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Soil Organisms in a Natural Forest and in a Plantation Forest. 1947

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SOIL ORGANISMS IN A NATURAL FOREST AND IN A PLANTATION FOREST

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Forestry

June 1947

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Acknowledgment

Appreciation is given to Dr. Kenneth L. Jones, Department of Botany of the University of Michigan, for the helpful advice and encouragement which he generously gave in the preparation of this paper. The author is indebted also to Dr. Dow V. Baxter and Professor Leigh J. Young, School of Forestry and Conservation of the University of Michigan, for their suggestions and interest in gathering the data and presentation of the problem.

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SOIL ORGANISMS IN A NATURAL FOREST AND IN A PLANTATION FOREST

Introduction

More and more the forester is thinking of the forest in terms of a living, dynamic association of plants and animals. There was a time, early in the history of forestry in this country, when little scientific emphasis was given to the organisms beneath the forest floor and their influence upon vegetative growth. In the new age of systematic research, the silviculturist, the forest manager, the pathologist, and hosts of soil technologists have discovered their studies were not entities within themselves. Students of the soil have found that in endeavoring to encompass the span of the soils research already accomplished, they were unable to isolate fields of learning without borrowing from other soil concepts. This generality might be applied to any field of endeavor, but especially is it true in a soils study where so many and varied lines of research cross and recross. Though many an individual researcher has sensed a delight in creating his own sphere of knowledge, he has unmistakably confronted a situation where he demands a broader concept of forest soils. In certain instances he has altered his approach of study to warrant his needs, while other

researchers have clung to their narrow spheres. Fortunately, there is no individual forester who possesses the overall insight to peruse all aspects of the soil, every time he wants to plant, to cut, or estimate a stand of timber. This minute study is left to the research scientist; the forester seeks a general knowledge that intimately affects his work in terms of the results of a forest enterprise. He seeks a practical and workable approach in conjunction with his needs, so that when confronted with a particular situation he will be versatile enough to recognize how the soil possibilities of an area fit into his plans. For example, the techniques and practices used in logging affect the soil and the regeneration on it; the silviculturist with his thinning must visualize his cuts in relation to soil factors; nurserymen need to know soil characteristics and fertilizer values, while the pathologist thinks in terms of species and their resistance to disease, all of which are a site characteristic. Forest managers desire a knowledge of soil capacities in order to evaluate stands more closely.

This study is concerned with the soil as a mass of living debris, including certain bacteria, actinomycetes, fungi, algae and protozoa. These living microbes are not a complete list of the soil flora and fauna. However, they exert a profound influence upon the genetical development of soil profiles as well as an effect upon forest vegetation.

There are other organisms such as nematodes, earthworms,

arachnids, insects and mammals, all of which have a relationship to soil processes. Their influence, however, is not so profound as regards biological transformations which are brought about in a large degree by the bacteria, fungi, and actinomycetes in forest soils.

No attempt has been made to enable the reader to analyze the results of these experiments by a strict formula reaction that will hold true in all cases. Nevertheless, the results are a function of two independent bodies, the soil and the forest, both of which are integrated parts of the same dynamic system. In all comparative studies certain variables and incidental factors enter in; no direct correlation between the soil and the forest stands described can be expected to hold true in all situations. Since no identical studies like the one here presented, have been made, interpretation of data are only indicative of principles and not absolute processes. The writer is fully aware of the limitations of a specific case study and yet certain principles and relationships have been investigated. In many instances the study has revealed similarities that closely approach the results of other investigators who have made studies not necessarily of the type here presented.

Objectives

This paper has the following objectives:

(1) To present quantitative data concerning certain soil organisms in two individual forest stands of

Red Oak (Quercus borealis var. maxima), one a native uneven-aged stand and the other an even-aged plantation stand.

(2) To interpret quantitative differences in the soil population between the two stands involved, by noting presence or absence of certain organisms and to give considerations for these differences.

(3) To present in a minor qualitative way, certain of the genera represented in the two soils at varying horizons.

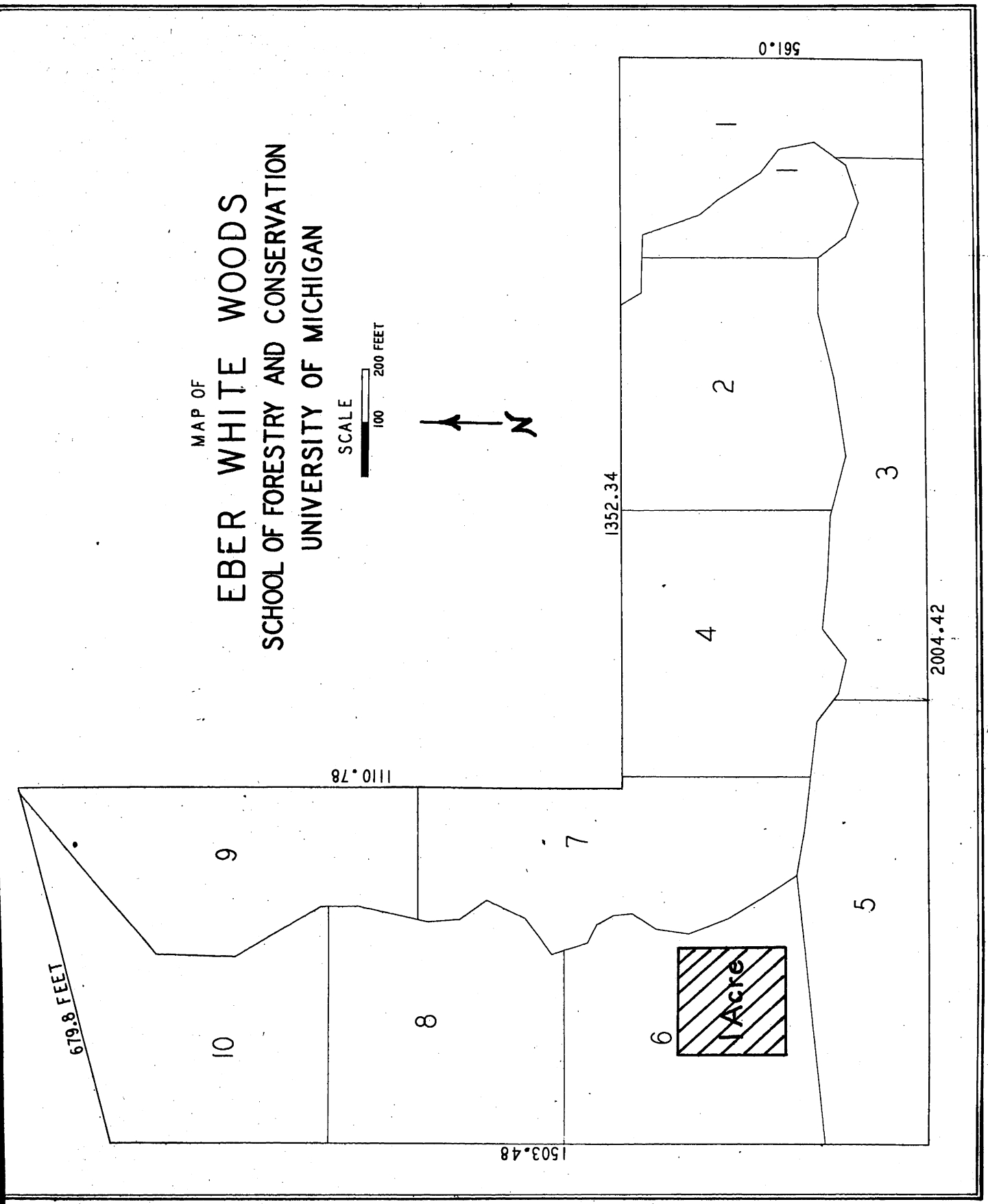
(4) To illustrate some indirect relationship between the age of the forest and the appearance of soil organisms in regard to comparable depths.

(5) To describe briefly the technique used in the isolation of certain organisms from the soil, so that this procedure may be applicable and easy reference to soil sampling for similar studies.

Description of Sampling Areas Involved in the Study

In order for the reader to visualize the soil conditions and site characteristics of the two areas involved, it becomes necessary to give in a general way some of the soil type and series characteristics of each area in orienting the approach. This enables the reader to correlate the results with the possible conclusion considerations. In addition to references cited for the soil type of the area in general, a representative profile of each area has been dug and horizons

MAP SHOWING SAMPLING AREA



measured in each forest in order to be more specific when various horizons are mentioned in the results. The soil profiles should be used as continual reference and kept in mind when soil organisms and their numbers are cited.

Eber White Woods

Location: This tract of forest land in which samples were taken has a total area of 41.2 acres containing ten compartments of 4.2 acres each. It is located one-half mile west of the city limits of Ann Arbor, Michigan. The plot in which the soil samples were collected is indicated by location as one acre within Block No. 6 shown on the accompanying map of the area.

Topography: The topography of the entire Woods is generally regular, with slight rolling areas in the southern part, but sloping off rather steeply towards the northwest. Block No. 6, where the samples were taken, has good drainage and level terrain.

Soil Type: For a general consideration of the soil type which is representative of the sampling area, soil origin is here considered. The entire region around Ann Arbor is a glacial terminal moraine underlain by Coldwater shale and Berea sandstone (5). The soil is primarily Miami silt loam described as follows: "The cultivated soil consists of the following layers:

1. A gray brown silt loam to plow depth
2. A layer of 2 to 6 inches of light gray or

grayish yellow silt loam which is floury or pulverulent when dry.

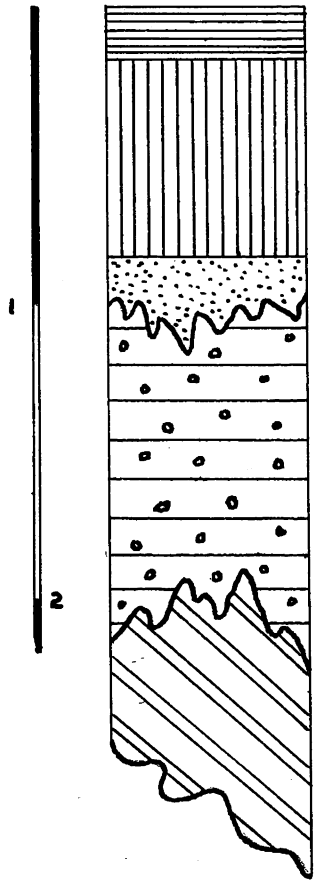
3. A layer of 18 to 24 inches of yellowish brown, firm, more clayey material which is plastic and impervious when moist and jointed and granular when dry.
4. Parent material of massive, compact, moderately gritty and stony, but a comparatively impervious bluish-gray clay which continues to a depth of several feet.

The content of organic matter is not high but the supply is fairly durable. The average content of moisture is comparatively high as both the subsurface layer and substratum are rather impervious and highly retentive of moisture. In general, the surface soil of the virgin soil is slightly acid or neutral, the second layer is medium or strongly acid, and acidity decreases with depth, until an alkaline reaction is obtained at a depth of 24 to 36 inches. The organic layers of the virgin soil are very thin, containing undecomposed woody matter.* (6)

Along with this general survey of the soils concerning the vicinity in question, a more specific idea of the soil and its profile within the forest area, was deemed advisable. Profiles were dug in three scattered locations within each forest stand; data concerning depth of horizon and soil type was combined and averaged into a representative profile.

The representative profile of the sampling area in Eber White Woods was found as follows:

REPRESENTATIVE PROFILE OF BLOCK NO.6 (EBER WHITE WOODS)



Depth	Horizon	
2"	A_0	Raw humus, loose leaf litter, and undecomposed organic debris.
6"-8"	A_1	Incorporated humus, mineral matter mixed with humus, and dark brown in color.
1"-2"	A_2	Not present as a continuous horizon.
18"-24"	B	Enriched layer, accumulated, yellowish brown, clayey material, plastic and impervious when wet.
24" up	C	Parent material, moderately gritty and stony, comparatively impervious.

Since the activity of microorganisms has a distinct relationship to the chemical and physical properties of the soil, a table showing the various percentage constituents at differing horizons is here presented: (6)

TABLE I

CHEMICAL ANALYSIS OF MIAMI SILT LOAM, WASHTENAW COUNTY, MICHIGAN

Horizon	Depth	SiO_2	Al_2O_3	Fe_2O_3	CaO	MgO	P_2O_5
A_0 & A_1	0-3"	71.4	10.6	2.55	1.5	.90	.17
A_2	3-8"	79.2	10.6	2.80	1.2	.84	.07
B	8-32"	66.8	11.2	3.75	1.00	1.68	.08

Species, Age and Diameter of Trees on the Sample Plot:

The dominant species represented on the sample plot is Red Oak (Quercus borealis var. maxima) with an understory of reproduction less than ten feet high consisting of White Ash (Fraxinus americana), Sugar Maple (Acer saccharum), Basswood (Tilia americana), and some Black Cherry (Prunus serotina). Little reproduction of red oak has occurred. A dense mat of wildflowers is a part of the forest floor during the spring and summer months. The stand is uneven-aged and a proper distribution of age classes has not been accomplished.

Some appreciation of the diameter class and age distribution by classes, can be learned from the following table:(10) Compartments 6 to 10 are representative of the area on which the samples for the study were taken. The age of the stand will be close to 160 years.

TABLE II

COMPARTMENTS 6 TO 10 INCLUSIVE OF EBER WHITE WOODS(ALL SPECIES)

Dia. Breast High in Inches	Total Basal Area Per Acre in Square Feet (1942-43)	Board Foot Content (1942-43)
1-3"	6.95	
4-7"	7.01	
8-11"	12.03	453
12-22"	37.04	3817.0
23" up	12.20	1713.0
	75.23	5983.0

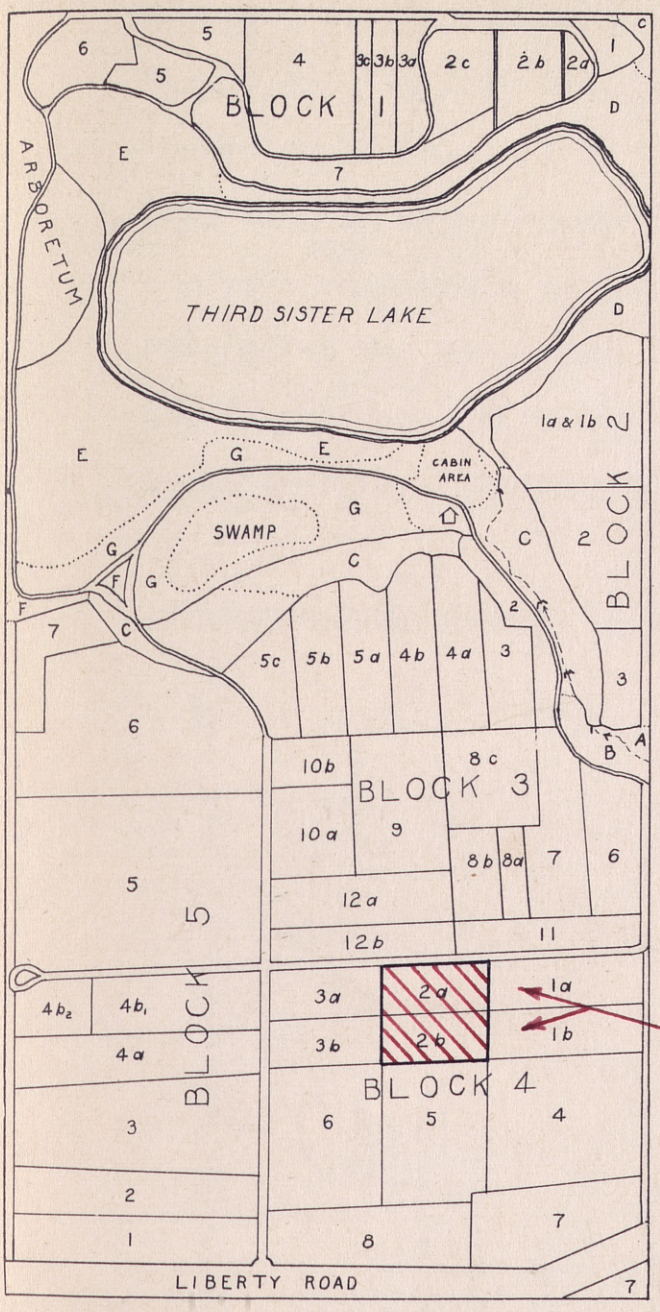
Saginaw Forest

Location: This tract of planted forest land in which samples were taken has a total area of 80 acres with various sized compartments. It is located west of the city limits of Ann Arbor, Michigan and approximately 1½ miles from Eber White Woods. The plot in which the soil samples were collected is indicated by location as the E½ of Lot No. 3A in Block No. 4 and is shown on the accompanying map of Saginaw Forest. The sample area is one acre.

Topography: The topography of the entire Forest is generally regular excepting a swamp area and lake near the center of the tract from which the topography rises slightly to the north and south. Lot No. 3A, where the samples were taken, has good drainage and a regular terrain.

Soil Type: As in Eber White Woods the same general

MAP SHOWING SAMPLING AREA



Block	Lot	Species	Stock	Date	Acres
1	1	Scotch Pine	2-0	Sp.'04	.24
	2a	Austrian Pine	2-0	"	.12
	2b	White Pine	2-0	"	.54
	2c	" "	2-0	"	.57
	3a	Douglas Fir	2-0	"	.37
		Western Y. Pine	2-0	Sp.'08	
	3b	Tulip Poplar	2-0	Sp.'04	.28
		White Pine		Sp.'08	
	3c	Douglas Fir	2-2	Sp.'21	.23
	4	White Pine	2-0	Sp.'04	1.63
	5	Western Y. Pine	2-0	Sp.'08	.75
	6	Scotch, Austrian + W.Y. Pine	2-0	Sp.'06	.61
	7	Scotch Pine	2-0	Sp.'08	.91
		Catalpa	1-0	Sp.'04	
2	1a+1b	Norway Spruce	3-0	Sp.'04	1.68
	2	Norway Pine	3-1	Sp.'23	1.04
	3	Scotch Pine	2-2	Sp.'22	.34
3	1	Black Locust	1-0	Sp.'04	.53
	2	Hickory	1-0	Sp.'07	
		Black Locust			
		Elm			
	3	Scotch Pine	2-2		
		Japanese Red Pine	2-2	Sp.'27	.53
	4a	Scotch Pine	2-2	Sp.'26	.77
	4b	" "	2-2	Sp.'24	.64
		Japanese Red Pine	2-2	Sp.'25	
	5a-5b-5c	Black Locust	1-0	" '06	1.86
		Norway Spruce	2-2	" '15	
	6	Basswood	1-0	" '06	.75
	7	W. Yellow Pine	2-1	" '38	.85
	8a	Sugar Maple	1-0	" '06	.24
	8b	" "	1-0	" '06	.45
	8c	Norway Pine	2-2	" '21	.90
	9	Corsican Pine	2-0	" '30	1.30
	10a	Red Oak	1-0	" '06	.76
	10b	White Oak	1-0	"	.35
	11	Wh. + Burr Oak	Seed	"	.62
	12a	Bl. Walnut	1-0	"	.46
	12b	" "	Seed	Fall '06	.61
4	1a	Wh. Oak	Seed	Fall '06	.74
		Wh. Pine			
	1b	Chestnut	Seed	Fall '06	.74
		Wh. Pine			
	2a+2b	Red Oak	1-0	Sp.'08	1.02
		Scotch + Wh. Pine			
	3a+3b	Red Oak	Seed	Fall '06	
				Sp.'07	1.08
	4	Bl. Walnut	"	" '09	
		Pine-Oak	2-2		1.87
		Larch-Spruce	2-2		
	5	Red Oak	1-0	Sp.'07	1.45
	6	" "	Seed	"	1.54
	7	Nor. Spruce	2-2	" '14	1.03
	8	Red Oak	1-0	" '08	1.17
5	1	W.Y. Pine	2-0	Sp.'09	1.07
	2	" " "	2-0	" '37	.97
	3	Nor. Spruce	3-0	Fall '11	2.21
	4a	Cottonwood	Cuttings	Sp.'12	1.05
	4b1	W.Y. Pine	2-1	" '15	1.00
	4b2	Nor. Pine	2-2	" '21	.37
	5	W.Y. Pine	2-0	" '09	4.04
	6	" " "	2-0	" '12	2.76
		Filled Douglas Fir		" '18	
	7	Nor. Spruce	2-0	" '37	.64

A = Box Elder B = Douglas Fir C = Oak-Hickory
D = Soft Maple, Willow, Aspen E = Elm, Soft Maple Swamp
F = Nor. + Wh. Spruce G = Wh. Cedar

SAGINAW FOREST

SCHOOL OF FORESTRY AND CONSERVATION

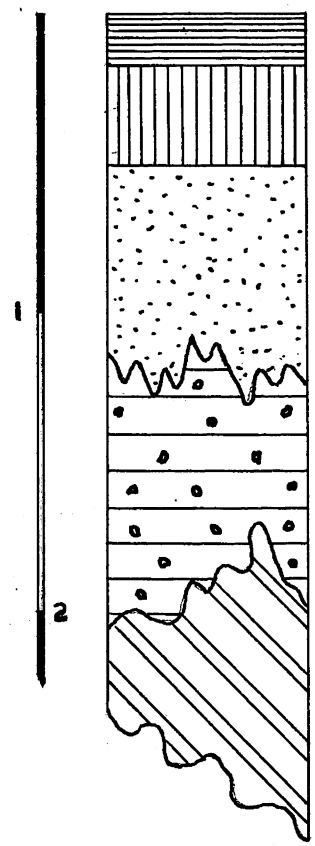
UNIVERSITY OF MICHIGAN

Scale: 0 100 200 ft.

Jan. 1939

classification and origin of soil may be ascribed to Saginaw Forest. It is primarily Miami silt loam and is a deep, fresh, clay loam with little organic matter (10). This was the condition of the soil in 1907 prior to planting and was at that time an agricultural land. The table of chemical analysis applicable to the previous forest soil will also be indicative for this area. Specifically, the profile of the sample plot used differed in some respects from the Eber White profile. The plantation profile is as follows:

REPRESENTATIVE PROFILE OF E½ LOT 3A BLOCK NO.4 (SAGINAW FOREST)



Depth	Horizon	
2"	A_0	Raw humus, loose leaf litter, undecomposed organic debris.
3"-4"	A_1	Incorporated humus, dark brown loam high in organic matter, good tilth, earthworms, and mineral matter mixed with humus.
7"-8"	A_2	Partially leached layer, light brown silty loam.
12"-24"	B	Enriched layer, accumulated, yellowish brown, clayey material, plastic and impervious when wet.
36" up	C	Parent material, moderately gritty and stony, and comparatively impervious.

Species, Age and Diameter of Trees on the Sample Plot:

Data comparable to that presented for Eber White Woods is now tabulated for the Saginaw Forest. The dominant species represented on the sample plot is Red Oak (Quercus borealis var. maxima) with little reproduction growing in the stand. There is no dense mat of wild flowers here and very little herbaceous understory. The stand is even-aged. From the following table which is comparable to Table II, an idea of size and diameter is learned. This plot is 42 years of age.

TABLE III (10)

E½ of Lot No. 3A in Block No. 4 (SAGINAW FOREST) One Species

Year	Diameter Breast High in Inches	Total Basal Area Per Acre in Square Feet
1943	5.6" (avg.)	84.55

Intermediate Summary

Before approaching a more detailed procedure for the isolation of organisms in these two forest stands it is appropriate to recognize at this point the more pertinent facts concerning the data thus far presented. An attempt has been made to minimize extreme variables and to select certain uniformities before proceeding further:

Significant Uniformities

1. Total basal area per acre as noted from the tables is approximately the same for both stands as of 1943. This, of course, indicates total basal area per acre for all species on the plots; no differentiation as to species in relation to basal area has been attempted.
2. Both stands cited are dominantly Red Oak (Quercus borealis var. maxima).
3. The size of each sample plot is the same (1 acre) for both stands, having soil of the same general origin, soil type, general chemical analysis, and similar topography.
4. Collecting dates for each soil horizon have been constant; collecting procedures and sampling methods used are comparable for both plots.
5. The stands are in close proximity to each other so that climatic factors such as weather and temperature are fairly comparable.
6. Moisture contents between the two forest soils did not differ more than 3% between any two horizons and no moisture content exceeded 5% at any horizon.
7. Laboratory procedures, pH of the media and other culturing techniques were carried out uniformly.

Significant Differences

1. The Eber White Woods plot is an uneven-aged stand with diameters as high as 23 inches; the Saginaw plot is an even-aged pure stand with an average diameter of 5.6" (1943) and no diameter exceeds 7 inches.
2. Acidities, or pH values, vary within horizons for each stand and also vary between stands at the same horizons.
3. Trees on the Eber White plot have been estimated up to 160 years of age and over, while the Saginaw plot is a stand only 42 years of age. The age differential is thus approximately 4 to 1 between the sample plots.

Cultural Procedure

Field Technique

The samples were collected in the autumn of 1946 and extractions were taken from all horizons within the period September 20, 1946 to October 5, 1946. In order for the sampling method to be uniform, 10 gram samples were collected from twenty different spots within each 1 acre oak stand. These twenty spot samples were taken at approximately thirty feet apart in one direction and forty feet in the opposite direction. Samples for only one horizon were collected in a day, making a total of 20-10 gram samples from each tract from one horizon in

one day. Similarly, the remaining horizons were extracted on other days. The 20 samples were then combined into one composite sample for each horizon on each tract, thus assuring a representative sampling. Prior to removing the individual samples, the paper containers used for collecting were sterilized to kill all organisms; the trowel used for extracting was cleaned and sterilized by ignition of alcohol each time a sample was taken. This was done in order to be certain of no contamination from other horizons.

Laboratory Technique

The laboratory procedure used in isolating the desired organisms from each soil and from each other divides itself into two parts:

1. Soil dilutions of each composite soil were dispensed and prepared as follows:

   The addition of 50 grams of soil to 500 cc. of sterile distilled water giving a dilution of 1:10. After the coarse particles had settled out after shaking, 10 cc. of the 1:10 dilution was pipetted into 90 cc. of sterile distilled water. Successive transfers were made resulting in a series of dilutions. These were 1:10, 1:100, 1:1000, 1:10,000, 1:50,000, 1:100,000 and 1:1,000,000. All work was done aseptically.

2. Selection of media and culturing procedure:

For bacteria, actinomycetes and fungi:

From the above dilutions 1 cc. of each was dispensed by sterile pipettes into a selective media and poured aseptically into sterile petri dishes. This mixture of agar and soil dilution was allowed to harden and the cultures were placed in the culture room at 28° C. The bacteria were permitted to grow one week before observations were made and the fungi were allowed four days before observing. The medium used for bacteria was Nutrose agar; for fungi a Peptone-glucose acid agar was used of pH between 3.8 and 4.0 so that bacteria would not develop. (See list of media used).

For protozoa:

In the case of isolating protozoa the medium was poured first into petri dishes and allowed to harden and the soil suspensions were added along with a small quantity of boiled and cooled tap water. Observations were made in twelve days after culturing at 28° C; the medium used was Ashby's.

For algae:

Soil suspensions of each dilution were added to flasks containing 90 cc. of sterilized

Detmer's medium. These flasks were placed in the greenhouse and examined every two days throughout the course of the study.

Number of replicates run:

In all cases where inoculations were made, glassware was thoroughly sterilized and the media used was autoclaved prior to each soil "run" in order to prevent contamination.

Six replicates of each dilution were run for the bacteria and fungi in addition to controls. With protozoa three replicates were run for each dilution plus controls and the algae were run singly with a control. Replicates were run to assure uniformity of procedures and results.

Moisture content:

Moisture contents were obtained for each horizon immediately before the isolations were made. This was done by heating the samples at 90° C. until all water was lost; the percentage figures obtained were indicative of the amount of water lost in each sample.

Acidity (pH) of the samples:

The relative acidities for each horizon were obtained by coloremetric tests.

Media Used in Culturing Organisms (See laboratory procedure)

Fungi - Peptone glucose acid agar

Agar	25.0 gms.
KH_2PO_4	1.0 gm.
$MgSO_4 \cdot 7H_2O$	0.5 gm.
Peptone	5.0 gms.
Glucose	10.0 gms.
Distilled Water	1.0 liter
Reaction pH	3.8 to 4.0 (adjusted)

Bacteria and actinomycetes - Nutrose agar

Agar	12.5 gms.
Nutrose	2.0 gms.
Glucose	1.0 gm.
K_2HPO_4	0.2 gm.
$MgSO_4 \cdot 7H_2O$	0.2 gm.
$FeSO_4 \cdot 7H_2O$	trace
Tap Water	1.0 liter
Reaction pH	6.8 (no adjustment)

Protozoa - Ashby's media

K_2HPO_4	0.2 gm.
$MgSO_4 \cdot 7H_2O$	0.2 gm.
NaCl	0.2 gm.
$CaCO_3$	5.0 gms.
$CaSO_4 \cdot 2H_2O$	0.1 gm.
Mannitol	10.0 gms.
Agar - agar	15 - 20 gms.
Tap Water	1.0 liter

Algae - Detmer's media

$Ca(NO_3)_2$	1.0 gm.
KCl	0.25 gm.
$MgSO_4 \cdot 7H_2O$	0.25 gm.
KH_2PO_4	0.25 gm.
$FeCl_3$	trace
Tap Water	3.0 liters

Approach to Interpretation of Results

Fungi: The results presented are primarily quantitative. For each dilution the numbers shown in the tables represent the number of colonies in each sample. Assuming that each colony developed from a single spore or hyphae, the number per gram can be computed by multiplying the number on the plate by the dilution. Adjustment with reference to moisture content must be made so that the number of organisms present are based on dry weight of the soil.

The fungus identification results are a minor qualitative result done in order to arrive at some idea of the genera represented in each horizon.

Bacteria: The results are entirely quantitative since no effort is made to identify the colonies. Actinomycete numbers are computed per gram based upon dry weight in identically the same way as the bacterial and fungal numbers.

Protozoa: An effort is made at identification so as to arrive at the presence or absence of protozoa. Their numbers per gram do not show any real correlation to results since their numbers fluctuate over short periods of time. Identification reveals the groups represented.

Algae: The results portray abundance in particular horizons with regard to presence or absence of algae; also identification shows what genera are represented.

Following the tables of fungus results is a photograph of the 1:1000 dilution which demonstrates how numbers of fungi vary with the two soils studied. Graphs supplement the data and show the results of average numbers plotted over horizon depth for each stand. Graphs have been made for fungi, bacteria and actinomycetes to reveal comparable trends and differences between the two stands.

Interpretation of Results

In order to show a relationship between the soil organisms of these two red oak forests, an explanation of the role of fungi in reference to what they do, is necessary. A relationship exists between the soil and forest growth and is quite variable and complex. The part that each group of organisms play in the study involved is presented, following a brief description of their role in general.

Numbers of Fungi in Forest Soils

Fungi are free of chlorophyll and derive their energy from the decomposition of dead or living matter, organic in character. Their prevalence in the soil is closely tied up with the presence or absence of decomposing organic matter.

They occur in soil either as free molds or as symbiotic fungi forming mycorrhizae with the roots of higher plants (9). The forms with which the present study is concerned are the ordinary filamentous fungi (Phycomycetes, Ascomycetes and the Fungi Imperfecti) which occur as free molds. Fungi occur abundantly in the soil, particularly in soils of high organic content where the acidity is high. This means that the fungi can withstand greater acidities than the bacteria and actinomycetes. It is rather difficult to estimate their abundance; it is even less possible to find a basis for comparing the relative abundance of fungi and bacteria in the soil and their capacity for causing a certain amount of transformation in the soil. Numbers of fungi will increase with moisture content provided there is a good aeration, and a supply of organic matter with proper soil reaction (7). The greatest numbers of fungi are found in the upper few inches of soil but will occur to a depth of at least four or five feet. It is evident that the role of fungi in forest soils cannot be over-emphasized. Fungi are largely responsible for the decomposition of proteins, cellulose, hemicellulose and most of the other carbohydrates (9). The composition of the fungus flora of the soil changes with a change in the nature of the soil, both quantitatively and qualitatively (7). It is important to note that although numerically, as determined by the number of single cells, the fungi are fewer in the soil than the bacteria, although

their actual abundance, as measured by the amount of cell substance produced, may be considerably greater than that of bacterial growth. This residue of mycelia adds to the nitrogenous content of the soil complex. As a rule, most fungi grow under aerobic conditions where most of the cell substance is produced.

Numbers and Genera of Soil Fungi in a Natural vs. Planted Red Oak Stand

Significant results (Refer to graph, photographs and numerical data)

1. The relative number of fungi occurring in both forest soils was markedly the same in all horizons studied, except in the A_0- Surface horizon, where the greatest number was found in the natural stand. This similarity can be seen by reference to the graphs and photographs illustrating the 1:1000 dilution.
2. The greatest number of fungi per gram of soil for both forest stands occurred in the A_1- 2" horizon, where the number of colonies rises to 118,560 per gram based on dry weight of the soil. This number of fungi represents the most reliable date of the dilutions made, and gives some idea of the numerical abundance of fungi.
3. The number of genera of fungi represented are similar in both stands at the surface level, A_0 ,

where the greatest difference in number of fungi occurs. The number of colonies in each genus was greatest in the natural forest stand.

4. Acidities at the A_1 - 2" horizons differ considerably between the two stands, yet the numbers of fungi per gram are approximately equal at this depth.
5. Moisture contents were nearly equal for both forest soils and very low.

Conclusions regarding fungus results:

Since there is no useful method yet devised to determine how much of the fungus material exists in the soil as mycelium or as spores by this isolation method, there is no way of determining what percentage of the colonies are due to spores and what percentage is due to pieces of mycelium. With this limitation in mind, the fact remains that the greatest number of fungi (spores and mycelium) were found at the A_1 - 2" horizon in both forest stands. Thus, no direct correlation between these numbers and the potential activity of the horizon can be made, unless it can be determined what percentage of these colonies are spores and mycelium respectively. The decompositional activity and exchange reactions of the particular horizon will depend upon the percentage of viable cells not actually entering into the reaction, and upon their functions as viable cells instead of mycelia. However, indications point to the fact that greatest activity occurs in the A_1 - 2" horizon in both

stands insofar as the statistical results are correct. Two other facts help to bear out this conclusion. The pH values differ for both stands at this level yet the numbers remain comparatively the same; also the moisture contents are equal at this horizon. Thus, the quantitative difference must be attributed to some other factor than those of acidity or content of moisture. Here again, numbers alone cannot verify the entire process but are merely aids to indications.

Since soil fungi are active in the decomposition of proteins and various complex carbohydrates such as cellulose and hemicelluloses, their numbers at the A_1- 2" level may be greater due to these substances being present in more available forms. In addition, the results indicate that the quantitative differences at the surface horizon, A_0 , are greater in the natural stand where the age differential is 4 to 1, thus allowing a longer period of time for more complete decomposition. Similarly, as with the A_1- 2" horizon , the moisture content and pH values do not differ appreciably. Supporting this contention is the fact that the results show that in the horizons below the A_1- 2" depth, the numbers of fungi fall off in both stands with depth, thus indicating less organic activity at the lower levels.

The genera represented in both stands at the A_0- Surface horizon do not vary considerably. In both instances (natural and planted stands) there were only three genera represented, Mucor, Zygorhynchus and Penicillium. The latter genus had

the greatest relative number in both stands at this horizon, the greatest number of colonies occurring in the natural forest stand. At the A_1-2" level two more genera were present, Sporotrichum and Aspergillus, in addition to the above three genera.

To date, more than sixty genera of fungi have been reported to be found in the soil and probably many more could be demonstrated except that the methods are not sufficiently developed as yet to isolate and identify certain organisms. In interpreting the results found here a more thorough discussion of the physiology of these fungi would be needed in order to make a more detailed correlation between their presence and their numbers. The presence of a certain organism in large numbers does not necessarily indicate its great abundance in the soil, but may be a result of local development from abundant spore formation. A physiological study of each organism in relation to its energy requirements would give a better idea of the specificity of the organism, but even then many variables would be present. Before any claim could be made as to the reason for a particular genus of fungus being present at one horizon and absent in another, a study would have to be repeated many times under differing conditions; even then the results would be limited.

However, in light of the results obtained it is apparent that the great difference in numbers of organisms

in the A_0- Surface horizon between both stands cannot be attributed entirely to a physiological difference in the genera represented since the genera are the same in both forest stands. This is, nevertheless, a possibility since fungi are more resistant to acidity than the other groups of organisms studied; but since the pH values at the surface do not vary appreciably in the case study, there is a possibility that the genera represented are a result of some other factor. No direct correlation can be made between the genera represented and the numbers found at corresponding horizons in this study.

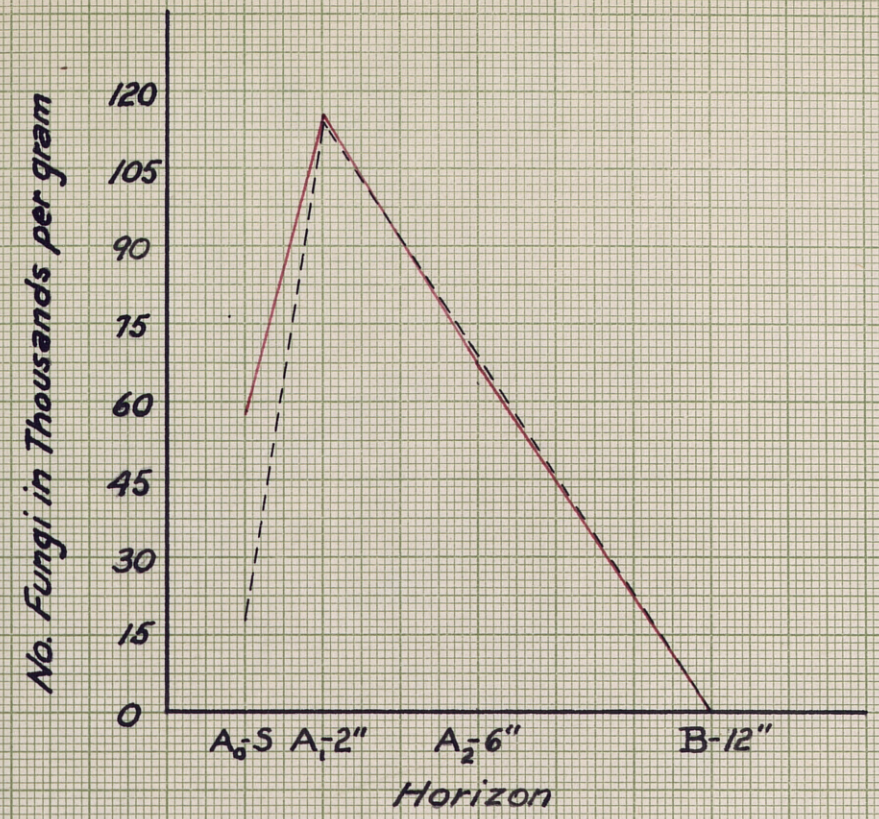
Thus, from the experimental results obtained, indications are to the effect that differences in numbers of soil fungi between a natural and a planted red oak forest stand, are more related to the availability of oxygen and organic matter as a result of decomposition processes than to a pH or moisture content relationship. First, this number difference may be attributed to the age differential of the stands involved since the moisture contents and pH values were fairly constant for both stands at the levels indicated. This is not to infer that the number difference is a direct result of the pH value, since fungi occur in a range of acidities; the range in the case study differ considerably, yet the numbers are nearly equal. Secondly, the number of colonies is related to the amount of organic matter (high in this study) and to the soil transformations and availability

of energizing organic materials. Thirdly, the numbers of fungi fall off with depth in both stands which is closely tied up with the amount and availability of nitrogenous and non-nitrogenous organic matter and oxygen.

It can be stated from the study involved, that the greatest numbers of fungi in both a natural and a planted red oak forest stand, occurred in the A_1- 2" horizon. Also, the greatest difference in numbers of soil fungi between a natural and a planted red oak forest stand, occurred in the A_0- Surface horizon, where the larger number was found in the natural stand.

Numbers alone fail to reveal the role of microorganisms in regard to forest growth; as such, numerical results used in conjunction with certain constants, are indicators of soil activity insofar as chemical, physical and biological changes are concerned. From the results obtained it is apparent that the fungus flora and fauna of the soil will vary with a change in the nature of the soil, both quantitatively and qualitatively.

Number of Soil Fungi in Natural vs. Planted Red Oak Forests



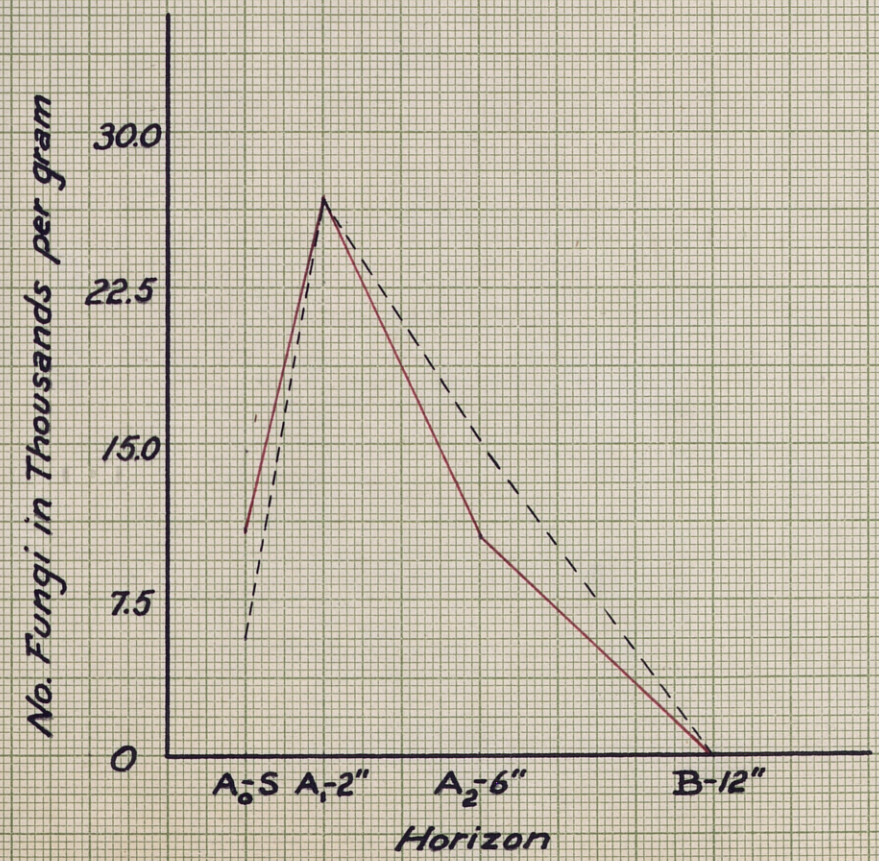


TABLE IV

NUMBER AND GENERA OF FUNGI IN A NATURAL AND A PLANTATION RED OAK FOREST AT VARIOUS DEPTHS

Natural Forest Soil

A_0 Surface Horizon

Dilution and Numbers			
Sample	1:1000*	1:10,000	1:50,000
1	56	14	2
2	74	0	2
3	59	15	2
4	57	8	2
5	61	13	1
6	47	12	4
Avg. no. colonies	59	10	2

Soil moisture content 5%
Soil pH 6.5

Dilution and Genera					
1:1000		1:10,000		1:50,000	
Penicillium	25	Penicillium	13	Penicillium	3
Zygorhynchus	7	Zygorhynchus	4	Zygorhynchus	1
Mucor	6	Mucor	3	Mucor	1

Date of culturing - December 7, 1946
Date of observation - December 11, 1946

Note: Average number of colonies is based upon dry weight of the soil.

$$\text{No. colonies per gm. dry wt.} = \frac{\text{Avg. no. colonies x dilution x 100}}{\text{100 - Moisture content}}$$

* See photograph

TABLE IV (cont.)

NUMBER AND GENERA OF FUNGI IN A NATURAL AND A PLANTATION RED OAK FOREST AT VARIOUS DEPTHS

Plantation Forest Soil

A_0 Surface Horizon

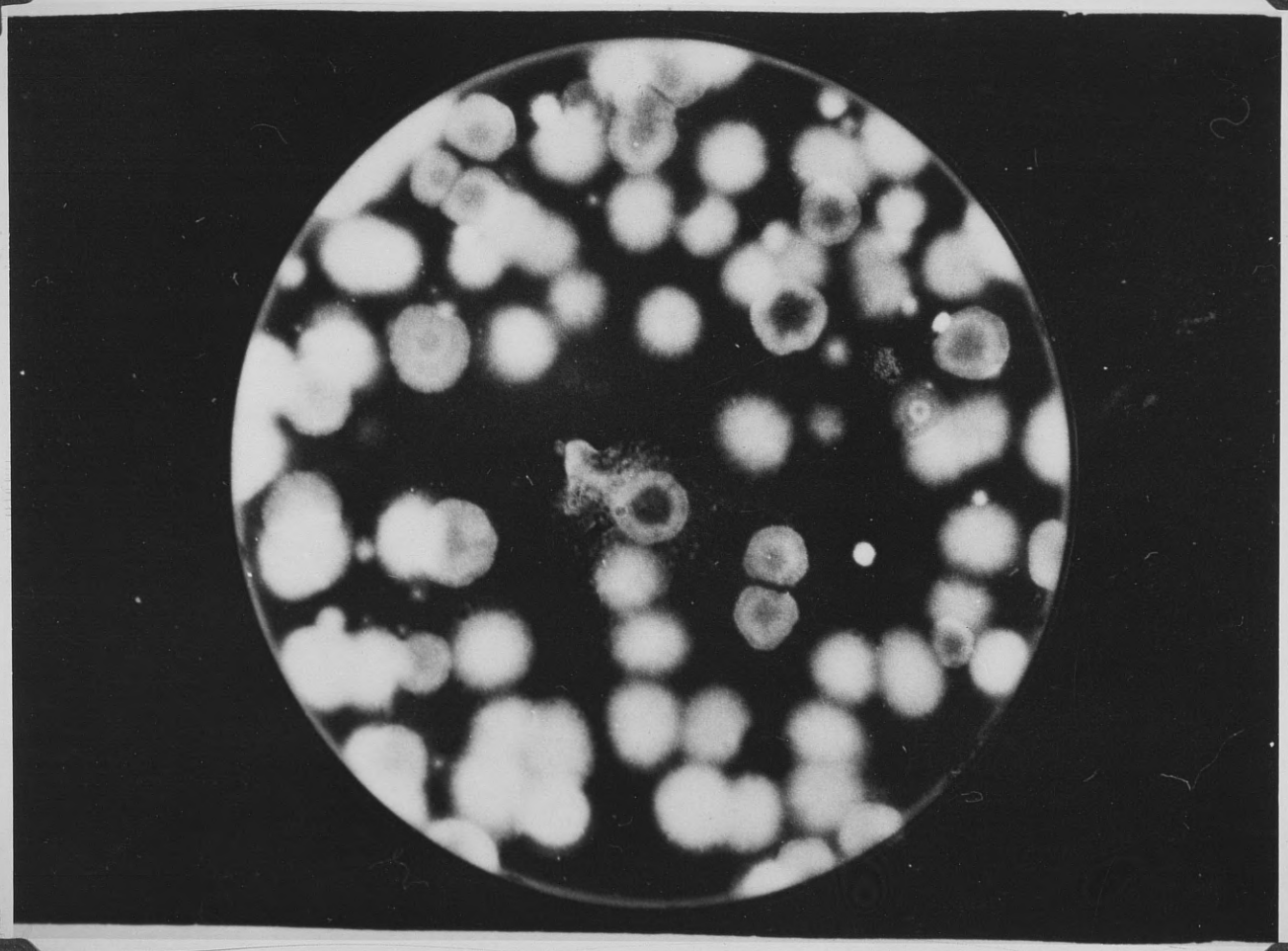
Dilution and Numbers			
Sample	1:1000*	1:10,000	1:50,000
1	19	12	0
2	18	3	0
3	27	8	0
4	16	6	0
5	12	5	0
6	14	7	0
Avg. no. colonies	18	5	0

Soil moisture content 4%
Soil pH 7.0

Dilution and Genera		
1:1000	1:10,000	1:50,000
Penicillium 11	Penicillium 7	Absent
Zygorhynchus 2		
Mucor 2		

Date of culturing - December 7, 1946
Date of observation - December 11, 1946

* See photograph



Natural Forest ↑ A_o - SURFACE HORIZON Plantation Forest ↓

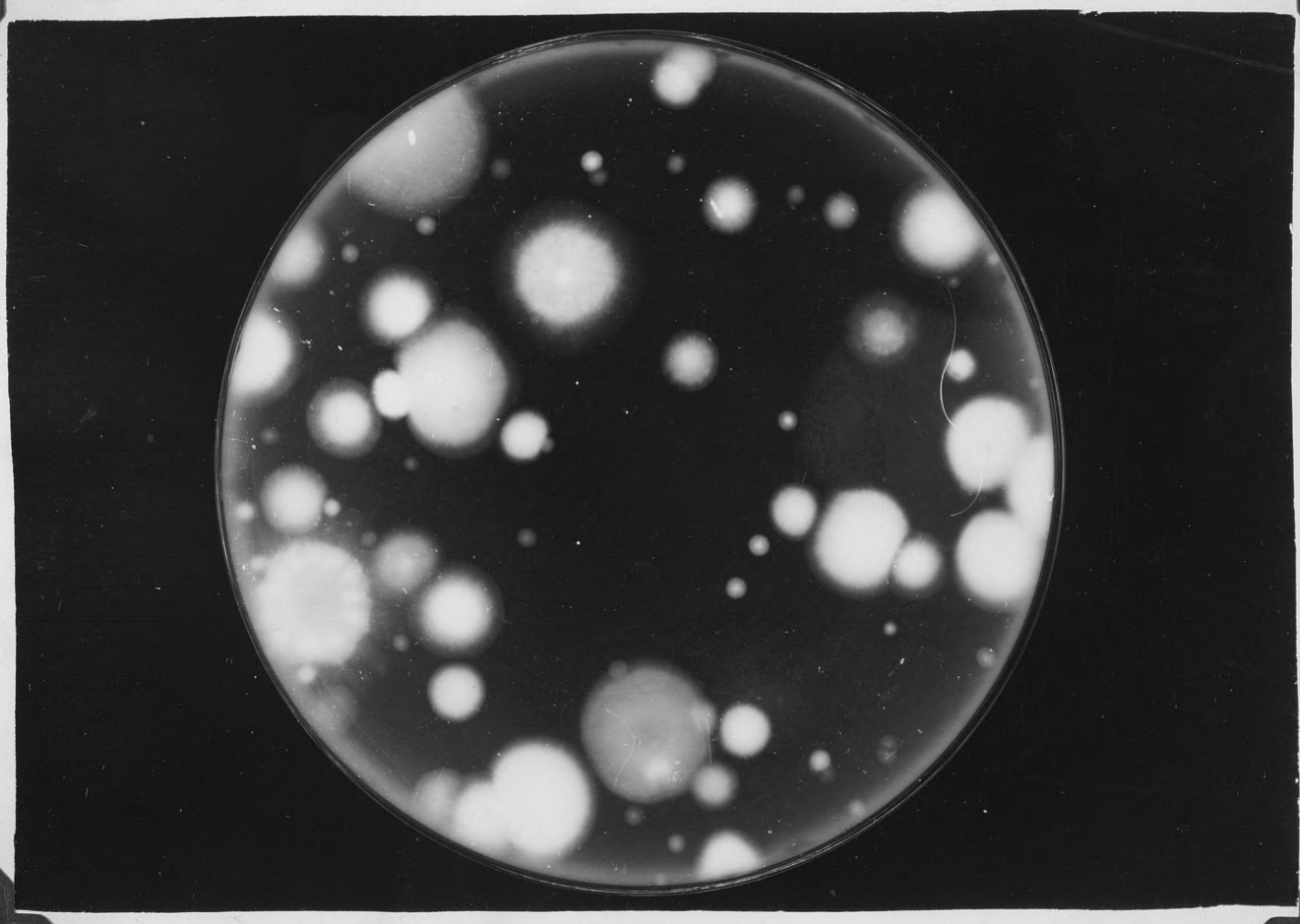


TABLE IV (cont.)

NUMBER AND GENERA OF FUNGI IN A NATURAL AND A PLANTATION RED OAK FOREST AT VARIOUS DEPTHS

Natural Forest Soil

A_1-2* Horizon

Dilution and Numbers			
Sample	1:100	1:1000*	1:10,000
1	Too high for count	114	25
2		110	20
3		108	29
4		118	24
5		122	26
6		112	28
Avg. no. colonies		114	25.3

Soil moisture content 4%
Soil pH 7.0

Dilution and Genera		
1:100	1:1000	1:10,000
Too high for ident.	Penicillium 85 Zygorhynchus 5 Mucor 1	Penicillium 21 Zygorhynchus 1 Sporotrichum 1

Date of culturing - December 26, 1946
Date of observation - December 30, 1946

* See photograph

TABLE IV (cont.)

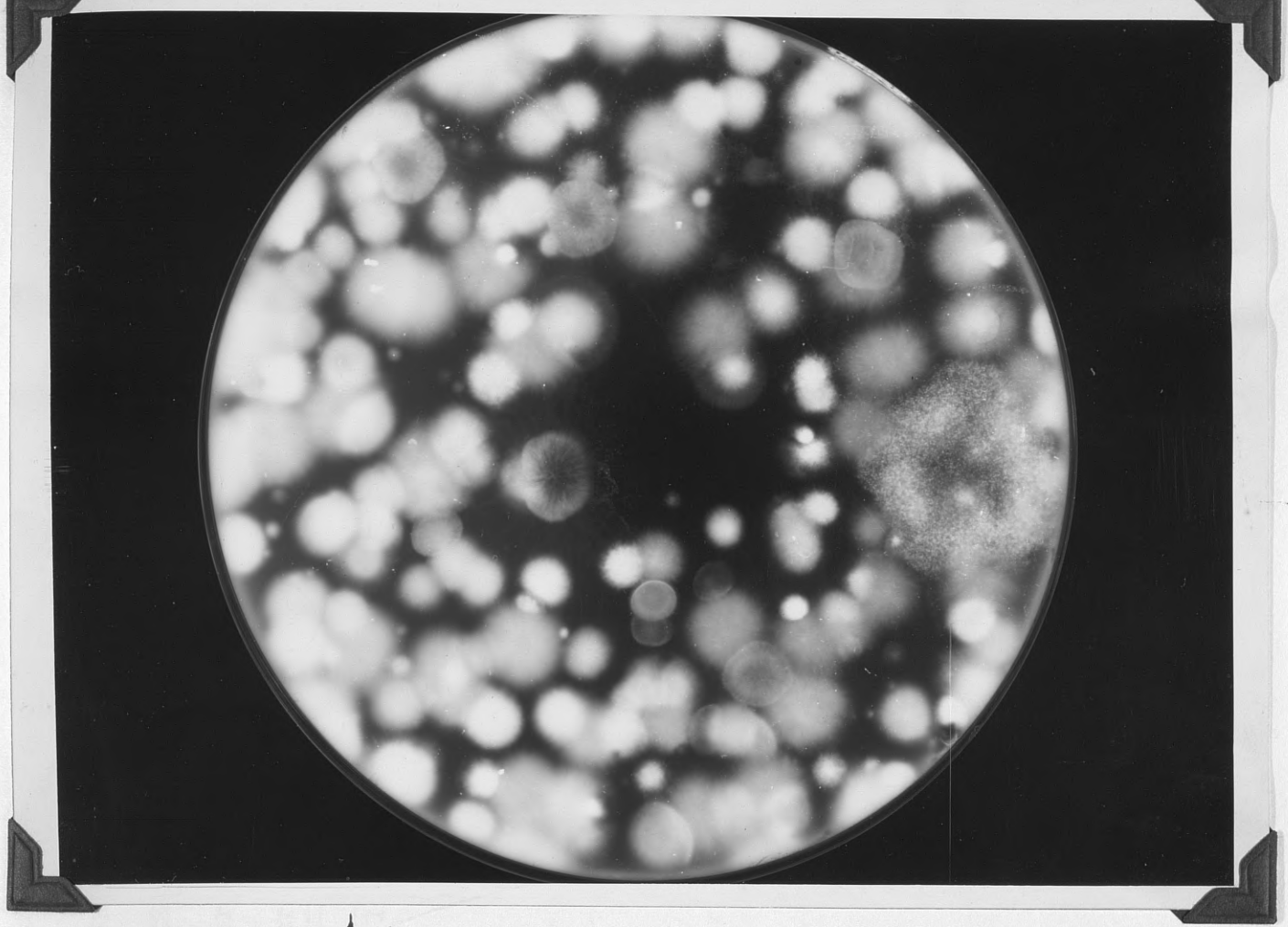
NUMBER AND GENERA OF FUNGI IN A NATURAL AND A PLANTATION RED OAK FOREST AT VARIOUS DEPTHS

Plantation Forest Soil

A_1-2" Horizon

Dilution and Numbers				Dilution and Genera		
Sample	1:100	1:1000*	1:10,000	1:100	1:1000	1:10,000
1	Too	113	28	Too	Penicillium 83	Penicillium 23
2	high	109	25	high	Mucor 4	Mucor 2
3	for	118	30	for	Zygorhynchus 3	Aspergillus 1
4	count	108	29	ident.		
5		122	27			
6		111	20			
Avg. no. colonies		113.1	26.5			
Soil moisture content 4%				Date of culturing - December 26, 1946		
Soil pH 5.5				Date of observation - December 30, 1946		

* See photograph



Natural Forest ↑ A_1 - 2" HORIZON Plantation Forest ↓

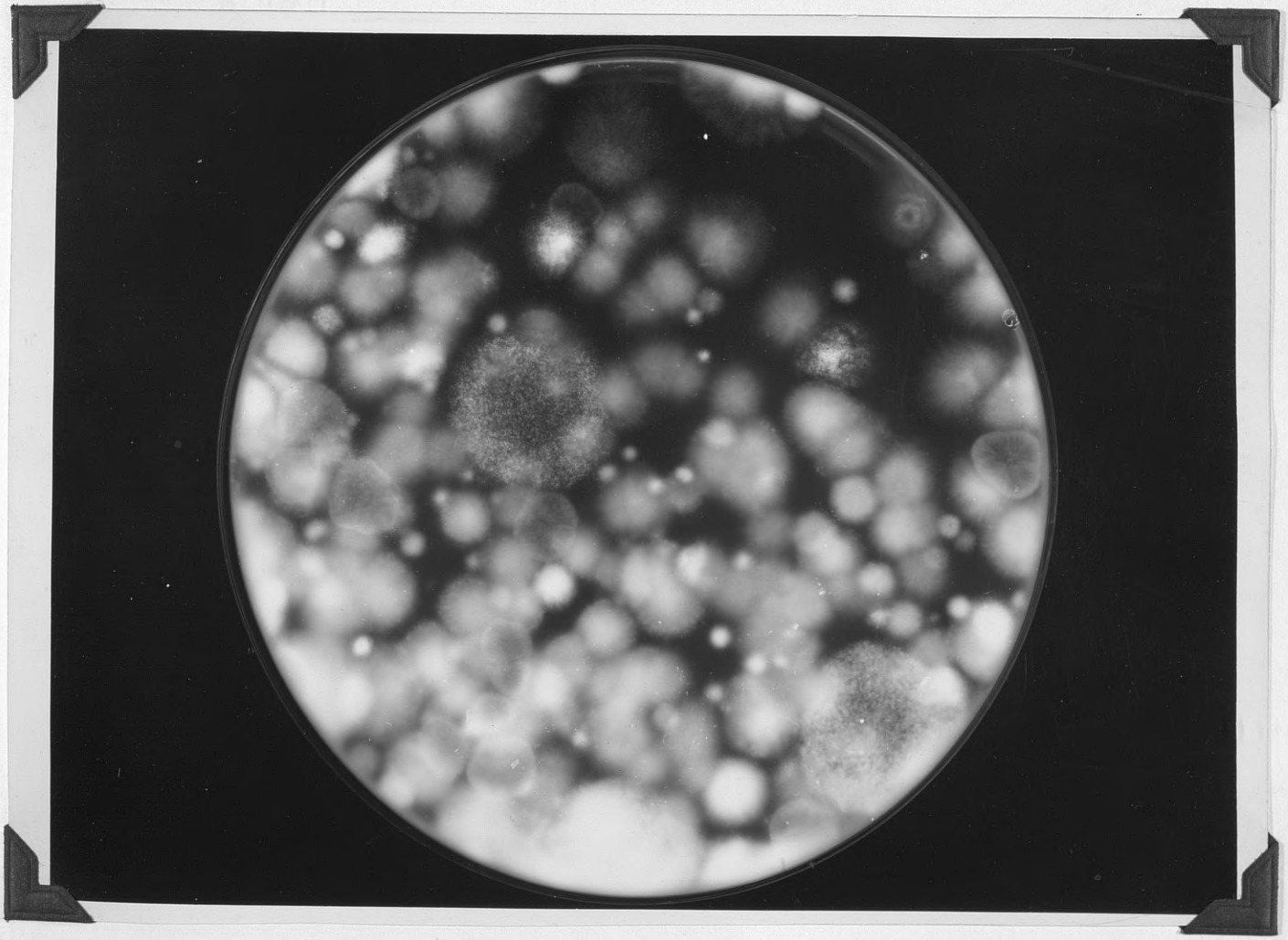


TABLE IV (cont.)

NUMBER AND GENERA OF FUNGI IN A NATURAL AND A PLANTATION RED OAK FOREST AT VARIOUS DEPTHS

Natural Forest Soil

A_2-6" Horizon

Dilution and Numbers			
Sample	1:100	1:1000*	1:10,000
1	Too	65	11
2	high	59	5
3	for	76	7
4	count	80	11
5		60	8
6		69	12
Avg. no. colonies		68.1	9

Dilution and Genera		
1:100	1:1000	1:10,000
Too high for ident.	Penicillium 56 Absidia 3 Zygorhynchus 1	Penicillium 7 Absidia 1

Soil moisture content 5%
Soil pH 5.5

Date of culturing - February 15, 1947
Date of observation - February 19, 1947

* See photograph

TABLE IV (cont.)

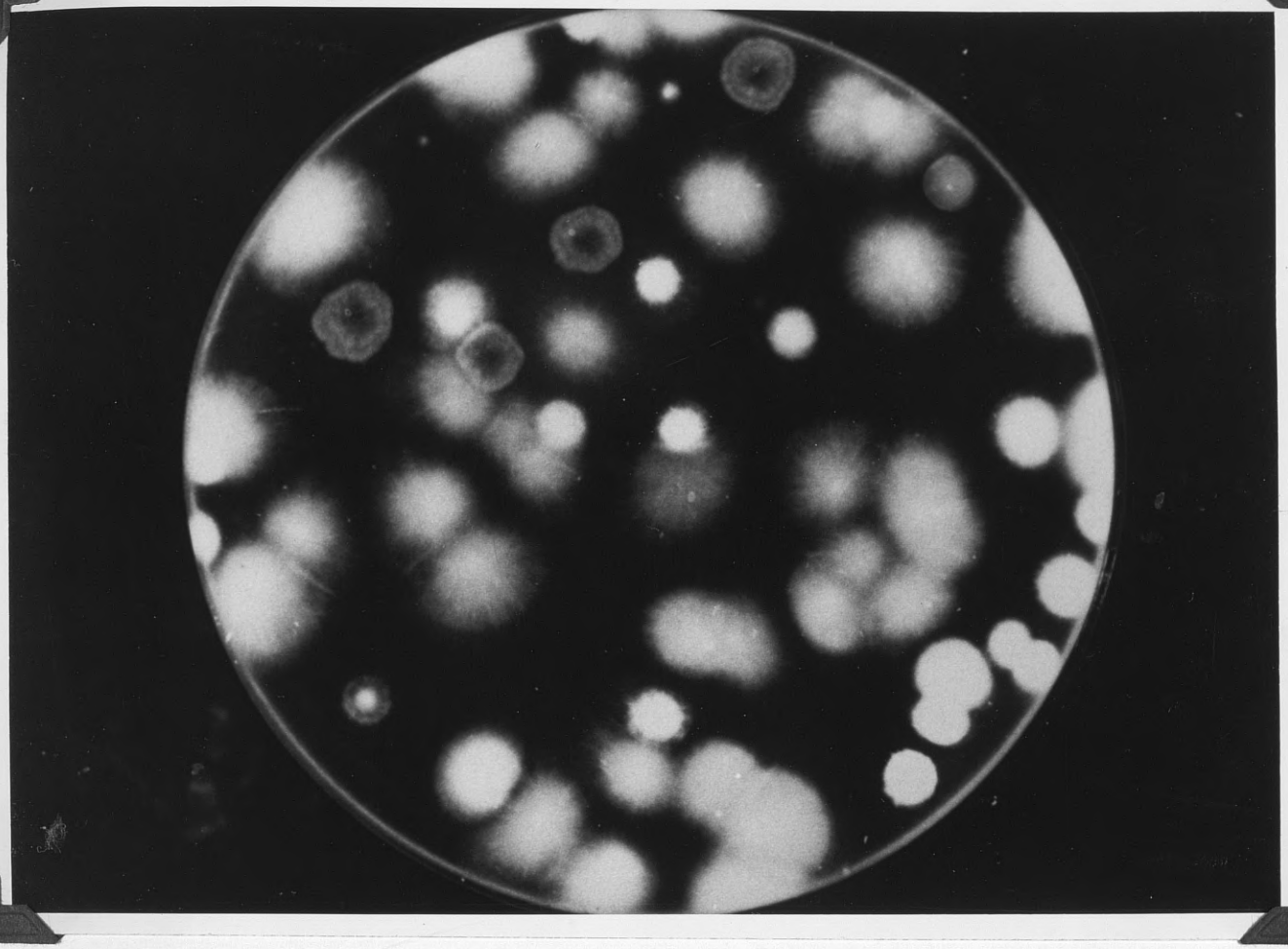
NUMBER AND GENERA OF FUNGI IN A NATURAL AND A PLANTATION RED OAD FOREST AT VARIOUS DEPTHS

Plantation Forest Soil

A_2-6* Horizon

Dilution and Numbers				Dilution and Genera		
Sample	1:100	1:1000*	1:10,000	1:100	1:1000	1:10,000
1	Too	71	17	Too high for ident.	Penicillium 62	Penicillium 10
2	high	73	17		Zygorhynchus 1	Zygorhynchus 1
3	for	66	14			Pachybasium 1
4	count	64	9			
5		77	20			
6		63	16			
Avg. no. colonies		69	15.5			
Soil moisture content 4%				Date of culturing - February 15, 1947		
Soil pH 5.0				Date of observation - February 19, 1947		

* See photograph



Natural Forest ↑ A_2 - 6" HORIZON Plantation Forest ↓

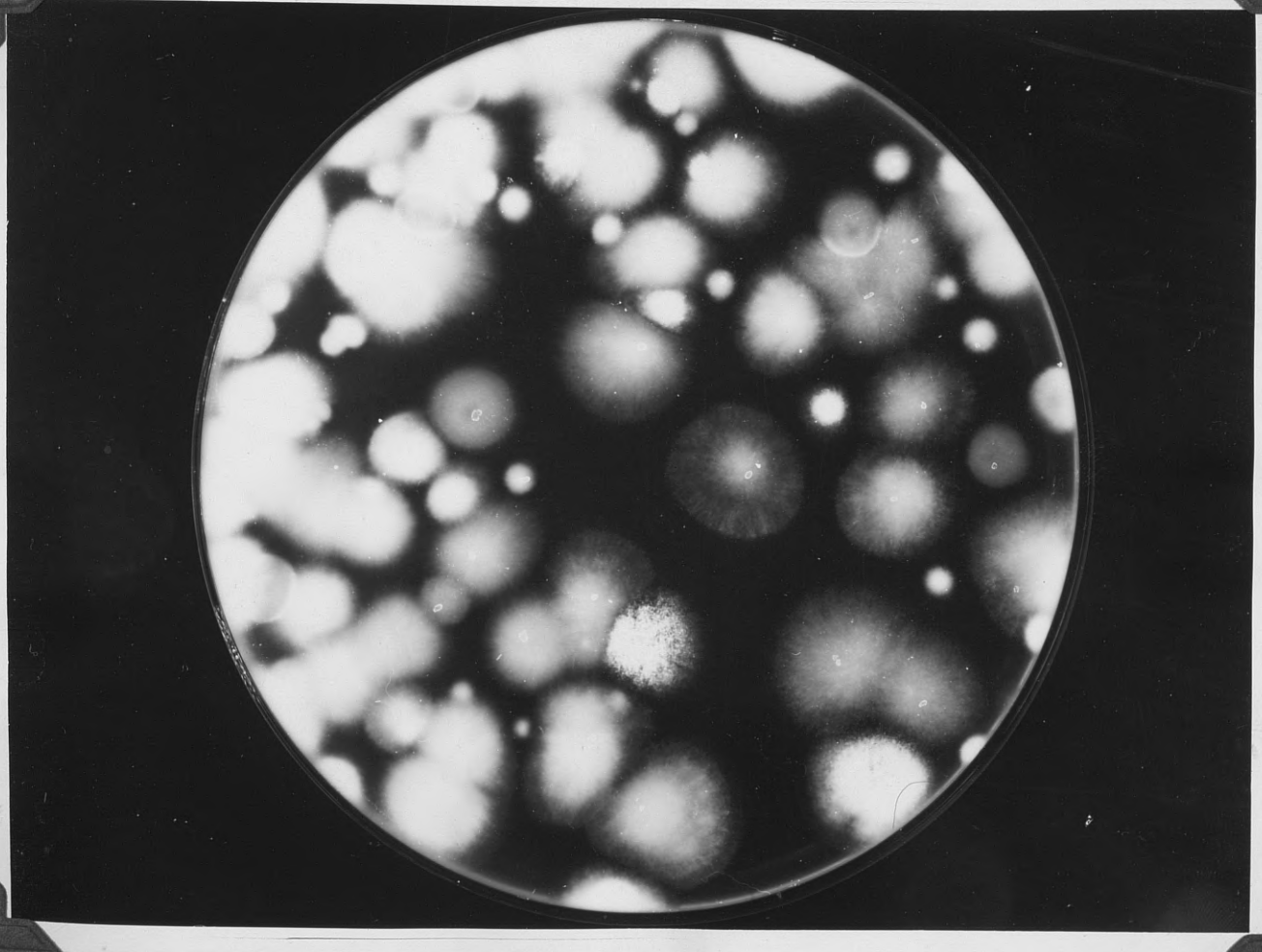


TABLE IV (cont.)

NUMBER AND GENERA OF FUNGI IN A NATURAL AND A PLANTATION RED OAK FOREST AT VARIOUS DEPTHS

Natural Forest Soil

B-12* Horizon

Dilution and Numbers			
Sample	1:1000*	1:10,000	1:100,000
1	1	0	0
2	0	0	0
3	1	0	0
4	0	0	0
5	0	0	0
6	1	0	0
Avg. no. colonies	.5	0	0

Soil moisture content 5%
Soil pH 5.0

Dilution and Genera		
1:1000	1:10,000	1:100,000
Penicillium 3	Absent	Absent

Date of culturing - March 8, 1947
Date of observation - March 12, 1947

* See photograph

TABLE (cont.)

NUMBER AND GENERA OF FUNGI IN A NATURAL AND A PLANTATION RED OAK FOREST AT VARIOUS DEPTHS

Plantation Forest Soil

B-12* Horizon

Dilution and Numbers			
Sample	1:100	1:1000*	1:10,000
1	7	0	0
2	10	0	0
3	9	1	0
4	7	1	0
5	10	2	0
6	11	1	0
Avg. no. colonies	9	8	0

Dilution and Genera		
1:100	1:1000	1:10,000
Penicillium 8	Penicillium 2	Absent
Absidia 1		

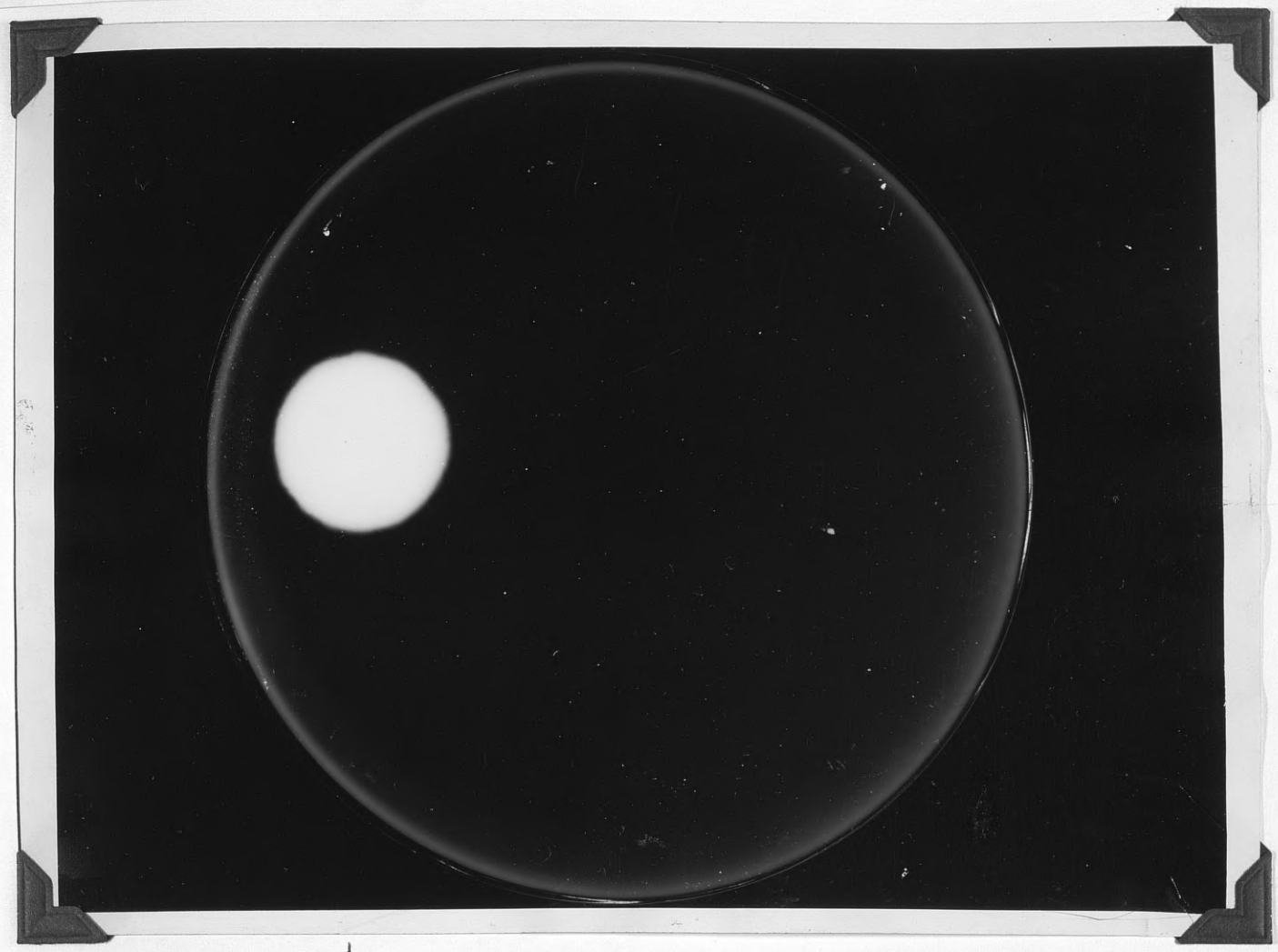
Soil moisture content 2%

Soil pH 5.0

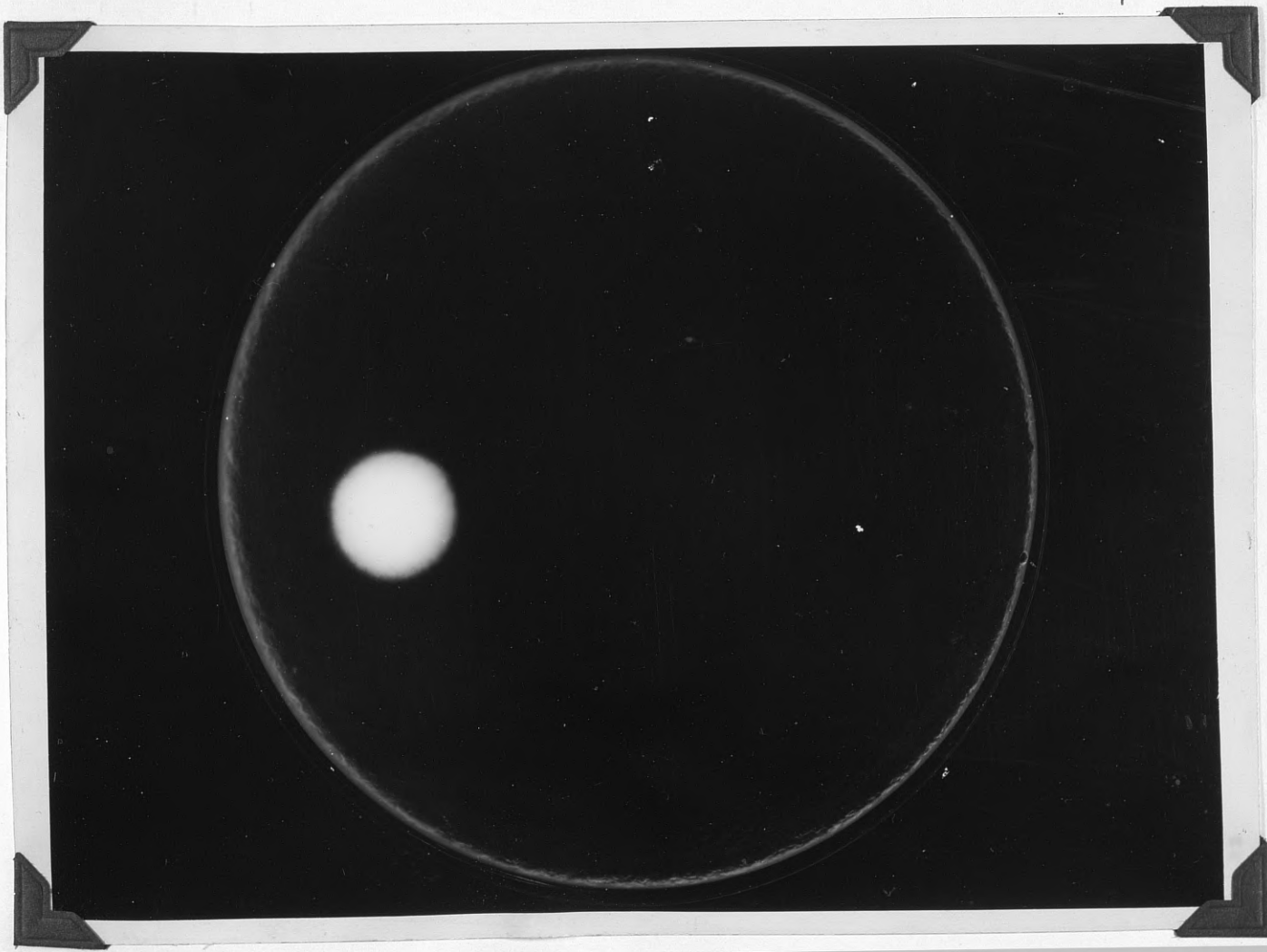
Date of culturing - March 8, 1947

Date of observation - March 12, 1947

* See photograph



Natural Forest ↑ B - 12" HORIZON Plantation Forest ↓



Numbers of Bacteria and Actinomycetes in Forest Soils

Bacteria as a group are the most abundant organisms in the soil. In their activities and numbers they exceed all other soil organisms except in the bulk of organic cell substance found in the soil as living and dead microbes. It is not exceptional to find hundreds of millions of bacterial cells in a gram of soil, particularly where the organic substances are present (8).

In this study the bacteria were isolated with the actinomycetes and it is appropriate to present them together since microscopically the spores of the actinomycetes appear like bacterial cells. However, as a group, they may be considered distinct because they have characteristics that resemble both fungi and bacteria. The bacteria are divided into two main groups based upon their method of deriving energy. These two main divisions are the autotrophic and the heterotrophic bacteria; the first named group derive their energy from the oxidation of simple organic substances and the latter derive their energy from complex organic substances. In the general subdivision of the soil bacteria the basis for classification is either upon their physiological activity or upon their morphological relationships. This study is not concerned with a specific study of these characteristics; no attempt has been made to segregate individual bacteria as to physiology and morphology; the relative numbers of bacteria and actinomycetes at different

horizons is the chief concern of the study. It is sufficient to say that studies in nitrogen fixation , autotrophic and heterotrophic bacteria, transformations of energy by bacteria, decomposition by bacterial activity and reduction processes are studies within themselves. Most of the bacteria developing on the plate method are heterotrophic, requiring combined nitrogen although still larger numbers may develop slowly or not at all.

Lohnis came to the conclusion that a determination of bacterial numbers in the soil is worthless as an attempt in interpreting soil phenomena (4). However, when the results are considered critically and compared carefully, it is found that the information obtained from the studies of numbers of microorganisms gives not only an interesting insight into the microbiological population of the soil, but also throws light on soil fertility (7).

Numbers of soil bacteria as determined by the plate method may vary with the soil type, season of the year, depth, moisture content and various environmental conditions. Soils from the same locality must be compared on the basis of knowledge concerning the physical and chemical condition of the soil, its origin and treatment. In comparing numbers of bacteria no single variable such as season of the year, can be considered alone without reference to moisture content, acidity and other factors (7). Most of the bacteria in the soil

develop best at reactions of acidity close to neutral (pH 7.0). The amount of free air with possible access to bacteria may determine the type of heterotrophic bacteria that develop, either aerobic or anaerobic. Temperature close to 20°- 30°C is favorable to growth of most bacteria of the soil though many forms will develop at higher temperatures. As far as moisture content is concerned, all bacteria require a considerable supply of water for active development and is often a limiting factor in numbers as well as kinds produced. These above mentioned optimum conditions should be kept in mind in any comparative study; in numbers computation it is important to know not only what the bacteria will do, but also under what conditions they are transforming organic and inorganic matter.

The media upon which the bacteria will grow is also well adapted for the growth of actinomycetes and their numbers are computed in comparison with the bacterial count. Since the actinomycetes are next to the bacteria in number of forms developing on the plate method they are important in the addition of mycelia to the soil, as are the fungi. Here again, large numbers do not necessarily indicate more abundant growth, but a result of a larger abundance of conidia (7). Actinomycetes are abundant in forest soils and the percentage of them in relation to the number of bacteria is important insofar as decomposition of organic matter in the soil is concerned. It is important to note that with an increase in

depth the actinomycetes decrease in number, but they increase in proportion to the other microorganisms. They are very sensitive to acidity and to an excess of moisture and the numbers decrease in soils devoid of decomposed material, in comparison with the bacteria. As a general rule, the less acid the soil, the higher is the relative abundance of actinomycetes. Most of the actinomycetes are aerobic and as a rule are more sensitive to changes in reaction and live over a narrower range of acidity and alkalinity than the bacteria (8).

Numbers of Bacteria and Actinomycetes in a Natural vs. Planted Red Oak Stand

Significant results (Refer to graphs and numerical data)

1. The numbers of bacteria per gram of soil is very low in all horizons of both forest soils.
2. The numbers of bacteria in both stands for all horizons is strikingly similar except at the B-12" horizon.
3. Numbers of bacteria per gram decrease with depth in both forest soils; using the most reliable dilution as observed data it was found that the greatest numbers of bacteria are found at the A_0- Surface horizon in both soils.
4. The number of actinomycetes per gram of soil is greatest in the natural stand in all horizons except the B-12" horizon.

5. In the natural forest stand actinomycete numbers are significantly great at the A_1- 2" and A_2- 6" horizons. Of these two horizons the greatest number occurs at the A_1- 2" level.
6. In the plantation forest soil the number of actinomycetes falls off gradually with an increase in depth for all horizons.
7. The percentage of actinomycetes in relation to the number of bacteria is very high in both forest soils. This percentage ratio difference increases with depth for the natural stand but for the plantation stand it is highest at the 2" and 6" levels.

Conclusions regarding bacteria and actinomycete results:

The relative numbers of bacteria in comparable forest soils may be altered by many variables. Certain considerations for number differences are given in reference to the variables present. For example, the season of the year is a factor in variation of bacterial numbers at the same depths. In the specific case studied the numbers of bacteria are very low in both stands. As the soils were collected at a time of the year (autumn) when the numbers of microbes are often highest, the low bacterial counts were not anticipated. It is not possible to isolate this factor as a single variable so it is considered in light of moisture content, acidity and other factors. The soil moisture contents for all horizons

were very low at this period of the year. In considering moisture content as a factor, the numbers of bacteria may be lower as a result of the actinomycete number being high since it is known that actinomycetes tolerate drier soils than do most bacteria. Few bacteria may be found in the soil at the end of a long dry period; the soils collected did not exceed in any horizon, more than 5% moisture content. In addition to this, the soils of acidity closest to neutrality will often give highest numbers of bacteria; the range of acidities in the results studied reveal that acidities ranged from pH 7.0 to 5.0, with only two horizons showing neutrality. These two factors, moisture content and acidity probably play the most important role in the resulting low bacterial count.

The similarity in numbers of bacteria in all horizons for both forest stands is striking, except at the B-12" horizon. This fact is even more significant since the age differential between the two stands is quite large. The similarity in numbers between the two forest soils is thus intimately related to comparable moisture contents and acidities within similar horizons.

Both soils indicate highest numbers of bacteria at the surface horizon with a gradual decrease in numbers with increased depth. Soils under shade (forest) have the highest numbers of bacteria at a depth of one inch, then the numbers decrease with depth (7). This fact is definitely indicated

in the results; the 2 inches of forest litter is undoubtedly a factor, along with the canopy of the tree crowns, in preventing the germicidal effect of the sun's rays from evaporating moisture directly at the surface. In more arid soils without such a protective layer, the numbers of bacteria at the surface would be less, due to desiccation effects. Thus, the results show that greatest bacterial activity is at or close to the surface in both red oak forest stands. In more arid soils the bacterial activity would penetrate much deeper into the subsoil.

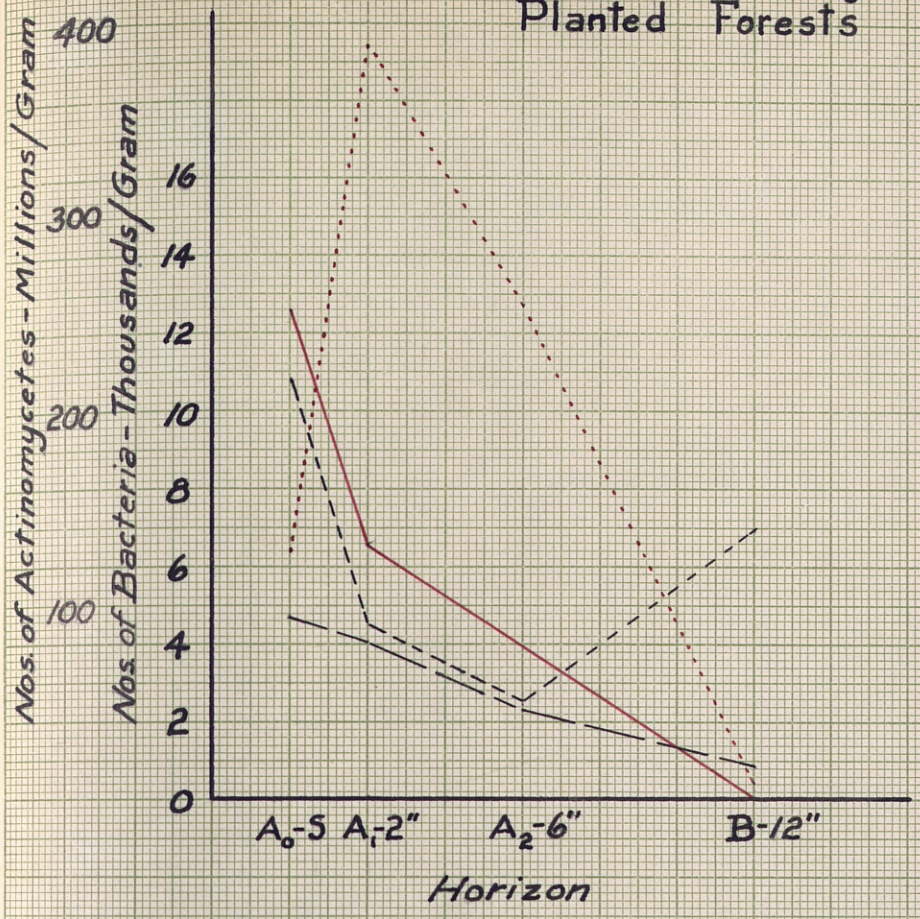
In regards to the actinomycetes, the greatest numbers are found in the natural forest stand at all horizons except the B-12" level. Hiltner and Stormer observed an increase in the numbers of actinomycetes in the autumn, relative to the other groups of microorganisms, due to the increase of the content in undecomposed organic matter in the soil (3). Investigators have shown that there is a higher percentage of actinomycetes in the fall over other microbes developing on plates. In forest soils such as the ones here observed, the percentage of actinomycetes in relation to bacteria is extremely high. Since the moisture contents and pH values do not vary considerably between the two soils at comparable depths, the larger number of actinomycetes found in the natural stand may be due to a richer accumulation of undecomposed organic matter available at corresponding horizons. Similarly, the fungi and bacteria showed some increase in

numbers in the natural forest soil but perhaps these organisms could not utilize the organic debris because of the arid conditions.

The number of actinomycetes in the plantation stand fall off rapidly with increased depth in all horizons in contrast to the natural stand where numbers increase with depth up to A_2- 6" level. A lack of undecomposed organic matter in the plantation stand is an important consideration here; this planted forest was begun on once cultivated land and the age or maturity of the forest is not far enough advanced to create a mature organic horizon. Actinomycetes are closely related to fungi and produce mycelia that require organic remains for energy requirements; the plantation stand is not far enough along in age organically to provide an abundance of organic matter as in the natural, more aged forest.

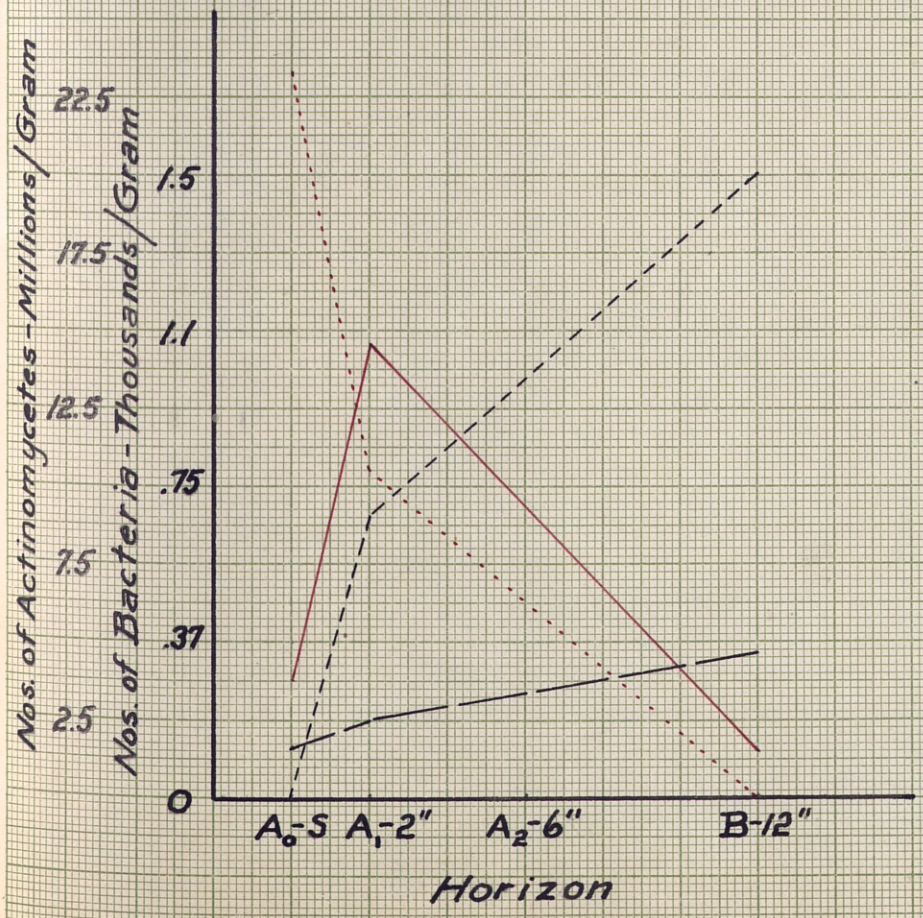
As depth increases the number of actinomycetes increases in proportion to the numbers of bacteria, in the natural forest soil. It is not known whether this is due to the washing down of the conidia, to the greater resistance of these organisms to the lack of oxygen, or to some other cause (7). In the results obtained in this study consideration is being given to the fact that there is less organic undecomposed matter in the plantation forest. The results show a greater increase proportionately in the natural stand than in the plantation stand; it is the author's contention that the greater proportionate increase is due to a greater amount of available oxygen and organic matter.

Numbers of Bacteria and Actinomycetes in Natural vs. Planted Forests



Bacteria:
——— Natural
- - - - - Planted

Actinomycetes:
····· Natural
— — Planted



Bacteria:
——— Natural
- - - - - Planted

Actinomycetes:
····· Natural
— — Planted

TABLE V (cont.)

NUMBER OF EUBACTERIA AND ACTINOMYCETES IN A NATURAL AND A PLANTATION RED OAK FOREST AT VARIOUS DEPTHS

Natural Forest Soil

A_0 Surface Horizon

	Dilution and Number Per Gram					
	1:10,000		1:100,000		1:1,000,000	
Sample	Eubacteria	Actinomycetes	Eubacteria	Actinomycetes	Eubacteria	Actinomycetes
1	17	111	0	56	0	1
2	17	132	1	6	0	0
3	0	169	0	67	0	1
4	16	166	0	7	0	0
5	15	101	1	4	0	2
6	10	112	0	1	0	0
Avg. no. colonies	12.5	131.3	0.3	23.5	0	0.6

Soil moisture content 5%

Soil pH 6.5

Date of culturing - December 7, 1946

Date of observation - December 14, 1946

TABLE V

NUMBER OF EUBACTERIA AND ACTINOMYCETES IN A NATURAL AND A PLANTATION RED OAK FOREST AT VARIOUS DEPTHS

Plantation Forest Soil

A_0 Surface Horizon

	Dilution and Number Per Gram					
	1:10,000		1:100,000		1:1,000,000	
Sample	Eubacteria	Actinomycetes	Eubacteria	Actinomycetes	Eubacteria	Actinomycetes
1	22	93	0	11	0	0
2	14	95	0	0	1	0
3	12	107	0	0	0	2
4	0	106	0	0	0	0
5	9	91	0	0	0	0
6	8	81	0	0	0	0
Avg. no. colonies	10.8	95.5	0	1.8	0.1	0.3

Soil moisture content 4%

Soil pH 7.0

Date of culturing - December 7, 1946

Date of observation - December 14, 1946

TABLE V (cont.)

NUMBER OF EUBACTERIA AND ACTINOMYCETES IN A NATURAL AND A PLANTATION RED OAK FOREST AT VARIOUS DEPTHS

Natural Forest Soil

A_1-2" Horizon

	Dilution and Number Per Gram					
	1:1000		1:10,000		1:100,000	
Sample	Eubacteria	Actinomycetes	Eubacteria	Actinomycetes	Eubacteria	Actinomycetes
1	Too	Too	9	450	2	11
2	high	high	6	375	2	6
3	for	for	6	390	1	11
4	count	count	7	380	0	15
5			5	350	1	16
6			7	400	0	5
Avg. no. colonies			6.6	390.8	1.0	10.6

Soil moisture content 4%

Soil pH 7.0

Date of culturing - December 26, 1946

Date of observation - January 2, 1947

TABLE V (cont.)

NUMBER OF EUBACTERIA AND ACTINOMYCETES IN A NATURAL AND A PLANTATION RED OAK FOREST AT VARIOUS DEPTHS

Plantation Forest Soil

A_1-2" Horizon

	Dilution and Number Per Gram					
	1:1000		1:10,000		1:100,000	
Sample	Eubacteria	Actinomycetes	Eubacteria	Actinomycetes	Eubacteria	Actinomycetes
1	Too	Too	8	78	1	4
2	high	high	2	85	0	3
3	for	for	5	71	3	3
4	count	count	7	88	0	5
5			4	91	0	0
6			2	87	0	0
Avg. no. colonies			4.7	83.3	.66	2.5

Soil moisture content 4%

Soil pH 5.5

Date of culturing - December 26, 1946

Date of observation - January 2, 1947

TABLE V (cont.)

NUMBER OF EUBACTERIA AND ACTINOMYCETES IN A NATURAL AND A PLANTATION RED OAK FOREST AT VARIOUS DEPTHS

Natural Forest Soil

A_2-6" Horizon

	Dilution and Numbers Per Gram					
	1:100		1:1000		1:10,000	
Sample	Eubacteria	Actinomycetes	Eubacteria	Actinomycetes	Eubacteria	Actinomycetes
1	Too	Too	7	950	4	360
2	high	high	10	1000	5	250
3	for	for	8	900	3	200
4	count	count	11	875	4	300
5			10	975	6	210
6			8	1060	3	230
Avg. no. colonies			9.0	960.0	4.1	258.3

Soil moisture content 5%

Soil pH 5.5

Date of culturing - February 15, 1947

Date of observation - February 22, 1947

TABLE V (cont.)

NUMBER OF EUBACTERIA AND ACTINOMYCETES IN A NATURAL AND A PLANTATION RED OAK FOREST AT VARIOUS DEPTHS

Plantation Forest Soil

A_2-6* Horizon

	Dilution and Numbers					
	1:100		1:1000		1:10,000	
Sample	Eubacteria	Actinomycetes	Eubacteria	Actinomycetes	Eubacteria	Actinomycetes
1	Too	Too	8	75	2	60
2	high	high	5	62	2	51
3	for	for	4	48	3	45
4	count	count	5	53	4	42
5			6	36	2	50
6			17	41	3	48
Avg. no. colonies			7.5	52.5	2.6	49.3

Soil moisture content 4%

Soil pH 5.0

Date of culturing - February 15, 1947

Date of observation - February 22, 1947

TABLE V (cont.)

NUMBER OF EUBACTERIA AND ACTINOMYCETES IN A NATURAL AND A PLANTATION RED OAK FOREST AT VARIOUS DEPTHS

Natural Forest Soil

B-12* Horizon

	Dilution and Number Per Gram					
	1:1000		1:10,000		1:100,000	
Sample	Eubacteria	Actinomycetes	Eubacteria	Actinomycetes	Eubacteria	Actinomycetes
1	2	46	0	9	1	1
2	2	54	0	0	0	1
3	1	67	0	3	0	0
4	1	49	0	4	0	0
5	0	52	0	4	0	0
6	0	43	0	11	0	0
Avg. no. colonies	1.0	51.8	0	5.1	.1	.3

Soil moisture content 5%

Soil pH 5.0

Date of culturing - March 20, 1947

Date of observation - March 27, 1947

TABLE V (cont.)

NUMBER OF EUBACTERIA AND ACTINOMYCETES IN A NATURAL AND A PLANTATION RED OAK FOREST AT VARIOUS DEPTHS

Plantation Forest Soil

B-12* Horizon

	Dilution and Numbers Per Gram					
	1:1000		1:10,000		1:100,000	
Sample	Eubacteria	Actinomycetes	Eubacteria	Actinomycetes	Eubacteria	Actinomycetes
1	27	210	11	31	1	5
2	33	185	8	24	2	4
3	22	225	3	18	1	3
4	25	190	6	21	2	7
5	28	200	7	25	2	4
6	29	220	0	0	1	4
Avg. no. colonies	27.3	205.0	7.0	24.0	1.5	4.5

Soil moisture content 2%

Soil pH 5.0

Date of culturing- March 20, 1947

Date of observation- March 27, 1947

Numbers of Protozoa in Forest Soils

The greatest amount of biological transformations are brought about by the bacteria, fungi and actinomycetes. There are certain instances under particuler soil conditions when the lower animal life, protozoa being most abundant, bring about certain processes in connection with soil transformations. It appears that conditions favoring the development of bacteria also favor the growth of protozoa. This is apparent when the fact is considered that bacteria are an important part of the protozoan diet (8).

Soils rich in organic matter and relatively high in moisture content generally produce the greatest abundance of protozoa. Of this fauna, the smaller flagellates and amoebae are more numerous than the larger ciliates. Optimum development occurs at slightly alkaline reactions though the protozoa appear to be as tolerant to acidity as the bacteria. There are protozoan forms existing as cysts and as active organisms. Under unfavorable conditions the soil protozoa encyst only to become active again under more favorable conditions. It is probable that the active stage is more common in the moister soils. As most forms of protozoa are larger than the bacteria they occur in soils in much smaller numbers, but occupy considerably more space. Greatest numbers of protozoa occur near the surface as with most other soil microorganisms.

Three main groups of protozoa are found abundantly in

the soil, the ciliates, flagellates and amoebae. Their chemical behaviors vary considerably and still is a matter of controversy. Some, at least, are capable of utilizing dead organic and inorganic substances from solution and from solid particles (8). As stated previously, many of them can ingest bacteria and use them as food. Much controversy has been made concerning soil fertility in regard to the relation of protozoa to bacteria; it would not be safe to assume that all protozoa depend upon bacteria for nutrition. It is true that they limit the bacterial population, but in what manner of complex relationship is still not confirmed.

A fairly accurate count for protozoa can be made by narrowing the dilutions so as to give a greater degree and range of accuracy. For example, if protozoa occur in a 1:10 dilution and do not appear in 1:100, there are between 10 and 100 protozoa in a gram of soil. The numbers of protozoa and bacteria are found to vary from day to day (1). Thus, numbers alone do not indicate actual relationships; the presence or absence of protozoa in relation to other microbes is often helpful as an indication of relative moisture content. The protozoa become active in the soil whenever there is excessive moisture present for a period of several hours (7). Since bacteria are chiefly used by protozoa as food the presence of bacteria in rich fertile soils will show abundant protozoa as well as bacteria, provided soil moisture is favorable.

Greatest numbers of protozoa are concentrated in the

top four or six inches of soil, where the bacteria are also at a maximum, while below twelve inches the soil is practically free from protozoa (2). Investigations have shown that protozoa may exist as cysts at very great depths.

Most of the protozoa are aerobic and excessive heat will destroy them. The range of optimum acidities favorable to protozoa was first believed to be close to neutrality but certain ciliates, flagellates and amoebae have been able to live and reproduce in very acid and alkaline media.

Presence and Absence of Groups of Soil Protozoa in a Natural vs. Planted Red Oak Stand

Significant results (refer to numerical data)

1. The abundance of all forms of protozoa observed decreased with depth of horizon in both forest stands.
2. The greatest abundance of all forms was found at the A_0- Surface horizon in both stands.
3. Amoebae were found at the surface in the plantation stand only.
4. Flagellates occurred in greatest abundance of the three forms observed.
5. The number of protozoa for all horizons in both forest stands was quite low.

Conclusions regarding protozoa results:

Since the abundance of all forms of protozoa decreased

TABLE VI

CULTURAL RESULTS OF PROTOZOA

A_0-Surface Horizon

Plantation Forest Soil		Natural Forest Soil	
Dilution	No. of sample and protozoa observed	Dilution	No. of sample and protozoa observed
1:10	1. Flagellates (some) and amoebae 2. Flagellates and amoebae 3. Flagellates (abundant) and amoebae	1:10	1. Flagellates (abundant) 2. Flagellates (abundant) 3. Flagellates (abundant)
1:100	1. Flagellates (abundant) 2. Flagellates (abundant) and amoebae 3. Flagellates and amoebae	1:100	1. None observed 2. None observed 3. None observed
1:1000	1. Flagellates and amoebae (abundant) 2. None observed 3. Amoebae	1:1000	1. Flagellates 2. None observed 3. None observed
Soil moisture content 4% Soil pH 7.0		Soil moisture content 5% Soil pH 6.5	

Date of observation - December 19, 1946
Date of culturing - December 7, 1946

TABLE VI (cont.)

CULTURAL RESULTS OF PROTOZOA

A_1-2" Horizon

Plantation Forest Soil		Natural Forest Soil	
Dilution	No. of sample and protozoa observed	Dilution	No. of sample and protozoa observed
1:10	1. Flagellates (abundant) 2. Flagellates (abundant) 3. Flagellates (abundant)	1:10	1. Flagellates (abundant) 2. Flagellates (abundant) 3. Flagellates (abundant)
1:100	1. Rare (unidentifiable) 2. None observed 3. Rare (unidentifiable)	1:100	1. Rare 2. Rare 3. Rare
1:1000	1. Rare 2. Rare 3. Rare	1:1000	1. None observed 2. None observed 3. None observed
Soil moisture content 4%		Soil moisture content 4%	
Soil pH 5.5		Soil pH 7.0	

Date of culturing - December 26, 1946

Date of observation - January 7, 1947

TABLE VI (cont.)

CULTURAL RESULTS OF PROTOZOA

A_2-6" Horizon

Plantation Forest Soil		Natural Forest Soil	
Dilution	No. of sample and protozoa observed	Dilution	No. of sample and protozoa observed
1:10	1. None observed 2. None observed 3. None observed	1:10	1. None observed 2. None observed 3. None observed
1:100	1. None observed 2. None observed 3. None observed	1:100	1. None observed 2. None observed 3. None observed
1:1000	1. None observed 2. None observed 3. None observed	1:1000	1. None observed 2. None observed 3. None observed
Soil moisture content 4%		Soil moisture content 5%	
Soil pH 5.0		Soil pH 5.5	

Date of culturing - February 15, 1947

Date of observation - February 26, 1947

TABLE VI (cont.)

CULTURAL RESULTS OF PROTOZOA

B-12" Horizon

Plantation Forest Soil		Natural Forest Soil	
Dilution	No. of sample and protozoa observed	Dilution	No. of sample and protozoa observed
1:10	1. Ciliates abundant (Colpoda) 2. Ciliates abundant (Colpoda) 3. None observed	1:10	1. None observed 2. None observed 3. None observed
1:100	1. None observed 2. None observed 3. None observed	1:100	1. None observed 2. None observed 3. None observed
1:1000	1. None observed 2. None observed 3. None observed	1:1000	1. None observed 2. None observed 3. None observed
Soil moisture content 2% Soil pH 5.0		Soil moisture content 5% Soil pH 5.0	

Date of culturing - March 8, 1947

Date of observation - March 20, 1947

with depth it is concluded that conditions most favorable for the growth of protozoa was at the surface. This result was related to the number of bacteria occurring at the surface, which, in both soils, was high near the surface horizon and decreasing with depth. The surface soil and the A_1- 2" horizon are rather abundant in organic matter and it is in these upper levels that the most protozoa occur. Also more acid conditions are prevalent at greater depths and though the moisture contents did not vary appreciably for both soils, the greatest number of forms is found in the horizons closest to neutrality. This ties in with the relative amounts of organic material at the upper horizons in relation to a greater protozoan fauna.

A detailed account of physiological needs of each form of protozoa would be an aid in trying to base a consideration for relative numbers and forms appearing and the possible reasons for their occurrence.

Low moisture contents in the results obtained is an important factor in basing conclusions for the relatively low numbers of protozoa for the horizons studied. Few attempts have been made to demonstrate whether protozoa actually injure important biological soil processes (7).

Algae in Forest Soils

The development of algae in the soil results in increasing the supply of organic matter and in temporarily

transforming soluble forms of nitrogen and minerals into organic or insoluble forms (8). There are three main groups found in the soil, namely, the Cyanophyceae or blue-green algae, the Chlorophyceae or green algae, and the Diatomaceae or diatoms. Of these three groups there is an abundance in the soil, but usually less abundant than the bacteria and fungi. The algae contain chlorophyll and develop this substance independent of the soil when they have free access to light; at lower depths the algae act similar to fungi, utilizing organic materials for energy. Algae hasten the solubility of minerals and in association with fungi aid in the weathering process. At lower levels the number of species represented is less, along with a decrease in numbers. They rarely occur in abundance in soils with a low moisture content; their distribution below the surface is controlled by factors similar to those which regulate the distribution of other organisms (8). It is impossible to generalize concerning the role that algae may play in soil processes (7).

Presence and Absence of Groups of Soil Algae in a Natural vs. Planted Red Oak Stand

Significant results (refer to numerical data)

1. A distinctly low number of algae occurs in both forest stands.
2. The number of groups of algae represented is limited

TABLE VII

CULTURAL RESULTS OF ALGAE

Natural Forest Soil

Horizon	Dilution	Number Present	Identification	Date of Culture	Date of Appearance
A_0-Surface	Absent	0	None	Dec. 7, 1946	
A_1-2"	Absent	0	None	Dec. 26, 1946	
A_2-6"	1:1, 1:10	10	Unidentifiable	Feb. 15, 1947	Mar. 7, 1947 - 1:1 Mar. 3, 1947 - 1:10
B-12"	1:1	1	Chlorococcum Protosiphon	Mar. 8, 1947	Mar. 20, 1947 - 1:1

TABLE VII (cont.)

CULTURAL RESULTS OF ALGAE

Plantation Forest Soil

Horizon	Dilution	Number Present	Identification	Date of Culture	Date of Appearance
A_0- Surface	Absent	0	None	Dec. 7, 1946	
A_1-2"	1:10, 1:100	100	Chlorococcum (abundant)	Dec. 26, 1946	Feb. 22, 1947- 1:10 Jan. 28, 1947- 1:100
A_2-6"	1:1, 1:10 1:100, 1:1000	1000	Chlorococcum	Feb. 15, 1947	Feb. 24, 1947- 1:1 Feb. 27, 1947- 1:10 Mar. 14, 1947- 1:100 Mar. 2, 1947- 1:1000
B-12"	Absent	0	None	Mar. 8, 1947	

to the green algae (Chlorophyceae). Of this group only two genera are represented in both forest soils.

3. There is an absence of algae until the A_1 - 2" horizon is reached in both stands.

Conclusions regarding algal results:

Since algae rarely occur in abundance in soils of low water content, this fact may be a partial explanation for the low number of algae present in both forest soils. From the results obtained, the first two inches of soil appear either too dry for development of algae or they may be absent as a result of competition by other soil organisms, since the fungi, bacteria and actinomycetes are more abundant at this level.

General Summary Regarding Activity of the Soil Organisms in a Natural vs. Planted Red Oak Forest

The results as a whole indicate the complex biological activity differences between a natural and a plantation forest stand. No complete analysis of chemical and physical activities in relation to these stands biologically, has been presented. Results to determine the amounts of carbon dioxide produced by biological activity would indicate more accurately than single determinations of a group of organisms , the amount and degree of biological activity. How-

TABLE VIII

SUMMATION TABLE SHOWING NUMBER OF SOIL ORGANISMS PER GRAM IN A NATURAL AND A PLANTED FOREST STAND AT ALL HORIZONS

Horizon	Forest	Water pct.	pH	Fungi	Bacteria	Actinomycetes		Protozoa	Algae
						Number per gm.	Pct. of total*		
Ao Surface	Natural	5%	6.5	61,950	131,250	1,378,650	91.3	1000	0
	Planted	4%	7.0	18,720	112,320	993,200	89.6	1000	0
A_1-2"	Natural	4%	7.0	118,560	68,640	4,064,320	98.5	100	0
	Planted	4%	5.5	117,520	48,880	866,320	94.7	1000	100
A_2-6"	Natural	5%	5.5	71,505	43,050	2,712,150	98.5	0	10
	Planted	4%	5.0	71,760	27,040	512,720	95.0	0	1000
B -12"	Natural	5%	5.0	525	0	53,550	100.0	0	1
	Planted	2%	5.0	510	71,400	244,800	78.0	10	0

* Indicates per cent of total bacteria and actinomycetes.

ever, the results thus obtained do show in an indicative manner, where the activity for producing carbon dioxide and subsequent breakdown of organic and inorganic matter, may occur in each of two different oak stands. The study indicates that since the two stands involved have not been materially altered as regard to the soil, all the agencies of microbiological phenomena tend to become adjusted to the environmental conditions existing in the soil at any one time. Some appreciation with reference to "areas of greater microbial activity" can be derived from this study. This information may aid in supplementing the knowledge learned by comparing other stands of a similar nature. Each forest has many variables and is a dynamic association. In a general way, some correlation has been shown in these results and certain principles found that tend towards the verification of previous studies made by other investigators.

SUMMARY

1. The number of actinomycetes per gram of dry soil occurring in a natural and a plantation red oak forest, is significantly greater in the natural forest at the A_1- 2" and A_2- 6" horizons.
2. The number of fungi per gram of dry soil occurring in a natural and a plantation red oak forest, is greatest in the natural forest soil. This difference is not large except at the A_0- Surface horizon.

3. Numbers of fungi, bacteria, protozoa and algae, per gram of dry soil in both a natural and a plantation red oak forest, decrease with an increase in depth of horizon. This indicates in both forest soils that at the upper horizons there is a greater amount of available nitrogenous and non-nitrogenous supply of organic matter plus oxygen.
4. For organisms such as protozoa and algae that require high moisture contents in addition to organic matter, the low numbers found in both a natural and a plantation red oak forest soil, may be attributed mainly to the low moisture content of these soils.

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