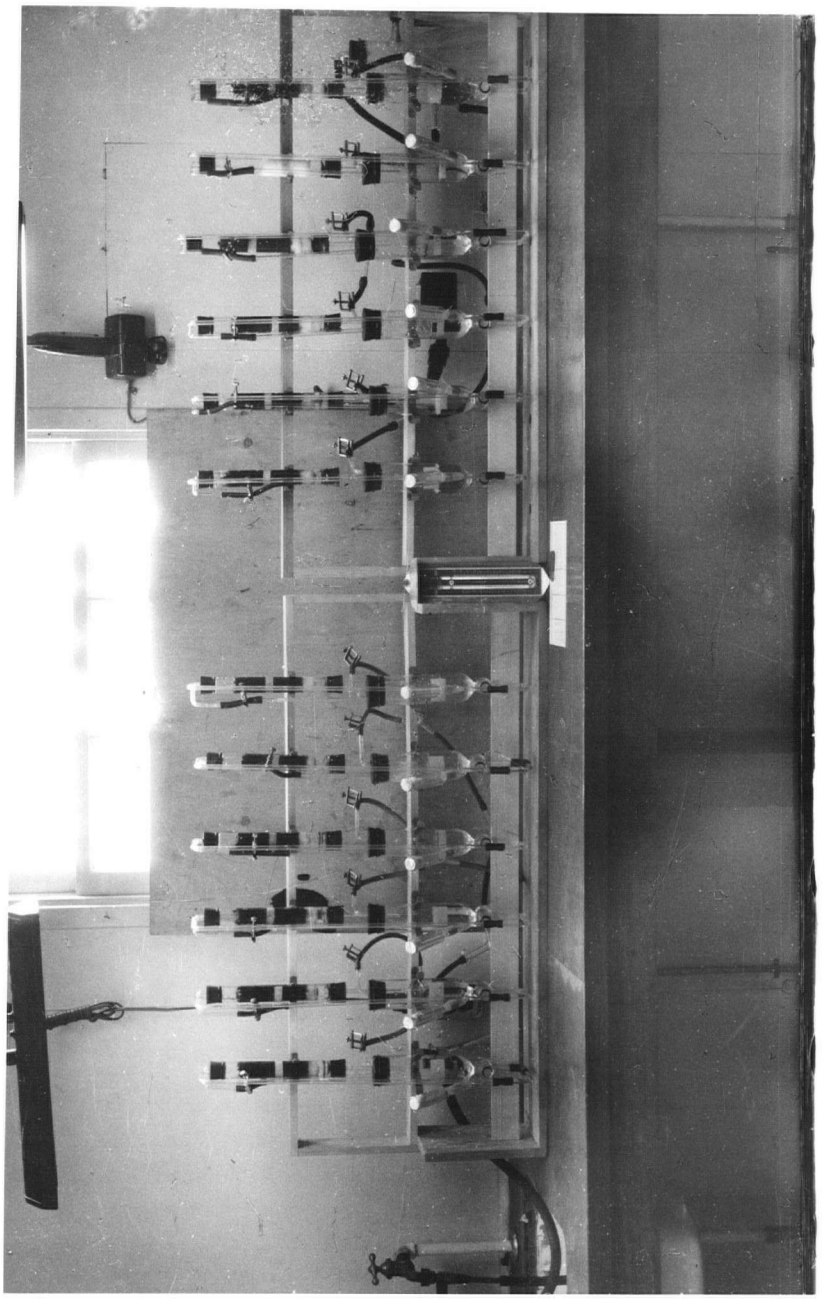
P - Main reservoir.

S - Soil tube.

T - By-pass tube.

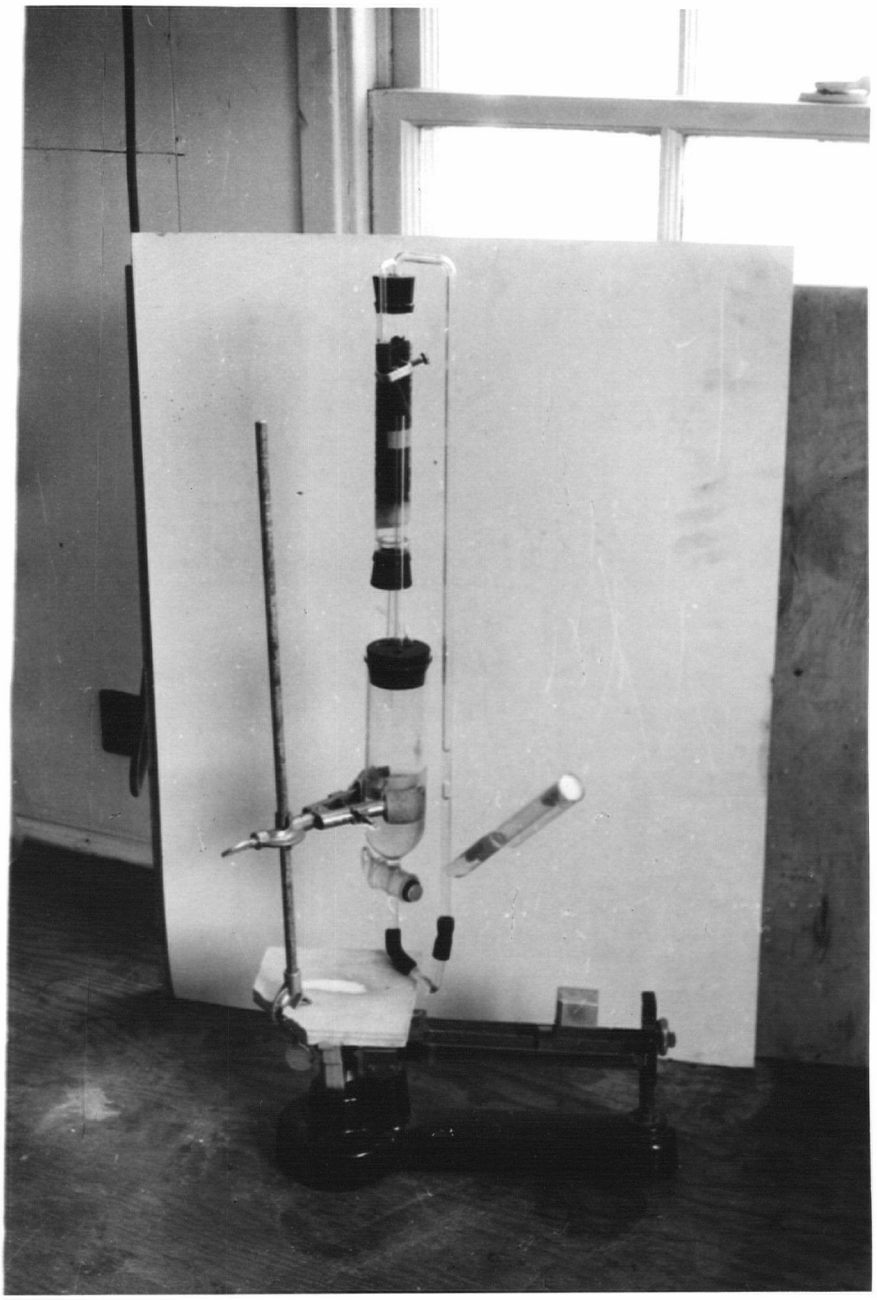
PERFUSION APPARATUS.

Fig. II



SET OF PERFUSION APPARATUSES AT WORK.

Fig. III



PERFUSION APPARATUS PREPARED FOR WEIGHING.

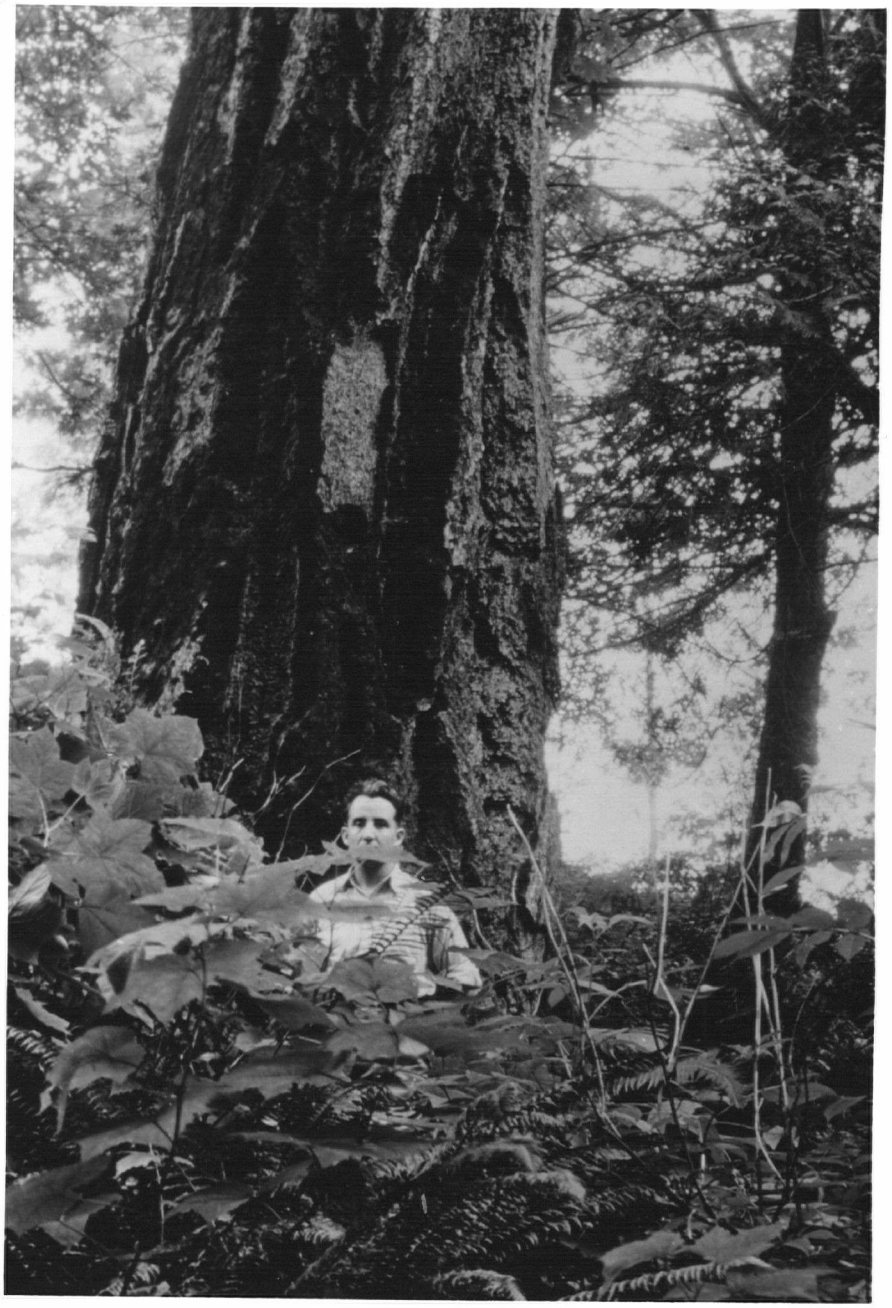
Fig. IV

Polystichum munitum, Thuja plicata, Sambucus pubens.

Fig. V

Pseudotsuga menziesii, Thuja plicata, Sambucus pubens, Rubus spectabilis, Rubus parviflorus, Polystichum munitum.

Fig. VI



Pseudotsuga menziesii, Polystichum munitum,

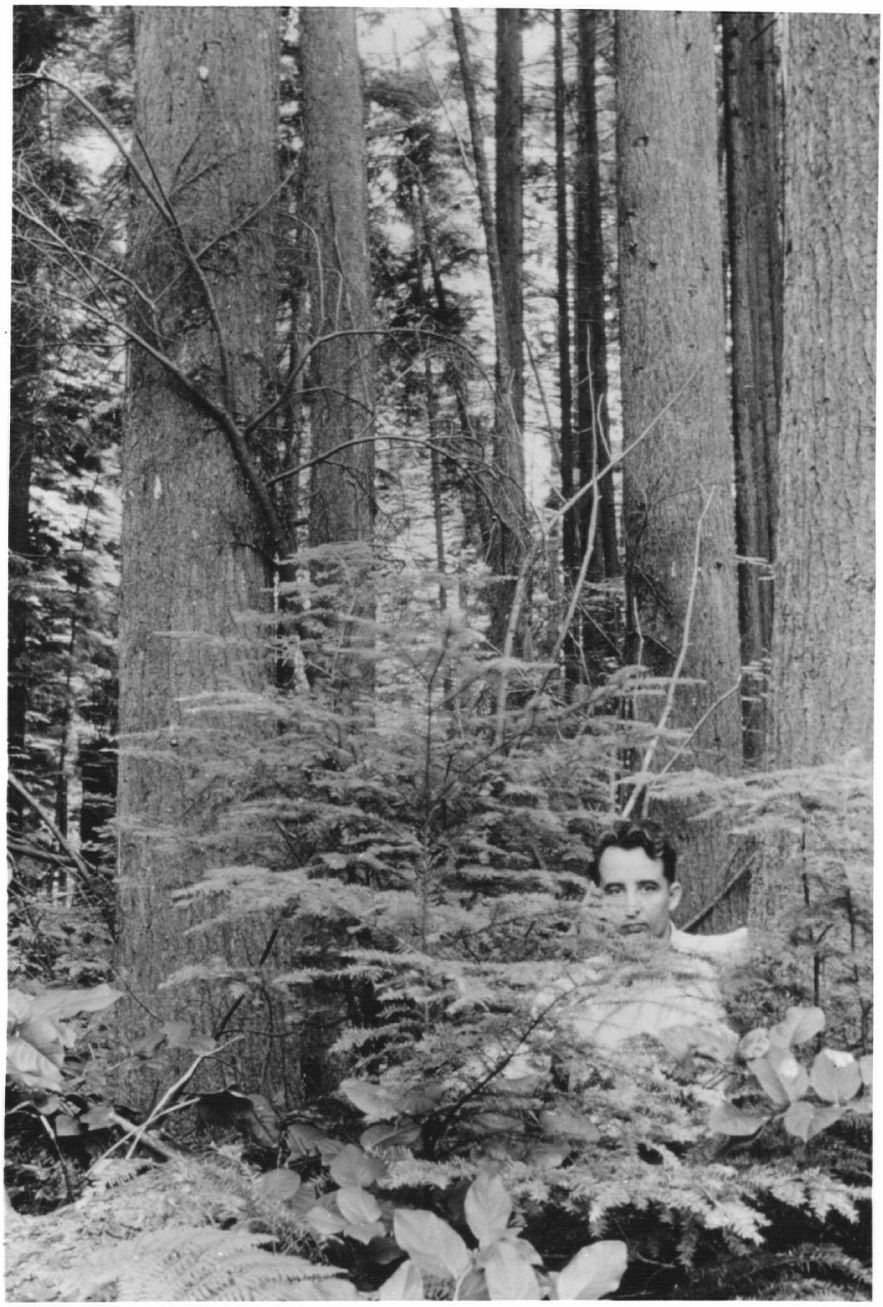
Rubus parviflorus.

Fig. VII

Gaultheria shallon.

Tsuga heterophylla, Pseudotsuga menziesii (stump), Thuja plicata, Cornus nuttallii, Gaultheria shallon, Vaccinium parvifolium, Vaccinium ovalifolium, Pteridium aquifolium.

Fig. IX



Tsuga heterophlla, Pseudotsuga menziesii (in the background), Gaultheria shallon, Vaccinium Parvifolium.