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J. G. LIPMAN

THE SOIL AND THE MICROBE

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An Introduction to the Study of the Microscopic Population of the Soil and Its Rôle in Soil Processes and Plant Growth

BY

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THIS BOOK IS DEDICATED TO

SIR JOHN RUSSELL

INVESTIGATOR AND WRITER, WHOSE BOOKS
ON SOIL FERTILITY AND PLANT GROWTH
HAVE DISSEMINATED WIDELY THE
KNOWLEDGE OF THE SOIL
AND ITS PRACTICAL
APPLICATION

FOREWORD

The soil is not a mass of dead débris, resulting simply from the physical and chemical weathering of rocks and of plant and animal remains through atmospheric agencies, but it is teeming with life. Every small particle of soil contains numerous types of living organisms belonging both to the plant and animal kingdoms, yet so small that they cannot be recognized with the naked eye. These organisms are, therefore, called microbes, microorganisms or microscopic organisms. These microbes comprise numerous types of bacteria, fungi, algae, protozoa, nematodes and other invertebrates which vary considerably in their structure, size, mode of living and relationship to soil processes.

In the cycles of transformation of elements in nature, the microbes play an important, if not a leading, rôle. Were it not for them, the soil would soon become covered with a considerable mass of undecomposed plant and animal residues; life would soon cease, since the very limited supply of carbon and available nitrogen, the most essential elements in the growth of living organisms, would become exhausted. It should be recalled that carbon dioxide, the source of carbon for the growth of plants, which in their turn supply the food for animals, is present in the atmosphere only in a concentration of 0.03 per cent. This is equivalent to 5.84 tons of carbon over each acre of land. A good yield of sugar-cane will consume about 20 tons of carbon in a single growing season; of course most of the surface of the earth supports less vegetation than this, and diffusion tends to create a uniform distribution of gases. It has actually been calculated that the plant world consumes 64.8 millions of tons of carbon annually, which amounts to 1/35 of the total carbon content of the atmosphere. The atmospheric supply of carbon dioxide is, however, constantly replenished from the decomposition products of the organic substances in the soil; only as a result of this does plant growth not cease entirely through a deficiency of an available supply of carbon. In the absence of microbes the available nitrogen would also become very rapidly exhausted, as can be appreciated from the fact that this nitrogen is never present in the soil in forms available to plant growth, as ammonia or nitrate, in amounts of more than a few pounds per acre. It is made available to plants only through the constant activity of the microbes.

The microorganisms, through their various activities, thus enable organic life to continue uninterruptedly on our planet. They keep in constant circulation the elements which are most essential for plant and animal life. They break down the complex organic molecules, built up by plants and animals, into the simple mineralized constituents, making the elements again available for the growth of cultivated and uncultivated plants which in their turn supply further food for animals.

Just as man and other animals, as well as higher plants, find their habitat on the surface of the soil or immediately below it, so do the microbes live largely within the upper few inches of the earth's crust, where they carry out their important activities, supplying a continuous stream of nutrients in an available form for the growth of higher plants. This surface pellicle of the earth is thus found to be the seat of numerous processes of incalculable importance in the life of man, animals, and plants, enabling them to carry out their normal existence on our planet. Just as man and animals are determined in their development by the supply of plant food, so is the growth of plants determined by the activities of microorganisms in the soil. The microbes were probably among the first living organisms which appeared on our planet millions of years ago. Although their presence in ancient rocks is largely speculative, it is reasonable to assume, from an appreciation of their specific physiological processes, that they may have lived normally on the earth long before it was a fit habitat for higher plants and animals.

Our knowledge of the soil microbes and their rôle in soil processes and plant growth has developed in the last fifty years. However, a large body of information has since accumulated which enables us to construct a clear picture not only of the microscopic population of the soil, of its numerous physiological reactions, but also of the relation of these processes to the origin and formation of soil, to the cycle of elements in nature, and to plant nutrition.

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CHAPTER I

THE SOIL AND THE PLANT

THE NATURE OF THE SOIL.—The upper layer of the earth's surface, varying in thickness from 6 to 18 inches in the case of some humid soils and up to 10 or 20 feet in the case of arid soils, possesses certain characteristic properties which distinguish it from the underlying rocks and rock ingredients. This very thin surface layer of the earth's pellicle is spoken of as the soil. It is distinguished from the lower layers by its mechanical, physical, and chemical properties, but especially by the presence of living organisms including a variety of microbes, lower animals, and roots of plants. Dead bodies of these organisms also occur in the soil in all stages of decomposition. The science of the soil is frequently spoken of as *Pedology*.

The type of soil that has developed upon the underlying rock is a result of climate and the organic life upon it or within it, including the action of higher plants, animals, and microorganisms. The soil is arranged in a series of characteristic layers or *horizons*, which make up the soil *profile*, which is a direct result of the conditions under which it has been developed. A soil profile is obtained by making a vertical cut through the soil, showing its various horizons (Fig. 1). The upper horizon is more or less dark colored on account of the presence of organic matter in different stages of decomposition. The color of the soil may become darker or lighter with depth, depending on the accessibility of air and movement of water through the profile, the penetration of roots, and the activity of microorganisms.

The soil is characterized morphologically by the texture, structure, color, and chemical composition of the various horizons. These horizons are designated by letters: A, usually at the surface, is that horizon from which certain material has been removed by mechanical or chemical means. B, is that horizon into which material has been carried. C, designates the parent material. These horizons are frequently subdivided into A_1, A_2, etc. The microbiological processes in the soil are carried out largely in the A horizons, and it is here that most of the plant remains become incorporated. A consideration of the composition of the various horizons reveals marked differences, especially in the content of organic matter, as shown in Table 1.

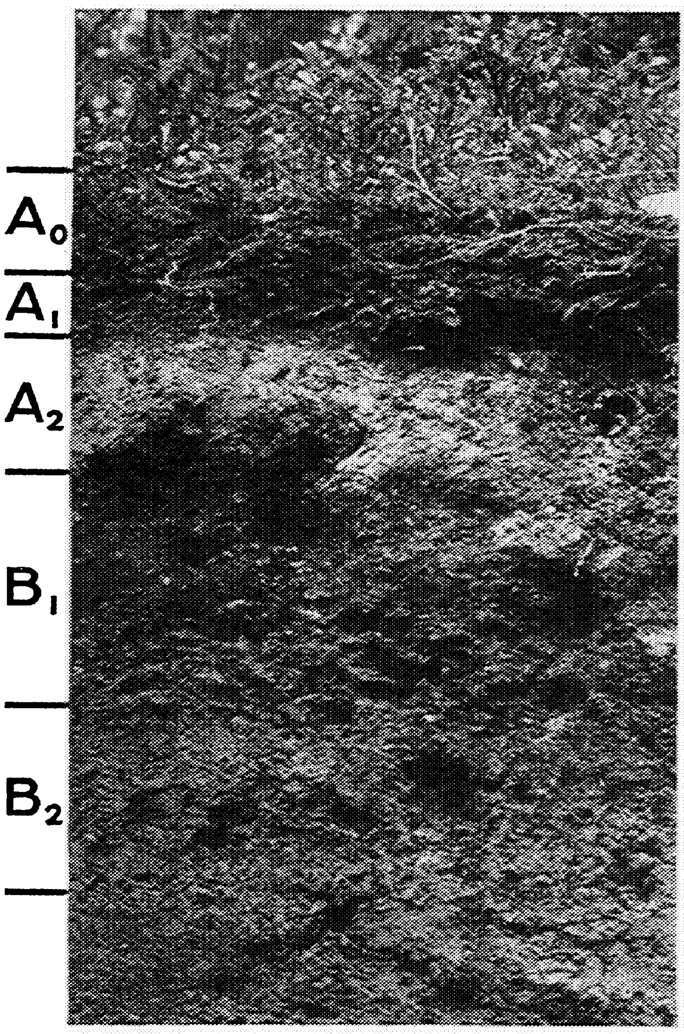

FIG. 1.—Profile of podsol soil with raw humus. The light-colored leached layer (A_2) so typical of a podsol, is clearly apparent (from Tamm).

WEATHERING OF ROCKS.—The surface of the earth is modified in physical appearance so slowly or in such ways that one is inclined to create a mental picture of the soil as a static or fixed formation. Violent natural changes are so few or so seldom noticed that the transformations which we may read in the rocks and soils as occurring through geologic ages appear to be widely separated from the times we live in. Only as our attention may become attracted by floods, earthquakes, volcanoes, or glaciers do we begin to appreciate the fact that physical forces are active in modifying the surface of the earth.

Such phenomena and others of a less apparent nature have been active in producing the earth's surface as we know it, and are active continually in creating new changes. The layer of fine

TABLE 1

COMPOSITION OF A GRAY FOREST SOIL* (FROM GLINKA)

Horizon	A_1	A_2	B	C
Description	Organic matter or peat-like material	Whitish horizon	Brownish-yellow	Granite
Soil Constituent	*Per Cent*	*Per Cent*	*Per Cent*	*Per Cent*
H_2O at 100° C	3.06	1.69	4.10	0.98
Organic matter	10.94	1.25	2.29	
Loss on ignition	12.78	5.02	6.00	1.21
SiO_2	66.86	74.01	63.60	74.87
Al_2O_3	13.38	13.78	17.10	13.82
Fe_2O_3	1.71	1.95	4.50	1.92
Mn_3O_4	0.04	0.04	0.08	0.04
CaO	1.38	0.92	0.69	0.63
MgO	0.14	0.13	0.45	0.40
K_2O	2.36	2.28	4.12	3.96
Na_2O	1.56	1.75	3.46	2.62

* Podsol.

material has all originated from the compact rocks by slow processes of disintegration due to weathering, encouraged by the action of waters, heat and cold, other atmospheric agencies, and biological factors. Some of this material remains superimposed upon the rocks from which it was formed; some becomes translocated by waters and winds and finds its resting place at regions far distant from the place where it originated. The disintegration of rocks finally leads to an accumulation of granulated material of the fineness of sands and clay. Soon after or even during rock disintegration, and also greatly assisting this process, vegetation springs up. The inorganic materials which are soluble in water or dilute acids, such as carbonic, are then removed from the soil by plants and percolating waters. Some of these materials again enter the soil and become incorporated with it on the death of the vegetation. Here also the plants undergo partial or complete decomposition by microorganisms.

The chemical processes involved in the weathering of rocks are those of hydrolysis, oxidation, hydration, solution, and carbonation, or carbonate formation. The following reactions illustrate the chemical changes involved in the weathering of orthoclase and olivine, two rock-forming minerals:

$$\underset{\text{Orthoclase}}{2KAlSi_3O_8} + \underset{\text{Water}}{2H_2O} + \underset{\text{Carbon dioxide}}{CO_2} = \underset{\text{Kaolinite}}{H_4Al_2Si_2O_9} + \underset{\text{Potassium carbonate}}{K_2CO_3} + \underset{\text{Silica}}{4SiO_2}$$

$$\underset{\text{Olivine}}{12MgFeSiO_4} + 26H_2O + 3O_2 = \underset{\text{Serpentine}}{4H_4Mg_3Si_2O_9} + \underset{\text{Silica}}{4SiO_2} + \underset{\text{Limonite}}{6Fe_2O_3 \cdot 3H_2O}$$

The soils thus owe their composition largely to the rocks from which they are formed. Rocks are not homogeneous substances, but aggregates of minerals which themselves are chemical entities and which vary in complexity, from the elements, as graphite (C) and free iron (Fe), to complex molecules like muscovite mica ($Al_3KH_2Si_3O_{12}$). The relative abundance of any one or groups of these minerals in the particular rock and the degree of their consolidation determine not only the nature of the rocks but also the soils which are formed from them.

TABLE 2

THE RELATIVE ABUNDANCE OF CHEMICAL ELEMENTS EXISTING IN GREATEST QUANTITIES IN THE EARTH'S CRUST (FROM CLARKE)

Element	*Per Cent*
Oxygen	47.33
Silicon	27.74
Aluminum	7.85
Iron	4.50
Calcium	3.47
Magnesium	2.24
Sodium	2.46
Potassium	2.46
All others	1.95
Total	100.00

The common soil minerals contain only 21 of the known chemical elements. Eight of these elements compose 98 per cent of the total mineral matter of the earth's crust, as shown in Table 2. The five elements, hydrogen, sulfur, carbon, titanium, and phosphorus occur in many important minerals, and each comprises from 0.1 to 0.5 per cent of the inorganic part of the soil. The remaining eight (fluorine, chlorine, zirconium, boron, nitrogen, barium, manganese, and chromium) make up together only 0.35 per cent of all soil mineral matter.

It is worthy of note that carbon, one of the most important elements in the life of plants and animals and which plays such an important rôle as a source of fuel and in the synthesis of hundreds of compounds used by man, makes up only a very small fraction of the surface of the earth, as well as of the whole lithosphere, hydrosphere and atmosphere. The constant circulation of this element in nature is necessary to keep life from becoming rapidly extinguished, and it is the soil microbes that bring about certain important phases of the transformation of this element.

Seventy-five per cent of all the solid surface of the earth's crust is composed of the two elements oxygen and silicon, while silica (SiO_2) as the compound and as combined in silicates makes up 60 per cent of the crust. This silica thus comprises the major part of the inorganic portion of the soil, which results from the disintegration of the rocks, due not only to its being the most abundant material in rocks but also as a result of its resistance to solution by water or dilute acids excreted by plants or formed by microorganisms in the soil.

Weathering agencies remove certain rock constituents quite rapidly, and others only in very small amounts even after long periods of time. Table 3 brings out further the changes which

TABLE 3

Chemical Composition of a Rock and of a Residual Soil Formed from It (from Clarke)

	Fresh rock	Residual soil
	Per Cent	*Per Cent*
SiO_2	60.69	45.31
Al_2O_3	16.89	26.55
Fe_2O_3 and FeO	9.06	12.18
MgO	1.06	0.40
CaO	4.44	Trace
Na_2O	2.82	0.22
K_2O	4.25	1.10
Ignition	0.62	13.75
P_2O_5	0.25	0.47
Totals	100.08	99.98

have taken place in the transformation of a micaceous gneiss to a soil, by the decomposition processes which occurred *in situ* under weathering agencies of a humid climate.

The general changes in the chemical composition during the process of rock weathering and the formation of the earth's crust consist in the separation of the silica and of the bases, the oxidation of the compounds of iron, the removal of bases by processes of leaching and replacement, the general hydration of the remaining silicates, aluminum and iron, and a very appreciable addition of organic matter coincident with the invasion of plants and of microorganisms.

In order to characterize soils as to texture they may be analyzed mechanically, to separate the particles into groups of certain sizes including various sands, silt and clay. Typical mechanical analyses of two soils are given in Table 4.

TABLE 4

CLASSIFICATION OF SOIL PARTICLES AND MECHANICAL ANALYSIS OF TWO SOILS*

Fraction	Size	Fine sandy loam	Clay
	Millimeters	*Per Cent*	*Per Cent*
Fine gravel	2.00–1.00	1	1
Coarse sand	1.0 –0.50	2	2
Medium sand	0.50–0.25	3	2
Fine sand	0.25–0.10	22	6
Very fine sand	0.10–0.05	35	11
Silt	0.05–0.005	24	41
Clay	0.005 and below	13	37

* On the basis of methods used at the U. S. Dept. of Agriculture.

EFFECT OF CLIMATE UPON CHEMICAL COMPOSITION OF SOIL.—Climate is the most important factor determining the type of changes brought about in the transformation of the rock constituents and therefore in the development of the soil. Among the various climatic factors, temperature and precipitation are of major importance. Differences in climate affect the rate of change, the course of mechanical and chemical transformation, the types and amount of vegetation, and the kinds of residues resulting and accumulating in the soil. In general, the cooler the temperature and the lower the precipitation, the slower the transformation of the soil materials. Table 5 presents certain differences obtained by the determination of the average composition of 466 soils in humid regions of the southern portion of the United States as compared with the composition of 313 soils from arid areas within the states of California, Washington and Montana.

TABLE 5

COMPARATIVE CHEMICAL COMPOSITION OF SOILS OF HUMID AND ARID REGIONS (FROM CLARKE)

	Humid Soil	Arid Soil
	Per Cent	*Per Cent*
Insoluble in HCl	84.03	70.57
Soluble SiO_2	4.21	7.27
Al_2O_3	4.30	7.89
Fe_2O_3	3.13	5.75
Mn_3O_4	0.13	0.06
MgO	0.23	1.41
CaO	0.11	1.36
Na_2O	0.09	0.26
K_2O	0.22	0.73
P_2O_5	0.11	0.12
SO_3	0.05	0.04
Water and organic matter	3.64	4.95
Totals	100.25	100.41

It is quite apparent that arid conditions are conducive to the accumulation of much more soluble substances than the humid environment. These arid soils are frequently very fertile and owe their scant plant growth entirely to the small amounts of water which reach them. It requires only the introduction of moisture to transform them from dry, brown deserts to flowering gardens.

The mineral compounds in the rocks undergo modifications which result in the solution and fragmentation of the large aggregates. Many of the more soluble substances initially present in the parent materials, such as chlorides and sulfates of the alkalies, are removed from the soil quite rapidly in humid regions and finally reach the ocean, which becomes a vast reservoir of these removed materials. In general the basic minerals enter solution more rapidly than the acidic rock components. This weathered soil material consists largely of silicates, aluminum and iron—a mere skeleton of the parent material, but enriched by a clothing of the organic residues of the decomposing vegetative cover.

The climate exerts even a more important influence upon the organic than upon the inorganic soil fraction. The soils in cool and moist regions are, as a rule, richer in organic matter than those in warm and dry regions. As shown in Fig. 2 the nitrogen contents of soils of cool regions are higher than the nitrogen contents of soils of warmer regions. Since there is generally a close relationship between the nitrogen content of a soil and the total amount of organic matter, these results may be interpreted as indicating the presence of greater amounts of organic matter in the cooler soils. Not only is the total organic matter in the soil influenced by climate, but also its chemical nature, such as the relation between the elements carbon and nitrogen.

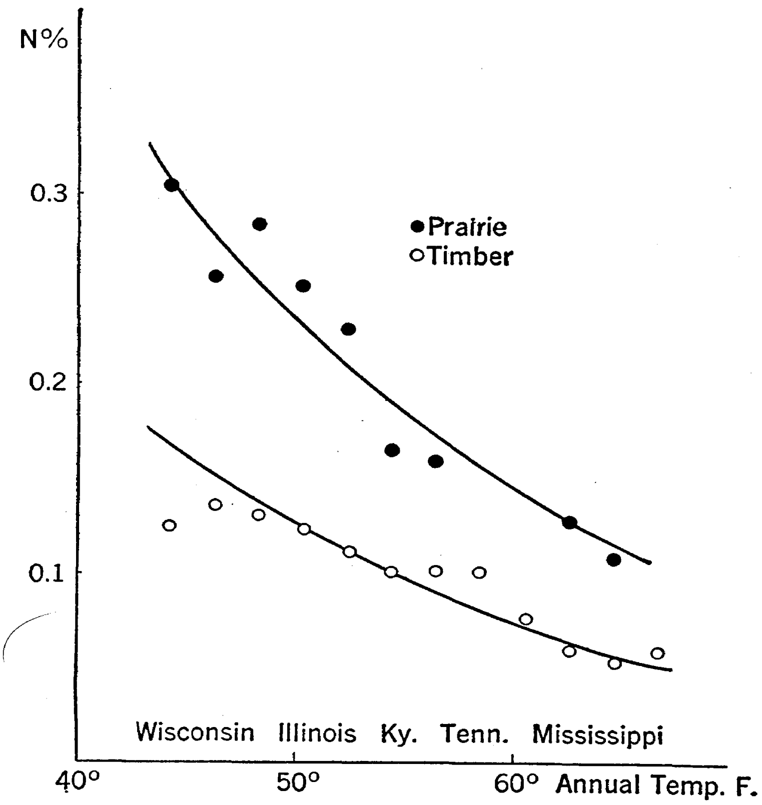

FIG. 2.—Influence of temperature upon the nitrogen content of prairie and timber soils (after Jenny).

We may consider then that the soil is continually exposed to a variety of influences which modify its physical structure, chemical composition, and even its location. Strictly non-biological factors may exert pronounced effects, but the development of higher plants and microorganisms is to a large degree responsible for the creation of fertile agricultural soils from the inorganic substances.

Soil Formation.—In the initial processes of formation of soils, lichens, mosses and other small organisms attack and weaken the rock constituents by removing certain of their more soluble elements or compounds. Changes in temperature cause expansion and contraction which open seams and gradually form small fragments which, when mixed with the decomposing organic remains of the first invaders, supply footholds for the development of larger plants. In turn they further disintegrate the rock materials by physical and chemical forces. Gradually the organic residues of these plants become mixed with the coarse and fine rock materials, thus giving rise to the beginnings of agricultural soils (Fig. 3).

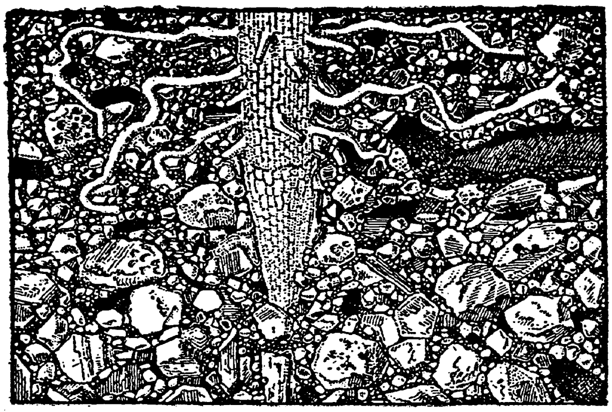

Fig. 3.—Physical structure of soil in relation to root development. Schematic representation of the solid soil particles, of the air spaces and of root development (from Francé).

The abrasion of soil particles is carried out further by the action of air, water and ice, thus adding to the disintegrated material. Much of this fine substance reaches the lowlands by water and ice removal and tends to fill in the valleys. Here, temperature conditions being more conducive to plant growth, a vegetation develops, the abundance of which is determined principally by the available moisture. The repeated development of plants and the incorporation of their remains with the soil, where they undergo partial disintegration, finally produces a material having very few characteristics in common with the rocks from which it originated. The greatest modification takes place near the surface, where pronounced disintegration and chemical changes have been produced. This region is exposed to the most marked processes of leaching and receives also the largest amount of plant residues. The deeper layers of soil, or the so-called subsoils or B horizons, contain much less organic matter and consequently consist almost entirely of mineral substances some of which may have originated from the surface and accumulated at deeper zones as the result of leaching. Below this material, large partially disintegrated aggregates of the parent rock are found superimposed upon the solid bedrock.

The organic substances are important in determining the physical properties of soils. They are largely colloidal in nature and thus have pronounced absorptive properties. This characteristic makes their presence particularly desirable in the coarse, open, sandy soils which are so readily leached by percolating water. To soils containing large amounts of fine particles, as clay, the organic residues give a more open granular structure, so necessary for proper cultural treatments, for the penetration of gases and for the movement of water.

The soil which has undergone changes through numerous generations is thus found to consist of an inorganic framework, of coarse and small particles, some existing separately but most occurring as aggregates, surrounded with a colloidal jelly-like layer, made up of very fine inorganic materials and substances of organic origin. This colloidal material is extremely fine, being smaller than 0.00004 of an inch in diameter. The average size of the colloidal soil particles appears to be close to 0.000004 of an inch. Such particles are only visible with the most powerful microscopes. It is these particles which determine to a large degree the physical properties of the soil. The tendency of soil particles to adhere in a plastic mass is largely determined by them. By reason of the large surface exposed per unit weight they have great absorptive capacity for gases, liquids and dissolved substances, and to them we owe the characteristic of soils to retain water and basic substances. The spaces between the solid particles are filled with water and gases. Diffusion tends to bring these gases to the same composition as the normal atmosphere, but there are always marked differences between the two. The speeds of decomposition of organic materials and permeability of the soils largely determine their differences. Since carbon dioxide is formed in large amounts in soils by the decomposition of organic materials, and since oxygen is consumed in the process, there are larger amounts of carbon dioxide and smaller amounts of oxygen in the soil air than in the normal atmosphere. Where penetration of air is greatly retarded, other gases such as methane and hydrogen, may appear in considerable quantities.

Movement of Water in Soil.—The soil air may be partly replaced by water, which forms films around the solid particles. At times, the water may entirely fill the pore spaces, pressing out all of the air. This film of water, or the free water, carries in solution the minerals which are dissolved from the inorganic soil constituents, the carbon dioxide and other substances produced from the decomposition of the organic matter. This film of water forms the soil solution. The growing plants obtain the nutrients necessary for their growth by absorbing them largely from the solution by means of their roots and root hairs, which penetrate between the inorganic soil particles.

Some of the plant nutrients are only slightly soluble in water or in the weakly acidified aqueous solution, and may be present, therefore, in the soil solution only in small amounts at any one time. However, as these minute quantities are removed by the growing plant or by drainage waters they are replaced by further solution from the crystalline or colloidal soil materials.

Water moves in soils in many ways, depending upon its abundance, the physical condition of the soil and the vegetation. Where large amounts reach the soil in a short period of time, much of it may disappear by running off from the surface into drainage channels. Some of the water penetrates the soil and, where there is more than the soil particles can hold about themselves, it sinks to the level of the water table by precolation. When moisture is not descending upon the soil there is loss by evaporation from the surface and transpiration from the leaves of the vegetative cover. This water may be drawn up from the lower levels of the soil by capillary forces as more and more water is lost from the surface. Heavy rainfall alone may not solve the problem of supplying water to plants. The solution lies rather in the proper distribution of the water in the soil over the period of time in which the plants develop. In humid regions the problem may become one of drainage; in regions of very low rainfall, irrigation may be resorted to. A combination of adequate drainage and irrigation systems makes possible almost complete control of the soil water, but unfortunately such equipment is too expensive for general practical application.

Organic Matter of Soil.—In addition to the solid inorganic particles or the mineral framework, the soil also contains solid organic particles, namely, roots and sloughed-off portions of roots, residues of stems, leaves and branches, as well as numerous organic complexes which have originated by the partial disintegration of the plant materials. To this is added a large quantity of organic matter in the form of bodies of microorganisms and their various decomposition products.

In addition to the mineral soils, which are predominantly inorganic (95 per cent), there are soils which are largely organic in nature. Here belong the peat soils, which originate from bogs. These soils contain 30 to 98 per cent organic matter and only 2 to 70 per cent inorganic material. The surface layers of certain types of forest soils are also predominantly organic in nature. These soils are characteristically organic, since the accumulation of plant materials takes place more rapidly than the decomposition of these residues by microorganisms, due either to saturation with water (as in peat bogs) or high acidity of soil combined with other factors unfavorable to the activities of the organisms.

Soil Phases.—The soil thus consists of three definite phases (see Fig. 4): (1) The solid phase, which, in the case of mineral soils, is composed largely (up to 99.5 per cent) of inorganic rock constituents and only to a limited extent (0.5 to 10 per cent) of organic materials; in the case of peat soils, the organic matter may make up 30 to 99 per cent of the solid phase of the soil constituents on a water-free basis. (2) The liquid phase, consisting of water which contains in solution various organic and inorganic materials. This water may be combined with the soil colloids as hygroscopic water which is held very tenaciously or it may be present as capillary film water or as free gravitational water.

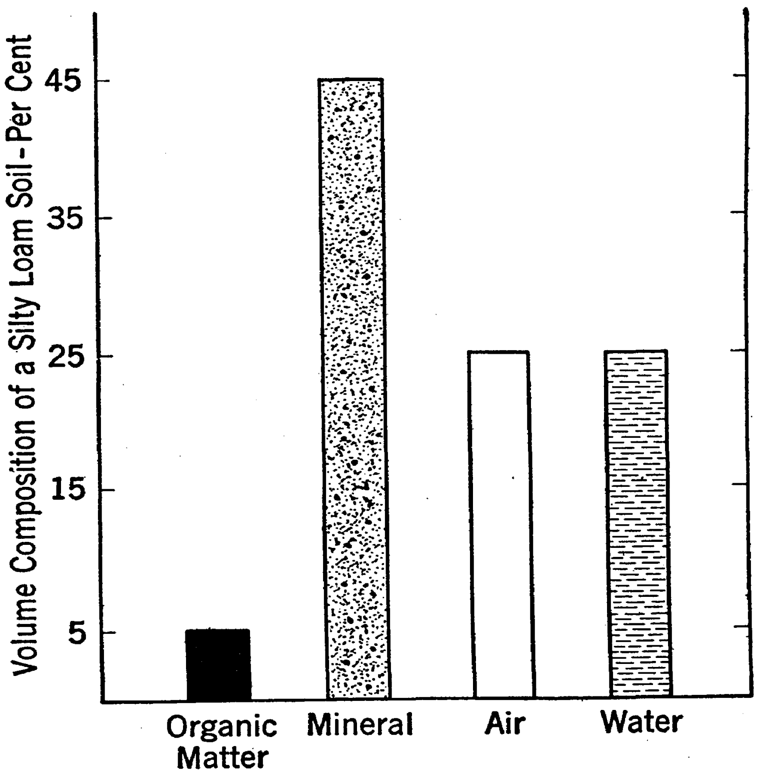

Fig. 4.—The relative distribution of materials which compose a soil under conditions favorable to plant growth.

It may make up 5 per cent (in the case of sands) to 93 per cent (in the case of peats) of the total volume of soil. (3) The gaseous phase, or the soil air, which may differ greatly in composition from ordinary atmosphere, because of the higher content of carbon dioxide, lower content of oxygen, and frequently the presence of some methane and hydrogen.

The existence of these three phases alone would hardly be sufficient to make the soil a medium fit for the growth of higher plants. The presence in the soil of another factor, namely, the microbes, makes the soil a living medium and renders it dynamic.

FIG. 5.—Schematic representation of the relation of microorganisms to the physical structure of soil (from Francé).

RÔLE OF MICROBES IN PLANT GROWTH.—The soil may be looked upon not only as a medium supporting growth of higher plants but as a complex natural environment inhabited by a population more diverse and active in a greater variety of affairs than the visible inhabitants of the earth. Economically the major interest in soils is concerned with the growth of higher plants, but, since there is such a close correlation between the development of plants and microbial activities, a comprehensive appreciation of the microbial life is of much more than academic interest.

These microbes are largely responsible for the numerous chemical changes constantly going on in the soil, principally as agents of destruction of the complex organic molecules synthesized by plants and transformed by animals. Coincident with this destruction is the construction or formation of new compounds. Some of the end products of such transformations are inorganic or mineral materials. There is no breaking down that is not accompanied by a proportionally equivalent building up. The activities of microorganisms upon the mineral constituents of soils are numerous and varied. These may be of the nature of oxidation, reduction, hydrolysis, and carbonation.

Were these microbes eliminated from the soil, the organic matter constantly added would accumulate until all the combined nitrogen, phosphorus, and some of the potassium would be in that form. These elements which are so important for the growth of cultivated and uncultivated plants would not be available to a new crop, simply because plants cannot utilize in any amounts materials in complex organic forms, but must have them supplied as simple mineralized substances. To understand these relationships we must know something of the nutrition of the plants and of their composition.

THE NATURE OF PLANT NUTRIENTS.—All plants require for their nutrition the elements, carbon, hydrogen, oxygen, nitrogen, phosphorus, potassium, calcium, magnesium, iron, sulfur, and possibly some other elements in small amounts (see Fig. 6). Very few of these elements, however, become factors limiting plant growth as a result of their presence in insufficient amounts in soils. This is due to the fact that either many of the elements are required by plants only in very small amounts or because some of these elements exist in soils in considerable abundance. Under continuous culture of soils in humid regions it is frequently necessary to replenish the store of three elements in particular—nitrogen, phosphorus, and potassium, and occasionally sulfur. These are required by plants in greater amounts than any of the other mineral elements obtained from the soil, and, therefore, become depleted most rapidly. To overcome this deficiency a great variety of substances are utilized. Nitrogen is added in such inorganic forms as compounds of ammonia, nitrate, cyanamid, as well as in organic substances such as urea, packing-house refuse, and guano. This last is the natural compost of excrements and carcasses of such animals as bats and sea-fowls. Besides nitrogen, it generally also contains considerable quantities of both phosphorus and potassium. The recently developed industries fixing nitrogen from the air have supplied products which largely replace the natural substances. These may be obtained as substances containing ammonia, cyanamid, nitrate, and urea. Certain plants, as the legumes, may draw upon the store of nitrogen in the air through the agency of bac-

	Element		Principal Source
Indispensable in appreciable amounts	Carbon	(C)	From the carbon dioxide of the atmosphere.
	Oxygen	(O)	From oxygen and carbon dioxide of the atmosphere and from water.
	Hydrogen	(H)	From water.
	Nitrogen	(N)	From nitrate or ammonia in soil. Can be obtained from gaseous nitrogen by leguminous plants.
	Phosphorus	(P)	From phosphates in soil.
	Potassium	(K)	From salts in the soil.
	Calcium	(Ca)	From salts in the soil.
	Magnesium	(Mg)	From salts in the soil.
	Iron	(Fe)	From ferrous or ferric salts in the soil.
	Sulfur	(S)	From sulfates in the soil.
Indispensable in small amounts, exerting stimulating effects or correcting soil conditions	Manganese	(Mn)	From salts in the soil.
	Silicon	(Si)	
	Boron	(B)	
	Zinc	(Zn)	
	Fluorine	(F)	
	Iodine	(I)	
	Chlorine	(Cl)	
	Aluminum	(Al)	
	Copper	(Cu)	

FIG. 6.—Sources of plant nutrients.

terial action. These plants are used quite generally as cover crops which, when plowed into the soil, greatly increase its nitrogen content. The return of barnyard manures to soils not only adds nitrogen but also both phosphorus and potassium.

Phosphorus is most frequently introduced into the soil as the relatively insoluble tri-calcium phosphate, as the soluble superphosphate, or as basic slag, which is a by-product of the steel industry. Ground bone may also be utilized as a source of this element. Potassium is used as the sulfate and chloride which are obtained from natural salt deposits.

Continuous cultivation of soils may lead to a depletion of the basic substances and to the development of an acid reaction, particularly where artificial fertilizers are injudiciously used. Both of these conditions are unfavorable to the development of many of the important agricultural plants. To offset these effects, calcium is applied in various forms such as the oxide, hydroxide, or carbonate. Its addition to the soil is not so much for the purpose of overcoming a deficiency of an element required for plant growth as to correct certain other unfavorable soil conditions.

An idea of the abundance of some of the most important constituents in soils may be derived from Table 6. Although

TABLE 6

Concentration of Important Constituents in Mineral Soils (from Lyon and Buckman)

Constituent	Percentage range	Average, per cent	Pounds per acre in upper 6 inches (on basis of 2,000,000 pounds of soil per acre)
Organic matter	1.00–10.00	4.00	80,000
Nitrogen (N_2)	0.05– 0.50	0.20	4,000
Phosphoric acid (P_2O_5)	0.01– 0.40	0.15	3,000
Potash (K_2O)	0.50– 4.50	2.00	40,000
Lime (CaO)	0.05– 1.50	0.40	8,000
Sulfur trioxide (SO_3)	0.05– 0.50	0.15	3,000

these data suggest that a soil may have sufficient nutrients to supply the requirements of large crops for many decades, this conclusion is not justified. Very small amounts of these substances are available to plants at any one time. Most of the nitrogen is locked up in organic compounds which are slowly decomposed by microbes. The phosphorus may exist in organic combination, in mineral materials such as apatite, and as other compounds of calcium, iron, or aluminum. Most of these compounds are only slightly soluble. Crops which are grown on soils which contain large total amounts of phosphorus frequently develop much better following the application of phosphatic fertilizers. Similarly, the potassium, calcium, and sulfur exist in combinations from which they are not liberated rapidly into the soil solution.

Some soils contain such an abundance of basic salts that plant growth is markedly depressed. The influence of this condition on plant development may be due to at least two effects: (1) In the so-called alkali soils of arid regions the concentration of soluble salts may be so great that the plants fail to grow. (2) In other soils the reaction may be so basic that iron will not go into solution in amounts sufficient to satisfy the requirements of the plants; this brings about a yellowing or mottling of the green surfaces of the plants, stunts the growth and may lead to death.

Absorption of Nutrients by Plants.—Carbon is taken from the carbon dioxide which is present in limited concentrations (0.03 per cent) in the atmosphere. Only green plants, which contain chlorophyll and which can utilize, by photosynthesis, energy of the sun, and a few bacteria, are able to use this source of carbon. The carbon dioxide is reduced in the leaves and combined with water to give formaldehyde, which is then built up to form carbohydrates. Soil activities are particularly important in this process, even if indirectly, by replenishing the carbon dioxide of the atmosphere by a constant stream of the gas originating from the activities of the microbes in the soil. Oxygen comes from the carbon dioxide obtained from the air, from the water drawn from the soil, and from numerous other substances. Hydrogen is derived principally from water.

Nitrogen in various combinations is absorbed by the plant roots from the soil. Although from 75 to 80 per cent of the gases of the atmosphere consists of elemental nitrogen, none of it can be used by the plants, since they can assimilate only combined nitrogen in the form of ammonia (NH_4^+) or nitrate (NO_3^-). Plants store in their tissues considerable quantities of nitrogen, which becomes available to subsequent plant growth only after these tissues undergo decomposition through the agency of the soil microbes. Very few plants (legumes) are able to make use of the vast store of gaseous elementary nitrogen, but even here they require the coöperation of soil organisms.

The elements phosphorus and sulfur are present in the soil both in organic and in inorganic forms. They undergo various transformations in the soil through the activities of microbes, either directly or indirectly, before they can be assimilated by plants. Here again, plants cannot assimilate appreciable amounts of phosphorus either in the form of organic compounds or in the form of insoluble rock phosphate. In the first case, it has to be decomposed by microbes; in the second case, it is made soluble by the various inorganic and organic acids formed by the soil microbes.

Potassium, iron, magnesium, and calcium are basic constituents of soils which are required by plants in certain amounts. Potassium frequently occurs in available forms in amounts insufficient to satisfy the requirements of plants. Calcium and magnesium are required by plants in smaller quantities, but they play important rôles in the neutralization of soil acids and in the coagulation of the dispersed soil particles. All of these elements are obtained by the plants from the soil, and all of them are changed from one form to another, directly or indirectly, by the soil organisms.

Higher plants may be grown in the absence of other forms of life and in the absence of organic materials under artificial conditions when they are supplied with the necessary salts as they are required. Such conditions never develop in the natural habitats. Here the soil organic matter acts as a reservoir from which many of the elements required for development of the plant are supplied throughout the growing season. The mineral fraction of the soil slowly furnishes other elements to the soil solution, partly as a direct result of microbial activities. This may be brought about either through the solvent action of carbonic or other acids or by the oxidation or reduction of these minerals.

Summary.—Four distinct relationships between soils, plants, and microbes should be considered: namely (1) the rôle of microbes in soil formation and soil transformation; (2) the rôle of microbes in the liberation of nutrients for plant growth; (3) the soil as a medium for the growth of microbes; (4) the rôle of higher plants in supplying nutrients for microbes.

Through their activities, microbes liberate large quantities of carbon dioxide, various organic acids as well as certain inorganic acids (notably nitric and sulfuric) which have a marked solvent action upon rocks and rock constituents and thus assist materially in the weathering processes which lead to soil formation. By assimilating certain chemical elements or compounds, by changing the nature of other chemical compounds, by the processes of decomposition of organic matter, as well as by the synthesis of new organic complexes, microbes play a very prominent part in the constant transformation of the soil.

The elements which plants require for their nutrition come directly or indirectly from the soil. The microbes play prominent rôles in transforming these elements into forms available for plants. Out of these elements the plants synthesize their tissues: the roots, the stems, the branches, the leaves, the grain, and the fruits. Only a part of these plant products are utilized by men and animals for their food. Another, frequently the larger part, is returned to the soil. Even the very bodies of men and animals that live by consuming plant substances will sooner or later also return to the soil, and with them a large part of the elements removed from the soil through the agency of the plants. These are acted upon by the soil microbes, liberating the important elements, carbon, nitrogen, phosphorus, sulfur, and potassium, into circulation, where they are again available for plant growth.

The soil is an excellent medium for the development of microbes since it contains all the elements essential for their activities. The plant and animal residues offer suitable sources of energy to keep the various microbes in a state of constant activity and reproduction. The relationships between soils and plants, on the one hand, and the microbes, on the other, are mutual. The soil and plant supply the medium for the growth of the microbes, and the energy and other nutrients for their activities and reproduction; the microbes carry out the processes which keep the elements in constant circulation, thus enabling the plants to develop with a limited supply of nutrients.

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CHAPTER II

THE MICROBE AND ITS ACTIVITIES

The Microbe and its Importance in the Soil.—The various forms of life which develop directly at the expense of the chemical constituents of the soil and the decomposing plant and animal residues are extremely numerous and diverse. Included among these forms are all plants, ranging from the large trees to the tiniest of microbes, with an almost limitless number of transition forms bridging this wide gap, and besides these are various representatives of the animal kingdom. Indirectly, all animal forms depend for their nutrition upon the elements which are liberated in the soil; the microbes of the soil keep the elements in circulation. Were it not for them, all plant and animal life would soon cease because of the exhaustion of the supply of available elements. A knowledge of these microbes and their activities is, therefore, of prime importance for the understanding of soil processes and plant growth.

The study of the nature and activities of soil microorganisms would be of considerable interest even if the existence of these organisms had no great influence upon the growth of other plants and animals. Their identity, habits of growth, and influences become of much wider interest and extreme importance with the realization that to these tiny living things we owe the continued development of higher plants. Studies of the reactions with which they are associated in their strife for existence, and the variety of influences which they exert directly and indirectly upon other living organisms, open a fascinating chapter in biology.

The microorganisms are the least differentiated of all forms of life—they are organisms either unicellular in structure or, at the most, simple aggregates of a few cells with comparatively minor differentiation. They are too small to be seen as individuals with the naked eye, and in some cases too small to be seen even with the aid of the most powerful microscope, and are known only through their activities, as in the case of the bacteriophage and filterable viruses. However, they vary considerably in the chemical processes that they bring about in the soil and in artificial cultures.

The importance of a study of the nature and development of higher plants is accepted without question because these plants are so evident in appearance and in general usefulness to man and his domesticated animals. In a similar manner, one has come to recognize the importance of those microorganisms which have been discovered to be the agents responsible for the various diseases of man, domestic animals, and plants, and the need for combating them. However, the microscopic forms of life in the soil are so obscured by their environment that they are not given such general consideration. They are frequently given no attention or considered to play an insignificant part in natural processes. The transformations with which they are concerned have been at times completely overlooked or called non-biological. Provided microbes were not generally distributed in soils, their presence in certain localities would be very apparent from the enhanced plant growth. Under the prevailing conditions, however, it might be considered that their very ubiquity tends to conceal their activity.

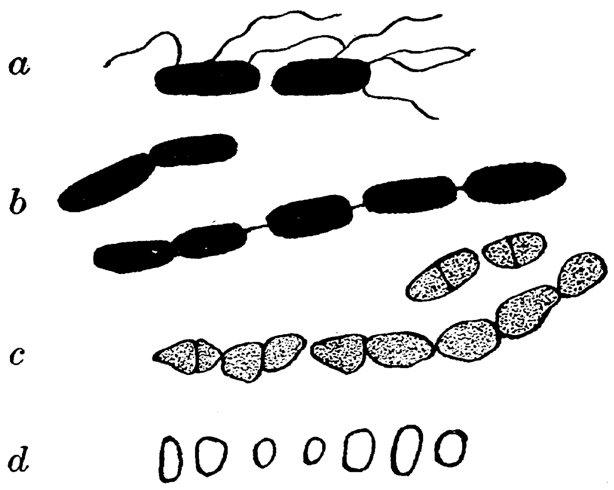

FIG. 7.—Heterotrophic, spore-forming bacterium, *Bacillus megatherium;* (a) young cells showing flagella, (b) young cells showing connections into chains of rods, (c) older cells, (d) variations in size and shape of spores (after Conn).

These soil organisms are largely saprophytic, acting upon the organic plant and animal residues and the inorganic soil constituents. Occasionally certain of the representatives may attack living tissues of the higher plants; the soil may also harbor a number of animal parasites. As compared, however, with the saprophytes, either in numbers, kinds, or importance, these occasional parasites are quite insignificant, except in certain special instances.

A qualitative survey of the microscopic organisms found in the soil discloses a heterogeneous assortment of forms belonging to no single classified group, either physiologically or morphologically. They vary considerably both in their general appearance and in the nature of the processes which they bring about. The individual organisms are so small that they cannot be seen with the naked eye and their structures can be studied only by means of powerful microscopes. However, a mass of growth of microorganisms, frequently referred to as a colony, can be obtained on

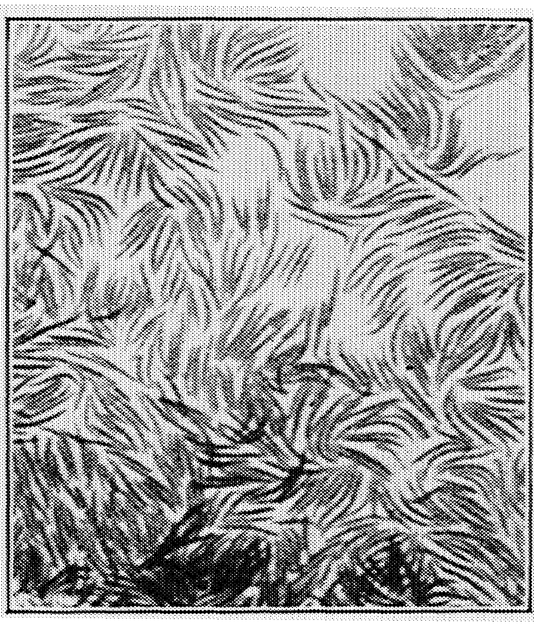

FIG. 8.—Aerobic cellulose-decomposing bacterium, *Cytophaga lutea* (from Winogradsky).

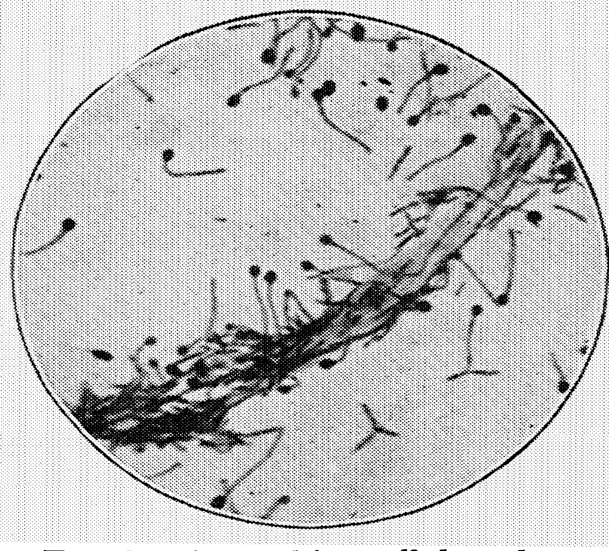

FIG. 9.—Anaerobic, cellulose-decomposing bacterium, *Bacillus cellulosae dissolvens* (from Khouvine).

natural or artificial substrates, large enough to be studied with the naked eye.

NATURE OF SOIL MICROBES.—Both in respect to numbers and abundance of cell substance in the soil, most of the microbes belong to the plant kingdom. These include various bacteria, actinomyces or ray fungi, a number of true fungi including the molds and the mushrooms, as well as various green and blue-green chlorophyll-bearing algae. The animal kingdom is numerically less abundant in the soil. Representatives include the protozoa, the nematodes or round-worms, the rotarians or wheel animalcules, a number of earthworms, and larvae of insects. In view of the fact that, in the case of some microbes, it is difficult to decide whether they should be classified with plants or with animals, they have frequently been referred to as *Protista* (first life). All these microbes have one thing in common, namely, they are all very small, and, with the exception of the higher animal forms, such as earthworms and larvae, and the fruiting bodies of mushrooms and certain other fungi, they cannot be seen with the naked eye.

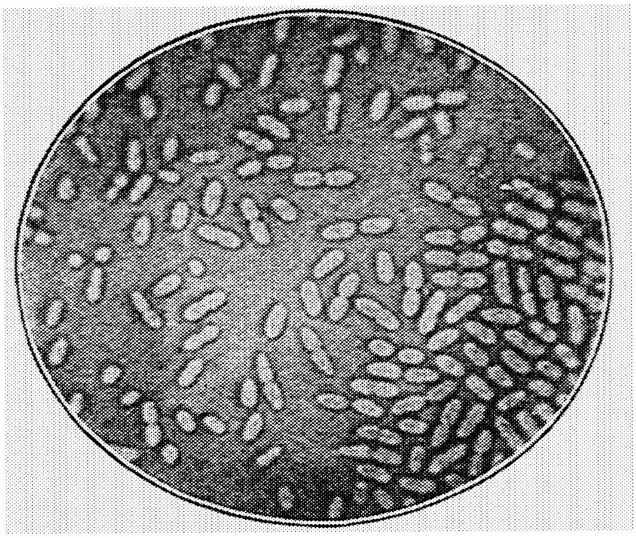

FIG. 10.—Aerobic nitrogen-fixing bacterium, *Azotobacter chroococcum* (from Krzemieniewski).

Various methods have been developed for the study of soil microbes. These include (1) microscopic methods for the study of the organisms as found in the soil; (2) cultural methods for the study of the activities of the microbes in the soil itself; (3) methods for the enumeration of different groups of soil microbes; (4) methods for the isolation and cultivation in pure culture of various microbes.

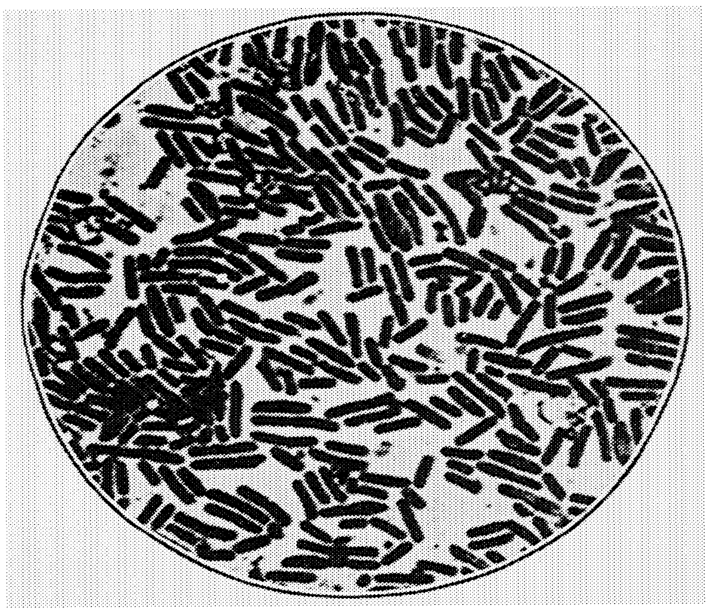

FIG. 11.—Anaerobic nitrogen-fixing bacterium, *Clostridium pastorianum* (from Omeliansky and Solounskoff).

In order to understand the rôle of a certain microbe in a known soil process, it is desirable to isolate this microbe from the soil, grow it in pure culture on artificial media, then reinoculate it into sterile soil and bring about the process in question under controlled conditions. A pure culture of a microbe is an isolated cell which reproduces a number of times giving identical cells. Such a culture allows an exaggeration of the activities of the particular microbe, so as to make accessible to observation the elementary principles of cell nutrition. It is not always possible or even necessary to isolate a soil organism in pure culture; valuable information concerning soil processes may be obtained even with so-called crude cultures. For physiological investigations, however, pure cultures of organisms are very desirable and frequently necessary.

NUTRITION OF SOIL MICROBES.—The microbes bring about in the soil various transformations which result in the liberation of plant food in forms available to cultivated and uncultivated plants. They find the soil a favorable medium for their activities, and also obtain from the soil a sufficient supply of energy and nutrients required for growth and reproduction. Most of the numerous microbes which inhabit the soil are useful, while some are or may become harmful from the standpoint of the practical agriculturalist. Since the activities of soil microbes are so closely associated with plant growth, an economic management of the soil requires a thorough understanding of these activities and their direction into the proper channels, so as to reduce the injurious effects to a minimum and increase the useful activities to a maximum. Frequently the development of toxic or unfavorable conditions for plant growth may be completely avoided or quickly overcome if, in the treatment of the soil, the activities of the soil microorganisms and not alone the higher plants are taken into consideration.

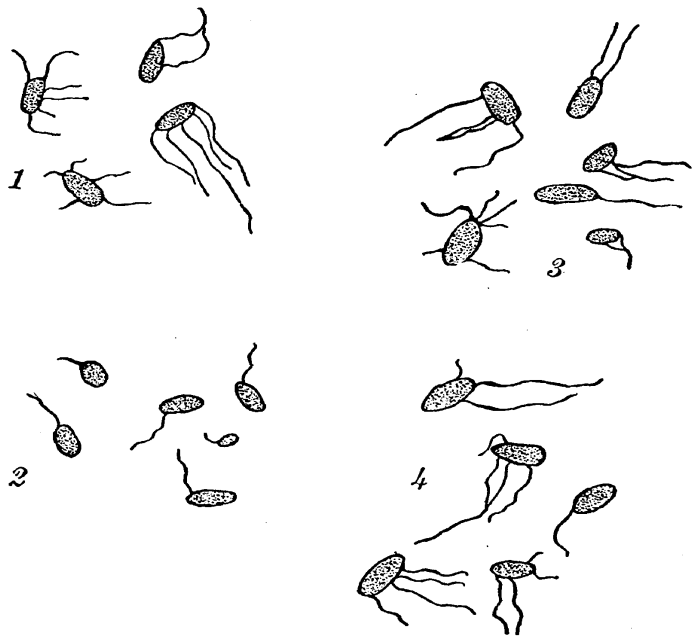

FIG. 12. — Symbiotic nitrogen-fixing bacterium; different strains of *Bacterium radicicola* obtained from: (1) garden bean, (2) goats rue, (3) vetch, (4) alfalfa (after Shunk).

In nutrition, microorganisms have much the same requirements as higher forms of life, but their existence as single cells is much simpler than that of their multicellular associates. All microbes require for their growth and synthesis of cell substance, supplies of energy and several nutritive elements, essential for the building up of their cells, including carbon, hydrogen, oxygen, nitrogen, phosphorus, potassium, sulfur, and a few others.

With the exception of the algae, all the microbes of the soil are devoid of chlorophyll. They are thus compelled to derive their energy either from the oxidation of simple inorganic substances, as in the case of the limited groups of autotrophic bacteria, or from complex organic substances, as in the case of the majority of bacteria or the heterotrophic organisms, and of all fungi and protozoa. The great majority of soil organisms are thus dependent upon the complex organic substances of the soil for their carbon and energy supply. The algae and autotrophic bacteria can obtain their carbon from the carbon dioxide of the atmosphere, the former using by means of their chlorophyll the energy of the sun, and the latter using energy which is liberated in the oxidation of simple inorganic substances, such as ammonia, sulfur, or hydrogen. The elements hydrogen and oxygen are present in sufficient amounts in water and in the gases of the atmosphere to supply all the needs of the microbes. Frequently, however, conditions arise (such as deficiency of elementary oxygen) under which microbes obtain their oxygen from inorganic compounds of nitrate or sulfate.

One of the most important elements in the nutrition of microbes is nitrogen. This is utilized by the microbe either in the form of complex organic substances, such as proteins and amino acids, or as simple inorganic compounds, such as nitrates or ammonium salts. Certain limited groups of bacteria are capable of utilizing the gaseous atmospheric nitrogen. The other elements are obtained by the microbes from the minerals of the soil, phosphorus being used in greater quantities than any of the others.

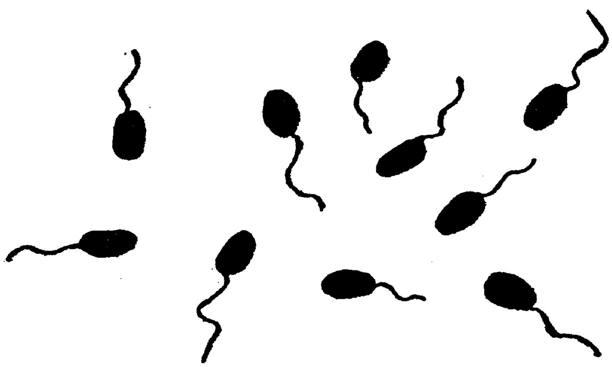

FIG. 13.—Nitrite-forming bacterium, *Nitrosomonas europea* (after Winogradsky).

In the utilization of the organic materials incorporated in the soil, the microorganisms make use of an elaborate store of enzymes, or organic catalysts. These may be active in attacking the insoluble protein or carbohydrate molecules, or in oxidizing, reducing, or hydrolyzing the numerous substances which serve as sources of energy and as materials for cell construction.

Bacteria of the Soil.—The most abundant group of organisms in the soil are the bacteria, exceeding both in numbers and in the variety of their activities all the other soil organisms, but not, however, in the bulk of the organic cell substance found in the soil in the form of living and dead microbes. The fact that bacteria are so abundant and that they participate in numerous soil processes is largely responsible for the frequent reference to the total microscopic population of the soil by the term *bacteria of the soil,* and reference to the whole subject of soil microbiology as *soil bacteriology.* These bacteria are so small that if 500 to 1000 of them were placed in one line, end to end, they would only extend for a length of one millimeter. They are in general about the size of colloidal particles, the maximum size of which is about 5 to 8 microns—one micron being 1/10,000 of a centimeter. It takes about 1,000,000 to 1,000,000,000 of them to weigh one milligram, or one-thousandth part of one gram; even then this mass of cell material will be found to consist of 80 to 85 per cent of water.

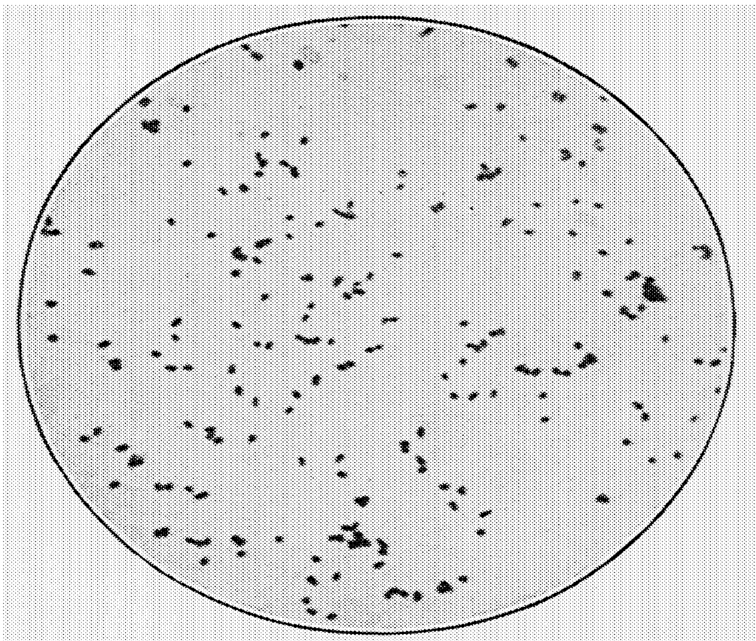

Fig. 14.—Nitrate-forming bacterium, *Nitrobacter* sp. (from Fred and Davenport).

It is not exceptional to find hundreds of millions of bacterial cells per gram of soil, particularly where organic substances have been added. Let us assume that there are one hundred million bacteria in one cubic centimeter of soil and that each cell occupies only one cubic micron of space. Since there are 1,000,000,000,000 cubic microns in one cubic centimeter, and since the one hundred million bacteria would occupy only 100,000,000 cubic microns, the cells would occupy only one-ten thousandth of the total volume of the soil. It is not surprising that the activities of such large numbers of living things may continue unnoticed in a small particle of soil and become revealed only by a careful search using a highly specialized technique.

These minute organisms are capable of bringing about very rapid and extensive transformations of the substrates upon which they are growing. A few hours are sufficient for the complete decomposition of a quantity of sugar to carbon dioxide and water, or of protein to ammonia, carbon dioxide, water, and other compounds. The extent of the development of these microbes is limited only by the supply of available energy, by the environmental conditions favorable or unfavorable to their development, and by the formation of certain products injurious to their own activities. Although modifications in the supply of oxygen, moisture, and of inorganic compounds, or a change in temperature, may affect the development of the soil population, the greatest response in activity is brought about by the incorporation of organic substances with the soil.

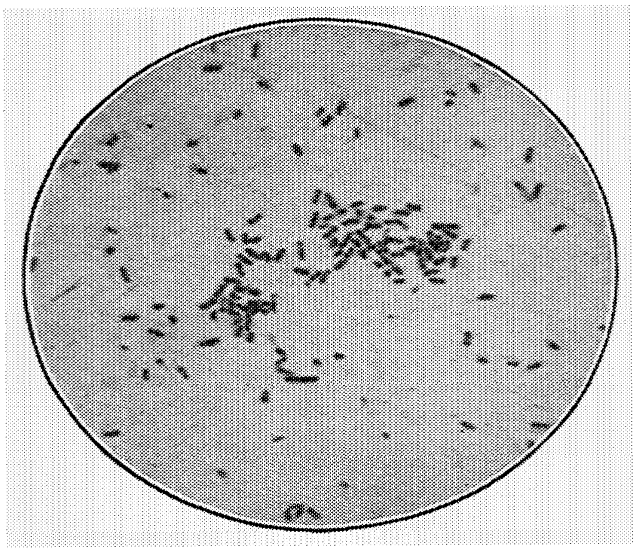

FIG. 15.—Sulfur-oxidizing bacterium, *Thiobacillus thiooxidans* (from Waksman).

In general, bacteria are not easily differentiated morphologically. The shapes which they may assume are very limited in number, and one must rely largely upon physiological distinctions for the identification of their numerous species. The soil bacteria are divided into three main groups: (1) The *cocci* or the spherical forms. (2) The *bacilli* or the rod-shaped forms, with rounded or square ends, differing both in length and in thickness. Some of them are able to form spores which are more resistant to unfavorable environmental conditions, such as dryness, changes in temperature, and action of antiseptics, than the vegetative cells; those rod-shaped bacteria which are unable to form spores are less resistant to adverse changes in environment. Some of these bacteria depend upon the movements of the fluids in which they live to transfer them, while others are able to migrate by means of their organs of locomotion, namely, the flagella. (3) The *spirilla* are thin spiral-shaped forms, which have different numbers of turns characteristic for the different species; those forms which produce only one-half turn are intermediate between rods and spirilla, and are spoken of as *vibrios*. Numerically, the rod-shaped forms are much the largest group of the soil bacteria.

Figs. 7 to 16 show several typical soil bacteria. Fig. 7 represents the general group of spore-forming, rod-shaped, heterotrophic bacteria, living by the processes of decomposition of various organic compounds in the soil. Figs. 8 and 9 represent two typical bacteria capable of decomposing cellulose, one being aerobic and one anaerobic in nature, living in the presence and in the absence of atmospheric oxygen, respectively. The aerobic and anaerobic non-symbiotic nitrogen-fixing bacteria are shown in Figs. 10 and 11. Species of symbiotic legume bacteria are shown in Fig. 12. The important groups of nitrifying bacteria, namely, the nitrite- and nitrate-forming organisms, are shown in Figs. 13 and 14. Bacteria capable of obtaining their energy from the oxidation of elementary sulfur to sulfuric acid are shown in Fig. 15. A bacterium which can reduce sulfates to sulfides is represented in Fig. 16. The activities of some of these bacteria and their rôles in soil processes will be described in subsequent chapters.

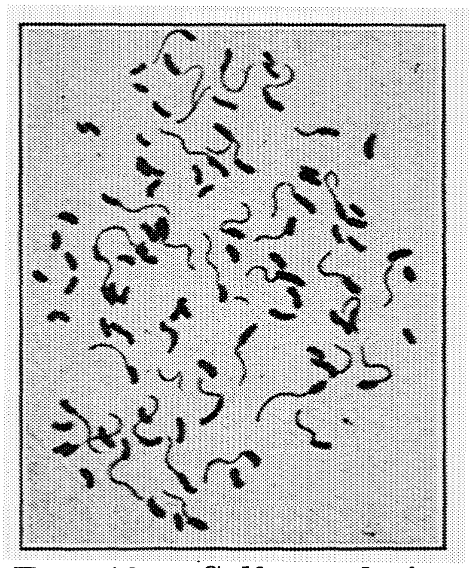

FIG. 16. — Sulfate-reducing bacterium, *Spirillum desulfuricans* (from Beijerinck and Omeliansky).

Nearly all the bacteria are so small that they are able to pass through ordinary filters. To remove them from liquids, filtration is accomplished by the use of Chamberland candles made of unglazed porcelain, or Berkefeld filters made of diatomaceous earth. The pore spaces of these filters are so small that they will prevent the passage of bacteria which are visible with the microscope. It has been established, however, that there are certain ultra-visible bacteria which can even pass through these filters. These are spoken of as *ultramicroscopic organisms*. Such tiny bits of life appear to be incapable of existing upon inert material and, so far as is known, can develop only in conjunction with living cells. The presence of such organisms in soil is speculative for the most part. There is, however, a certain kind of ultramicroscopic substance parasitic upon bacteria—the bacteriophage—which is of common occurrence in soil. It has been found that these organisms are about the size of a protein molecule, or only 20 to 30 millimicrons ($\mu\mu$) in diameter. Some appreciation of this infinitesimal size may be gathered from the fact that 1 $\mu\mu$ is equal to 1/1,000 μ, or 1/10,000,000 of a centimeter. It has also been shown that the ultramicroscopic organism causing mosaic diseases in plants may survive in soil for a short period of time.

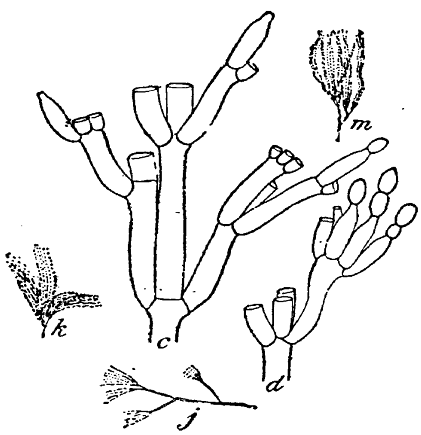

FIG. 17.—*Penicillium* sp.: (*c*, *d*) terminal sporulating filaments highly magnified; (*j*, *k*, *m*) lower magnifications of sporulating organs (from Thom).

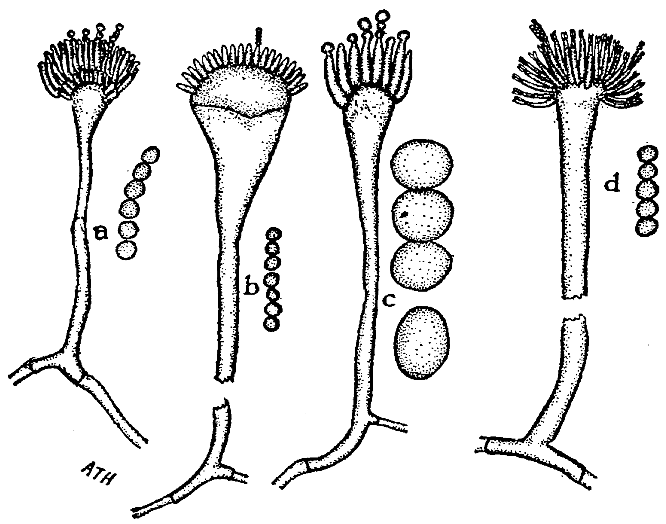

FIG. 18.—*Aspergillus* sp. showing various sporulating characteristics and differences in sizes of spores (from Henrici).

ACTIVITIES OF SOIL BACTERIA.—The activities of the bacteria, especially those concerned with the decomposition of organic matter, are frequently referred to by the layman as processes of "putrefaction," "fermentation," and "decay." These terms (with the possible exception of the use of the term "fermentation" as applied to the decomposition of carbohydrates under anaerobic conditions) are significantly important only historically. In times past they were used in reference to a variety of processes previous to an understanding of the nature of the chemical changes and the biological agents which were involved. These terms will, therefore, be carefully avoided in the following pages.

Some of the soil bacteria are strictly aerobic in nature, developing only under conditions where there is free access of air; some exist in the complete absence of oxygen, under so-called anaerobic conditions. Most of the soil bacteria develop best at reactions close to neutrality, where there is no appreciable excess acid or base. Some, however, can exist at a much higher acidity than others. Thermophilic bacteria, or those which are favored by high temperature, such as 50 to 60° C, also occur in soils, but the great majority of the soil bacteria grow best at reactions between 20 and 30° C. All the bacteria require a considerable supply of water to permit their active development. Under dry conditions, they either change to resistant spores, become dormant vegetative cells in the moist films which cover the soil particles, or die from the effects of desiccation. In the presence of excessive moisture where rapid circulation of oxygen is prevented, the aerobic microorganisms are depressed and the anaerobic cells find conditions more conducive to their growth.

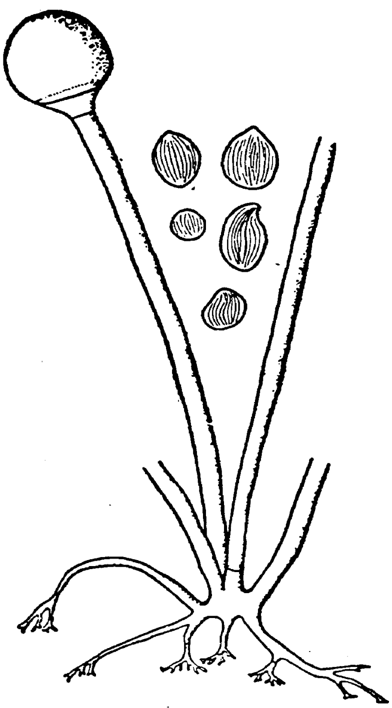

FIG. 19.—*Rhizopus* sp. (from Jensen).

As a result of microbial attack upon proteins, there are formed carbon dioxide, ammonia, and various incompletely decomposed organic substances. From the decomposition of carbohydrates, carbon dioxide, organic acids, and alcohols are produced. The oxidation of ammonia leads to the formation of nitrites which are oxidized to nitrates. These various substances may be further acted upon by soil organisms. The same organism may also produce different end products from different initial compounds, or from the same compound under different conditions. The numerous processes brought about by soil organisms are usually interdependent and follow one another. Irrespective of their source, these substances are waste products of the metabolism of the microbial cells, and many of the nutrients obtained by higher plants as a result of the activities of bacteria in the soil are actually waste products of the nutrition of these bacteria.

Among the soil processes in which bacteria are important and frequently play a predominant rôle, the following need only be enumerated here in order to suggest the diversity of the changes: the fixation of atmospheric nitrogen symbiotically by bacteria in association with leguminous plants, and non-symbiotically by the bacteria in a free-living condition in the soil; the decomposition of various organic constituents of plant and animal residues, including cellulose, hemicelluloses, sugars, proteins, amino acids, fats, and waxes, both under anerobic and aerobic conditions; the liberation of ammonia as a result of the decomposition of various proteins, protein derivatives, and other organic nitrogenous substances; the formation of nitrites and nitrates from ammonia; the reduction of nitrates to nitrites, to ammonia and to atmospheric nitrogen; the oxidation of sulfur and of hydrogen, as well as of incompletely oxidized compounds of sulfur and of iron, and the oxidation of various other simple inorganic and complex organic substances.

FIG. 20.—*Trichoderma* sp.: (*a*) arrangement of spores on sporulating hyphae; (*b*) higher magnification of one of the branches (from Henrici).

Truly, bacteria are active in all phases of transformation of inorganic complexes and of organic matter in the soil, this fact partly justifying the early conception that bacteria are the only significant soil organisms. However, as the important rôle of other groups of soil microbes is understood, it becomes evident why the general conception of the soil population was changed to include a consideration of many other soil microbes. The science

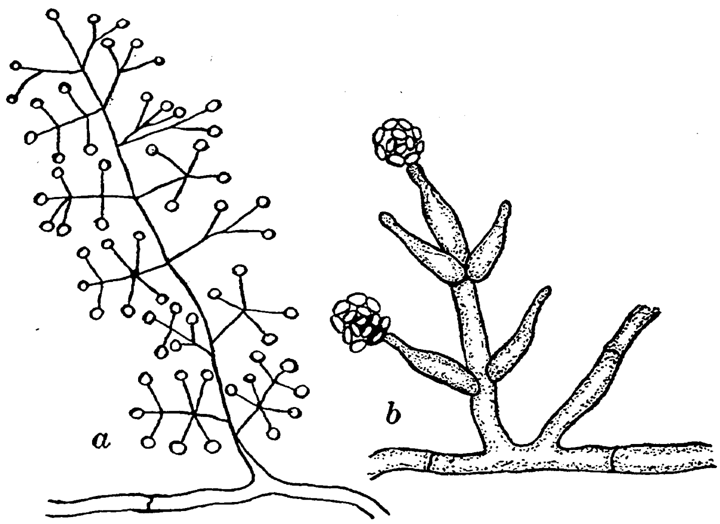

FIG. 21.—*Acrostalagmus* sp.: (*a*) arrangement of spores on sporulating branches, (*b*) higher magnification (after Goddard).

of *soil microbiology* may be interpreted as embracing a study of the microscopic soil population which is responsible for the numerous transformations occurring in the soil environment. However, many of the soil microorganisms are not limited to this environment alone but may exist also in a variety of other habitats.

THE FUNGI OF THE SOIL.—Fungi are characterized by their filamentous structure. This is termed mycelium, and consists of numerous hyphae, either unicellular or multicellular. This mycelium frequently attains considerable dimensions and forms a complicated, profusely branched, vegetative growth with specialized, spore-forming, fruiting bodies. Although numerically, as determined by the number of single cells, they are fewer in the soil than the bacteria, their actual abundance, as measured by the amount of cell substance produced, may be considerably greater than that of bacterial growth. They vary considerably in structure and size from the simplest yeasts and molds to the more complex forms, as the mushrooms and bracket fungi whose sporulating bodies can be seen with the naked eye. Fungi are devoid of chlorophyll, and consequently cannot obtain their carbon from the carbon dioxide of the air as green plants do.

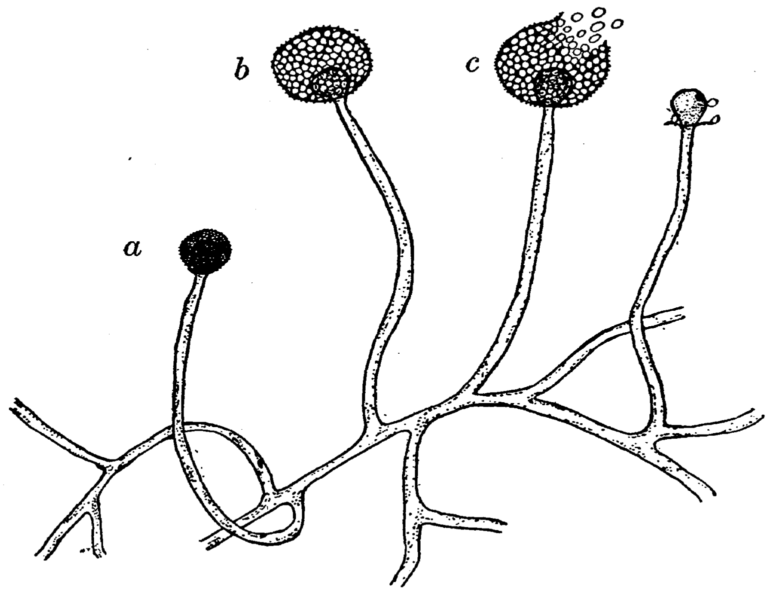

FIG. 22.—*Mucor* sp: (*a*) young sporulating head, (*b*) mature sporulating organ, (*c*) spores becoming liberated from sporangium, (*d*) columella after the scattering of the spores.

Fungi can be classified into three principal groups on the basis of their morphology: (1) *Filamentous fungi*, which are frequently spoken of as *molds*. These comprise various Phycomycetes, Ascomycetes, and Fungi Imperfecti or Hyphomycetes. The important soil genera, Mucor, Rhizopus, and Zygorhynchus are found in the first class; Aspergillus and Penicillium in the second, while a number of other forms, including Fusarium, Hormodendrum and Trichoderma, producing both colorless and black mycelium, belong to the third class. (2) The *yeasts*, which belong to the Ascomycetes, are found in the soil only to a limited extent. (3) The *mushroom fungi*, belonging to the Basidiomycetes, are found abundantly in forest and other soils in the form of a very extensive mycelium, sometimes producing fruiting bodies in the form of various mushrooms, toadstools, and puffballs. Many fungi belonging to this group also produce an associative growth with the roots of higher plants, especially forest trees, referred to as *mycorrhiza* (or fungus root). In such associations, the fungi assist the plants in obtaining their nutrients from the soil. As a rule, the fungi show such differences in structures that their classification into species is almost entirely based on morphological features; unlike the bacteria, little resort to physiological tests is used in their differentiation. Figs. 17 to 23 show a series of typical soil fungi.

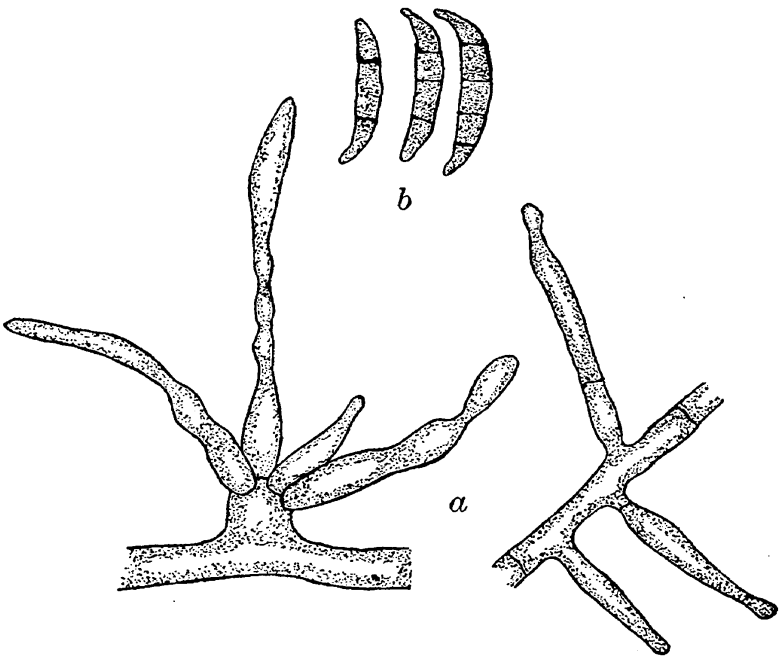

FIG. 23.—*Fusarium* sp: (*a*) hyphal segments, (*b*) spores (after Sherbakoff).

The fungi vary considerably in the nature of the processes which they bring about in the soil, but they are almost all associated with the decomposition of complex organic substances, non-nitrogenous as well as nitrogenous. They have the capacity for producing more cell material per unit of organic substance decomposed than the bacteria. They are usually able to grow only under aerobic conditions, although most yeasts and some Mucors can also live in the absence of free gaseous oxygen, without, however, making much increase in cell substance under such anaerobic conditions. Certain Basidiomycetes may grow extensively in anaerobic environments, providing portions of the growth are exposed to an abundant supply of oxygen. Their deep penetration into woody tissues and thick masses of decomposing organic materials illustrate this characteristic development.

The filamentous fungi are probably more tolerant to wide changes in reaction than any other large group of soil microorganisms. A great number develop equally well under acid and under alkaline conditions, although spore formation may be somewhat delayed at high alkalinity. They are particularly favored by a relatively humid aerobic environment.

The soil fungi are very active in the decomposition of proteins and of various complex carbohydrates, such as cellulose and hemicelluloses. Some fungi are far more active than bacteria in such transformations. As a group, they are more versatile than any others in their ability to decompose a great variety of organic compounds. Some of the most resistant substances succumb to the attacks of the fungi: these include a wide variety of nitrogenous compounds, cellulose, starch, pentosans, vegetable gums, paraffins, and lignin. Nearly all of the simple and complex organic compounds are attacked by one group or another of the soil fungi, and it is through these activities that they play a particularly prominent part in the transformations in the soil.

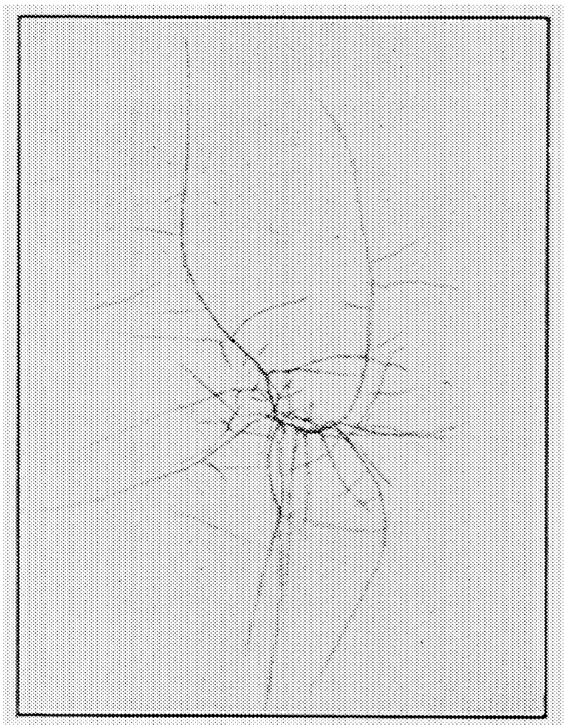

FIG. 24.—Young mycelium of Actinomyces (from Lieske).

THE ACTINOMYCES OF THE SOIL.—The actinomyces are similar to the bacteria in that they are of about the same size in cross-section. They are unlike the true bacteria and resemble the filamentous fungi in that they produce a very extensive unicellular filamentous network, very profusely branched and in many cases reproducing by sporulation on specialized structures. Microscopically these spores appear like bacterial cells. Sometimes the entire mycelial growth breaks up into fragments which, as individuals, are indistinguishable from bacterial cells. As a group, the actinomyces may be considered distinct, having characteristics which place them in a position intermediate between the bacteria and the filamentous fungi, although the majority of the soil forms somewhat more closely resemble the fungi than the bacteria. The mycelium is, however, much smaller in cross section than that of the filamentous fungi. The actinomyces are relatively abundant in the soil, making up from 10 to 60 per cent of the colonies developing on the plates which have been prepared from soil, by the use of artificial media. Actinomyces are active in the decomposition of a variety of organic substances in the soil.

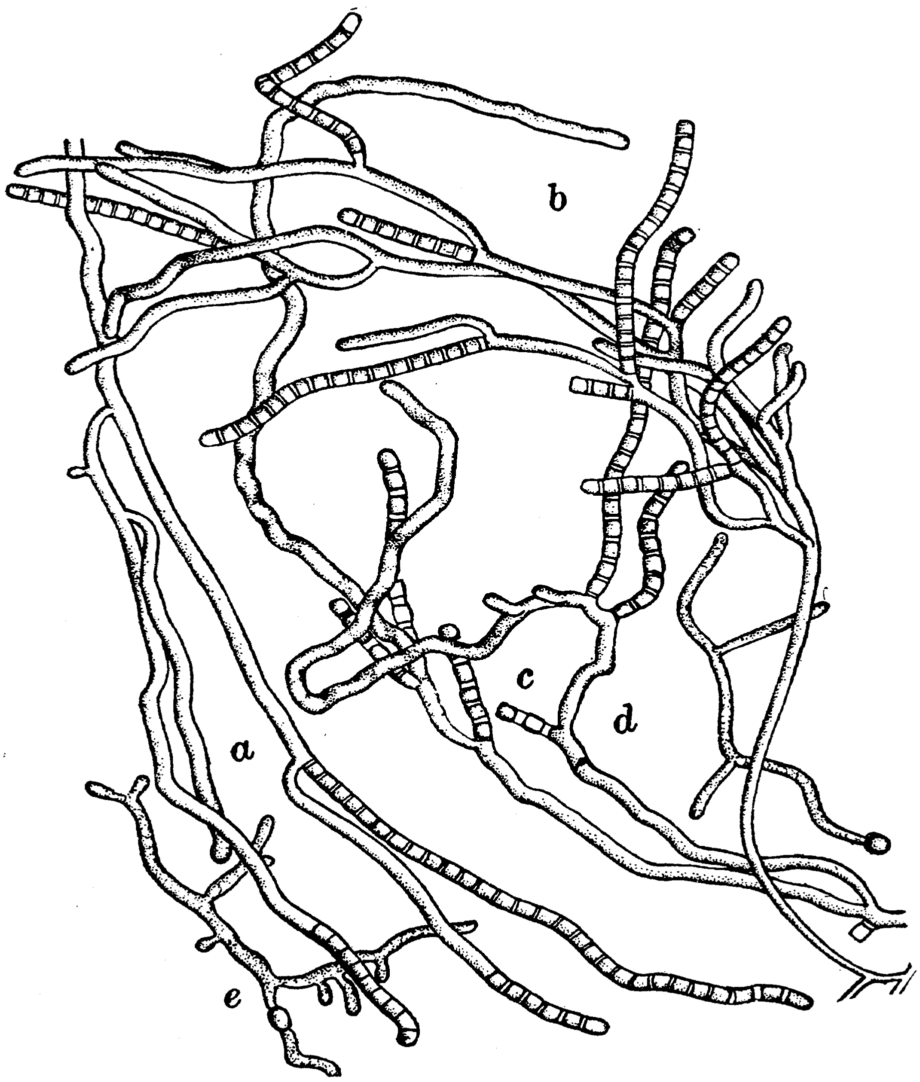

FIG. 25.—Branching mycelium of Actinomyces (from Drechsler).

Most of the actinomyces are aerobic, although possibly less strictly so than most aerobic bacteria. They are as a rule more sensitive to changes in reaction and live over a narrower range of acidity and alkalinity. They appear to make more extensive development than the bacteria in soils of low moisture content, but they develop quite well in fairly moist soil under aerobic conditions. Fig. 24 shows a typical actinomyces mycelium as it appears under the microscope, using low magnification. A much more complicated structure is shown in Fig. 25; this schematically represents highly magnified portions of growth.

THE ALGAE OF THE SOIL.—The chlorophyll-bearing microscopic plants, namely the algae, also form an extensive group of soil organisms. They vary greatly in size and in shape; but the soil forms are largely microscopic species, unicellular or filamentous in structure. Since they contain chlorophyll and are capable of utilizing the energy of the sun, they are independent of the energy sources in the soil so long as they have free access to light. They are capable of making an extensive growth, deriving from the soil the required nitrogen and minerals. They live both on the surface of the soil and at various depths beneath the surface. Below the soil surface they act in a manner similar to fungi, that is, living at the expense of the energy derived from the utilization of organic materials.

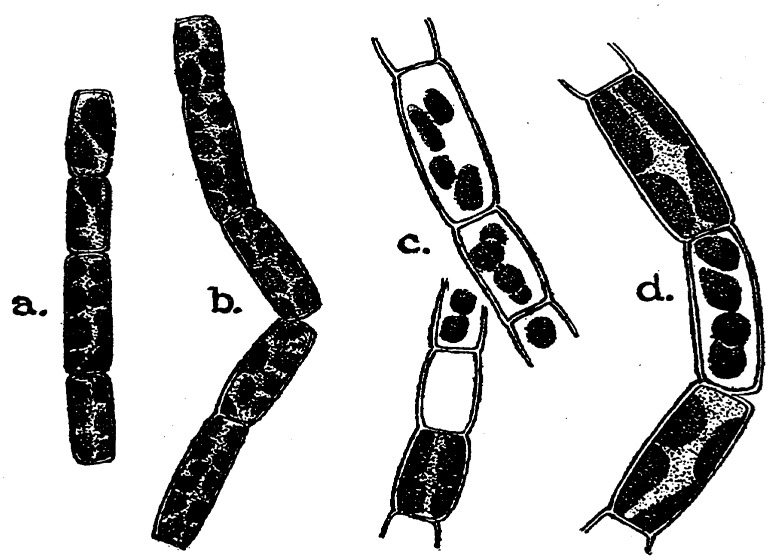

FIG. 26.—Soil alga, *Bumillaria exilis* (from Bristol).

Soil algae are divided into three groups: (1) *Cyanophyceae* or *blue-greens*; (2) *Chlorophyceae* or *grass-greens*; and (3) *Diatomaceae* or *diatoms*. Representatives of these three groups are found in the soil in considerable numbers, generally less abundantly, however, than the bacteria and the fungi. Their development 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. Figs. 26 and 27 show two of the most common types of soil algae. Fig. 28 shows a soil diatom.

The Protozoa of the Soil.—The protozoa are the most elementary forms of life belonging to the animal kingdom. They are all microscopic, unicellular, larger than bacteria and more complex in their activities. They move either by means of cilia, flagella, or pseudopodia. The nature of their locomotion is used as a basis for their classification. They may be conveniently grouped as follows: (1) The Ciliata, which include the *ciliates* or *infusoria*; (2) the Mastigophora, which include the *flagellates*; and (3) the Rhizopoda, which include the *amoebae*. They vary from naked forms to those which produce chitinous or siliceous envelopes. All three groups are abundantly represented in the soil, the smaller flagellates and amoebae being especially abundant. Representatives of the various groups of protozoa are shown in Figs. 29–33.

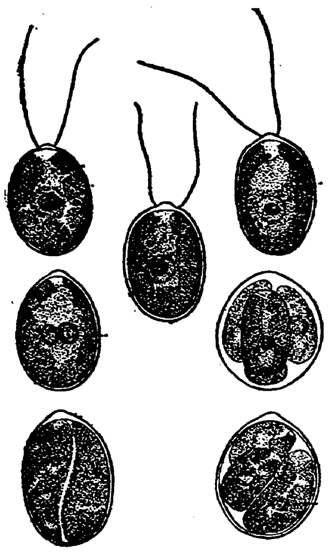

Fig. 27.—Soil alga, *Chlamydomonas communis* (from Bristol).

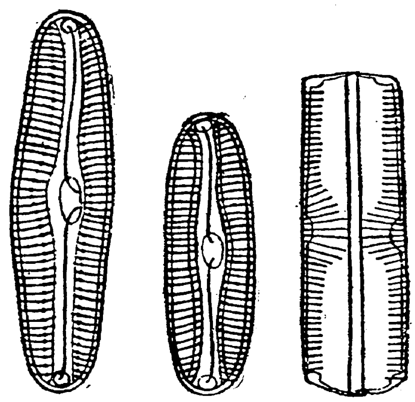

Fig. 28.—Soil diatom, *Navicula terricola* (from Bristol).

Physiologically the protozoa vary considerably. Due to the fact that it is very difficult to free protozoa from bacteria, the exact nature of the physiology of these infinitesimal animals is still a matter of dispute. Some of them at least are capable of utilizing dead organic and inorganic substances from solution and from solid particles. Many forms are capable of ingesting living bacteria and using them as food. A nutrient solution inoculated with some fresh soil first shows an extensive development of bacteria; this is soon followed by the appearance of numerous protozoa, which use the bacteria as food.

This succession of microscopic forms need not indicate that all the protozoa depend for their nutrition upon the bacterial cells and that they appreciably limit bacterial development. It simply points further to the complex interrelationships of the soil organisms. It gave rise to a theory which tended to explain soil fertility on the basis of the interrelationships of the protozoa and the bacteria. The protozoa feeding upon bacteria were supposed to limit soil fertility, since the bacteria were looked upon as the sole organisms bringing about the important processes of decomposition of soil organic matter. However, this theory has not found confirmation on further study and investigation.

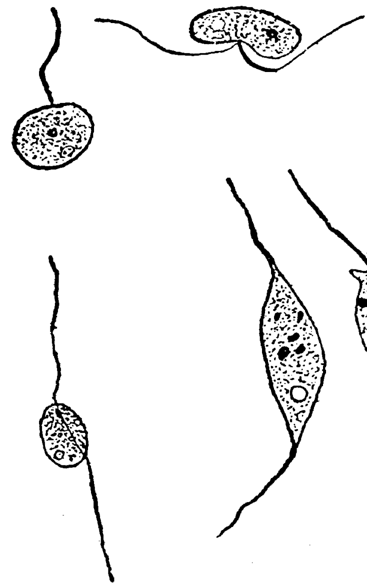

Fig. 29. — Soil flagellates (from Fellers and Allison).

Worms and Insects in the Soil.—The higher animal population is represented in the soil by numerous worms and insect larvae which inhabit the soil permanently and take an active part in soil processes. Some live in the soil only for certain periods of their life. Some of these groups are very abundant in most soils; others occur only in certain soils under a given set of conditions. Among the various worms, it is sufficient to mention nematodes and earthworms. The nematodes (Figs. 34 and 35) alone are found in billions per acre of soil. The insects may find in the soil a temporary habitat, by passing there a certain part of their life cycle; some of them spend in the soil the major part of their life, only coming to the surface occasionally. All these members of the animal population are very active in bringing about an intimate mixture of the soil and the organic matter, in macerating the organic matter and the fungus mycelium attacking it, and thus assisting in the disintegration of the dead materials. Some of them cause damage to living plants by attacking their roots or certain parts growing above ground.

Some nematodes are predacious in nature, feeding upon plant parasites.

Soil Organisms Causing Plant and Animal Diseases.—The soil also harbors a number of organisms which are causative agents of disease, either in plants or in animals. Some of them find in the soil only a temporary habitat, while others persist in the soil for long intervals. The animal pathogens are represented in the soil by such important organisms as *Clostridium tetani*, causing tetanus, *Clostridium botulinus*, causing food poisoning, and various organisms causing sporotrichosis and actinomycosis in man and animals. The number of plant pathogens which

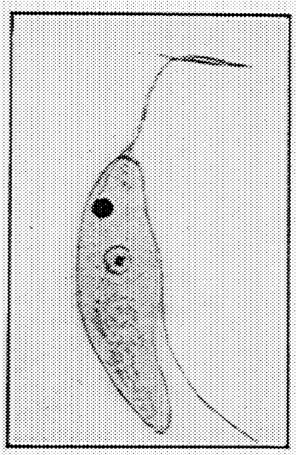

Fig. 30.—Flagellate, *Bodo caudatus* (from Martin and Lewin).

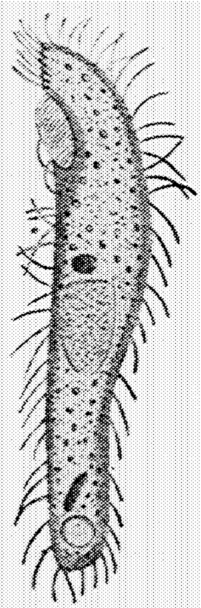

Fig. 31.—Ciliate, *Balantiophorus elongatus* (from Goodey).

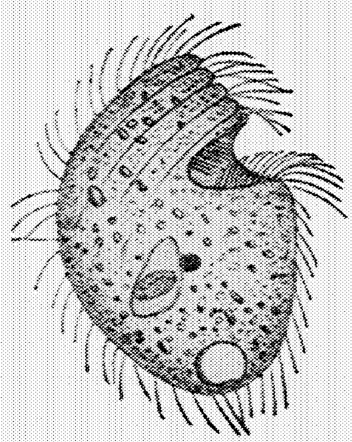

Fig. 32.—Ciliate, *Colpoda steinii* (from Goodey).

find a temporary or permanent habitat in the soil is quite large. This group includes organisms causing such bacterial diseases as various rots, wilts, leaf spots, and galls; such fungus diseases as numerous root rots, damping-off fungi and blights; such actinomycotic diseases as scab in potatoes and sugar beets and pox in sweet potatoes; various nematodes causing diseases which appear as swellings on the roots of a number of plants.

The soil microorganisms are frequently classified into beneficial and injurious forms, on the basis of their relation to man and his cultivated plants and domestic animals. The beneficial microbes are considered as taking part in the various transformations in the soil which result in the liberation of nutrients necessary for the growth of plants, which in their turn supply the food for domestic animals and man. The injurious organisms either attack the plants and animals directly, by causing various diseases, or injure them indirectly by bringing about transformations in the soil which are unfavorable to plant growth.

Such a strict classification of microorganisms as either beneficial or injurious forms is too arbitrary. Some organisms may be beneficial at one time and injurious at another, depending upon the soil conditions and plant growth. Even certain organisms, as various Fusaria, Pythia, and Rhizoctonia, which cause plant diseases, grow extensively in soil, leading a saprophytic existence and taking a part in the decomposition of organic matter. Other common soil saprophytes may cause nitrogen starvation of plants at one time and produce an abundance of nitrogen available for plant consumption at another time, depending upon the cultural treatment of the soil.

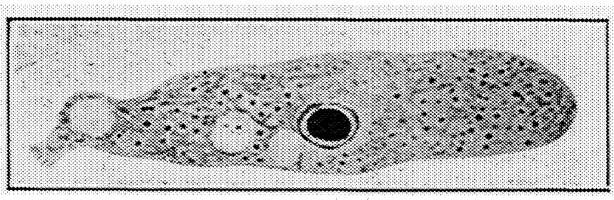

FIG. 33.—Soil amoeba, *Vahlkampfia soli* (from Martin and Lewin).

SYMBIOTIC RELATIONSHIPS.—Certain soil microorganisms live in definite symbiosis with plants. Included in this group are various fungi that form mycorrhiza with forest trees and other plants, such as orchids. The activities of these fungi are of decided importance in the nutrition of the specific plants; some of the higher plants may actually be parasitic upon the fungi which supply them with nutrients from the soil, or play another important part in their metabolism. In the case of legume bacteria, which form nodules on the roots of leguminous plants, the symbiosis appears to benefit both the plants and the bacteria.

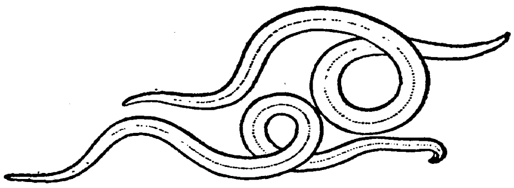

FIG. 34.—Parasitic nematode (from Cobb).

SUMMARY: THE COMPLEX SOIL POPULATION.—All the microorganisms living in the soil and included in the numerous groups of bacteria, actinomyces, fungi, protozoa, algae, and small invertebrate animals make up the *soil population*. They do not live in the soil in separate communities, but are variously intermixed, so that minute particles of soil contain many representatives of the various groups of soil microbes. The reactions produced by a single organism are far from simple, and the complexity of the mixed population makes the reactions taking place in the soil even more complicated. One set of conditions favors the development of certain groups in preference to others. Many microbes compete with one another for the available nutrients; many complete one another, one using as food the waste products of other organisms, some depending for their nutrients upon the activities of others.

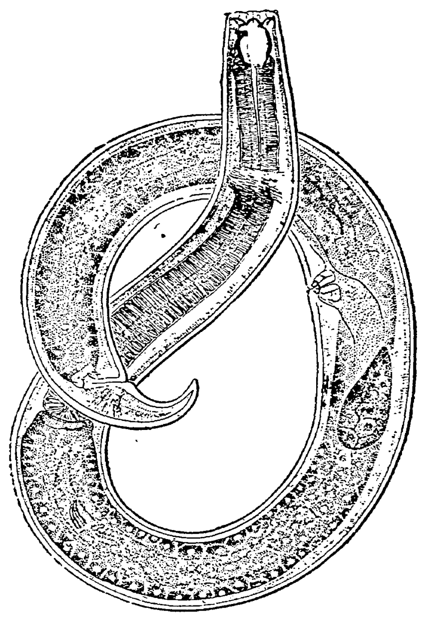

FIG. 35. — Beneficial soil nematode, *Mononchus papillatus* (from Cobb).

The sum total of the activities of the various organisms is the liberation of nutrients from the dead plant and animal débris into forms available for the nutrition of higher plants, as well as the storage of some of the nutrients in the microbial cells which contribute directly or indirectly to the soil organic matter. The environmental conditions modify the nature of the soil population; the nature of the organisms influences the form in which the nutrients again become available; the nature of these nutrients influences the nature of the plant vegetation which a given soil will support. So intimately interwoven are these numerous reactions that their products appear to be formed by a single reaction from a single body.

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CHAPTER III

THE SOIL POPULATION AND ITS DISTRIBUTION

THE OCCURRENCE OF MICROBES IN SOIL.—Because of the fact that microbes are so small in size and that many of them are capable of growing on a variety of foods in a wide range of environments, they are found universally distributed in nature. They are present in great abundance in water, in various foodstuffs, upon all growing and dead plants, in the digestive tracts of animals, upon dust particles, and in soil. Because of their great abundance in nature and the ease with which they are distributed by means of air, water, moving animals, living and dead plants, as well as through man's activities, it is reasonable to assume that in soils of the same composition in identical environments the numbers and abundance of types of microorganisms should be nearly identical.

However, microbes do not develop actively upon all substrates where they may be located. The mere fact that many bacterial cells are found upon clothing and dust does not indicate that they grow and develop upon these substrates; it merely indicates that clothing and dust are carriers of these microbes and that the organisms can be distributed in nature through these vehicles. Likewise certain microbes may be found in the soil merely because they have been introduced there by dust, waters, or through man's activities, as in the application of manures of domesticated animals. The fact that a certain microbe is found in the soil is no proof that it is a normal inhabitant of the soil. The presence of large numbers of *Bacterium coli* in a soil may merely indicate that this organism has recently reached the soil in animal excreta and not that it finds the soil a favorable medium for its development.

One should thus differentiate between microbes found in the soil as a result of their fortuitous introduction and those which lead a normal existence in the soil. If conditions are not favorable for the development of specific organisms, repeated introduction of even great numbers of these microbes will fail to establish them as permanent members of the soil population. The development of a specific microbe in a soil depends upon the chemical composition of the soil, especially the presence of nutrients essential for its growth, and the environmental conditions, such as temperature, moisture, and reaction. The soil and environmental conditions thus determine to a large extent the abundance and kinds of organisms that will inhabit the soil and the reactions which they perform there.

RELATION OF MICROBES TO PLANTS AND ANIMALS.—In their relation to plants and to animals, microorganisms can be considered as being either parasites or saprophytes. The parasitic forms are capable of growing upon the living plant or animal, causing a diseased condition of the tissues, leading to an abnormal condition of the host and possibly to death, unless the host is able to withstand the attack of the parasite.

The saprophytic microorganisms grow only upon dead plant and animal tissues or their decomposition products. They are thus of extreme importance in the transformation of complex organic substances into simple forms, making the nutrient elements locked up in those substances again available for plant nutrition. The typical soil population consists of microorganisms which are largely saprophytic in nature. However, certain plant and animal parasites may persist in the soil for some time, and a few are even capable of leading a normal existence there. Certain microbes are capable of acting parasitically upon other microbes which are either beneficial or injurious to plants. The saprophytic microorganisms are widely distributed in the soil and occur in greater or less abundance practically everywhere. There is no soil known, ranging from the Sahara Desert to the polar regions, that should be free from saprophytic microorganisms. Certain specific groups of organisms may of course be absent, where conditions are unfavorable for their development.

QUALITATIVE AND QUANTITATIVE DISTRIBUTION OF MICROBES IN SOIL.—In most soils we find much the same qualitative distribution of organisms, but there are very marked differences in the quantitative relationships. A change of soil conditions, such as an increase in acidity or alkalinity, a change in food supply, moisture content, aeration, or other physical or chemical soil conditions, is followed by marked changes in the qualitative and quantitative distribution of the soil microflora and microfauna.

The microbes occur in greatest abundance in the surface layer of the soil (Fig. 36). This layer varies in thickness from a few centimeters, as in the case of humid soils, to two or three meters, as in the case of arid soils. The greatest abundance of individuals and variety of microorganisms are found either at the very surface of the soil, as in forests, meadows, and other shaded soils, or just below the surface, as in the case of the open cultivated

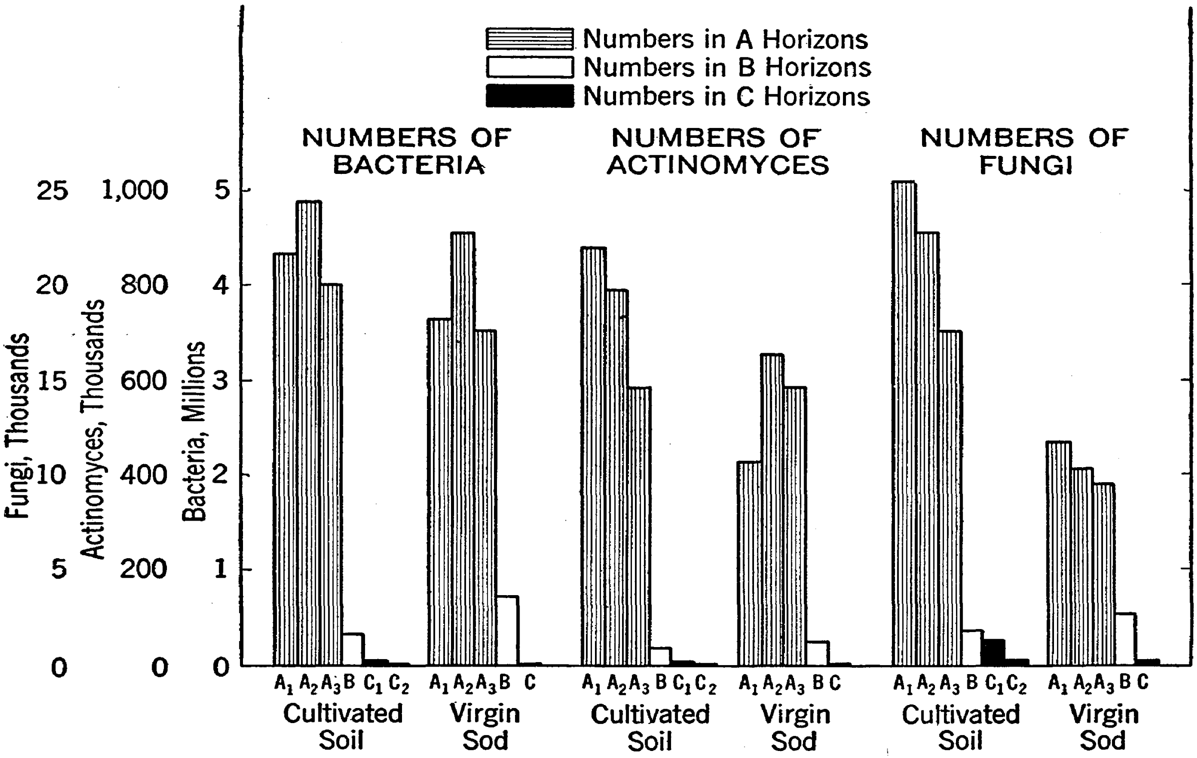

FIG. 36.—Vertical distribution of microbes in soil (after Brown and Benton).

soils. The numbers of microorganisms diminish with depth, the rapidity of the diminution varying with the soil conditions, especially the distribution of organic matter and degree of aeration. The decrease in numbers and activities of microorganisms is very rapid in the shallow, humid soils; in the case of the deep arid and semi-arid soils, the decrease is comparatively slow.

Although there are certain differences in the abundance of organisms from place to place in the soil, the general distribution of microorganisms in any limited soil mass is more or less uniform. However, the relative abundance of the different types of organisms found in different soils varies considerably, depending upon the composition of the soil and the environmental conditions. The physical characteristics of the soil (whether it is sand, loam or clay, porous or compact, well drained or poorly drained), the reaction, abundance and nature of organic matter, the amount of rainfall, average temperature, period of the year, nature of the vegetation, type of cultivation, and numerous other factors—all influence the nature, abundance, and activities of microorganisms in the soil. Largely as a result of the influences of these factors,

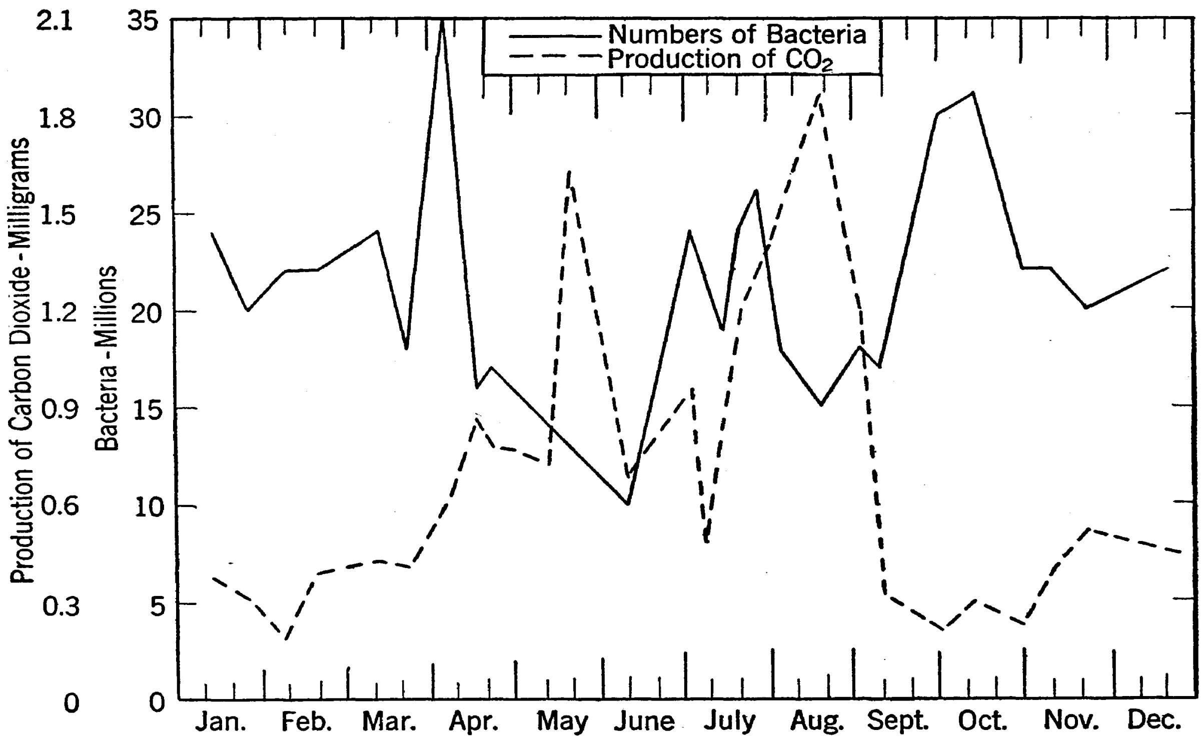

FIG. 37.—Abundance of bacteria and carbon dioxide in soils at different seasons of the year (after Russell).

marked differences are found in the numbers of different groups of microorganisms during different seasons of the year (Fig. 37). Differences may be found even within a few days or a few hours.

It is important to keep in mind the fact that, since the physical and chemical soil conditions even at the same depth of soil are not homogeneous, the distribution of microorganisms throughout the soil as a whole is not uniform. There are pronounced differences in the abundance of microorganisms in different samples of soil from an apparently uniform field. Twice as many organisms may be found in one gram of soil taken at one spot of the field than in another gram taken a few feet or even a few inches distant from the first. Results obtained from studies of the influences of environmental factors on the abundance of microorganisms in field soils should therefore be interpreted with an appreciation of the natural variation in the normal distribution of these organisms in the soil.

METHODS FOR DETERMINING NUMBERS OF MICROORGANISMS IN THE SOIL.—There is no one method available at the present time which can measure the total abundance and activities of soil microorganisms. The various methods commonly employed are adapted to the enumeration of only a certain few groups of organisms commonly found in the soil or to the measurement of one product of the development of some microbes. The organisms which are counted make up only a part, frequently a very small one, of the total soil population, and the product measured is but one of many. The methods used for counting soil organisms are based either upon the observation of microorganisms in stained preparations of soil, or upon their development in solid or liquid culture media which are specially prepared for the cultivation of these organisms. In either case only a part of the total soil population is measured. In the case of stained preparations, some of the organisms are either destroyed in the process of preparing the specimen, as in the case of the protozoa, or are not visible at all, or are found in clumps, so that the individual cells cannot be counted. In the case of those methods which are based upon the development of microbes in culture media, the greatest difficulty is experienced in finding any single medium which will permit the growth of a large fraction of the soil inhabitants.

Each medium is more or less selective as far as the growth of certain organisms is concerned, but some media permit the development of fewer forms than others. Some media are purposely made favorable for the development of only those microbes that are capable of bringing about a specific transformation. Such media can be used for the determination of the abundance of specific *physiological groups* of organisms which play known rôles in certain important soil processes.

METHODS FOR STUDYING ACTIVITIES OF SOIL MICROBES.—For the determination of the activities of soil organisms, the methods commonly employed are based upon a knowledge of those soil processes which are considered to be essential to soil fertility. These may deal with the transformation of a single element of known importance to plant growth, such as the elements, nitrogen, carbon, or sulfur, or with a single change in a group of transformations in which a certain element or compound is involved In the decomposition of cellulose in the soil, for example, either the disappearance of cellulose, the formation of intermediary substances such as organic acids, or the formation of an end product of the reaction, such as carbon dioxide, can be used as an index of the microbial action. In studying the degradation of a protein in the soil, the disappearance of the protein, the formation of amino acids, the accumulation of ammonia, and frequently even the transformation of ammonia into nitrate have served as measures of transformation. Many different groups of organisms are concerned in such reactions, and the value of any single measurement depends upon the object of the study.

Cellulose and proteins are only two groups of chemical complexes contained in the organic matter which is added to the soil by the growing plants, either in the form of residues of the aerial portions of the plant, such as leaves, needles, stems, and fruiting parts, or in the form of the subterranean portions, namely, the roots. The quantity of organic substances reaching the soil is also increased through the introduction of stable manures, green manures, various animal and plant residues, and such artificial organic preparations as urea and cyanamid. In the decomposition of the organic complexes, various organisms are concerned which in their development bring about numerous processes of oxidation and reduction, decomposition and synthesis, which are of extreme importance to soil fertility.

To measure the activities of all these organisms, just as to determine their total abundance, is beyond the scope of any single method or group of methods. The nearest approach to the ideal method is that which allows the determination of an end product of the sum-total of the activities of a large fraction of the soil microbes. One usually studies only those transformations which appear to be of greatest economic importance to man, who utilizes these activities for the growth of his crops and the nutrition of his animals. However, microorganisms participate in a great many processes which take place in the soil of which little or nothing is known. With the advance of our knowledge of the soil microbes and their activities, the relationships of soil microorganisms to the transformation of soil constituents will be more accurately appraised and their activities will be more profitably utilized.

Summary of Methods.—The present methods for studying soil microorganisms can be listed as follows:

(1) Those methods which are used for the determination of the number of microorganisms in the soil as well as the specific nature of some of these organisms. These methods can be conveniently subdivided:

(*a*) Direct microscopic methods.

(*b*) Cultural methods, based upon the development of microorganisms upon solid media in plates or in tubes. By adjusting the concentration and nature of specific nutrients, such as the energy source, nitrogen supply, and other inorganic salts, by modifying the hydrogen-ion concentration and air supply, these media can be prepared so as to allow the development of either large numbers of a variety of microbes, or only representatives of specific groups.

(*c*) Cultural methods, based upon microbial growth or upon a specific chemical transformation brought about by microbial development in a medium containing a specific chemical substance, after inoculation with high dilutions of soil. Either liquid or solid media are employed for such use.

(2) Methods which are devised primarily for obtaining information about the activities of the total soil flora and fauna, or of only one specific transformation, irrespective of the nature of the organisms concerned.

(3) Methods for determining the availability in soil of elements essential for plant growth. These will be discussed in Chapter IX.

Direct Microscopic Methods.—The direct microscopic methods have been devised with the idea of determining the total abundance of organisms in the soil in order to supplement cultural methods which are selective. A small particle of soil or a dilute soil suspension is first mixed with a dilute solution of agar or gelatin, dried and fixed upon a slide, and then stained with acid dyes such as rose bengal or erythrosine. The bacteria can be distinguished and counted with the aid of a microscope, since they are stained deep red, while the organic and inorganic soil particles are either not stained at all or stained yellowish or brown. By the use of a graduated slide, a calibrated microscope, and a definite volume of the known soil suspension, for making the stained preparation, the approximate number of bacteria in any mass of soil can be determined. This method has the advantage that it stains all the soil bacteria, irrespective of their nutritive peculiarities, and thus permits the determination of their abundance in the soil. It also permits the recognition of the relative abundance of various

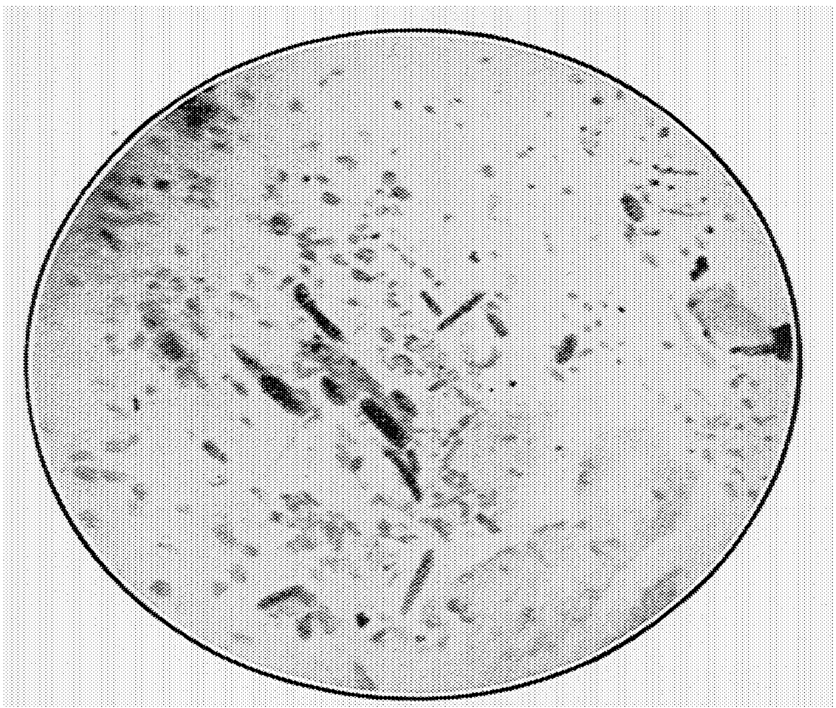

Fig. 38.—Presence of bacteria in soil, as indicated by direct staining of soil (from Cholodny).

morphological groups of soil organisms, as well as the physical relationship between the soil and its microbiological population. On the other hand, there are certain factors which limit the usefulness of the method. Some of the bacteria occur in aggregates, which makes their counting difficult; there is also great variation in the distribution of the cells in the stained preparation, thus decreasing the accuracy of the enumeration; actinomyces spores can seldom be distinguished from bacteria by this procedure; many of the small bacteria are difficult to distinguish from soil particles, and only a minute portion of the soil (one-millionth or one-five-millionth of a gram) can be examined by the microscope at any one time. Figs. 38 and 39 are preparations of soil showing the occurrence of some of the microbial cells in stained specimens.

The results obtained by this method indicate that bacteria exist in the soil not only in millions but in tens and hundreds of millions per gram. Most of the bacteria found by the direct microscopic method are non-spore-forming, rod-shaped or spherical cells. Many of the non-spore-forming organisms are found within the colloidal films which surround the soil particles, while the spore-forming organisms occur in the spaces between the individual particles. The cells may occur in large aggregates or colonies enclosed in an abundance of capsular material. The protozoa, fungus mycelium, and portions of actinomyces growth are frequently disintegrated by the process of staining. Table 7 shows the large numbers of bacteria and fungi which have been observed in soil by the direct microscopic method.

TABLE 7

NUMBERS OF MICROORGANISMS IN ONE GRAM OF SOIL AS DETERMINED BY THE DIRECT MICROSCOPIC METHOD (FROM RICHTER)

Type of soil	Depth	Numbers of Bacteria		Azotobacter cells	Pieces of fungus mycelium
		Cocci	Bacilli		
Forest	Surface	1,379,000,000	1,212,000,000	1,000,000	47,000,000
	10 cm.	991,000,000	466,000,000	31,000,000	34,000,000
	20 cm.	281,000,000	169,000,000	....	7,000,000
Brown loam	Surface	870,000,000	376,000,000	84,000,000	5,000,000
	10 cm.	569,000,000	106,000,000	1,000,000	3,000,000
Sandy soil	Surface	519,000,000	192,000,000	79,000,000	3,000,000
	10 cm.	407,000,000	153,000,000	23,000,000	19,000,000
	20 cm.	269,000,000	139,000,000	8,000,000	3,000,000

PLATE METHOD FOR COUNTING MICROBES.—The plate method for counting bacteria as well as certain other soil microbes makes use of the fact that media containing agar and gelatin can be kept liquid at temperatures which are not destructive to microbes and form gels at temperatures favorable to growth of the organisms. A highly diluted suspension of soil is prepared in sterile water in such a manner that the liquid contains only a limited number of microbes; these are as completely dispersed in the water as possible. When a small portion of this suspension is added to the medium which has been brought to the liquid condition (by warming), mixed well and the medium then allowed on cooling to solidify in Petri dishes, the individual cells of the various organisms become fixed in separate positions on the dish. If the medium contains all of the nutrients required for the growth of these microbes and if other conditions, such as reaction, temperature, and circulation of gases are favorable, the organisms multiply

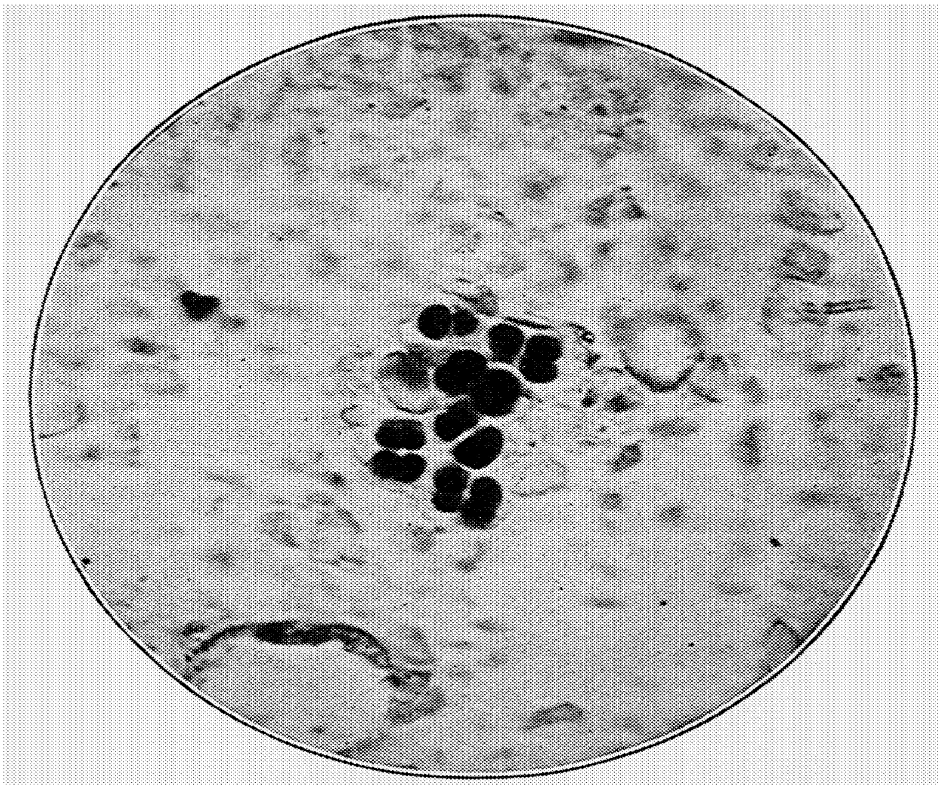

FIG. 39.—Azotobacter cells in soil, as demonstrated by direct staining (from Winogradsky).

rapidly, with the result that colonies are formed from each of the original cells present in the soil suspension. After incubation for a few hours or days, the colonies become visible to the naked eye, and their total number can be determined by simple enumeration (Fig. 40). If the dilution has been so conducted that a known amount of soil was added to the medium, one can calculate, from the number of visible colonies which appear on the plate, the number of organisms per unit of soil which are able to develop under this particular set of conditions.

The plate method possesses several distinct advantages. Determinations can be made of the abundance in the soil of such organisms as are capable of developing upon the media which are used. Since the various microbes form typical colonies, the method allows the determination of the abundance of specific

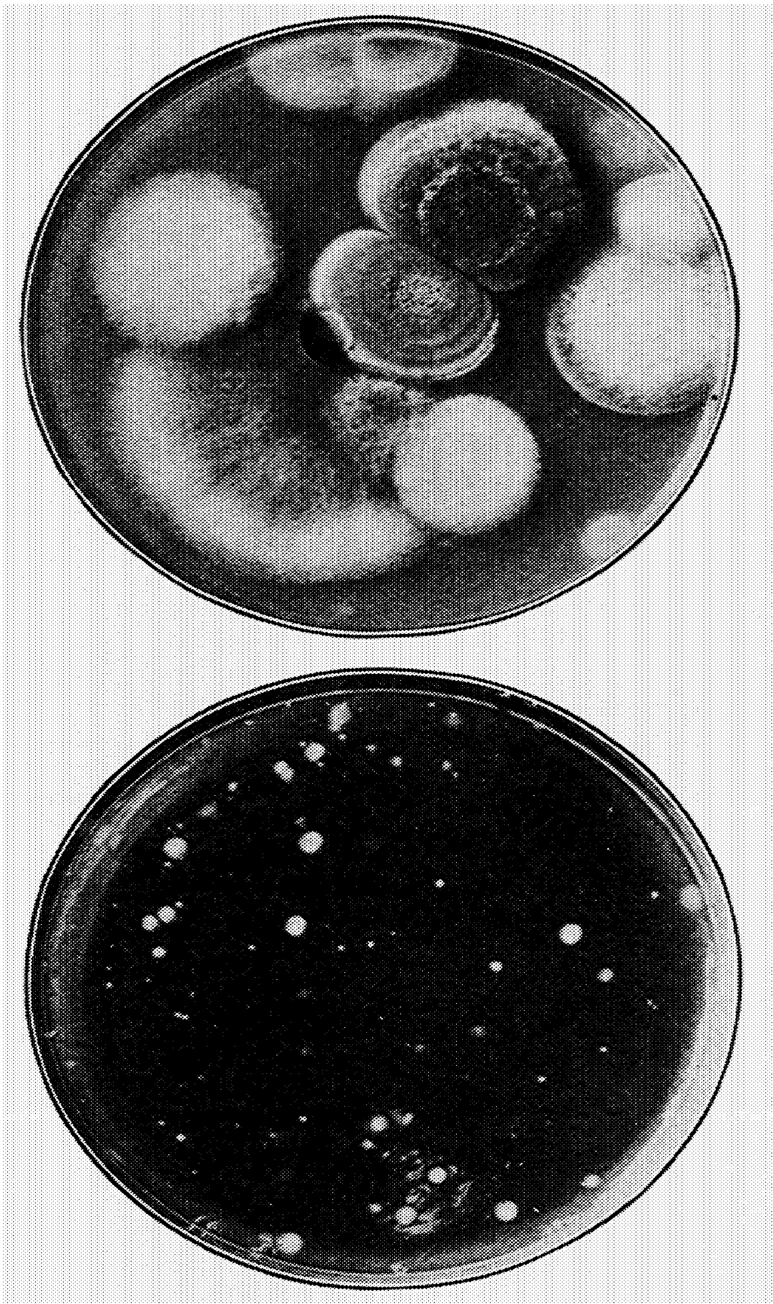

Fig. 40.—Colonies of bacteria and actinomyces (below), and fungi (above), developing on plates as used in counting these organisms in soils.

types of organisms in the soil. Each medium is selective for certain groups of microbes, and each set of conditions under which the organisms are allowed to develop upon the medium is selective for certain organisms. A medium containing proteins will favor the development of proteolytic bacteria; a medium containing starch or cellulose will favor the growth of organisms capable of utilizing starch or cellulose. The incubation of the plates under aerobic conditions will favor the development of aerobic organisms; under anaerobic conditions the development of anaerobic bacteria is favored. At high temperatures (50 to 65° C) thermophilic organisms develop. An acid medium favors the development of acid-tolerant microbes. The value of the method as an agency for measuring the total numbers of microbes in the soil is further limited by the fact that not all of the cells of even those organisms for which the medium is suited develop and form colonies. Frequently only 3 to 10 cells out of 100 will grow into colonies. Many fungi and protozoa fail to grow even upon the most carefully prepared media. Many bacteria and fungi require special media and conditions of incubation which are adapted to a highly specific type of development.

Elective Culture Method.—To meet the peculiar nutrition of the numerous soil organisms, the third method, namely, the *solution* or *elective culture* method, has been introduced. It consists in adding a definite quantity of soil to definite volumes of sterile water, thus diluting the soil to a desired degree, then adding measured quantities of the various dilutions to media favorable for the development of the particular organisms. The cultures are then incubated under favorable conditions, and observations are made to determine at what dilutions growth of the specific microbes has taken place. One can thus measure the approximate number of the specific microbes in a given quantity of soil.

To determine, for example, the number of aerobic cellulose-decomposing bacteria in a certain soil, the following method has been used. A series of flasks or tubes containing a liquid or solid medium favorable for the development of cellulose-decomposing bacteria is prepared and sterilized. A medium containing cellulose in the form of filter paper or cotton and the other nutrients as inorganic salts in dilute solution was found favorable for this purpose. The soil is diluted with sterile water so that 1 cc. quantities of the dilution contain the following portions of a gram of soil in suspension: 1/100, 1/400, 1/1,000, 1/5,000, 1/10,000, 1/25,000. One cubic centimeter portions of the suspensions are added to the flasks or tubes of the medium, and the flasks are incubated at 25 or 28° C. After a few days, the cultures are examined to determine whether or not growth of microbes has taken place. Development will be readily detected by the disintegration of the paper in the culture, especially in contact with the surface of the liquid. If the flask that has been inoculated with 1/5,000 of a gram of soil shows evidences of growth, and those flasks or tubes to which smaller amounts of soil were added show no growth, one is justified in concluding that one gram of the particular soil contains at least 5,000 cells of bacteria which are capable of decomposing cellulose under aerobic conditions. Certain formulae have been devised which can be used for accurate determinations of the number of specific organisms in a given quantity of soil from the number of cultures giving positive and negative growth.

It may be necessary to modify the method greatly so as to encourage the growth of the various specific microbes found in the soil. For the development of protozoa, sterile portions of nutrient meat extract agar media are placed in sterile Petri dishes; these are inoculated with 1 cc. portions of various dilutions of soil and incubated for at least 28 days. The surface of the agar is kept moist by frequent additions of sterile water. At various intervals, a loopful of material is removed from the surface of the agar and examined under the microscope to determine the presence of protozoa in general and of specific forms in particular.

For the development of nitrogen-fixing bacteria, media are employed which are free from combined organic or inorganic nitrogen, but which contain the mineral elements required for growth and a source of energy, such as simple sugars or higher alcohols. Thus, by modifying the nature and composition of the culture media and conditions of growth, the development of various soil microbes can be favored.

In spite of the fact that all of the methods mentioned previously are limited in their usefulness for determining the abundance of the microscopic organisms in soils, they become quite useful for comparative studies of different soils when interpreted in the realization of their deficiencies.

Abundance of Bacteria in Soil.—The numbers of bacteria found in different soils depend entirely upon the method employed for their enumeration. When the plate method is used, the total number of bacteria is found to vary from 10,000 per gram, as in the case of desert sands, sand dunes, and very poor sandy soils, to

50,000,000 frequently found in well-manured and cultivated soils. The nature of the medium employed for preparing the plates is of considerable importance in this connection, peptone-meat-extract media giving a smaller number of organisms than the so-called synthetic media, since the former media favor the rapid

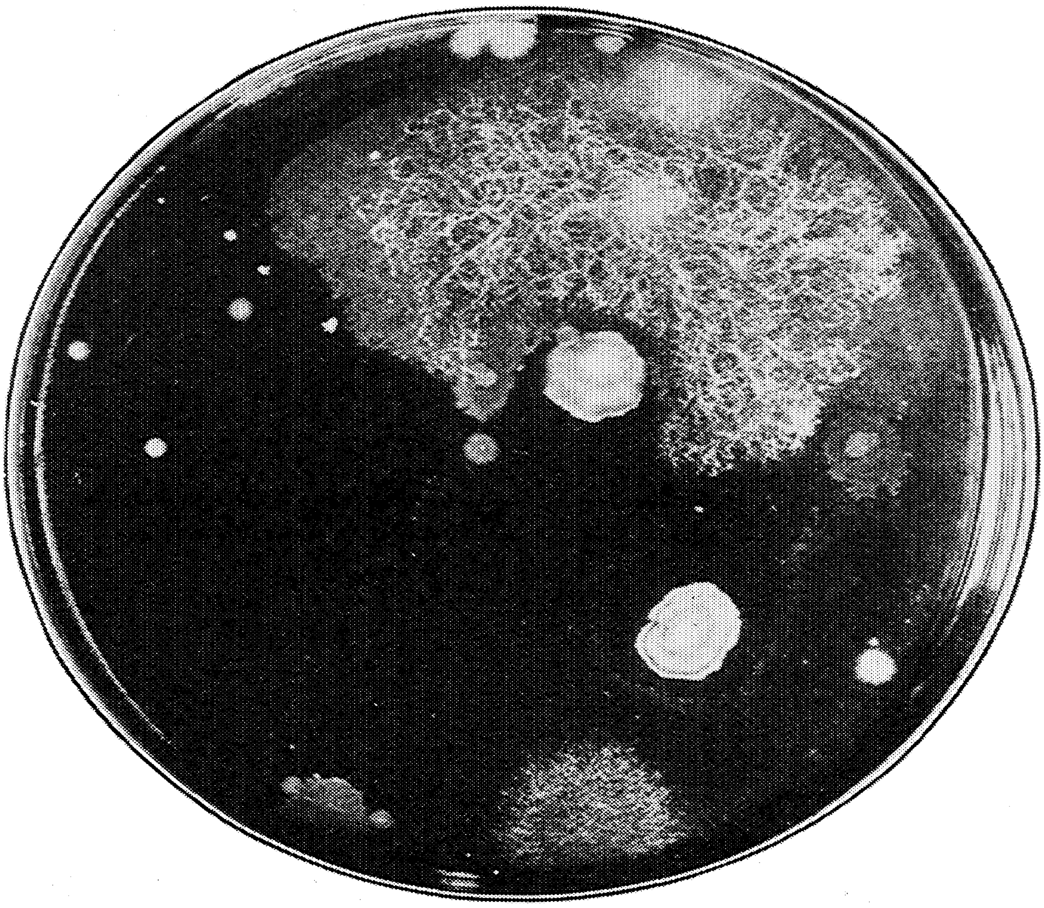

FIG. 41.—Plate preparation showing development of spreading colonies of bacteria (from Lipman).

development of certain spreading colonies which rapidly overgrow the plate (Fig. 41).

When the direct microscopic examination of stained soil preparations is used, the bacteria per gram of soil are numbered in the hundreds of millions or even billions (Table 7). By the use of elective culture methods, lower numbers are found and the organisms are limited to those microbes favored by the specific media. Table 8 gives a comparison between the abundance of bacteria determined by the use of various elective culture methods and the plate method. All these methods give, however, only a fraction of the total bacterial population of the soil.

TABLE 8

NUMBERS OF BACTERIA IN ONE GRAM OF SOIL AS DETERMINED BY PLATE AND DILUTION METHODS (FROM DÜGGELI)

Soil type	Garden	Field	Deciduous forest	Lowland
Numbers of aerobic bacteria developing on gelatin plate	8,116,000	10,640,000	1,422,000	1,010,000
Numbers of anaerobic bacteria	622,000	820,000	44,020	80,200
Aerobic nitrogen-fixing bacteria	2,620	6,040	0	0
Anaerobic nitrogen-fixing bacteria	8,200	4,420	460	440
Nitrifying bacteria	2,620	2,201	2,000	2,020
Anaerobic cellulose-decomposing bacteria	401	420	1	1

INFLUENCE OF SOIL CONDITIONS AND TREATMENT UPON THE DISTRIBUTION OF BACTERIA.—The physical and chemical composition of the soil, as well as the environmental conditions, determine the relative abundance of the microbes present at any given time in the soil. In general, light sandy soils, poor in organic matter, contain fewer microbes than fertile clays and loams rich in organic matter. The number of microbes need not necessarily indicate their potential activity, since such processes of decomposition and oxidation as are of the greatest importance for plant growth may be as active, if not more so, in the lighter than in the heavier soils. As soon as available nutrients are added, the response in microbial development becomes even greater in sandy soils than in heavy clay soils; there is a more rapid increase in the numbers of microbes and quicker transformation of the microbial foods.

Undrained peat and water-logged soils contain considerably smaller numbers of fungi, actinomyces and aerobic bacteria than the corresponding drained soils, although the number of anaerobic bacteria may be greater in the former. Acid soils, especially acid forest or raw-humus soils, are very rich in fungi and poor in bacteria. The fungus development is so extensive that the soil is profusely permeated with fungus mycelium (Fig. 42). As soon as an acid soil is limed, there is a rapid increase in the abundance of bacteria.

In general, the numbers of bacteria, as determined by the common plate method, range from 1,000,000 to 20,000,000 per gram of soil. These figures refer only to the aerobic bacteria that are capable of developing upon certain solid media. They are

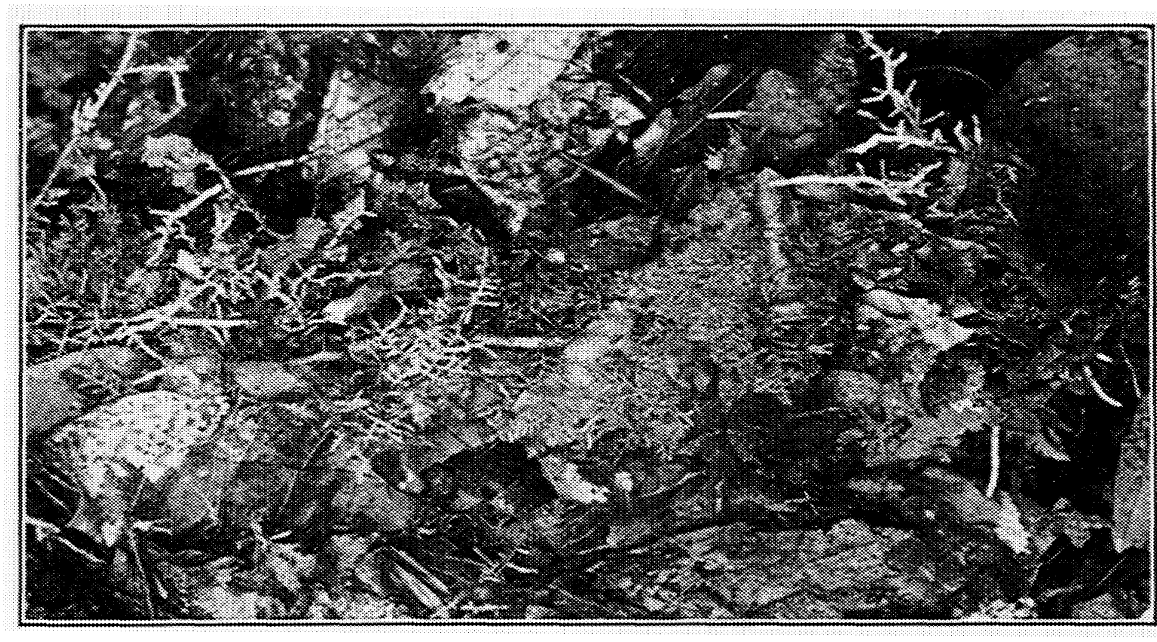

FIG. 42.—Fungus development on surface litter of forest soil (from Rayner).

only relative figures and should not be interpreted as representing a constant number of organisms in a particular soil; they refer only to the relative abundance of certain types of cells at the time that the determination was made. It should be kept in mind that these numbers change greatly from time to time, even without any apparent alteration in the conditions of the soil. Much greater changes occur when the environment is considerably modified.

Injudicious cropping without fertilization will in time greatly deplete the soil organic matter and the potential sources of nutrient substances for plants, and will lead to depressions in the abundance of microorganisms. Certain fertilizer practices, as continued application of ammonium sulfate without calcium carbonate, increase the acidity of the soil to such a degree as to greatly lower the numbers of bacteria. On the other hand, the application of inorganic fertilizers considerably increases the microbial population through both direct and indirect factors. The effect may be direct, by satisfying the deficiencies of certain elements required for microbial growth. It may be indirect, by affecting the physical condition of the soil, by modifying the soil solution, creating a greater or less solvent action, or by changing the soil reaction.

The environmental factors affecting the abundance of bacteria in soil as well as their distribution at different depths are numerous, most important being the factors of organic matter, moisture, reaction, temperature, and air penetration.

INFLUENCE OF ORGANIC MATTER.—Additions of organic materials to the soil probably exert more pronounced effects upon the microbial population than any other treatment, especially under humid conditions, but the microbial response is different with different substances, depending upon the relative ease with which they are decomposed and the types of organisms which are able to attack them. The effects of introduction of plant or animal residues are pronounced upon the physical condition of the soil, by binding loose sandy soil more closely together, and by bringing heavy clay soil to a more porous state. These effects are in general desirable for the development of both plants and microorganisms. Soil conditions resulting from the introduction of plant substances will generally be associated with extensive development of the microbial inhabitants. Soils not receiving any fresh additions of organic matter become progressively depopulated, irrespective of other soil conditions.

What course the microbial changes follow will be largely determined by the nature of the organic materials added. Tree products are quite resistant to decomposition, and produce marked effects on the organisms only over a considerable period of time. The lower carbohydrates and protein substances produce effects which are very quickly apparent but which persist for comparatively short periods. Owing principally to the chemical composition and abundance or residues which they may leave in the soil, different plants affect the development of soil organisms differently, as will be shown in Chapter IV.

TABLE 9

INFLUENCE OF MOISTURE CONTENT OF SOIL ON NUMBERS OF BACTERIA (FROM ENGBERDING)

Per cent saturation of the moisture-holding capacity	Moisture content, per cent	Numbers of bacteria per gram of dry soil	Relative numbers
30	6.51	9,980,000	33.3
50	10.85	11,890,000	39.7
65	14.10	16,410,000	54.8
80	17.35	29,960,000	100.0
100	21.69	25,280,000	84.4

INFLUENCE OF MOISTURE.—Bacterial development is generally at a maximum when the moisture content is relatively high (Table 9). The maximum development of the aerobic bacteria, which are concerned with some of the most important soil processes, takes place when the moisture content is between 50 and 70 per cent of the moisture-holding capacity. This corresponds to the moisture content best adapted to development of most cultivated plants. Although bacterial activity in air-dry soil, with 2 to 5 per cent moisture, is almost negligible, drying of soil has a striking effect on the growth of both microbes and higher plants, after the dried soil has been remoistened. Drying renders the soil substances much more available for decomposition, due to physical and chemical changes in the soil organic matter, to the killing of many microbial cells by the desiccation process, and to modifications in the nature of the soil population. Whatever the responsible factors may be, the intermittent drying and moistening of the surface soil is quite beneficial in rendering nutrient elements available for plant growth. Fig. 43, taken from laboratory studies, indicates the course of microbial development following the moistening of a dried soil. Over the same period of time, the soil which had not been desiccated would have shown no pronounced increase or decrease in abundance of bacteria or fungi or in the formation of carbon dioxide.

In humid regions the root systems of plants are confined much nearer to the surface than in semi-arid zones, consequently the plant residues are more generally concentrated near the surface

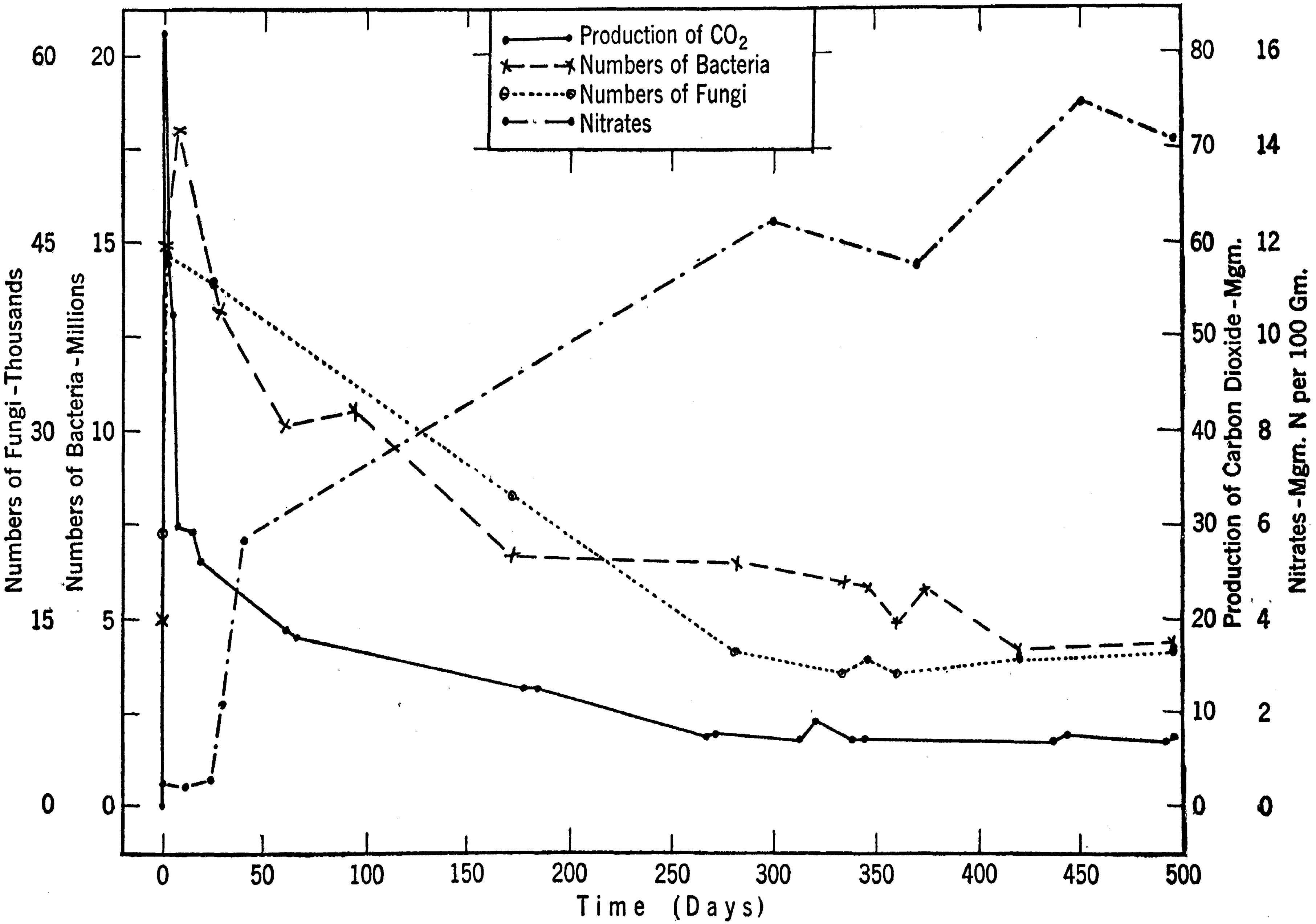

FIG. 43.—Response of microbial development following the remoistening of a dried soil (after Waksman and Starkey).

in regions of heavy rainfall and may appear more abundantly in the deep soil layers in regions of scant precipitation. The factor of major importance in determining the conditions in the dry regions is the lack of sufficient moisture at the surface to support a vigorous growth of either the plant or the microbial population. In compact soils the physical condition is such as to resist the deep penetration of roots and, even though there were an abundance of organic matter at considerable depth, the material would be incompletely disintegrated on account of the lack of oxygen penetration.

The influence of climate is quickly impressed upon the bacterial activities in the soil. Even when soils are brought from one region to another there is a rapid modification, chemically, biologically, and physically (Table 10). The numbers of bacteria of arid soils usually increase when the soils are placed under humid conditions, and bacterial numbers in humid soils decrease when soils are placed under arid conditions.

TABLE 10

INFLUENCE OF CLIMATE ON BACTERIA IN SOILS (FROM C. B. LIPMAN)
Numbers of bacteria per gram of soil in the surface foot

	California Soil	
In California	In Kansas	In Maryland
813,000	5,067,000	5,467,000
	Kansas Soil	
In California	In Kansas	In Maryland
1,507,000	5,167,000	1,370,000
	Maryland Soil	
In California	In Kansas	In Maryland
6,140,000	880,000	546,000

INFLUENCE OF REACTION.—Among the environmental factors influencing the development of bacteria in soil, the reaction, that is, the degree of acidity or alkalinity, is of particular importance. When a humid soil is treated with acid-forming fertilizers, such as ammonium sulfate which on oxidation by nitrifying bacteria gives nitric and sulfuric acids, or with sulfur which on oxidation by sulfur-oxidizing bacteria gives sulfuric acid, the total number of bacteria, as well as the development of certain specific types, will decrease. When an acid soil of humid regions is treated with lime, the numbers of bacteria rapidly increase.

As a result of this, the idea prevails that bacteria are more favored by an alkaline reaction while fungi are more abundant in acid soils. The results given in Table 11 show the effects upon bacterial development of differences in reaction resulting from differences in treatment of soil. These results bring out further the fact that the reaction is not the sole limiting factor in the development of microorganisms. Plot 5A is slightly more acid in reaction than 9A, but still contains a much larger number of bacteria because it contains more organic matter, as shown by the higher nitrogen content. Plot 9A is more acid than plot 7B, but still it contains a greater number of bacteria, because of the higher organic matter content.

When a black alkali soil having a *p*H value of 9.6 to 10.0 is treated with sulfur, sulfuric acid, aluminum sulfate, or other acid-reacting substances, thus making the soil less alkaline, an increase in the number of bacteria takes place, because of the fact that conditions become more favorable for their development.

Influence of Season of Year.—The greatest numbers of bacteria are usually found in the soil in the spring time and in the fall (Fig. 37). During the winter months the soil becomes so altered chemically and physically by the low temperatures that there is a pronounced acceleration of microbial development when the soil habitat becomes warm again. After this period of unusual activity, the microorganisms decrease in numbers somewhat and fluctuate from this lower level, in response to changes in their environment brought about by rainfall, plant development, and cultural treatments. In the fall of the year, the organisms reach a second peak in development which may be explained in part by the incorporation of considerable amounts of organic material accompanying maturity of plants, in part by the higher moisture content resulting from decreased transpiration and evaporation. During the winter months, when the soil is frozen, the organisms are quite inactive, although a large portion of the microbial population persists in spite of the adverse conditions (Fig. 44).

Appreciable changes in temperature, in the moisture films about the soil particles, or in the amount of available food materials, are followed by changes in the abundance and activities of the microscopic population of the soil.

Development of Actinomyces in Soil.—Qualitatively, the actinomyces react to changes in the soil environment in much the same way as most of the soil bacteria do. They respond markedly to the addition of organic materials, such as root residues or other plant and animal remains, and may be concerned with the slow decomposition of some of the most resistant organic substances

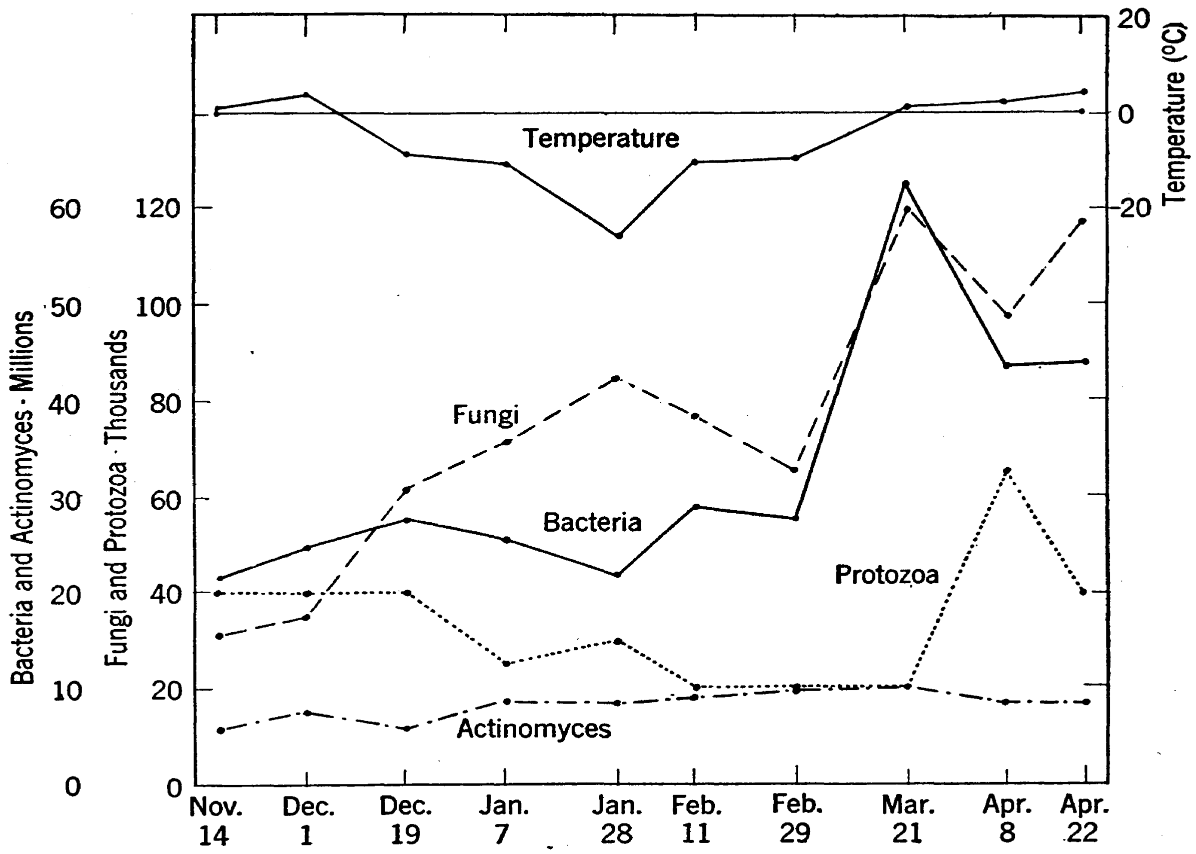

Fig. 44.—Microorganisms in soils during the winter and spring months (after Lochhead).

in the soil. They are favored by much the same moisture content as the bacteria, but may develop better than the bacteria when the soil is relatively low in moisture. They are more sensitive than bacteria to soil acidity, and develop best at neutral or slightly alkaline reactions. These organisms appear to be more constant in abundance than either the bacteria or fungi and show less marked fluctuations due to environmental changes. Although they decrease in abundance from the surface to the deeper layers of soil, the decrease is more moderate than that of the numbers of bacteria. Consequently, at the deep layers of many soils the plate count reveals a proportionally large abundance of actinomyces. This may be due to their greater tolerance to low concentrations of oxygen as well as to their ability to thrive upon the organic substances of a complex nature which may be washed into these low levels. The earthy odor of plowed sod land is due to certain aromatic substances elaborated by the actinomyces.

Since actinomyces are essentially aerobic organisms they are absent in peat bogs, except near the surface, since the bog conditions favor the development of anaerobic organisms. Most actinomyces are sensitive to acidity, and are, therefore, practically absent from raw-humus forest soils and unlimed highmoor peats. Certain few species of actinomyces, however, are known to thrive at reactions even more acid than pH 4.0. Since they are quite resistant to dry conditions they are found abundantly in arid and semi-arid soils as well as in sandy soils.

In general, the numbers of actinomyces range from a few thousands to many millions per gram of soil. However, in view of the fact that they occur in the soil both in the form of spores and vegetative mycelium, a large part of which may not develop readily upon artificial media, the numbers determined by the plate method are only relative, and serve merely to indicate the approximate abundance of these organisms in a given soil under a given set of conditions.

From observations of the actinomyces in differently treated plots of soil which were originally the same (Table 11), it is apparent that, when the soil had become acid following repeated additions of ammonium sulfate, the numbers of actinomyces diminished even to such a low level as 370,000 per gram. In the same soil kept at pH 6.7 by liming (19B), the organisms were as numerous as 2,820,000 per gram. However, with the actinomyces as with the bacteria, the organic matter content of the soil is an important factor in determining their abundance. In Plot 5A, with a pH of even 5.4, as many as 2,920,000 actinomyces were found per gram of soil.

Development of Fungi in Soil.—Fungi occur in soil in the form of both vegetative mycelium and spores; this is true both of soils acid in reaction and rich in organic matter, or neutral and even alkaline in reaction and containing little organic residues. As

TABLE 11

INFLUENCE OF SOIL TREATMENT UPON THE DISTRIBUTION OF BACTERIA, ACTINOMYCES, AND FUNGI (FROM WAKSMAN)

Number of plot	Treatment for a number of successive years	Reaction of soil, pH	Nitrogen content of soil, per cent	Bacteria	Actinomyces	Fungi
5-A	Mineral fertilizer and manure...........	5.4	0.1185	8,800,000	2,920,000	72,500
7-A	No fertilizer..........................	4.6	0.0785	3,000,000	1,150,000	59,700
7-B	Lime, no fertilizer.....................	6.4	0.0821	5,210,000	2,410,000	22,450
9-A	Mineral fertilizers+($NaNO_3$)...........	5.5	0.0975	7,600,000	2,530,000	46,450
11-A	Mineral fertilizers+($(NH_4)_2SO_4$)..........	4.1	0.0904	2,690,000	370,000	111,450
11-B	Mineral fertilizer+$(NH_4)_2SO_4$+lime......	5.8	0.0819	6,990,000	2,520,000	39,100
19-A	Minerals, no N........................	5.2	0.0872	4,000,000	1,340,000	61,000
19-B	Minerals, no N+lime..................	6.7	0.0784	7,300,000	2,820,000	26,200

seen from Table 12, it is likely that fungi exist in soils in the vegetative state to a much greater extent than in the form of spores. It has been observed that, when vegetative growth is rapidly desiccated in soils, it undergoes destruction; under the same conditions fungus spores are unaffected. When soils containing the soil organisms in their normal condition are rapidly desiccated, the numbers of fungi are very largely reduced. Therefore, it seems likely that when one counts colonies of fungi on agar plates prepared from soil dilutions, one is counting colonies which have developed from pieces of mycelium of the organisms. What the sizes of the pieces of mycelium may have been at the time of the determination is unknown. Undoubtedly the individual pieces varied greatly in size.

TABLE 12

Influence of Desiccation of Soils on Development of Colonies of Fungi on Agar Plates (from McLennan)

Preparation	Average Number of Colonies per Plate	
	Plated before desiccation	Plated after desiccation
Only spores present in the soil	31.0	30.0
Only mycelium present in the soil	20.0	0.8
Field soil No. 1	10.6	0.6
Field soil No. 2	11.6	1.0
Field soil No. 3	8.8	1.1
Field soil No. 4	25.2	3.0
Field soil No. 5	199.0	13.7
Field soil No. 6	326.3	5.6

The value of the plate method for determining the abundance of fungi in the soil is further limited by the fact that many fungi fail to grow on the media commonly used for their determination. In this respect there is a similarity to the plate method for determining the numbers of bacteria. The results of these fungus counts should, therefore, be interpreted only as rough quantitative measurements of certain representatives of the fungus population.

The types of fungi which are found in different cultivated soils are much the same, but the relative abundance of any of the species is determined by the environmental conditions. One of the most important factors affecting their growth and development in the soil is the abundance of organic food. However, even in soils low in organic substances there may be relatively extensive fungus development; such a condition develops most frequently in quite acid soils. This does not necessarily signify that fungi develop best in soils under extremely acid conditions, for it has been observed that they develop quite well over a wide range of reaction. Since the bacteria and actinomyces may be at a low order of activity in the acid environment, the fungi, which can develop under these conditions as well as in neutral soils, have less competition for the available food supply and consequently appear in greater abundance.

The results of determinations of the numbers of fungi in variously treated plots, as shown previously in Table 11, are illustrative of the above conclusions. Limed soils contain fewer fungi than unlimed soil, while soils treated with acid fertilizers contain the largest numbers of fungi. In soils more nearly neutral in reaction, there is such a large number of microorganisms which are capable of developing under these conditions, that the factor of competition for food by the cells may keep the fungi from making an extensive development.

The fungi are favored by a relatively high oxygen content in the air since they are distinctly aerobic organisms. Consequently, we would expect to find fungi developing best under the aerobic conditions close to the soil surface. The fact that there is greater abundance of organic matter in the surface soil is also responsible for the most extensive fungus development being restricted to the superficial layers.

From the response of the actinomyces and fungi to the soil reaction it becomes apparent that frequently the total abundance of these organisms, and also the portion of the total soil population composed of either fungi or actinomyces, are functions of the soil reaction. However, the responses of these two groups to reaction are opposite, one appearing in greatest abundance in acid soils, the other in more nearly neutral or basic soils.

Development of Protozoa in Soil.—To a large degree, the biological transformations in soil are brought about by the bacteria, actinomyces, and fungi. The lower animal life may be of particular importance under certain soil conditions and in connection with some processes, but it would appear that it exerts minor influences upon the activities brought about by microorganisms in a normal soil environment.

The conditions which favor the development of bacteria also appear to favor the growth of protozoa. The explanation becomes apparent when the fact is considered that bacteria are an important part of the protozoan diet. In fertile soils rich in organic matter and relatively high in moisture, the protozoa are found in greatest abundance. The small flagellates and amoebae are considerably more numerous than the ciliates. They develop best at slightly alkaline reactions, but appear to be as tolerant to acid conditions as most of the soil bacteria. Under unfavorable conditions, the soil protozoa go into a dormant state in the form of cysts, which again become active when the environment is favorable. The active trophic stage of protozoa is probably more common in moist soils. Although they may occur in the soil in much smaller numbers than bacteria, as shown in Table 13, their cells occupy considerably more space, as brought out in Table 14. As with most of the other members of the soil population, the protozoa occur in greatest numbers near the surface.

TABLE 13

NUMBERS OF PROTOZOA IN AMERICAN SOILS (FROM SANDON)

	*p*H	Flagellates	Amoebae	Ciliates
Irrigated land, no manure	7.5–8.0	450	3,596	225
Irrigated land, 30 tons of manure	7.5–8.0	14,380	28,770	114
Humid Sassafrass soil, unmanured, unlimed	5.3	121	165	0
Humid Sassafrass soil, manured	5.2	6,780	95,067	145
Humid soil (Penn loam), under alfalfa	....	10,785	10,785	98
Humid soil (Penn loam), fallow after corn	....	4,795	81,495	263

DEVELOPMENT OF ALGAE IN SOIL.—Algae occur in greatest numbers in cultivated land and to depths of even 40 to 50 cm. below the surface. As chlorophyll-bearing organisms, they are capable of utilizing the rays of the sun photosynthetically when growing on the surface, but, at lower depths of soil, they function as heterotrophic organisms. Since their distribution below the sur-

TABLE 14

COMPARATIVE NUMBERS AND WEIGHTS OF CELL SUBSTANCE OF PROTOZOA AND BACTERIA IN THE SOIL (FROM CUTLER)

	Period of Low Numbers		Period of High Numbers	
	Number per gram of soil	Pounds per acre	Number per gram of soil	Pounds per acre
Flagellates	350,000	78	770,000	174
Amoebae	150,000	156	280,000	294
Bacteria	22,500,000	24	40,000,000	40

face is controlled by factors similar to those which regulate the distribution of other organisms, they are more numerous near the surface, and have fewer species represented and less total number of individuals in the deeper layers. Rarely do they appear in abundance in soils of low water content. Grassland appears to be a more favorable environment for their development than the more highly cultivated soils. It has been estimated that, per volume, they occupy about three times as much space per unit of soil as the bacteria, and about one-third the space occupied by the protozoa. Naturally they can hardly be present in such abundance without exerting profound changes in certain soil processes.

According to Bristol-Roach, the actual numbers of algae in the cultivated layers of agricultural soils range from 700 per gram to many thousands, the numbers changing with depth of soil, season of year, and soil treatment (Table 15).

TABLE 15

INFLUENCE OF MANURE AND SOIL DEPTH UPON THE DISTRIBUTION OF ALGAE IN ENGLISH SOILS (FROM BRISTOL-ROACH)

Depth	Numbers per gram of soil	
	Unmanured	Manured
Surface inch	16,000	62,000
Second inch	10,000	28,000
Fourth inch	28,000	56,000
Sixth inch	4,000	15,000

Lower Invertebrates in Soil.—The lower invertebrates occur in greatest abundance in light soils, other conditions being alike. As with the other soil organisms, environmental conditions greatly affect their prevalence; probably these effects are even more marked than with those forms which do not have the ability to migrate appreciable distances. The abundance of organic matter is a particularly important factor. In unmanured field soil cultivated to wheat, Morris found 4,885,400 of the larger invertebrate animals per acre. In the manured soil there were 14,795,000 individuals. However, these figures represent only a very small fraction of the total numbers of invertebrate animals present in the soil. The nematodes alone have been found in hundreds of millions of cells per acre of land (Table 16).

TABLE 16

Abundance of Nematodes in Soils (from Cobb and Steiner)

Soil	Number per acre in the surface 6 inches
Missouri corn field	648,000,000
New Jersey corn field	129,600,000
New Hampshire corn field	99,600,000
Vermont corn field	580,000,000
Acid forest soil in Virginia	320,000,000 (in surface 3 cm.)
Utah sugar-beet field	12,044,111,000 (in surface 2 feet)

The effects which these small animals exert are probably more physical than chemical. They aid in the decomposition of organic substances to some extent, but it is doubtful that they play rôles which could be compared with the bacteria and other related microorganisms.

The Activity of the Soil Population as a Whole.—Many methods have been used to determine the response of the total soil population to changes in its environment. Of these methods the determination of the amounts of carbon dioxide formed in a certain unit of time has proved to be most valuable. The carbon dioxide of soils originates almost entirely through biological agencies. Practically all of the soil microbes produce carbon dioxide as one of the principal waste products of their development, and the amounts are closely proportional to the degree of their activity.

Consequently, results of determinations of the amounts of carbon dioxide produced give information which indicates, more accurately than determinations of the numbers of any single group of organisms, the level of biological activity, irrespective of the organisms that are concerned.

The method does not discriminate between those organisms which can be counted by plate methods and those failing to grow on artificial media. Although one cannot distinguish what organisms are responsible for producing the carbon dioxide, one has in this method a device for determining to what extent microbial activity as a whole becomes depressed or favored by particular soil conditions.

In general, those factors, considered in the preceding pages, which favor development of aerobic bacteria, actinomyces, and fungi, also favor rapid formation of carbon dioxide. High carbon dioxide evolution is associated with productive soils, and factors which increase crop production also increase formation of carbon dioxide. Such factors as relatively high temperature and moisture content, thorough aeration, presence of an abundance of organic matter, inorganic plant nutrients, and lime, all exert favorable effects on biological activity as measured by the rate of formation of carbon dioxide.

The resultants of some of these effects are indicated in the seasonal variation of the gas formation which is shown in Fig. 37.

The rate of formation of nitrates, oxygen absorption by soil, and heat evolution, as a result of the activities of microorganisms, can also be used as measures of the activity of the soil population, but none of these is as simple as the determination of evolution of carbon dioxide.

SUMMARY.—The soil is inherently a very complex system, where chemical, physical, and biological systems are active. All of these agencies tend to become adjusted to the environmental conditions existing in the soil at any one time. If a soil is kept under uniform conditions, the speeds of the numerous processes taking place in the soil also tend to become uniform. Any influence which tends to disturb this adjustment in the soil conditions alters these processes, which will now tend to become adjusted to the new conditions. Thus, a change in soil reaction causes a series of changes in the biological condition of the soil; after a lapse of time, the new population becomes adjusted to the new reaction.

The magnitude of the effects of different factors in bringing about changes among the microorganisms of the soil, including numbers, types, and activities, varies greatly. Cultivating a soil shows only slight effects on the population. Addition of inorganic fertilizers increases the activities slightly more. Of the various soil treatments in humid regions, the introduction of organic substances affects the numbers and activities of microorganisms more than any other.

The soil in its natural environment is constantly exposed to fluctuating conditions of temperature, moisture, pressure, and air movements, numerous factors being active at any one time. Furthermore, addition and removal of organic and inorganic materials, through the agency of man, animals, and plants, are always occurring. These factors are undoubtedly responsible for the perpetuation of the active biological conditions in the soil. They exert their action either directly upon the microbes, or only indirectly, by first influencing the plant and the soil constituents, and these in their turn modifying the development of the microorganisms.

The composition of the soil microbial population, both qualitatively and quantitatively, is thus a resultant of numerous factors, which can be traced to the soil and atmospheric agencies, as well as to the nature of the plant and animal populations.

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CHAPTER IV

RÔLE OF MICROBES IN THE DECOMPOSITION OF ORGANIC SUBSTANCES IN THE SOIL

PRINCIPLES OF DECOMPOSITION OF ORGANIC MATTER BY MICROBES.—Undoubtedly the most important function of microorganisms, from the point of view of man's economy, is their ability to bring about the decomposition of organic plant and animal remains not only in soils but in all natural environments. The simplification of the chemical structure of the organic materials, or their transformation from complex to simpler forms, leading finally to the liberation of completely oxidized inorganic substances in forms available to higher plants, is brought about by most of the microorganisms in the soil. The end products of the complete decomposition of non-nitrogenous materials are carbon dioxide, water, and synthesized microbial cells. The nitrogenous substances give also, in addition to these, ammonia, and other simple compounds of nitrogen, as well as compounds of sulfur and phosphorus.

The attack of any complex compound by microorganisms seldom results directly in complete decomposition even under aerobic conditions; various intermediate products are usually formed. The incompletely decomposed residues left from the action of certain organisms are further attacked by other members of the soil population which have the ability to decompose them. Sooner or later the initial material completely loses its identity and is transformed principally to the completely oxidized end products, and partly to a variety of synthesized substances in the form of microbial cells. The various soil organisms differ from one another greatly in their physiological characteristics; what may be a readily available food for one species would not be available to others. The great diversity of nutrition exhibited by the many soil microbes makes possible the rapid digestion of whatever material may be added or formed in the soil.

Scarcely any organic compounds are unlikely to be introduced into the soil. The numerous substances synthesized by the great variety of higher plants are continuously added in the form of plant roots, stubble, leaves, twigs and branches. Immature plants are also abundantly incorporated with the soil as green manures. Plant residues are added to the soil in the form of stable manures. Various waste products reach the soil subsequent to threshing, milling, and utilization of various cereal crops, as well as cottonseed and linseed. Partly digested plant products, which have been used in animal feeding, are contained in the manures. Various animal products are also introduced into the soil, other than the partially digested excreta. Those most commonly added are by-products of industry, such as dried blood, meat scraps, bone meal, hair, hoofs, and feathers. There are also large numbers of small invertebrate and vertebrate animals which find in the soil a temporary or permanent habitat. Finally there is a continuous addition of considerable quantities of microbial cell substance to the soil. Consequently, we may feel secure in assuming that there may be added to the soil any compound contained in higher plants, animals, or microorganisms, as well as any substance which might be formed from these compounds as they are attacked by the microorganisms in the soil.

Composition of Plant and Animal Substances.—The plant and animal residues comprise a large number of chemical compounds which can be classified conveniently into the following groups:

I. Carbohydrates.
  1. Monosaccharides.
    a. Hexoses ($C_6H_{12}O_6$), such as glucose, fructose, mannose.
    b. Pentoses ($C_5H_{10}O_5$), such as arabinose and xylose.
  2. Disaccharides ($C_{12}H_{22}O_{11}$), the most important of which are sucrose and maltose.
  3. Trisaccharides ($C_{18}H_{32}O_{16}$), raffinose.
  4. Polysaccharides.
    a. Starch, glycogen, inulin, and dextrins.
    b. Cellulose.
    c. Hemicelluloses and polyuronides.
      (1) Hexosans, as mannans and galactans.
      (2) Pentosans, of the structure $(C_5H_8O_4)_x$.
      (3) Pectins and other uronic acid compounds.

II. Lignins.

III. Tannins.

IV. Glucosides.

V. Organic acids, salts, and esters.

VI. Fats, oils, waxes, and related compounds.

VII. Resins.

VIII. Nitrogen compounds.
1. Proteins.
2. Amino acids.
3. Amines.
4. Alkaloids.
5. Purines.
6. Nucleic acids.

IX. Pigments.
1. Chlorophyll—green coloring matter.
2. Carotinoids—pigments of leaves, stems, flowers, and fruit.
3. Anthocyanins—pigments of leaves, fruit, and flowers.

X. Mineral constituents.
1. Bases, especially Ca, Mg, K, Fe.
2. Phosphates.
3. Chlorides.
4. Sulfates.
5. Silicates.

The various plant constituents can be divided into the following groups, which are the most extensive and important, since they make up the larger part of the plant and influence the nature and rapidity of its decomposition:

(1) The water-soluble constituents, including the simple carbohydrates, starches, amino acids, and various organic acids.

(2) The hemicelluloses, which are condensation products of hexoses, pentoses, or both of these with uronic acids.

(3) Cellulose, which is a condensation product of glucose.

(4) Lignin, the exact chemical nature of which is still unknown. It is known to comprise a benzene ring group, several methoxyl and hydroxyl groups, and an aldehyde group. The following formula is typical for this complex:

$$C_{40}H_{30}O_6 \cdot (OCH_3)_4 \cdot (OH)_5 \cdot CHO$$

In the plant it forms absorption or chemical compounds with cellulose. The young plant contains largely cellulose in its structural tissues, but with the maturing of the plant, the cellulose fibers absorb (or combine with) lignin, thus forming the ligno-cellulose of the plant tissues.

(5) Proteins, composed of various amino acids. These are the most important nitrogen constituents of the plant.

(6) Fats, oils, and waxes, which are esters of alcohols and one or more higher fatty acids.

(7) The ash or the mineral constituents of the plant.

A typical series of analyses of various plant products is given in Table 17.

TABLE 17

PROXIMATE COMPOSITION OF NATURAL ORGANIC MATTER (FROM PRINGSHEIM)

Substance	Cellulose	Pentosan	Lignin	Protein	Fats and waxes	Ash
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Hay	28.50	13.52	28.25	9.31	2.00	6.05
Oat straw	35.43	21.33	20.40	4.70	2.02	4.81
Wheat straw	34.27	21.67	21.21	3.00	0.67	4.33
Corn-cobs	37.66	31.50	14.70	2.11	1.37	1.80
Corn-stover	30.56	23.54	15.13	3.50	0.77	6.15

The remaining substances not given in the above table include the water-soluble constituents (sugars, organic acids), various hemicelluloses, in addition to the pentosans, the pigments, and other substances present in smaller fractions.

Not only do various plants vary in their composition, but the composition of the same plant varies at different stages of growth, as seen in Table 18.

FACTORS AFFECTING DECOMPOSITION.—The various chemical complexes are decomposed in the soil with different degrees of rapidity and frequently by different organisms. Since different plants vary so greatly in the relative abundance of the various constituents, the speeds of their changes and the types of the residues are very dissimilar even under the same environmental con-

TABLE 18

COMPOSITION OF THE RYE PLANT (STEMS AND LEAVES) AT DIFFERENT STAGES OF GROWTH* (FROM WAKSMAN AND TENNEY)

Stage of plant growth	Fats and waxes	Water-soluble sub-stances	Pen-tosans	Cellu-lose	Lignin	Total nitrogen	Ash
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
10–14 inches high....	2.60	34.24	16.60	18.06	9.90	2.50	7.66
Just before heads begin to form.....	2.60	22.74	21.18	26.95	11.80	1.76	5.90
Just before bloom...	1.70	18.16	22.71	30.59	18.00	1.01	4.90
Mature plant.......	1.26	9.90	22.90	36.29	17.10	0.24	3.90

* Calculated on the dry basis.

ditions. To understand why certain plant residues may be decomposed when they are introduced into a soil and become an available source of nutrients to higher plants, and why other plant materials, or the same material under different conditions, may accumulate and fail to undergo destruction, is a problem involving a consideration of many factors. The chemical nature of the complex, the other substances with which it is associated in the plant structure, the nature of the soil (such as physical structure, chemical composition, aeration, moisture content, reaction, and temperature) with which the materials are incorporated, are all important factors determining the types of organisms active in the transformation and the nature of the resulting material. Under some conditions there are such accumulations of organic matter as peat; in other instances, grassland formations or forests develop. Further, the environmental conditions determine the kind of natural vegetation and consequently the nature of the organic substances which become added to the soil.

DECOMPOSITION OF SUGARS AND THEIR DERIVATIVES.—After organic matter reaches the soil, sugars and other water-soluble substances are the first to be decomposed. These substances largely disappear within the first few days subsequent to their attack by various bacteria and fungi. A part of these is completely oxidized to carbon dioxide and water, while some are incompletely decomposed with the formation of a number of various organic acids and alcohols. Some fungi decompose the sugars with the formation of glucuronic, citric, oxalic, fumaric, and succinic acids, as shown in the following reactions:

$$\underset{\text{Glucose}}{C_6H_{12}O_6} + O_2 = \underset{\text{Glucuronic acid}}{CHO\cdot(CHOH)_4\cdot COOH} + H_2O$$

$$\underset{\text{Glucose}}{C_6H_{12}O_6} + 1\tfrac{1}{2}O_2 = \underset{\text{Citric acid}}{CH_2\cdot COOH\cdot COH\cdot COOH\cdot CH_2\cdot COOH} + 2H_2O$$

$$\underset{\text{Glucose}}{C_6H_{12}O_6} + 4\tfrac{1}{2}O_2 = \underset{\text{Oxalic acid}}{3(COOH)_2} + 3H_2O$$

$$\underset{\text{Glucose}}{C_6H_{12}O_6} + 6O_2 = 6CO_2 + 6H_2O$$

Bacteria decompose sugars in a manner quite different from that of the fungi, and the resulting end products are determined principally by the presence or absence of free oxygen. Lactic, butyric, acetic, propionic, formic, and valerianic acids, methane and hydrogen are some of the products formed under anaerobic conditions; frequently butyl alcohol, ethyl alcohol, and acetone are also produced under these conditions.

$$\underset{\text{Glucose}}{C_6H_{12}O_6} = \underset{\text{Lactic acid}}{2CH_3\cdot CHOH\cdot COOH}$$

$$\underset{\text{Glucose}}{C_6H_{12}O_6} = \underset{\text{Butyric acid}}{CH_3\cdot CH_2\cdot CH_2\cdot COOH} + 2CO_2 + 2H_2$$

$$\underset{\text{Glucose}}{C_6H_{12}O_6} = \underset{\text{Ethyl alcohol}}{2CH_3\cdot CH_2OH} + 2CO_2$$

In the presence of oxygen, the sugar molecule is broken down, through the lactic acid, acetaldehyde, and pyruvic acid stages, to carbon dioxide and water, as shown by the following reactions:

$$\underset{\text{Glucose}}{C_6H_{12}O_6} = \underset{\text{Pyruvic acid}}{2CH_3\cdot CO\cdot COOH} + 2H_2$$

$$\underset{\text{Pyruvic acid}}{CH_3\cdot CO\cdot COOH} + H_2 = \underset{\text{Lactic acid}}{CH_3\cdot CHOH\cdot COOH}$$

$$\underset{\text{Pyruvic acid}}{CH_3\cdot CO\cdot COOH} + H_2 = \underset{\text{Acetaldehyde}}{CH_3\cdot CHO} + H_2 + CO_2$$

$$\underset{\text{Acetaldehyde}}{CH_3\cdot CHO} + H_2 = \underset{\text{Ethyl alcohol}}{CH_3\cdot CH_2OH}$$

$$\underset{\text{Pyruvic acid}}{CH_3 \cdot CO \cdot COOH} + H_2O = \underset{\text{Acetic acid}}{CH_3 \cdot COOH} + \underset{\text{Formic acid}}{H \cdot COOH}$$

$$CH_3 \cdot CH_2OH + 3O_2 = 2CO_2 + 3H_2O$$

$$CH_3 \cdot COOH + 2O_2 = 2CO_2 + 2H_2O$$

$$H \cdot COOH + \tfrac{1}{2}O_2 = CO_2 + H_2O$$

The starch content of plants, with the exception of a number of various seeds and other reserve food bodies, is very small. The decomposition of the starches is very rapid, the process being similar to the decomposition of the sugars. Some of the microbes, especially the anaerobic bacteria, attack the starch molecule with the formation of various organic acids, alcohols, aldehydes, acetone, hydrogen, and carbon dioxide. Numerous soil microbes are able to produce an enzyme, diastase, which hydrolyzes the starch to maltose, and another enzyme, maltase which hydrolyzes the disaccharide maltose to two molecules of glucose.

DECOMPOSITION OF HEMICELLULOSES.—Hemicelluloses are present in considerable amounts in higher green plants and to some extent in microscopic green and chlorophyll-free plants, where they may occur as reserve food material. On hydrolysis, they are changed to simple sugars, hexoses or pentoses:

$$(C_5H_8O_4)n + nH_2O = n(C_5H_{10}O_5)$$

$$(C_6H_{10}O_5)n + nH_2O = n(C_6H_{12}O_6)$$

The most abundant group of hemicelluloses are the pentosans, present in quantities ranging from 7 per cent in pine needles to 32 per cent in corn-cobs.

Hemicelluloses, especially pentosans, are readily decomposed by a large number of fungi, actinomyces, and bacteria. In the degradation of fresh plant materials in the manure heap or in the soil, the decomposition of the pentosans proceeds somewhat more rapidly than that of cellulose. However, the organic matter in certain peats and in mineral soils is apt to be free from cellulose, while still containing considerable quantities of hemicelluloses. This is due to the greater resistance of some of the hemicelluloses to decomposition and to the formation of new hemicelluloses through the synthesizing activities of the microorganisms. Such substances formed by the organisms are commonly referred to as gums and slimes, which are produced by a variety of bacteria and fungi, either as excretion products of the cells, in the form of capsules, or as cell constituents.

Decomposition of Cellulose.—With certain few exceptions, cellulose forms the largest single group of constituents of plant materials added to the soil. Such fibrous materials as flax, hemp, cotton, and jute are largely made up of pure cellulose. Cellulose is not present in a free state in any great abundance in the vegetative structure of the plant, but is combined with lignin, cutin, and pectin, the compounds thus formed giving rise to ligno-celluloses, cuto-celluloses, and pecto-celluloses.

Cellulose is an amorphous polysaccharide, giving on hydrolysis with strong mineral acids or with appropriate enzymes the disaccharide cellobiose, which in its turn is readily hydrolyzed to glucose. When acted upon by soil microorganisms, sugars can be demonstrated as intermediate products only in exceptional cases. Cellulose can be readily decomposed by a large number of organisms which are present in normal soils, including various fungi, bacteria, and actinomyces, and possibly also some protozoa. The nature of the microbes responsible for the decomposition of cellulose varies with the conditions; it is different in soil than in the manure pile or in the peat bog; the chemistry of the process of cellulose decomposition depends upon the environmental conditions and the organisms concerned in the process.

Fungi Decomposing Cellulose.—The fungi capable of decomposing cellulose include a large number of organisms found among the Ascomycetes, Basidiomycetes, and Fungi Imperfecti. The following genera have been shown to contain one or more species capable of decomposing cellulose: Trichoderma, Aspergillus, Penicillium, Fusarium, Cephalosporium, Verticillium, Sporotrichum, Monosporium, Alternaria, Hormodendrum, Humicola, Chaetomium, various Polyporynae, and Agaricinae. These fungi are especially active in the so-called raw-humus forest soils, which are acid in reaction and are only seldom favorable for the development of cellulose-decomposing bacteria. The forest humus, comprising the upper few centimeters of forest soil, consists largely of a mass of partly or completely disintegrated leaves, needles and their residues; this is so much interwoven with colorless and brown fungus mycelium that, on examining the mass of partly disintegrated organic residues under the microscope, it is difficult to say whether the residue from the plants or the mycelium of the fungi is more predominant. Even in field, garden, and greenhouse soils, where the reaction is quite favorable for the development of the other cellulose-decomposing organisms, the fungi also play an active part in the disintegration of this abundant plant constituent, especially at the early stages of decomposition. This is brought out in Table 19, which shows the influence of the addition of cellulose to the soil upon the development of fungi, the latter being determined by the plate method.

TABLE 19

INFLUENCE OF CELLULOSE UPON THE NUMBERS OF FUNGI IN SOIL (FROM WAKSMAN AND STARKEY)

Nature of soil	Reaction of soil, *p*H	$NaNO_3$ added to cultures	Numbers of Fungi in 1 gm. of Soil	
			Soil without cellulose	Soil with 1 per cent cellulose
Unlimed, unmanured	5.1	−	115,700	160,000
Unlimed, unmanured	5.1	+	115,700	4,800,000
Limed, unmanured	6.5	−	20,000	47,000
Limed, unmanured	6.5	+	20,000	290,000
Unlimed, manured	5.5	−	87,300	320,000
Unlimed, manured	5.5	+	87,300	3,100,000

Accompanying the decomposition of the cellulose the fungi assimilate considerable quantities of nitrogen, which are required by the organisms to enable them to synthesize their cell substance. There is a fairly definite ratio between the amount of cellulose decomposed and the nitrogen required, which is between 30 : 1 and 50 : 1; in other words, for every 30 to 50 parts of cellulose decomposed, the organisms require 1 part of available nitrogen. A large part of the carbon of the cellulose decomposed is also used by the fungi for the synthesis of their mycelium. Since the mycelium or microbial cell substance has a definite chemical composition and the ratio between its carbon and nitrogen content is more or less constant, and since the cellulose decomposed is used both as a source of energy and as a source of carbon for the building up of the microbial cell substance, the ratio between the carbon used for energy and the carbon used for cell synthesis is constant for a given organism under a given set of conditions. There is also a ratio between the amounts of cellulose decomposed and the nitrogen assimilated. For every 100 units of cellulose decomposed, 20 to 30 units of cell substance are synthesized, which contain about 1.5 to 2.5 units of nitrogen. Since cellulose is free from nitrogen, this element has to be obtained from some other compounds to enable the microbes to bring about the decomposition of the cellulose. The amount of available nitrogen in the soil very frequently becomes the limiting factor in the decomposition of cellulose. The effect of the presence of available nitrogen on development of fungi is apparent from Table 19. The influence which soil reaction and available nitrogen may have is shown in Table 20.

TABLE 20

INFLUENCE OF REACTION AND AVAILABLE NITROGEN UPON DEVELOPMENT OF CELLULOSE-DECOMPOSING BACTERIA AND FUNGI (FROM DUBOS)

Nature of soil	Treatment	Soil reaction, *p*H	Mgm. of C given off as CO_2 from 500 mgm. cellulose in 36 days	Cellulose decomposed, per cent	Number of cellulose-decomposing bacteria	Number of fungi
Unlimed.....	Control	5.4	....	....	0	87,000
Unlimed.....	Cellulose added	5.4	63.6	55	50,000	353,000
Unlimed.....	Cellulose and $(NH_4)_2SO_4$ added	5.4	122.8	96	10,000	2,700,000
Limed.......	Control	6.8	....	....	1,000	43,000
Limed.......	Cellulose added	6.8	60.4	53	5,000,000	110,000
Limed.......	Cellulose and $(NH_4)_2SO_4$ added	6.8	130.2	99	10,000,000	490,000

The beneficial effects which additions of animal manures and many different fertilizer materials exert on the decomposition of cellulose may frequently be ascribed to the greater amounts of available inorganic nitrogen which these substances put to the disposal of the microbes in the soil. Since phosphorus, and to a less extent potassium, sulfur, and other nutritive elements, are also required for the synthesis of microbial cell substances, these elements must be present in the soil in appropriate amounts in available forms to permit the microbes to use the cellulose as food. Phosphorus is required in greater amounts than any of the other mineral substances, and may comprise two-fifths of the total ash of microbial cells. However, phosphorus (calculated as P) may be required for the synthesis of cells in only about one-tenth the amounts that nitrogen is needed. Other minerals are required in much smaller quantities.

Bacteria Decomposing Cellulose.—The bacteria decomposing cellulose can be divided conveniently into two groups, representatives of which differ considerably in their morphological characters and physiological activities: (1) aerobic bacteria and (2) anaerobic bacteria. Among the aerobic forms there are spore-forming and non-spore-forming rod-shaped bacteria, spherical organisms and spirochaete-like or flexuous organisms (see Fig. 8). It is characteristic of all that free atmospheric oxygen is required for development, just as in the case of the cellulose-decomposing fungi, but, while the fungi will grow readily in very acid soils of a *p*H 4.0 and even less, the aerobic bacteria will not grow at an acidity greater than *pH* 5.6–6.0, as shown in Table 21.

TABLE 21

Effect of Reaction of the Soil on the Number of Cellulose-Decomposing Bacteria (from Dubos)

Soil reaction, *p*H	Number of cellulose-decomposing bacteria	Soil reaction, *p*H	Number of cellulose-decomposing bacteria
8.7	0	6.5	25,000,000
8.5	250,000	6.0	250,000
8.2	25,000,000	5.2	0
7.5	25,000,000	4.5	0
7.0	25,000,000	4.0	0

Neither fungi nor aerobic bacteria will grow in marshes or undrained peat soils which are anaerobic in nature. In forest, field, and garden soils, both groups of organisms will develop, the reaction of the soil determining the group that will predominate. Soils more acid than *p*H 5.5 will show largely a flora of fungi which will be the active agents in cellulose decomposition; soils less acid than pH 5.6 will favor the development of aerobic bacteria, without, however, being unfavorable to fungi (see Table 20).

Many of the aerobic bacteria use cellulose as the exclusive source of energy and are unable to develop upon the simple sugars, indicating that this process of decomposition may not proceed through the sugar stage or at least does not lead to the accumulation of the lower carbohydrates. Bacteria decompose cellulose, leaving a mucilaginous mass consisting of bacterial cells imbedded in certain gum-like compounds (hemicelluloses) synthesized by the organisms. Small amounts of various acids and certain yellow and orange pigments are also formed. The aerobic bacteria also require considerable quantities of nitrogen and phosphorus for the decomposition of the cellulose, for the same reasons that they are required by fungi.

Anaerobic bacteria (see Fig. 9) capable of decomposing cellulose occur in arable soils only in limited numbers. They are found abundantly in peat and marsh soils, in rivers, in manure piles, and in other habitats where fresh organic matter is abundant and free access of air is prevented. Some of the anaerobic bacteria are thermophilic in nature, being capable of active growth and decomposition of cellulose at 60° C. Both the organisms that grow at normal temperatures and those that grow best at high temperatures (55 to 65° C) can bring about very rapid decomposition of cellulose with the formation of various organic acids, alcohols, and gases, such as methane, hydrogen, and carbon dioxide. Omeliansky found that out of 3.3 gm. of cellulose decomposed, there were produced 2.2 gm. fatty acids, 1.0 gm. CO_2, and 0.014 gm. hydrogen. A thermophilic bacterium produced from the decomposition of 42 gm. cellulose, 21.6 gm. acetic acid, 10.3 gm. ethyl alcohol, and 11.9 gm. CO_2, in addition to considerable quantities of hydrogen. In addition to acetic acid, formic, lactic, and butyric acids have been found as products of decomposition of cellulose by anaerobic bacteria. The formation of several of these products was explained by the following reactions:

$$\underset{\text{Cellulose}}{(C_6H_{10}O_5)n} + nH_2O = 2n(\underset{\text{Pyruvic acid}}{CH_3 \cdot CO \cdot COOH}) + 2nH_2$$

$$\underset{\text{Pyruvic acid}}{CH_3 \cdot CO \cdot COOH} + H_2 = \underset{\text{Lactic acid}}{CH_3 \cdot CHOH \cdot COOH}$$

$$\underset{\text{Pyruvic acid}}{CH_3 \cdot CO \cdot COOH} = \underset{\text{Acetaldehyde}}{CH_3 \cdot CHO} + CO_2$$

$$\underset{\text{Acetaldehyde}}{2CH_3\cdot CHO} + H_2O = \underset{\text{Ethyl alcohol}}{CH_3\cdot CH_2OH} + \underset{\text{Acetic acid}}{CH_3\cdot COOH}$$

$$\underset{\text{Ethyl alcohol}}{2CH_3\cdot CH_2OH} = \underset{\text{Acetic acid}}{CH_3\cdot COOH} + \underset{\text{Methane}}{2CH_4}$$

$$\underset{\text{Lactic acid}}{2CH_3\cdot CHOH\cdot COOH} = \underset{\text{Butyric acid}}{CH_3\cdot CH_2\cdot CH_2\cdot COOH} + 2CO_2 + 2H_2$$

Various bacteria are capable of decomposing cellulose in the absence of atmospheric oxygen if nitrate is present; under such conditions the nitrate is used as a source of oxygen. The nitrate is reduced to gaseous nitrogen, and the oxygen thus liberated is used for the decomposition of the cellulose.

A number of actinomyces decompose cellulose but at a comparatively slow rate. The nature of the reaction is still unknown.

Influence of Soil Conditions upon Cellulose Decomposition.—The above considerations tend to explain why cellulose is decomposed in different soils with different degrees of rapidity. The soil conditions, such as moisture, reaction, aeration, organic matter content, and supply of available nutrients, influence the types of organisms present in the soil and the types that take an active part in the decomposition of the cellulose; these organisms in their turn influence the nature of the chemical reactions brought about and the nature of the products formed. However, the microbes which have the capacity of decomposing cellulose are so numerous in the soil, they are of such various forms, and they differ so greatly in physiological requirements, that, wherever sufficient inorganic nutrients are available and wherever there is adequate moisture and the temperature conditions are favorable, cellulose is rather quickly decomposed. Either under moderate or excessive moisture, at acid, neutral, or basic reactions, at moderate or high temperatures, some microbes will find conditions conducive to their activity. However, the mechanism of the decomposition and the chemistry of the products formed will be different. The formation of organic substances through the synthesizing agencies of microorganisms contributes one of the sources of the soil organic matter frequently referred to as *soil humus*.

Fig. 45 shows the comparative growth of the three major groups of microorganisms, namely the bacteria, fungi, and actinomyces, during the decomposition of cellulose at different moisture contents.

LIGNIN AND ITS DECOMPOSITION.—The lignin in plants increases in abundance as the plants become older; this complex makes up a considerable portion of the mature plants. Since lignin is very resistant to decomposition by the common soil fungi and bacteria, it tends to accumulate with the advance in decomposition of the plant materials, while the sugars, proteins, hemicelluloses, and

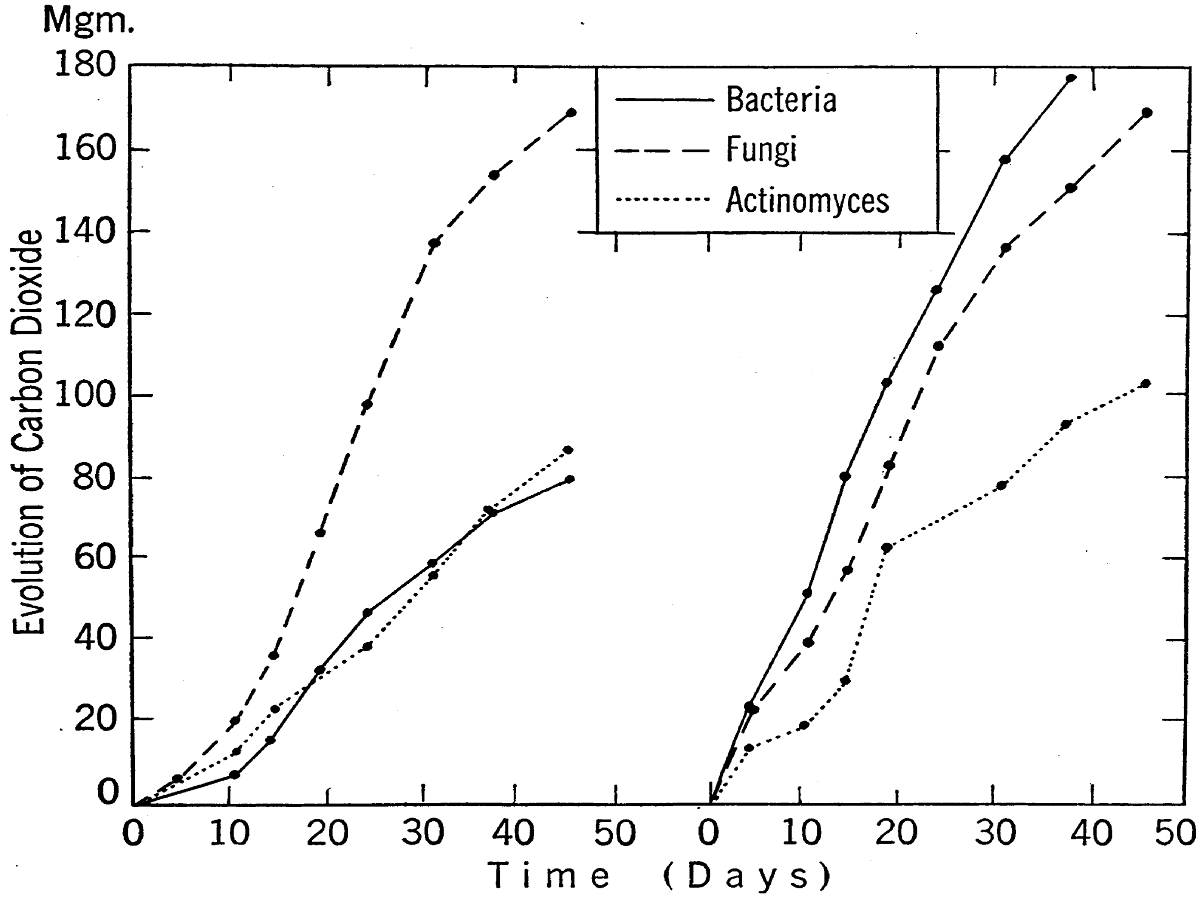

FIG. 45.—Comparative growth of cellulose-decomposing bacteria, fungi, and actinomyces in a neutral soil. Left curves at 30 per cent of the moisture-holding capacity of the soil; curves at right at 50 per cent of the moisture-holding capacity (after Dubos).

cellulose are quite readily decomposed. Only certain groups of Basidiomycetes (species of Polyporus, Merulius) and certain Actinomyces are capable of attacking the lignin; however, even the action of these organisms is much slower than in the decomposition of other plant constituents. Under anaerobic conditions, as in peat soils, the lignin remains undecomposed and rapidly accumulates.

Fig. 46 shows the relative resistance to decomposition of the lignin as compared with cellulose and hemicelluloses, when alfalfa is allowed to decompose under aerobic and anaerobic conditions.

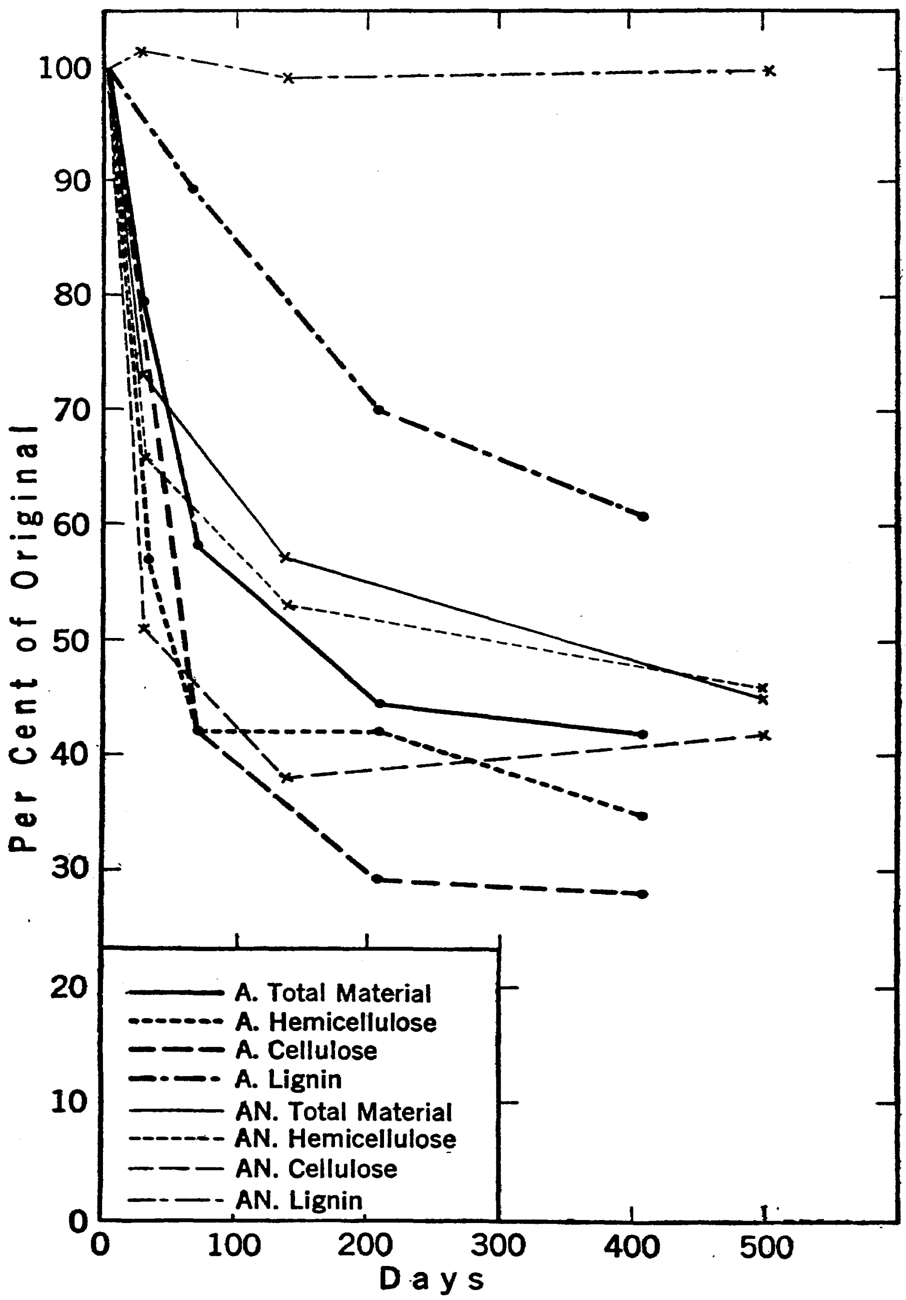

FIG. 46.—Course of decomposition of total plant material (alfalfa) and of the hemicelluloses, cellulose, and lignin under aerobic (A) and anaerobic (AN) conditions (from Tenney and Waksman).

DECOMPOSITION OF FATS.—Fats are esters of the higher acids and a tribasic alcohol, glycerol. The decomposition of a typical fat proceeds as follows:

$$\begin{array}{l} CH_2 \cdot OOC \cdot C_{17}H_{33} \\ | \\ CH \cdot OOC \cdot C_{17}H_{33} + 3H_2O = \underset{\text{Glycerol}}{CH_2OH \cdot CHOH \cdot CH_2OH} \\ | \\ \underset{\text{Triolein (a fat)}}{CH_2 \cdot OOC \cdot C_{17}H_{33}} \qquad\qquad\qquad + \underset{\text{Oleic acid}}{3C_{17}H_{33}COOH} \end{array}$$

The glycerol and fatty acids are further decomposed in the soil by a number of various fungi and bacteria, giving lower acids, carbon dioxide, and water. The decomposition of fats occurs principally under aerobic conditions.

DECOMPOSITION OF OTHER PLANT CONSTITUENTS.—A detailed discussion of the decomposition of proteins is given in the following chapter, where other transformations of nitrogen are considered. The subject is of great importance, since nitrogen forms one of the most important elements in the protein molecule and since nitrogen is apt to be the factor most limiting abundant crop growth. The numerous organic substances present in plants in quantities ranging from mere traces to one or more per cent, are sooner or later decomposed by one or more groups of soil organisms, and either destroyed completely or transformed into other organic complexes. Even such substances as various aldehydes, vanillin, toluene, phloroglucinol, phenol, cresol, naphthalene, and their derivatives, are decomposed more or less rapidly in the soil by specific or non-specific organisms. As a result, compounds which might otherwise be toxic to plant growth become innocuous and may even lead to the formation of substances beneficial to plant growth. It is necessary, however, that the soil conditions, such as proper aeration, reaction, and moisture, should be favorable to the development of the particular organisms.

Methane originates as an intermediate decomposition product under anaerobic conditions, and may be attacked by a variety of aerobic bacteria which oxidize it to carbon dioxide and water:

$$CH_4 + 2O_2 = CO_2 + 2H_2O$$

Some bacteria which are able to oxidize methane are also active in decomposing benzene and paraffin. Numerous fungi and non-spore-forming bacteria decompose paraffin, petroleum, vaseline, benzene and kerosene under aerobic conditions. The disappearance of these substances under anaerobic conditions is much slower. The decomposition of such compounds, like the transformation of other non-nitrogenous substances, necessitates the presence of considerable nitrogen available from other sources.

The changes which take place in the plant constituents may be shown in the graphic representation of the decomposition of cornstover (Fig. 47). It is apparent that there is a rather rapid decomposition of the water-soluble constituents, hemicelluloses and cellulose, and a slow destruction of the lignin. The crude protein, on

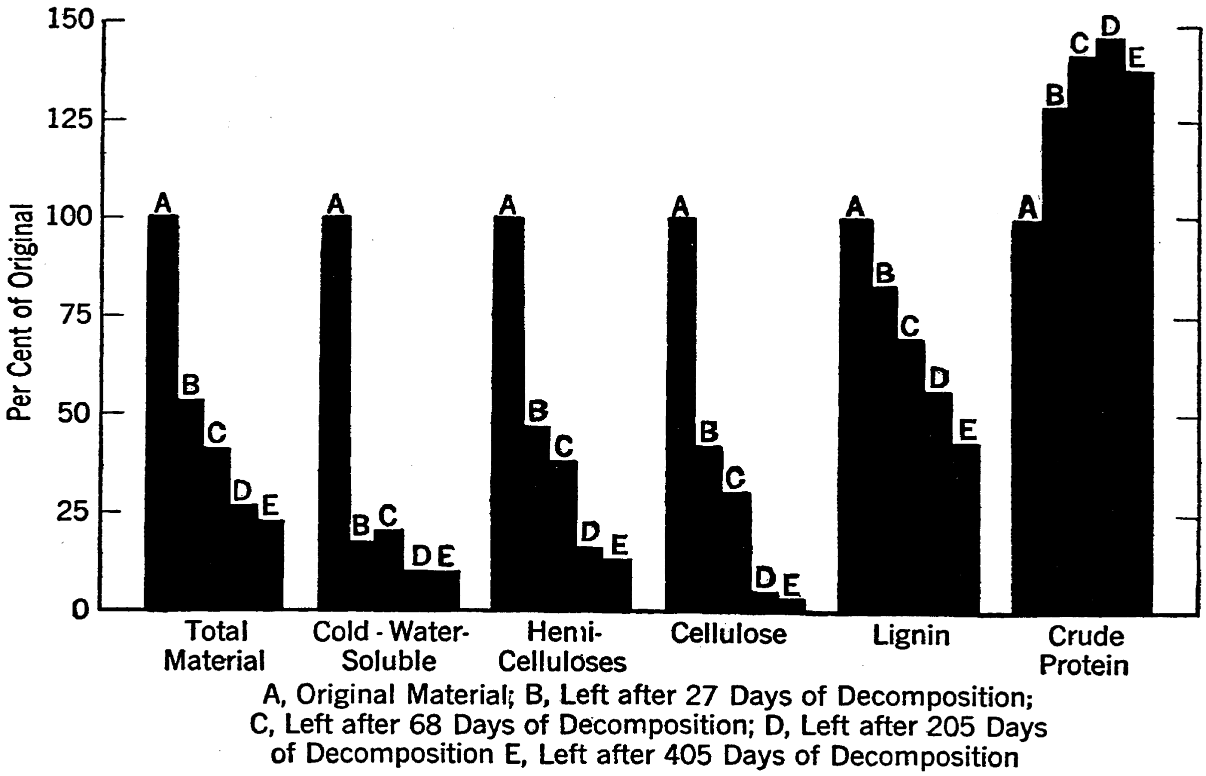

FIG. 47.—Course of decomposition of various chemical constituents of cornstover under aerobic conditions (after Tenney and Waksman).

the other hand, shows a pronounced increase resulting from the synthesis of an abundance of microbial cells at the expense of the water-soluble nitrogen. This does not indicate, however, that the protein constituents are resistant to decomposition. Under the aerobic conditions, the material as a whole had decomposed to one-third of the original amount in the period of 68 days.

DECOMPOSITION OF THE PLANT AS A WHOLE.—The rate at which plants or organic fertilizers decompose is regulated to a large extent by the relative abundance of the various constituents which compose the organic matter and the state of aggregation of these constituents. Some plant residues largely disappear in a few weeks, while others still retain their original appearance after several months in the soil. Generally, however, certain portions of practically all organic materials decompose somewhat rapidly within a few days subsequent to their addition to the soil. This period is followed by a uniform but rather abrupt retardation in the speed of decomposition which is followed by a slow but continuous decomposition of the remaining material. Even a year or more after the addition of the organic matter, the soil is still in a more active biological condition than it was before the treatment.

Although differences exist between the rates with which soils of different fertility are able to decompose the same organic materials, these differences are not very pronounced after the first few days. As a rule, however, conditions favorable to development of cultivated plants, such as relatively high temperature and water content, presence of sufficient available inorganic substances, and reaction close to neutrality, also exert favorable effects on decomposition. Consequently, the more fertile the soil, the more rapid the mineralization of the added organic matter.

Organic materials are applied in farming practice with the object of producing beneficial effects on plant growth. Whether or not such effects are produced will depend principally upon the reactions which occur during decomposition of the organic substances. As has been stated previously, the application of organic materials results within a very short period in an enhanced development of the microorganisms in the soil. This is not confined to any one group, but generally applies to all soil microbes; the extent of stimulation differs with the various species of bacteria, fungi, actinomyces, and protozoa, and depends upon the composition and the amount of the organic material added. The increase in abundance of cells is also accompanied by increases in their activities, as measured by the formation of carbon dioxide and other end products.

Coincident with the decomposition of organic matter containing small amounts of nitrogen, there is an immediate and appreciable decrease in the amounts of ammonia and nitrate nitrogen originally contained in the soil, as is shown in Table 22. In this experiment, the substances were added in such amounts as to introduce the same quantity of nitrogen in each case. As a result of the application of appreciable amounts of organic matter containing only small relative concentrations of nitrogen, growth of higher plants may be greatly depressed.

TABLE 22

INFLUENCE OF NATURE OF ORGANIC MATTER UPON NITRATE FORMATION IN SOILS (FROM LYON, BIZZELL AND WILSON)

Treatment	Nitrogen content	Amount of material added to 28 pounds of soil	Total NO_3-N in leachings
	per cent	*gm.*	*mgm.*
Soil alone	....	....	950.0
Soil + oat roots	0.45	133.3	207.3
Soil + timothy roots	0.62	96.8	398.4
Soil + corn roots	0.79	75.9	510.6
Soil + clover roots	1.71	35.1	924.4
Soil + dried blood	10.71	5.6	1751.1

Associated with these changes there is an increase in the abundance of insoluble nitrogen, organic in nature; this increase is practically quantitatively equal to the decrease in the water-soluble nitrogen, such as ammonia and nitrate. In the decomposition of such materials as corn-stover and oat straw, increases of 200 to 300 per cent in the organic nitrogen are not exceptional. Under such conditions there is seldom any loss of total nitrogen; as a result of the creation of conditions favorable to nitrogen fixation, there may even be an absolute increase in the total nitrogen.

Somewhat similar changes occur with sulfur and phosphorus as with nitrogen. There is a reduction of the amounts of these elements found in inorganic forms with, however, no loss in the total amounts found in the soil.

These effects are all directly associated with the activities of the microbes which are responsible for the decomposition of the organic materials; they are also concerned with the normal nutrition of the microbial cells. The nitrogen, whatever its combination, is built up from an inorganic into organic cell substance; there is a direct proportion, in which, for a unit of organic matter decomposed or per unit of energy liberated, a definite amount of nitrogen is assimilated and a definite amount of cell substance synthesized. A great abundance of soil microorganisms, including numerous bacteria, actinomyces, and fungi, are able to assimilate nitrogen provided as ammonia, nitrate, or in simple organic combination; the extent of the assimilation of inorganic nitrogen depends upon the amount of available energy material and its relative content of organic nitrogen.

Metabolism of Microbes and Decomposition of Organic Matter.—Knowing the nature of the organisms effecting the decomposition of an organic material, the composition of the microbial cells, the energy balance of these cells, the composition of the organic matter undergoing decomposition and the environmental conditions, it is possible to interpret in a very definite manner the end products which will be formed and the speeds with which these will accumulate. Any one of these five variables may show differences over a considerable range, but sufficient is already known to interpret in general terms what may be expected to take place.

Fungi use the organic substance as a source of energy and as a nutrient (a source of carbon); they assimilate, in the mycelium and spores, from 20 to 50 per cent of the carbon contained in the organic compound decomposed. The rest of the carbon goes off as carbon dioxide or is left as incompletely decomposed material. For the sake of simplicity let it be assumed that 35 per cent of the carbon of the organic matter decomposed is assimilated by the fungi. Bacteria assimilate much less of the carbon, utilizing the available energy less economically. They may assimilate from 1 to 30 per cent of the carbon contained in the organic material decomposed. An average of 7 per cent may typify conditions for many bacteria. Actinomyces are intermediate between the bacteria and fungi, assimilating from 15 to 30 per cent of the carbon.

Accompanying the carbon assimilation, there is a consumption of considerable amounts of nitrogen, which is largely synthesized in the form of protein complexes. The carbon content of the cells may be between 45 and 54 per cent. For convenience it may be assumed that dry microbial cell substance contains 50 per cent of carbon. The nitrogen content and the ratios of carbon to nitrogen in the cells may be assumed to be as follows:

	Nitrogen content, per cent	Ratio of carbon to nitrogen
Fungi	3–8, av. 5	10.0 to 1
Bacteria	8–12, av. 10	5.0 to 1
Actinomyces	7–10, av. 8.5	6.0 to 1

It is apparent that fungi assimilate much less nitrogen per unit of carbon, but, as has been mentioned above, they use much more of the total carbon contained in the material during the decomposition process.

The following calculations show what would happen as the fungi decompose such substances as cellulose, straw, alfalfa, and dried blood. For the sake of simplicity, it is assumed that all of the organic material is decomposed. This is not absolutely the case, but the errors introduced by this assumption will not greatly modify the results which are calculated.

Cellulose contains 45 per cent carbon. In 100 pounds of cellulose there are 45 pounds of carbon, 35 per cent of which is assimilated, as assumed previously.

$45 \times 0.35 = 15.75$ pounds of carbon assimilated.

For every ten parts of carbon there is one part of nitrogen assimilated, or 10 per cent as much nitrogen as carbon assimilated.

$15.75 \times 0.10 = 1.575$ pounds of nitrogen assimilated.

Since there is no nitrogen in cellulose, there would be a deficit of nitrogen, which would have to be added to the medium in which the decomposition was taking place.

Some straws contain about 37 per cent of carbon and 0.5 per cent of nitrogen. In 100 pounds of straw there would be 37 pounds of carbon and 0.5 pound of nitrogen. The following figures indicate the result of its decomposition:

$37 \times 0.35 = 12.95$ pounds of carbon assimilated.
$12.95 \times 0.10 = 1.295$ pounds of nitrogen assimilated.

Since there was 0.5 pound of nitrogen in the straw originally, if this was all used by the fungi, $1.295 - 0.50 = 0.795$ pound of nitrogen would appear as a deficit which would have to be supplied to the fungi in order that decomposition of the straw take place.

Alfalfa may be assumed to contain 40 per cent of carbon and 3 per cent of nitrogen. In 100 pounds there would be 40 pounds of carbon and 3 pounds of nitrogen.

$40 \times 0.35 = 14.00$ pounds of carbon assimilated.
$14.00 \times 0.10 = 1.40$ pounds of nitrogen assimilated.

$1.40 - 3.00 = -1.60$ pounds of nitrogen, meaning that there would be 1.60 pounds of nitrogen in excess of the requirement. This excess of nitrogen would appear as ammonia as the decomposition progressed, and would represent a waste product of the nutrition of the fungi.

Dried blood contains about 40 per cent of carbon and 10 per cent of nitrogen. There would be 40 pounds of carbon and 10 pounds of nitrogen in 100 pounds of the material.

$$40 \times 0.35 = 14.00 \text{ pounds of carbon assimilated.}$$
$$14.00 \times 0.10 = 1.40 \text{ pounds of nitrogen assimilated.}$$

$1.40 - 10.00 = -8.60$ pounds of nitrogen, meaning that there would be 8.60 pounds of nitrogen in excess of the requirements. Here there would be a great excess of nitrogen over that required for the nutrition of the fungi.

Similar calculations may be made to indicate the results of the decomposition of the same organic materials by bacteria. Less of the carbon is assimilated (only 7 per cent), and there is a greater amount of nitrogen required per unit of carbon used—ratio of C to N is 5.0 to 1, or one-fifth as much nitrogen as carbon is required.

It is quite evident that, in the decomposition of organic materials, such as cellulose and mature straw, there is a marked deficit in nitrogen which must be supplied from some source, such as inorganic ammonium salts, nitrates, or available organic nitrogenous compounds. With organic materials, as in the case of leguminous plants, which contain from 1.5 to 3 per cent of nitrogen, there will generally be more nitrogen than is necessary to satisfy the requirements of the organisms, and consequently there would soon be some ammonia formed. This may not always occur, since the plant is not homogenous but a complex aggregate of compounds which decompose with unequal rapidity. It has been observed that when the plant materials contain less than 1.7 per cent of nitrogen there will be a more or less extended deficiency of nitrogen for decomposition to proceed rapidly. With materials containing more than 1.7 per cent of nitrogen no deficiency appears; in fact, there will be more nitrogen than is required by the microorganisms; this excess will appear as ammonia (later as nitrate), and the amount of the excess will be determined by the excess of nitrogen over 1.7 per cent which the organic material contains.

The assimilation of nitrogen by microorganisms should not suggest that the nitrogen is permanently stored up in microbial cells. Cells of the microorganisms lead a transient life in the soil; they develop and die within short intervals, depending upon the environmental conditions. Upon their death they become decomposed with the liberation once more of at least a part of the nitrogen and of the other elements which were assimilated during growth. It can be appreciated that, in the presence of even small amounts of nitrogen and large amounts of nitrogen-free organic matter, there would eventually be no deficiency of nitrogen but even a liberation of most of the nitrogen as ammonia. The process will proceed somewhat slowly, since the organisms must die and be decomposed over a considerable period of time.

Fig. 48 shows schematically the course of the changes under ideal conditions, or what might be called the perfect case, where the total plant and the microbial cells which are formed are all completely decomposed. In the calculations the assumption has been made that the organisms causing the decomposition assimilate into their cell substance one-third of the carbon of the material decomposed and eliminate the other two-thirds as carbon dioxide. It is further assumed that the cells of the organisms have a ratio of carbon to nitrogen of 10 to 1. The nitrogen of the original organic material (12.5 pounds) permits the organisms to transform 125 pounds of carbon into their cells, since they assimilate ten times as much carbon as nitrogen. Also, since they assimilate only one-third of the carbon that they attack, 250 pounds of carbon will be eliminated as carbon dioxide. This leaves 625 pounds of carbon in the form of residual undecomposed organic matter. Providing the microbial cells suffer complete decomposition, 42 pounds of carbon and 4.2 pounds of nitrogen will be synthesized into the new cells which cause this decomposition (A). Since only one-third of the carbon is used by the cells, two-thirds of the carbon, or 83 pounds, will be eliminated as carbon dioxide. Since the cells which were decomposed contained more nitrogen than the new cells required for growth, 8.3 pounds of nitrogen will be eliminated as ammonia. This ammonia permits decomposition of more of the undecomposed organic matter. As a result of this decomposition, 166 pounds of carbon go to carbon dioxide, 83 pounds of carbon are assimilated by microbial cells (B), and 376 pounds of carbon still remain undecomposed (C).

If the total constituents of the microbial cells (A) and (B) are

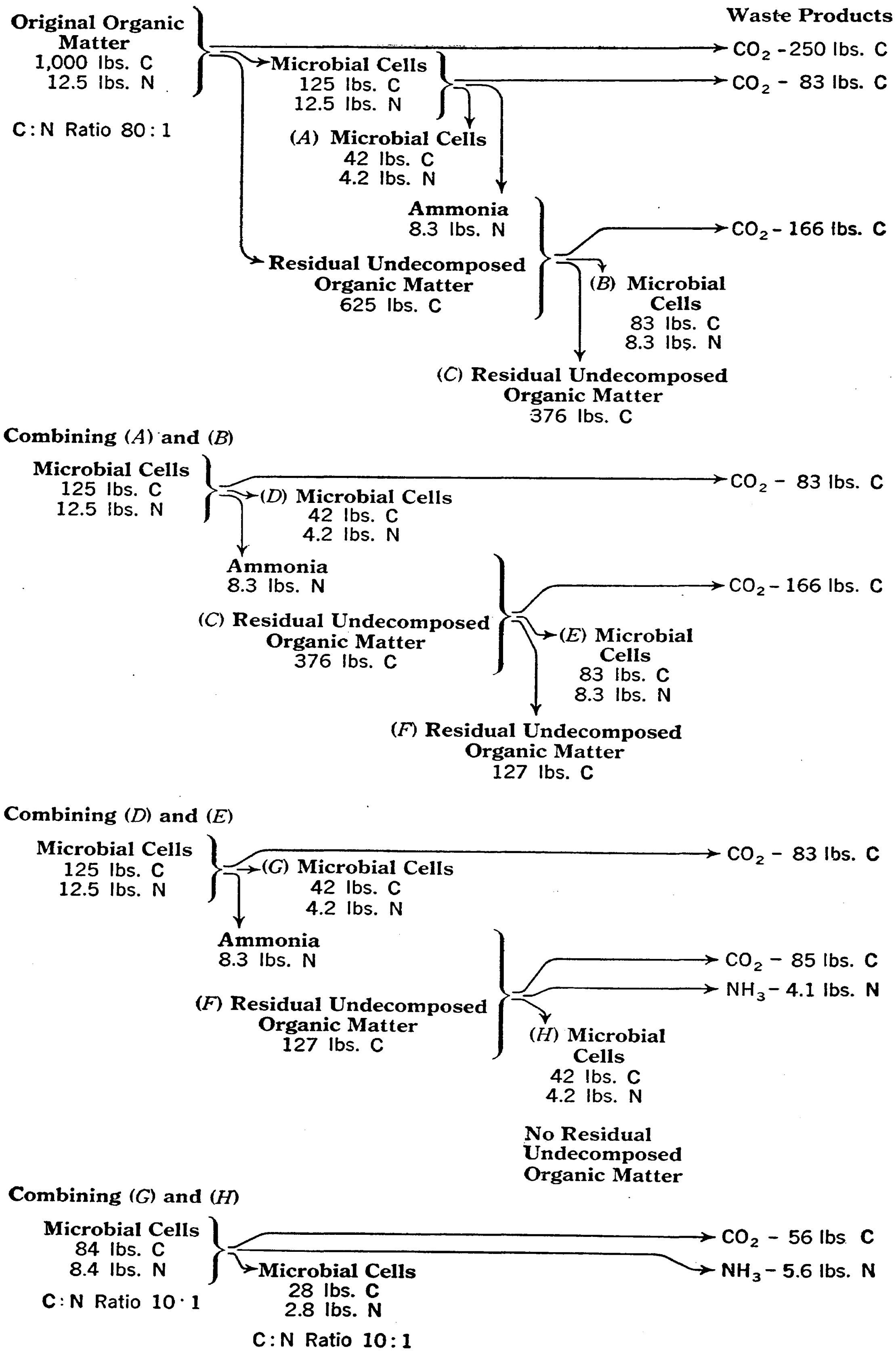

FIG. 48.—Schematic representation of the changes in the carbon and nitrogen of organic matter during the decomposition of material containing small amounts of nitrogen. (Braces in each case indicate decomposition taking place.)

combined at this stage we account for 125 pounds of carbon and 12.5 pounds of nitrogen. Continuing with the decomposition as previously, there is a progressive increase in the amount of carbon accounted for as carbon dioxide and less present in residual undecomposed organic matter. After decomposition of the microbial cell substance represented by (D) and (E) there are eliminated 8.3 pounds of nitrogen as ammonia, which are more than sufficient to permit the organisms to decompose the 127 pounds of residual undecomposed organic matter remaining at this time (F). Consequently, the excess nitrogen remains as ammonia, which may now be considered as a waste product since it will not be further used by the organisms causing the decomposition. From this point on, more and more ammonia will be added to the waste products, and the remaining organic matter will be accounted for entirely as microbial cells. It can be seen from this outline that eventually ammonia will be liberated from the decomposition of organic materials having as wide an initial ratio of C to N as 80 to 1. This entails the repeated mineralization of the nitrogen of the microbial cells, and eventually leads to a C : N ratio of 10 to 1, which persists at this figure as long as there is any organic matter remaining.

In normal soils there is practically always a rather close relationship between the amounts of organic carbon and nitrogen present. Even when organic materials of wide ratios of carbon to nitrogen are added to soils, there is a tendency for these ratios to become narrower with time, until they become about 10 to 1, at which level the ratio remains practically indefinitely, although the total amounts of organic carbon and nitrogen may be continually decreasing. In the light of the diagrammatic conversion of organic matter pictured above, it may be apparent why the ratio becomes 10 to 1 and does not become lower. Some variations from this ratio frequently occur, as determined by differences in the organisms causing the decomposition and by accumulations of resistant plant residues of wide C : N ratios.

Summary. Decomposition of Organic Matter in Soil and Formation of Soil Humus, or Soil Organic Matter.—A brief summary of the facts discussed in this chapter allows us to obtain a clear idea of the general processes involved in the decomposition of organic materials of plant and animal origin added to the soil and the formation of the dark-colored substances which comprise the soil organic matter, giving to the soil its characteristic properties, and frequently spoken of as *soil humus*.

When a ton of fresh organic matter (on a dry basis), in the form of plant stubble, green manures, stable manures, or organic fertilizers, is plowed under or worked into the soil, the microorganisms immediately become active, decomposing first the water-soluble substances, then the starches, the proteins, the hemicelluloses, and cellulose. Within 10 to 20 days, under favorable conditions of moisture, aeration, and temperature, only about 1,000 to 1,200 pounds may be left out of the 2,000 pounds originally added. These 1,000–1,200 pounds comprise only a part of the cellulose, pentosans and fats, most of the lignin, a large part of the waxes and cutins, and a large quantity of substance synthesized by the microorganisms of the soil. Most of the original protein has disappeared, and in its place there has been formed fresh microbial protein, some ammonia and nitrate, depending upon the relative nitrogen content of the original plant material. If the nitrogen was present in amounts less than 2 per cent, only a small amount of the nitrogen becomes mineralized; if the nitrogen content of the original plant material was greater than 2 per cent, a large part may become liberated as ammonia or nitrate within 20 days under favorable conditions.

Another month has passed, and the residual organic material represents a new picture. If the original material was in a green state, or if there was an abundance of nitrogen available, only 400 to 600 pounds may be left out of the original ton of dry material. This will consist largely of lignin from the original plant or its transformation products, some fats and waxes, considerable synthesized microbial cell substance in the form of proteins, hemicelluloses, chitin, etc. This mass of residual and synthesized materials is referred to as soil organic matter or *humus*. If the original plant material was in the form of cereal straw or cornstover, which contain very little nitrogen (about 0.5 per cent), the decomposition of the plant cellulose and hemicelluloses would have been slower, since the transformation would have been controlled by the amount of available nitrogen and by the rapidity with which the soil microorganisms were able to use the nitrogen in the soil and in the plant.

The organic matter in the soil is not uniform in composition. It constantly undergoes numerous changes. The condition of this organic matter is not static but dynamic, being different from what it was yesterday, and it will be different to-morrow from what it is to-day, the changes being both qualitative and quantitative. The soil organic matter gives off a constant stream of carbon dioxide, diminishing from day to day, if the soil is left undisturbed. Rain and dryness, heat and frost, will constantly modify the conditions of the soil, influencing variously the activities of the microorganisms, the nature and composition of the soil organic matter. The nature of the growing plants and the management of the soil still further modify the nature and amount of the soil organic matter. That the organic matter of the soil which receives little or no added material becomes depleted is apparent from the fact that about 30 mgm. of carbon dioxide may be produced per kilogram of soil of average fertility every day for about 200 days of the year. This would mean approximately a ton and a half of carbon per year for each acre of 2,000,000 pounds. Boussingault stated that in eleven years, one-half of the carbon in a certain soil had been converted to carbon dioxide.

A knowledge of the chemical composition of the plant and animal residues undergoing decomposition, of the microorganisms bringing about the decomposition of the various chemical constituents, and of the environmental conditions under which the decomposition is taking place, is essential before we can understand the nature and rapidity of liberation of the nutrient elements in forms available for plant growth and of the formation and nature of the residual organic matter or soil humus.

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CHAPTER V

TRANSFORMATION OF NITROGEN BY SOIL MICROBES

Sources of Nitrogen in Soil.—The atmosphere is the source of supply of nitrogen in the soil; a continuous exchange is operating between the nitrogen that now exists in various combinations in the soil itself and what remains above the earth in the atmospheric cloak. The gaseous covering of the atmosphere contains the relatively inert molecular form of nitrogen. This becomes available to green plants only as it is changed into combined forms by certain specific microbes or by strictly non-biological processes, such as union of nitrogen and oxygen to form nitrate through the agency of electrical discharges, through catalytic combination of nitrogen and hydrogen to form ammonia, or by a reaction between nitrogen and calcium carbide to form calcium cyanamid. Natural electrical discharges add small amounts of inorganic nitrogenous compounds to the earth; the commercial exploitation of fixation processes has resulted in the further addition of vast quantities of combined nitrogen to soils. However, the principal natural mechanism by which plants obtain their combined nitrogen and by which the lost quantities of nitrogen are restored to the soil is that operated by some of the microbes which make the soil their natural habitat.

The mere presence of nitrogenous compounds in the soil does not solve the problem of satisfying the requirements of higher plants for this element. Green plants require certain very specific nitrogen compounds, and the production of a continuous supply of these substances is largely the result of the activities of microorganisms. The nitrogen compounds in the soil, from which the supply of nitrogen made available for plant growth originates, are varied and many are quite complex in composition. They comprise largely organic compounds produced as a result of the growth of green plants and animals, as well as of the numerous microbes. These compounds include proteins, proteoses, peptones, peptides, nucleic acids, amino acids, amides, and urea, as well as the alkaloids and heterocyclic compounds. These various substances are attacked by soil microbes before they become available to higher plants. In certain processes, microbes diminish the supply of combined nitrogen in the soil; they occasionally form molecular nitrogen or nitrous oxide from nitrate and thus decrease the quantity of nitrogen in the soil.

These numerous interrelated processes resulting from the presence and activities of microscopic and higher forms of life in and upon the soil for untold thousands of years have resulted in the accumulation of considerable quantities of nitrogenous organic matter in the soil.

The nitrogen content of the superficial 8 to 12-inch layer of soils of humid regions varies from 0.025 per cent, in the case of the very poor sandy soils, to practically 4 per cent in the case of certain peat and forest soils, 90 per cent or more of which may consist of organic compounds. The nitrogen content of most field soils ranges from 0.05 to 0.5 per cent, with an average of about 0.1 to 0.2 per cent. Practically all of this nitrogen is found in the soil in complex organic forms, and only a fraction of 1 per cent of the total nitrogen of the soil is generally present in an inorganic form, either as ammonia, nitrite or nitrate. The nitre spots of certain arid irrigated soils are exceptions.

The explanation for the lack of any abundance of nitrogen in the form of inorganic salts is found in the following two facts: (1) Transformation of ammonia to nitrate occurs more rapidly under most soil conditions than the formation of ammonia; and (2) nitrates are so soluble that they do not persist long in the soil in humid regions, and are either absorbed by the root systems of plants or become washed out by percolating water. It is, therefore, obvious why it is necessary to provide some means for creating a continuous supply of nitrate during the period of active absorption by the growing plants. Nature has invested such a mechanism in the microbial inhabitants of the soil. Their action is generally supplemented or enhanced by cultural treatments of intensive agricultural practice.

Practically all of the nitrogen that is brought into the soil by natural agencies, as in the roots and stubble of cultivated and uncultivated plants, animal residues, and dead animals, is in the form of organic compounds, largely proteins and their derivatives.

The same is true of the forms of nitrogen in the stable manures and green manures that are added to the soil in varying quantities and, in the case of some soils, at quite frequent intervals. Various commercial organic fertilizers, such as guano, dried blood, cottonseed meal, and tankage, contain their nitrogen in the form of organic compounds. Most of the nitrogen added to the soil in artificial fertilizers is in the form of inorganic compounds, largely nitrates, ammonium compounds, and amides. However, even some of the synthetic fertilizers, such as cyanamid and urea, require the previous interaction of microorganisms before the nitrogen is made available to growing plants.

Transformation of Nitrogen in Soil.—As long as the nitrogen is in an organic form it remains to a large extent unavailable as far as the growth of higher green plants is concerned. The organic complexes must be mineralized or decomposed, and, after a series of transformations, the nitrogen finally becomes liberated as ammonia. Many plants can absorb their nitrogen in this form. Others require their nitrogen in the form of nitrite. The transformation of the ammonia first to nitrous acid (nitrite), then to nitric acid (nitrate), is carried out by specific bacteria, under favorable conditions, in the soil. The nitrate may be used by the plants; it may be assimilated by microorganisms in the presence of available energy; it may be reduced to nitrite, to gaseous oxides of nitrogen, to ammonia, and to atmospheric nitrogen; finally, the nitrate may be leached from the soil.

The various transformations to which nitrogen is exposed in soil are illustrated in Fig. 49.

Fixation of Atmospheric Nitrogen.—While practically all living organisms rely for their nitrogen upon organic compounds, like proteins and their derivatives, or upon inorganic compounds, such as nitrates or ammonium salts, there are certain microbes which are able to make use of elementary (molecular) nitrogen to supply the need of this element for their growth. Such an assimilation of nitrogen by organisms from the molecular state is termed biological nitrogen fixation.

The ability of utilizing gaseous atmospheric nitrogen and *fixing* it in their bodies in the form of complex organic and simple inorganic compounds is limited largely to two groups of bacteria. These include: (1) the non-symbiotic bacteria which lead a free existence in the soil and obtain their energy from various organic compounds found in the soil, preferably sugars, higher alcohols and organic acids; (2) the symbiotic nitrogen-fixing bacteria which live upon the roots of certain specific plants, namely, the Leguminosae, in specialized bodies called nodules, and cause the fixation of gaseous nitrogen, which becomes available to the plants during their growth. The nodule-forming bacteria are also capable of leading an independent existence in the soil, their ability to fix nitrogen under these conditions being still a matter of dispute. It seems likely, however, that while developing independently of the plant these symbiotic bacteria fix no appreciable quantities of nitrogen.

In addition to these two groups of

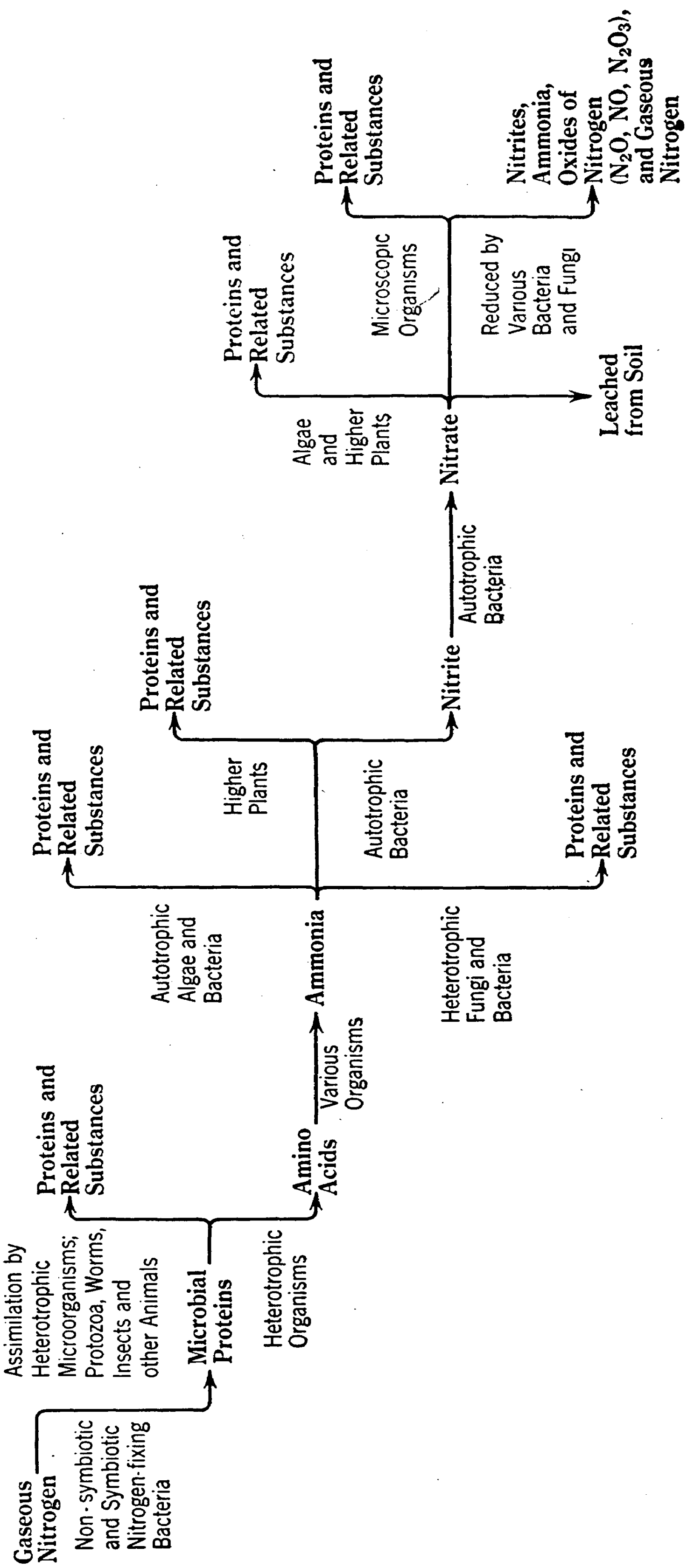


FIG. 49.—Schematic representation of transformations of nitrogen in soils.

bacteria, a number of other bacteria may be capable of fixing small quantities of nitrogen, especially when these microbes have been recently isolated from the soil. However, neither the amount of nitrogen fixed nor the specificity of their nature can place these organisms on an equivalent basis with the other two groups of bacteria. There are indications that certain highly specialized forms of fungi, such as species of Phoma, which cause the formation of endotrophic mycorrhiza, may fix some nitrogen. Certain of the blue-green algae, as species of Anabena

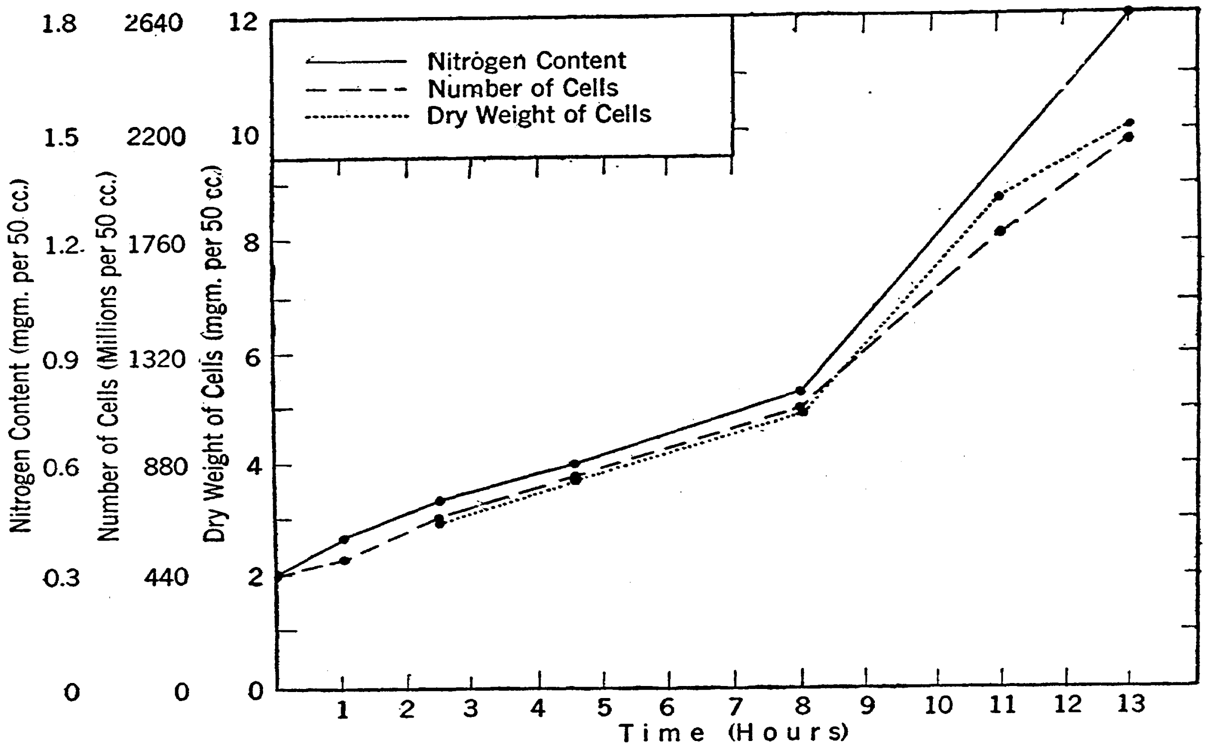


FIG. 50.—Correlation between fixation of nitrogen and growth of bacterial cells (after Meyerhof and Burk).

and Nostoc, may also be able to fix relatively large amounts of nitrogen under certain conditions, but the grass-green algae have no such capacity.

The fixation of nitrogen is thus a physiological inheritance of a comparatively small number of microorganisms with the principal representatives included among the bacteria.

NON-SYMBIOTIC NITROGEN-FIXING BACTERIA.—Those bacteria capable of fixing atmospheric nitrogen which lead a free existence in the soil are found distributed in two groups: (1) Azotobacter—large, coccus-like, non-motile, aerobic organisms. This group is represented in the soil by the following species: *Az. chroococcum, Az. agile, Az. beijerinckii, Az. vinelandii, Az. vitreum,* and *Az. woodstownii* (see Fig. 10). The first species, *Az. chroococcum,* occurs much more abundantly in soils than any of the other forms. (2) Clostridium—spore-forming, rod-shaped organisms, anaerobic in nature, that is, capable of existing in the complete absence of free oxygen; the nitrogen-fixing species of this organism are generally referred to as *Cl. pastorianum* (or *Bacillus amylobacter*), but it is likely that there is a large group of such forms embracing numerous closely related forms, most of which produce butyric acid when utilizing carbohydrate material.

For the fixation of nitrogen, these organisms require a source of energy, some available forms of phosphorus and potassium, sufficient calcium to keep the reaction from becoming acid, and smaller amounts of various other minerals. These nutrients with nitrogen from the atmosphere are used for the growth of the cells; as a result of growth there is an increase in the fixed forms of nitrogen in the environment where the cells develop. The amount of nitrogen fixed is closely related to the amount of energy available (as well as to the available phosphorus when the available energy is not a limiting factor). This is shown in Table 23. The amount of nitrogen fixed is very intimately associated with the extent of cell development, the fixed nitrogen being built up into cell constituents. Fixation of nitrogen is, therefore, an index of the growth of the cells and an increase in the cell substance (see Fig. 50). Cells may remain alive and decompose the available energy source without fixing nitrogen; such a condition may exist if the cells are merely respiring without increasing in numbers.

TABLE 23

Influence of Amount of Glucose on Fixation of Nitrogen by Azotobacter (from Hunter)

Glucose concentration, per cent	Nitrogen Fixed (mgm.)		
	2 Days	4 Days	6 Days
0.6	6.37	9.75	9.70
1.1	7.41	17.29	18.72
1.5	9.1	17.68	26.0

As shown by Fig. 51, the largest amount of nitrogen is fixed per unit of food material used during the early periods of cell development of a culture. This decreases rapidly as the culture ages. In the young culture most of the cells are using the food materials for growth and multiplication, and nitrogen fixation is proportionately efficient. In the later stages much of the energy is consumed merely to support respiration of many of the cells, and few cells are multiplying. Eventually a stage is reached where multi-

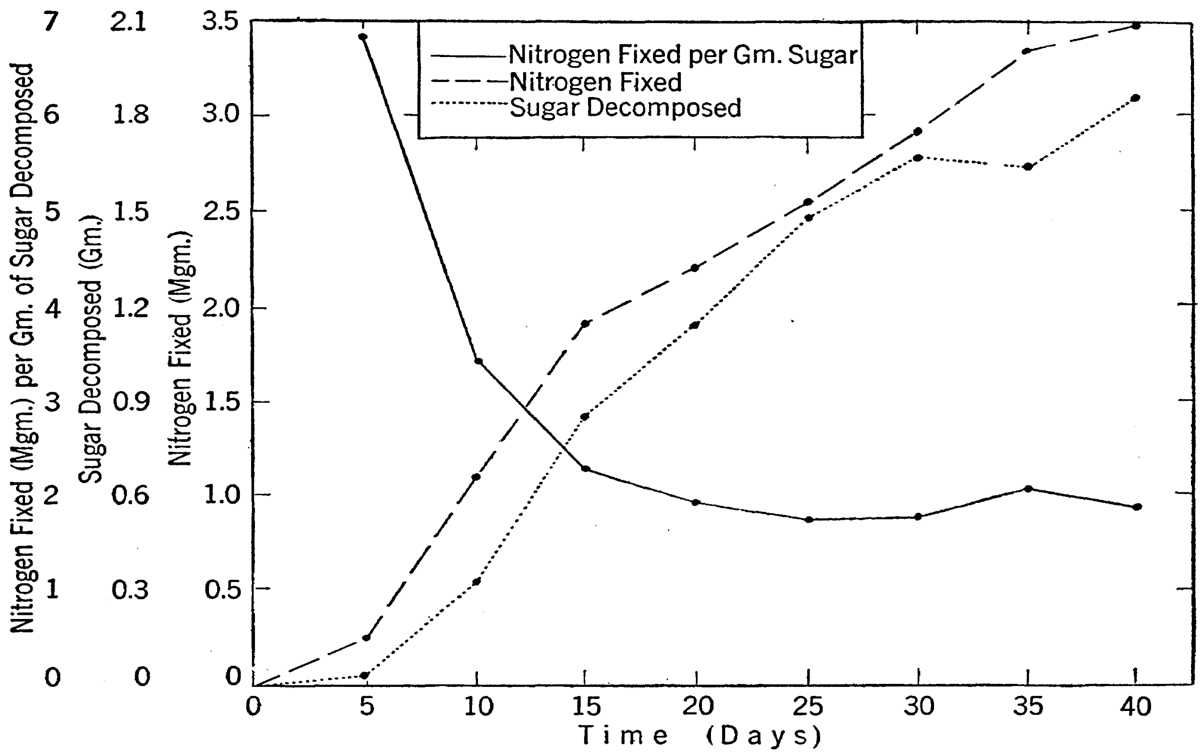


FIG. 51.—Relationship between carbohydrate decomposed and nitrogen fixed (after Omeliansky).

plication ceases entirely and no nitrogen is fixed, even though food is being consumed to keep alive the cells which already exist.

For similar reasons, the lower the initial concentration of the energy source in the absence of fixed forms of nitrogen, the greater are the amounts of nitrogen fixed per unit of energy material decomposed. This is apparent from Table 24.

Since under soil conditions there is generally a comparatively small amount of energy materials present at any one time, it is likely that a relatively large amount of nitrogen is fixed per unit of carbohydrate consumed during the process. In fact, where nitrogen fixation does occur in soils it appears to be a relatively economical process.

TABLE 24

INFLUENCE OF CONCENTRATION OF MANNITOL ON FIXATION OF NITROGEN BY AZOTOBACTER (FROM LIPMAN)

Concentration of mannitol, per cent	Nitrogen fixed per gram of mannitol decomposed, (mgm.)
0.1	10.5
0.2	8.3
0.5	6.4
1.0	4.68
1.5	3.22

The energy used by Azotobacter and Clostridium may be obtained from sugars (glucose, galactose, maltose, sucrose), higher alcohols (glycerol, mannitol), starches, dextrins, a number of organic acids, and certain hemicelluloses; the Clostridium uses the latter two sources only to a limited extent. Cellulose, lignin, and some of the hemicelluloses cannot be utilized by any of these nitrogen-fixing bacteria as sources of energy.

IMPORTANCE OF NON-SYMBIOTIC NITROGEN FIXATION.—In view of the fact that the natural organic materials added to the soil in the plant residues and in the various organic manures are largely made up of cellulose, lignin, proteins, and hemicelluloses, while the content of soluble carbohydrates is very limited, being not more than 2 to 10 per cent of the total organic matter added to the soil, the amount of energy available for the nitrogen-fixing bacteria is very small. The problem is complicated still further by two facts: (1) The abundant flora of other bacteria and fungi in the soil will also readily utilize the soluble carbohydrates, if available forms of nitrogen are present. (2) In the presence of available forms of fixed or combined nitrogen, such as organic nitrogenous substances, ammonium salts, or nitrates, even the nitrogen-fixing bacteria fail to fix nitrogen. This is not due to the inability of the bacteria to develop under such conditions, but rather to the utilization of the nitrogen which already exists in fixed forms to supply their requirements. Nitrogen is used from the atmospheric sources only in the absence of other available sources of supply (see Table 25). Growth of most higher plants in soils is dependent upon a rather extensive and continuous supply of nitrogen in such fixed forms as ammonium salts or nitrate. Since the presence of such nitrogen in soils would prevent fixation processes, it is likely that fixation does not occur very extensively in soils while plants are growing. This does not eliminate the possibilities of fixation in some localized regions of the soils or at certain periods when considerable amounts of plant residues may become introduced into soils.

TABLE 25

Influence of Nitrates on Transformations of Nitrogen by Azotobacter (from Bonazzi)

	Period of incubation, days	Nitrogen Present in Culture Media (mgm.)		
		Nitrate	Organic	Total
Control	5	8.66	0.47	9.13
Inoculated	5	1.12	6.53	7.65
Control	8	8.66	0.47	9.13
Inoculated	8	0.88	7.41	8.29
Control	13	8.66	0.47	9.13
Inoculated	13	0.72	8.59	9.31

Associated with the decomposition of 100 parts of available organic matter free from nitrogen there may be fixed about one part of nitrogen. Assuming that an acre of soil received a liberal application of plant residues, such as 2 tons of water-free material, the amount of carbohydrates available for the use of non-symbiotic nitrogen-fixing bacteria would not exceed 800 pounds. Assuming further that all this energy was used by the specific bacteria, only about 8 pounds of nitrogen would be fixed per acre per year if the aerobic organisms were active. Less would be fixed by the anaerobic organisms. Considering the fact that the 4,000 pounds of dry organic matter would contain 20 to 60 pounds of nitrogen, some of which will be liberated through the activities of the numerous soil fungi and bacteria, the evidence that any appreciable amounts of energy would be left for the use of nitrogen-fixing bacteria and that these would have to use the nitrogen from the atmosphere is not very conclusive. Under field conditions, however, some nitrogen may be added to soils through the agency of bacteria. Fixation has been noted even when animal manure was the organic substance with which the soil was treated. The soil conditions and the nature of the growing crop probably exert the determining influences in many cases. It has been stated that in some soils as much as 40 pounds of nitrogen become added to soils annually as a result of the activities of non-symbiotic nitrogen-fixing bacteria. Certain soils in regions of deficient rainfall show particularly striking increases in nitrogen.

Due to the fact that it is almost impossible to detect even appreciable increases in the nitrogen content of soils (such as 40 pounds per acre per year), the actual agricultural importance of the non-symbiotic organisms is practically unknown. With the numerous facts in mind, it may be assumed that, in most soils, the increase in the nitrogen content as a result of the activities of these nitrogen-fixing bacteria is rather limited.

There are two conditions not yet considered under which the activities of these organisms may tend to increase the store of combined nitrogen in the soil: (1) In the neighborhood of growing roots of plants there is an excretion of soluble carbohydrates and addition of other residues to the soil which may serve as food for the bacteria. Plants rapidly consume most of the available combined nitrogen from this portion of the soil. These two factors, namely, the presence of available sources of energy and a nitrogen minimum, would favor the rapid development of Azotobacter and Clostridium and lead to nitrogen fixation. (2) A similar relationship may exist between certain green algae and the nitrogen-fixing bacteria. The former *fix* the carbon from the carbon dioxide of the air (by photosynthetic agencies at the surface of the soil) and liberate some of the synthesized carbohydrates, which can be used as sources of energy by the nitrogen-fixing bacteria; the latter *fix* the nitrogen and liberate some of it in a combined form which can be used by the algae.

The reaction of the soil is one of the factors of major importance in determining the presence or absence of Azotobacter, as shown in Table 26. Under the great diversity of soil conditions, the reaction may limit the distribution of the organisms more frequently than any other condition. If the reaction is more acid than *p*H 6.0, Azotobacter is seldom present; when the soil is limed and the reaction is changed to *p*H 6.0 or above, Azotobacter will develop quite readily, when present or when introduced. The Clostridium types can resist a somewhat greater acidity than Azotobacter (such as *p*H 5.2) and occur under a greater variety of conditions and in considerably greater numbers.

TABLE 26

SOIL REACTION AND OCCURRENCE OF AZOTOBACTER (FROM GAINEY)

Reaction (*p*H)	Number of soils tested	Azotobacter	
		Present, per cent	Absent, per cent
Above 7.50	32	100	0
7.00 to 7.49	60	97	3
6.50 to 6.99	45	90	10
6.00 to 6.49	47	80	20
5.50 to 5.99	122	20	80
5.00 to 5.49	43	14	86
4.50 to 4.99	44	7	93
Below 4.50	3	0	100

Soil conditions which appear to be aerobic, that is, well-aerated soils with a good crumb structure and with optimum moisture content, may be a very favorable habitat for the anaerobes. The conditions within the aggregates of soil particles or close to decomposing portions of soil organic matter may be far from aerobic. Aerobic organisms may consume the oxygen about their cells during growth and create sufficiently anaerobic conditions to permit neighboring cells of Clostridium to develop. Such associative development of various organisms is probably more common in soils than development of single species of organisms entirely separated from one another. At least, one need not postulate the fixation of nitrogen by the anaerobic types only under bog conditions or in water-logged soils. In fact, the fixation of small amounts of nitrogen in soils by the group of non-symbiotic nitrogen-fixing bacteria may be as much the result of the action of the Clostridium as of the Azotobacter.

Large amounts of phosphorus are required by Azotobacter cells for their growth. Phosphorus is by far the most important of the mineral substances absorbed during growth of the cells. The amount of the element required by the bacteria is in direct proportion to the amount of nitrogen fixed. This is borne out by a simple analysis of the composition of the microbial cell which, in a washed condition (freed from adhering capsular material, largely hemicelluloses), contains about 10 per cent nitrogen and 5 per cent P_2O_5. It has been calculated that for every gram of glucose used by Azotobacter as a source of energy, 2.5 mgm. of phosphorus are required. This offers a good method for determining the amount of available phosphorus in the soil. For this purpose a definite amount of soil is added to a definite amount of culture solution, free from phosphorus, and the solution is then inoculated with Azotobacter; the amount of nitrogen fixed under these conditions is taken as an index of the quantity of available phosphorus in the given quantity of soil added to the solution. A very practical procedure for determining the available phosphorus in soil, based upon this phenomenon, is discussed in detail later.

Symbiotic Nitrogen Fixation.—It has been known since olden times that the growth of leguminous plants, such as clover, alfalfa, peas, or beans, leaves the soil richer for the succeeding crops. The reason for this phenomenon was not known, but the practical farmer recognized that the growth of such a crop was equivalent to the application of manure as far as the succeeding crop was concerned. It was only toward the middle of the nineteenth century that it became an established fact that this beneficial effect of a leguminous crop is due to an increase in the supply of soil nitrogen; the leguminous plants were found capable of utilizing the nitrogen gas of the atmosphere, changing it thereby into combined forms in some mysterious fashion. It was soon established that when the soil in which the legume is grown is previously heated or sterilized, the leguminous plant behaves like a cereal plant and is unable to use the gaseous nitrogen. When a little fresh soil is added to the previously sterilized soil, the legume is once more able to draw upon the store of gaseous nitrogen (Fig. 52). After a series of investigations, to which botanists, bacteriologists, agronomists, and chemists have contributed, it became definitely established toward the end of the last century that a minute bacterium is responsible for this process. The bacterium in question was finally (1888) isolated in pure culture by the Dutch bacteriologist Beijerinck, and was named *Bacillus radicicola.* This organism provokes the development of certain growths,

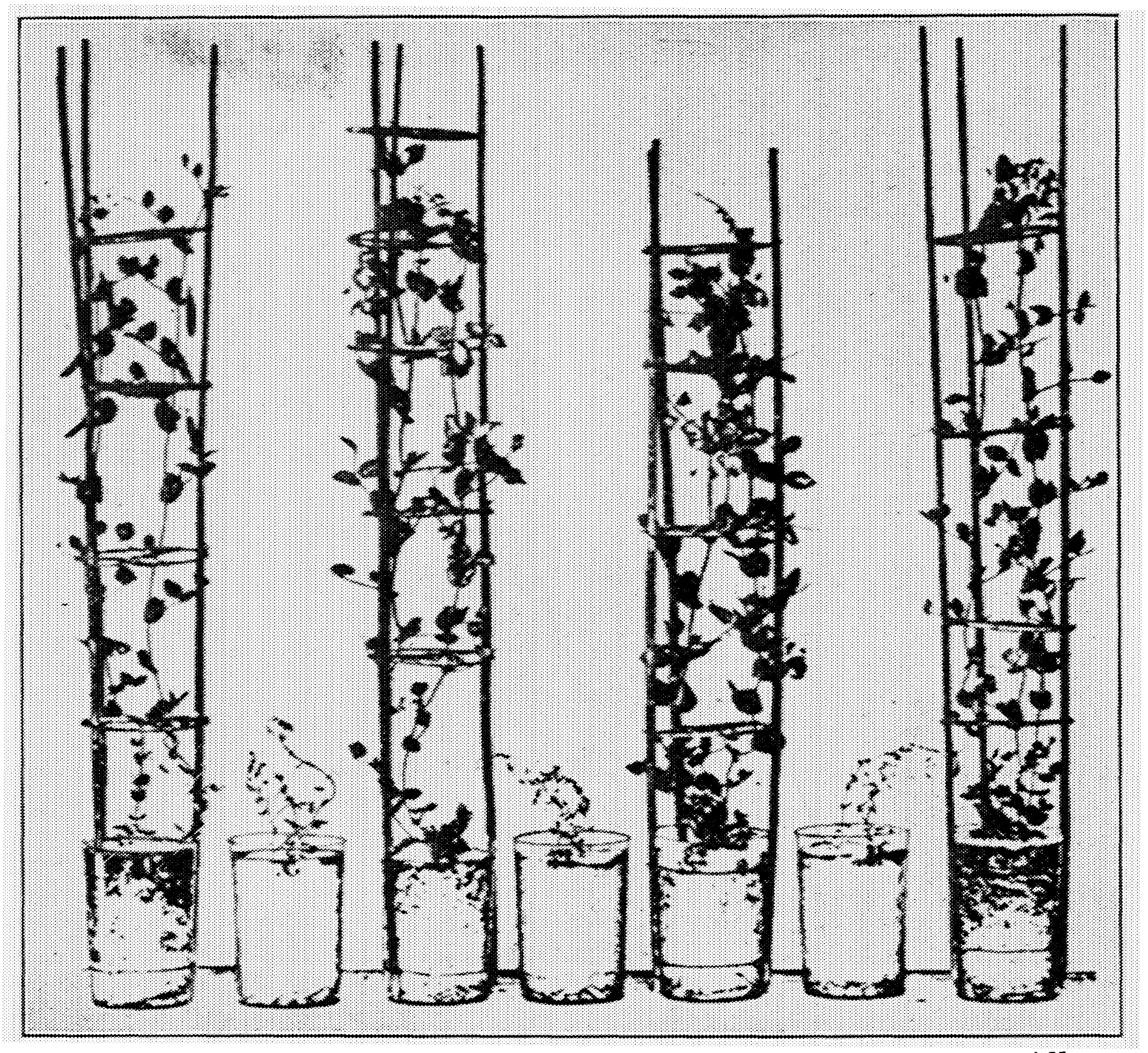


FIG. 52.—Influence of the legume organisms on growth of peas. All are growing in sterilized sand, but in the four pots placed alternately, which show good growth, extract of garden soil has been added (from Hellriegel and Wilfarth, after Russell).

called nodules, on the roots, and in these newly formed biological laboratories causes the fixation of nitrogen to take place.

Since the organisms are not contained within the seeds of the legumes they must enter the plant during its growth in order to develop their effects. Infection takes place through the root hairs, and the organisms appear to become attracted to these roots by some substance which is excreted by the young roots. The infecting organisms are probably coccoid cells, either non-motile (preswarmers) or motile (swarmers). Once within the tissues the bacteria multiply rapidly at the expense of the food material of the plant. They occur at this stage as short rods enclosed in a mass of mucoid material. The mass of bacterial growth continues

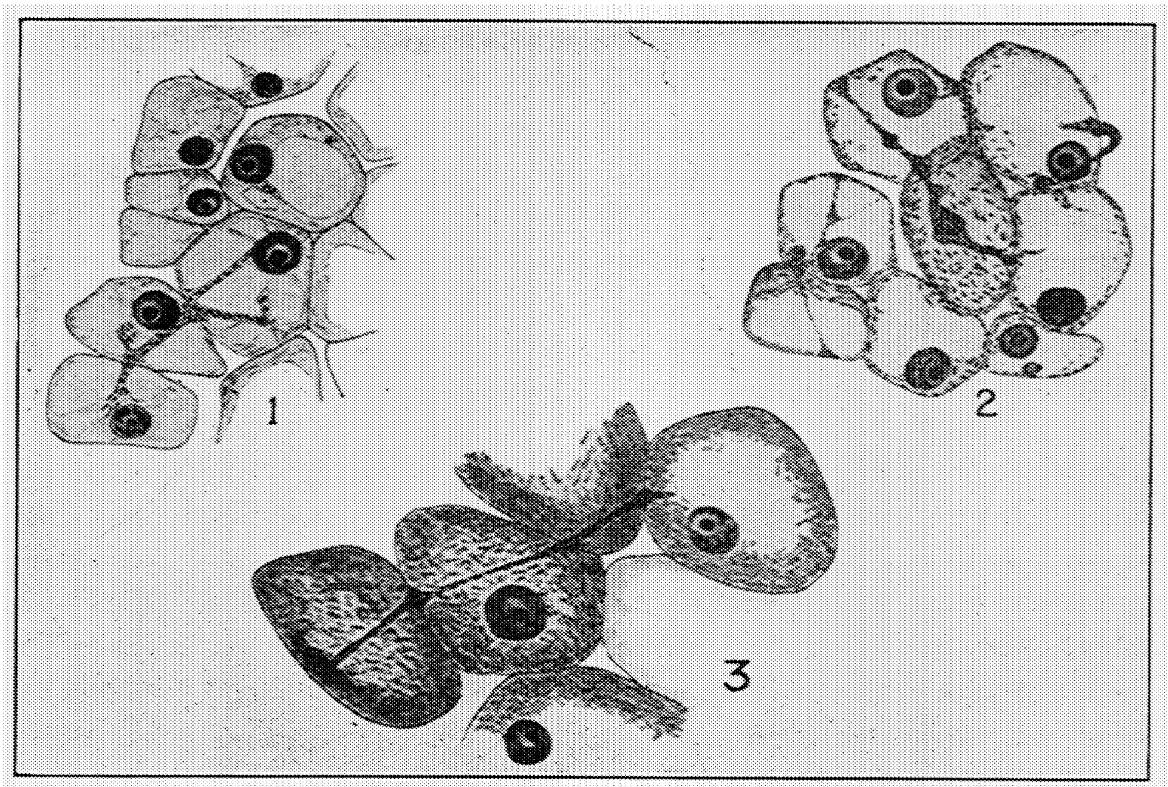


FIG. 53.—Infection of root cells of a legume by the legume bacterium: (1) cells from the young nodule tissue showing small rod-shaped cells in the infecting strands; (2) cells from older nodule tissue; many bacteria have separated from the infecting strand and are still existing as thin rods; (3) cells from quite old parts of the nodule tissue; the cells have become filled with bacteria in the swollen "bacteroid" condition (from Brenchley and Thornton).

to pass through cells of the cortex of the root in the form of a filament with many branches, called the infection thread or strand. The penetration of the microorganisms results in growth and multiplication of the inner cortical cells, which push outward to form the young nodule. These cells, in turn, become invaded by the infection threads (see 1, Fig. 53). As the nodule develops, vascular tissue which conducts nutrient materials in the plant grows outward into the nodule and favors active division of the plant cells and growth in the terminal region. The plant cells at the base of the young nodule swell and become vacuolated and occupied with bacteria freed from the infecting strand (see 2, Fig. 53). These bacteria appear as thin rod forms. As the nodule ages, the bacterial cells in these older plant cells become swollen with protoplasmic constituents concentrated unevenly; these are known as the bacteroids (see 3, Fig. 53).

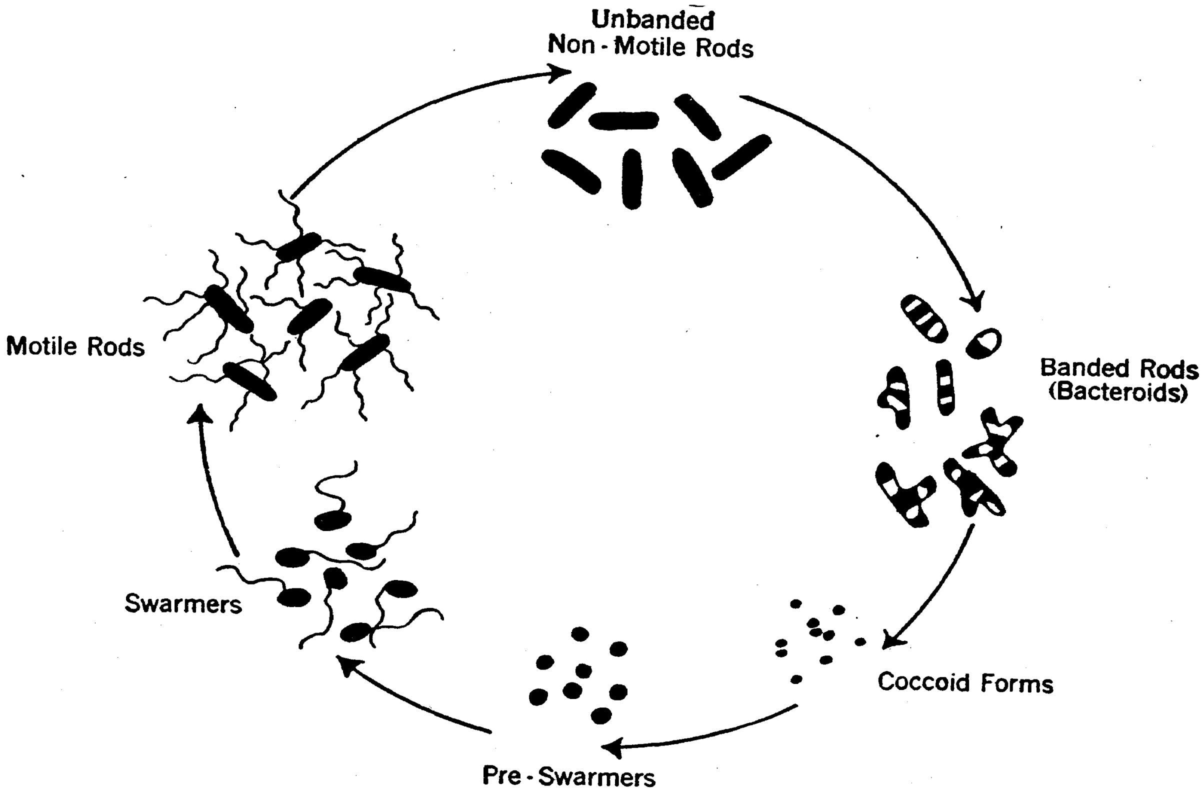


FIG. 54.—The life cycle of *Bacillus radicicola* (after Thornton and Gangulee).

The changes in appearance of the bacterial cells appear to be closely related to the food supply in the nodule. Where food materials, presumably carbohydrates, are supplied in abundance, the bacteria retain the appearance of rod-shaped cells which take stains uniformly. As the food supply decreases, in the older regions of the nodule, the bacteria become banded rods and finally bacteroids. These morphologic changes are characteristic of bacteria passing from stages of youth to old age and senescence (see Fig. 54). Thus, if a culture of a legume organism is introduced into a certain culture medium, it soon appears in the " preswarmer " or non-motile coccoid stage. The cells increase in size until the diameter is about doubled, but they still remain non-motile. These cells become ellipsoidal and develop high motility in which stage they are known as "swarmers." The swarmers elongate to form the rod-shaped cells which remain motile for a time, but gradually become non-motile as the food becomes consumed. When the food material becomes exhausted or other such unfavorable conditions develop, the rods appear as banded cells with the protoplasmic contents concentrated in several locations. Eventually many of these cells become branched, swollen, or otherwise changed from the rod shapes into the characteristic bacteroids. The bands of cell material may give rise to small cocci once more when placed under suitable conditions of adequate food supply.

This relationship between the nodule organism and the host plant has been called symbiosis, since presumably both organisms benefit from the association. The plant produces carbohydrates through its photosynthetic activities and supplies such food and other nutrients to the bacteria as enable them to develop extensively. The bacteria, through some agency, enable the plant to derive nitrogen for its development through the transformation of gaseous nitrogen to fixed compounds. Under certain conditions, however, the relationship may not be one of symbiosis but of actual parasitism. If the plant is unable to make normal growth on account of a lack of certain essential nutrients, providing that the bacteria enter the roots, the plant tissue may not react to develop a normal nodule, but will initiate development of nodular tissue which soon becomes extensively invaded by the bacteria, completely destroying the tissue.

The mechanism of the fixation of nitrogen is little understood. The bacteria related to the process appear to be quite unable to fix nitrogen while growing independently of the host plants. Likewise the legume fails to fix nitrogen while growing free from the bacterium. It is only in the associative development that appreciable fixation of the nitrogen occurs, and then only when the vascular system of the root develops into the nodule and furnishes carbohydrates for the growth of bacterial and plant cells; that system must also carry away the products of cell development, including presumably nitrogenous compounds resulting from the fixation. Whether the bacteria fix the nitrogen under the conditions existing in the nodule, or whether the plant fixes the nitrogen in the presence of the bacteria, or whether both share equally in the reaction, is speculative. There are thus many points of

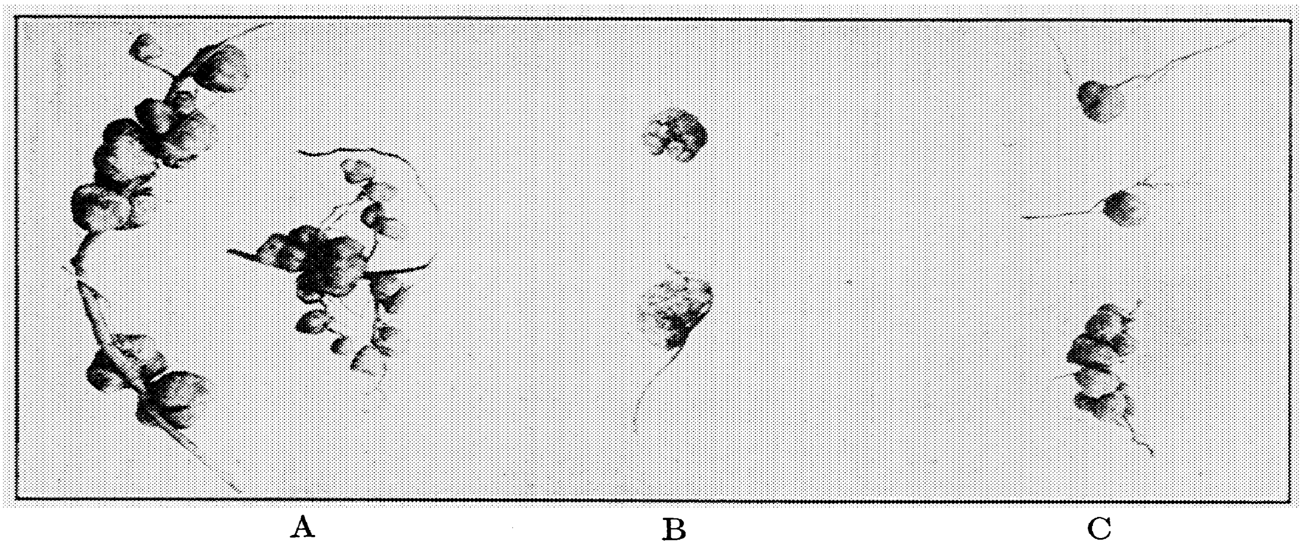


FIG. 55.—Nodules of cowpeas (A), soybeans (B), and lima beans (C) (from Löhnis and Leonard).

difference between fixation by symbiotic and non-symbiotic bacteria, but the actual chemical reactions involved may not be unlike.

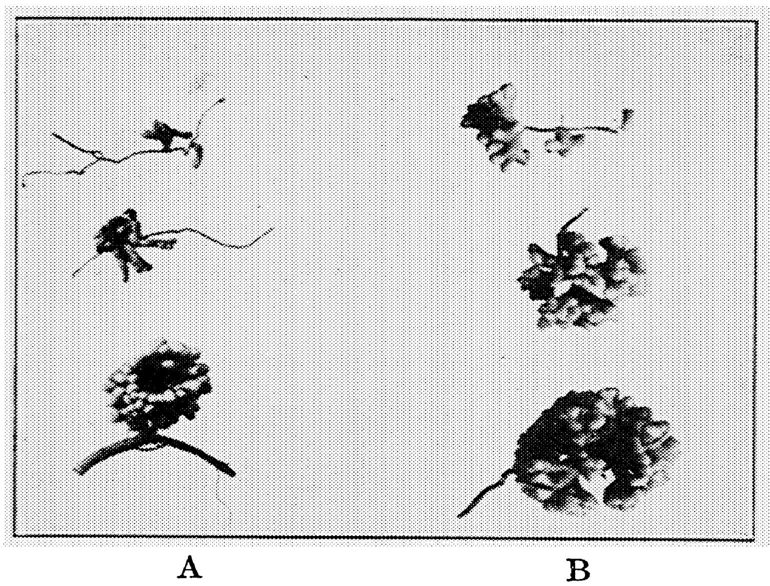


FIG. 56.—Nodules of alfalfa (A) and vetch (B) (from Löhnis and Leonard).

The nodules produced on roots of different legumes may be quite different in appearance, but the general shape is quite constant in any one species of plant. On some of the beans the nodules are fairly large and spherical, and seldom occur in clusters (Fig. 55). Nodules on many of the clovers and alfalfa are small elongated swellings, sometimes branching or occurring in clusters (Figs. 56 and 57). The nature of the plant and the soil conditions affect the location of the nodules. Under conditions particularly favorable for inoculation, the nodules may appear in abundance close to the main root near the surface of the soil. Where the moisture content of the soil is low, as in the semi-arid regions, nodulation may be prominent at a considerable distance from the surface of the soil. The abundance and size of the nodules are good indexes of the extent of fixation of nitrogen. Where the nodules are large and numerous, nitrogen fixation proceeds rapidly; where the nodules are smaller and more scattered, fixation is much slower.

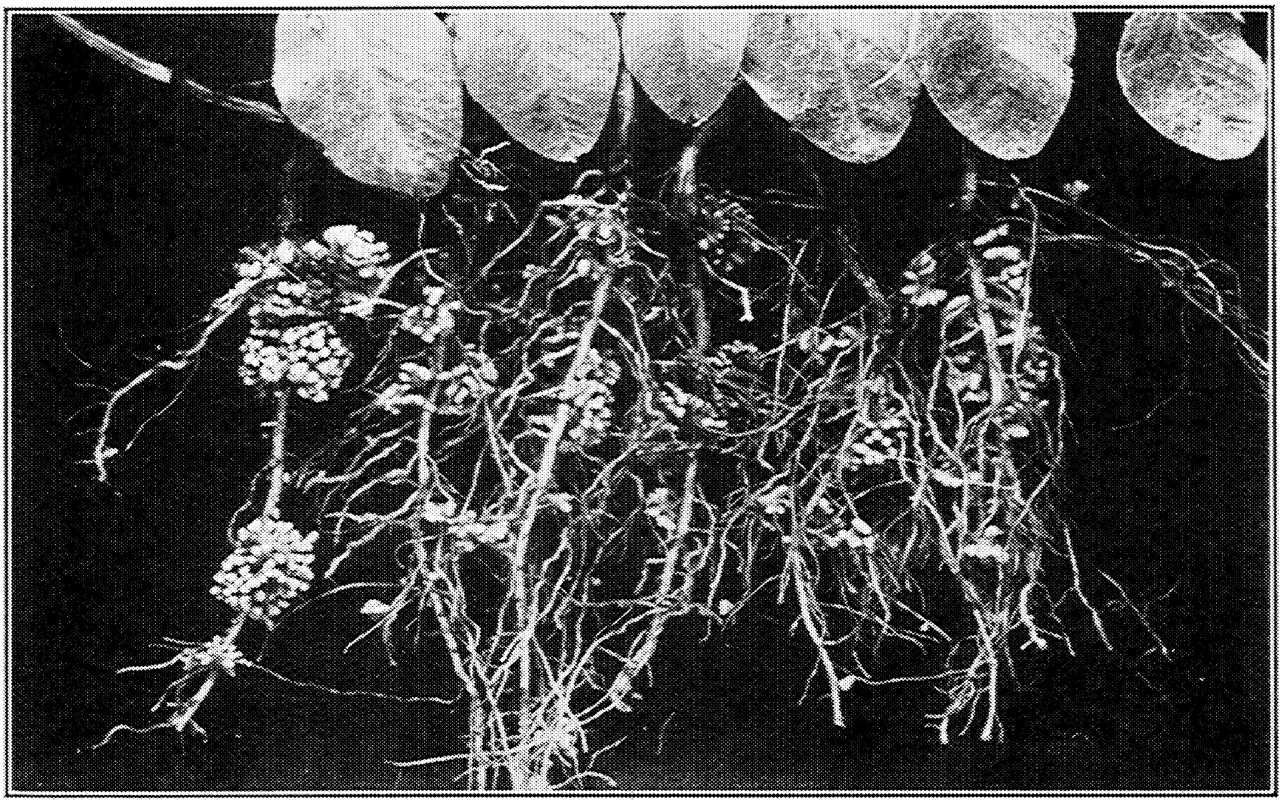


FIG. 57.—Nodulation on roots of peas (from Wilson and Leland).

NATURE OF THE BACTERIA AND CLASSIFICATION OF LEGUMINOUS PLANTS.—The bacteria can be readily isolated from the nodules, and grown upon artificial culture media and studied carefully. It has been found that not all leguminous plants can be equally infected by the same microbe. In fact, the bacteria infecting legumes include a number of different species or strains, each of which can inoculate only one or more leguminous plants. At least twelve species or strains of the legume bacteria have been established, each of which will inoculate legumes belonging to only one of the groups listed below. Consequently the number of groups is the same as the number of different cultures of nodule bacteria required to inoculate these legumes.

(1) Alfalfa, white sweet clover, yellow sweet clover, hubam, bur clover, yellow trefoil, and fenugreek.
(2) Red, white, crimson, alsike, bersem, and cow clovers.
(3) Garden, canning, and field peas; hairy, spring, and wild vetches; broad bean; lentil; sweet pea; and perennial pea.
(4) Cowpea, peanut, Japan clover, velvet bean, lima bean, partridge pea, wild indigo, acacia, dyer's greenwood, and tick trefoil.
(5) Garden, field, navy, kidney, wax, and scarlet runner beans.
(6) Lupines and serradella.
(7) Soybean.
(8) Hog peanut.
(9) Lead plant.
(10) Trailing wild bean.
(11) Black or common locust.
(12) Wood's clover.

These varietal differences between the bacteria capable of forming nodules upon the roots of various plants can be demonstrated by direct inoculation upon the plants, and also by cultural and serological tests. Morphologically the organisms belonging to the different races are quite alike (except for some differences in the formation of flagella), but physiologically they are quite different.

Influence of Nitrate upon Nitrogen Fixation.—The amount of nitrogen fixed by inoculated legumes is influenced to a considerable extent by the amounts of nitrate in the soil on which the plants are growing. Nitrate-nitrogen is readily absorbed by legumes, and when present in abundance in the soil may supply the entire nitrogen requirements of the plants. Since absorption of nitrate dominates fixation of nitrogen, it is only when the nitrate available to the plants is less than the nitrogen requirements of the plants that appreciable fixation occurs. This is apparent from Figs. 58 and 59. The amounts of nitrogen in the plants are not very different irrespective of the amounts of nitrate supplied to them. However, the amounts of nitrogen fixed are inversely proportional to the amounts of nitrate available.

The presence of some nitrate in the soil appears greatly to benefit growth of certain leguminous plants and fixation of nitrogen. This is particularly the case with legumes, such as alfalfa and the clovers, which produce tiny seeds. The reserve nitrogenous food material in the seeds is insufficient to enable the plants to develop

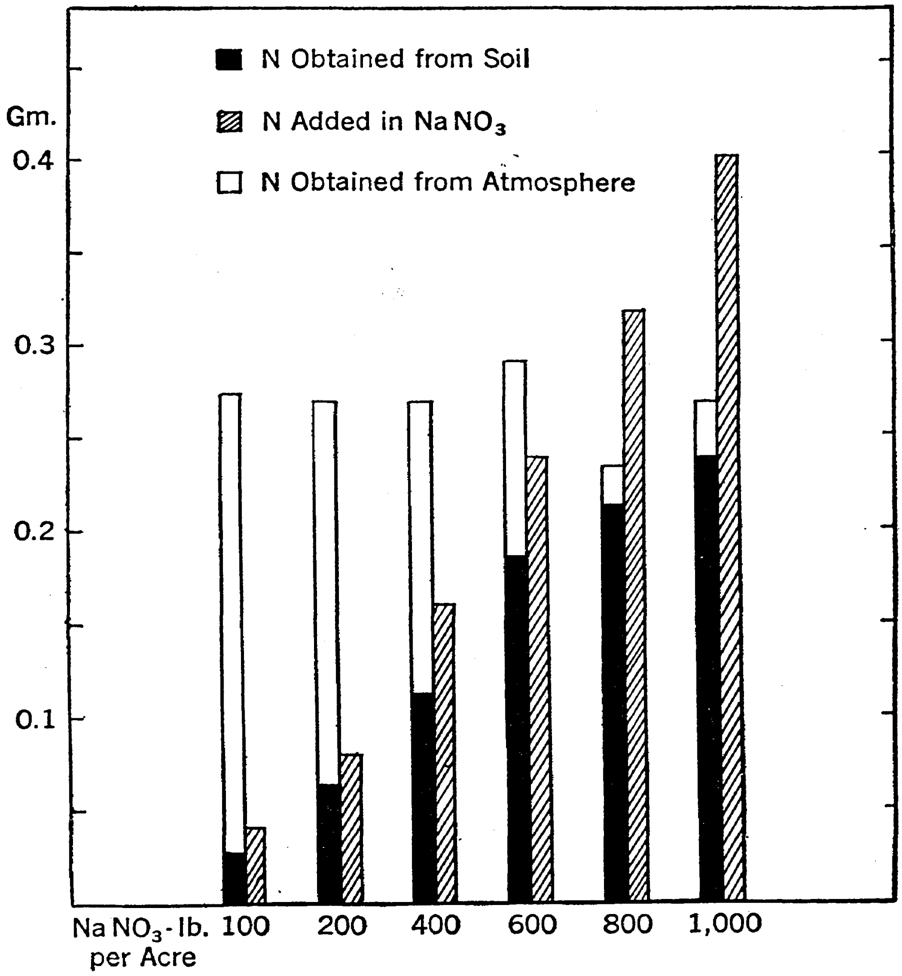


FIG. 58.—Relation between soil and atmospheric nitrogen obtained by a crop of inoculated alfalfa growing on soil variously treated with sodium nitrate (from Giobel).

substantial root systems without drawing upon some nitrogen from the soil. It is during this stage that nitrates greatly favor the plants. After proper root development has taken place, inoculated plants finding no combined available nitrogen in the soil develop as well as those which are supplied with an abundance of nitrate. Legumes having larger seeds, as beans and peas, are not appreciably affected by the presence or absence of available forms of soil nitrogen even in the early stages of development, if they are properly inoculated, since the reserve nitrogenous food materials in the seeds is generally sufficient to enable the plants to become established. Subsequent to this initial development, the degree of inoculation largely regulates the extent to which the legumes may draw upon the atmospheric nitrogen for their growth.

The explanation for the depressing effects of nitrate on nitrogen fixation by legumes is not clear, but certain suggestive relationships exist. Nitrate does not prevent penetration of the root hairs by

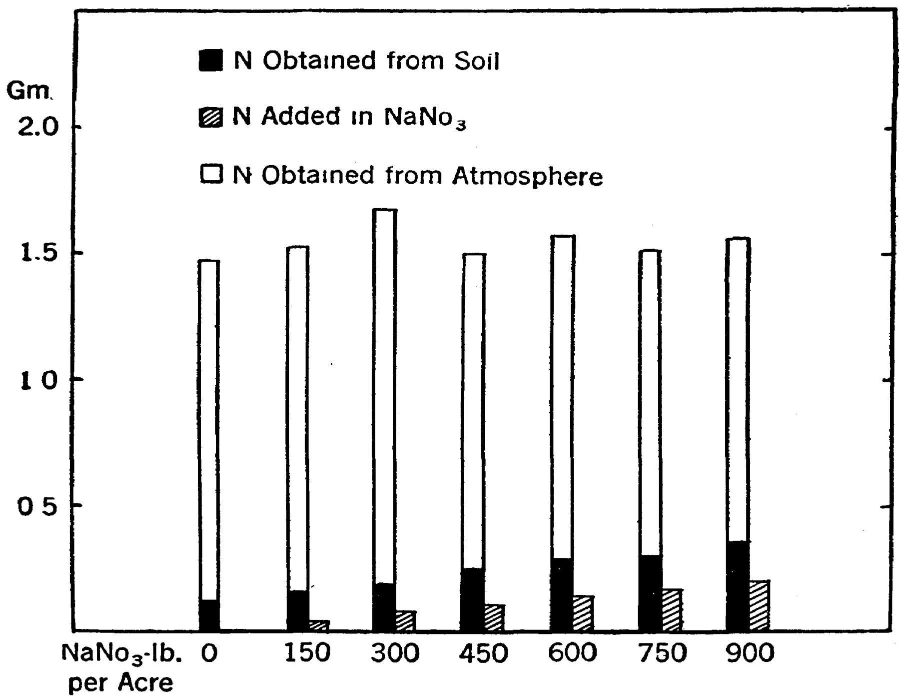


FIG. 59.—Relation between soil and atmospheric nitrogen obtained by soybeans growing on soil variously treated with sodium nitrate (from Giobel).

the bacteria but, if very abundant in soils, may inhibit nodule formation. The bacteria are not themselves injured by the nitrate, but certain physiological responses of the plants to nitrate depress nodulation and subsequent fixation of nitrogen. Where nitrates are abundant in soils the plant juices also contain appreciable amounts of nitrate or other forms of nitrogen which are readily assimilated. Such a condition leads to rapid transformation of the carbohydrates in the plant juices to plant tissues, decreasing the concentration of carbohydrates to a low level. Since the carbohydrates are probably the principal food of the nodule bacteria, the decreased concentration of the food may be

TABLE 27

Amounts of Nitrogen Fixed by Various Legumes, in Pounds per Acre (from Giobel)

Location	Alfalfa	Clover	Crimson clover	Vetch	Peas	Soybeans	Velvet beans	Beans	Cowpeas	Serradella	Lupines	Mixtures
Canada	....	50–175										
Wisconsin	....	....	....	....	....	57						
Minnesota	118	70–133	....	....	....	106	....	....	86			
Illinois	160	106										
Connecticut	....	197	....	180	....	....	....	205	....	....	97–150	
New Jersey	150–175	....	200									
Delaware	....	....	139–188									
Alabama	....	....	139	79	....	....	213					
Louisiana	....	....	....	....	....	....	172					
Florida	....	....	....	....	....	....	141					
United States in general	....	....	....	....	....	....	....	....	....	....	....	122
Germany	....	....	....	....	....	....	....	288	....	....	120	
Germany	207	114	....	....	....	....	....	....	....	....	....	225
Germany	....	....	....	163	72	....	....	142–210	....	122–222	117–243	85–202
Germany	....	....	....	....	200	....	....	....	....	....	128–171	166
Germany	....	100–180	....	....	....	....	....	....	....	206	203	108–180

responsible for the very limited development of the organisms once they have entered the plant. The final result is a lack of nodule development and consequently no fixation of nitrogen within the plant.

AMOUNTS OF NITROGEN FIXED.—Since legumes absorb large amounts of nitrogen from the soil, if the nitrogen exists in available forms, it is not possible to state without qualifications how much nitrogen will be fixed through the use of legumes in agricultural practice. Under conditions where the legumes make good growth and the nitrogen supplied by the soil is small, there are usually between 100 and 200 pounds of nitrogen fixed per acre (Table 27). Even larger amounts of nitrogen may be fixed under particularly favorable conditions. However, when the crop is removed for hay or similar purposes, there may be practically no increase in the combined nitrogen of the soil, since most of the nitrogen of the plant (often as much as 75 per cent) is in the stems, leaves, and seeds, and only a small amount in the roots. The nitrogen contained in the roots may represent no more than such nitrogen as has been absorbed from the soil during growth. Where a large portion of the plants is not removed but becomes incorporated with the soil, there may be great increases in the combined soil nitrogen.

INFLUENCE OF SOIL CONDITIONS UPON NITROGEN FIXATION.—Although no other single factor appears to exert such a pronounced influence on the degree of nitrogen fixation as the nitrate content of soils, there are numerous other factors which influence the process. Among these may be mentioned, moisture, soil reaction, temperature, soil texture, and addition of fertilizer salts. In general, with the exception of the influence of nitrogen compounds, it may be stated that there is a close correlation between the effects of these factors on nodule formation and on the growth of the legumes. Those conditions most favorable to vigorous development of the host plants favor nodulation and nitrogen fixation. Conditions which are unfavorable to the plants lower nodulation and fixation of nitrogen.

With most legumes, growth is best at reactions close to neutrality, and nodulation is greatest under such conditions. Some legumes are quite acid tolerant, and good nodule development occurs in acid soils. In general, the degree of acidity which limits development of the bacteria is also one that is known to be injurious to the host plant (Table 28). Of the various fertilizing materials, phosphates and calcium exert the most pronounced influences on the legumes, although any element essential to plant growth which is present in insufficient amounts lowers nodulation.

TABLE 28

CRITICAL REACTION (pH) FOR THE BACTERIA OF SOME LEGUMES (FROM FRED)

Alfalfa and sweet clover	4.9
Peas and vetches	4.7
Clovers and beans	4.2
Soybeans	4.0
Lupine	3.2

Since nitrogen fixation is dependent upon the presence and activity of specific bacteria, it is essential that the organisms be present in soils in order that the plants may make use of atmospheric nitrogen. Where a legume has never been grown in a certain soil it is likely that the specific bacterium is not present, at least in sufficient numbers to thoroughly inoculate the plants. Consequently, the seeds or the soils in which the seeds are planted are frequently inoculated with the bacteria at the time of planting. Once the legume has been well nodulated during growth, the bacteria persist in the soil for a number of years, provided that the soil reaction is not too acid. In the absence of the host plant, the organism will survive longer in a neutral or slightly acid soil than in a more acid soil.

In addition to the leguminous plants, several non-legumes are also capable of forming nodules on their roots. These include such plants as the alder, sweet gale, and red root. The microbe responsible for the formation of these nodules was found to belong, in most cases, to the *Bac. radicicola* group, namely, to the same group which includes the organisms that cause nodule formation on the roots of legumes. However, it is still unknown whether or not nitrogen becomes fixed in these non-leguminous plants.

Some tropical plants, such as Pavetta and Ardisia, form nodules either on the upper or on the lower part of their leaves. Bacteria were found to be the responsible agents. These bacteria are aerobic, rod-shaped cells, developing abundantly in the intracellular spaces of the plants and said to be present even in the embryo sacs of the seed. With the germination of the seed, they develop through the plants, so that there is unbroken continuity of the association throughout the life cycle of the plants. In these plants, nitrogen fixation appears to take place, similar to that in the legumes, and advantage is taken of this fixation in India by using such plants as green manuring crops.

DECOMPOSITION OF PROTEINS BY MICROORGANISMS.—Whether the nitrogen is fixed in the soil through the agencies of nitrogen-fixing bacteria, whether it is introduced there in the tissues of plants, animals, and microorganisms in the numerous organic residues, or whether it is added to the soil in the form of organic fertilizers—it is not directly available to the growth of higher plants. This nitrogen occurs to a large extent in the form of proteins and their derivatives, and to a less extent in the form of other complex organic nitrogenous compounds. Before this nitrogen can be utilized for the growth of green plants, these complex organic substances have to be decomposed and the nitrogen changed to other forms. This process of liberation of nitrogen is carried on in the soil by numerous fungi, actinomyces, bacteria, and invertebrate animals, and is one of the most important activities of the microbes in the soil. It is also one of the processes common to a large portion of the microorganisms inhabiting the soil.

Proteins, the most important group of nitrogenous compounds in the living organism, are made up of chains of certain units or building stones, namely, the amino acids. So far, between 20 and 30 different amino acids have been isolated from the plant and animal organism and described. These amino acids can be combined in various ways, giving a number of complex bodies, which are typical of the proteins and their derivatives found in the living and dead animals, plants, and microbes. Living processes involve the building up of new tissue substance or cell material, of which the proteins are vital constituents; among the processes associated with the decomposition of the dead bodies of plants and animals is the decomposition of these proteins into simpler compounds.

Some of the amino acids are comparatively simple in composition; others are more complex, as shown by some typical formulae:

Glycocoll or glycine. $CH_2(NH_2) \cdot COOH$
Aspartic acid...... $CH_2 \cdot COOH \cdot CH(NH_2) \cdot COOH$
Arginine.......... $(NH)C(NH_2) \cdot NH \cdot (CH_2)_3 \cdot CH(NH_2) \cdot COOH$
Tyrosine.......... $C_6H_4(OH) \cdot CH_2 \cdot CH(NH_2) \cdot COOH$

These various amino acids combine to form simple and complex peptides. The greater the number of amino acids which are combined together, the more nearly does the complex approach the characteristics of pure proteins. The protein molecule itself is built up in a similar manner out of a large number of amino acids, and has the following general formula, where R may be any of a large number of radicals:

$$NH_2 \cdot CHR \cdot CO \cdot (NH \cdot CHR \cdot CO) \cdot NH \cdot CHR \cdot COOH$$

When a protein is treated with hot alkaline or acid solutions or with certain proteolytic enzymes, it is hydrolyzed to the individual amino acids of which it is constituted. This may be illustrated by the hydrolysis of a dipeptide as follows:

$$\begin{gathered} NH_2 \cdot CH_2 \cdot CO \cdot NH \cdot CH_2 \cdot COOH \\ + \\ H_2O \\ \downarrow \\ NH_2 \cdot CH_2 \cdot COOH + NH_2 \cdot CH_2 \cdot COOH \end{gathered}$$

The amino acids themselves are readily used by most of the soil microbes as sources of energy, while the nitrogen is liberated as ammonia. In the process of decomposition of amino acids, carbon dioxide, ammonia, organic acids, alcohols, and other compounds are formed. In addition to these substances, amino acids may also give rise, under anaerobic conditions, to amines, hydrogen sulfide, mercaptans, and a considerable variety of incompletely oxidized substances. Some amino acids, such as tyrosine, which contains a benzene group in the molecule, give rise to such compounds as cresol and phenol as intermediary products of decomposition. Although some of the amino acids are decomposed very readily, others are resistant to decomposition.

The nitrogen of the amino acid molecule finally appears as ammonia. A certain part of the nitrogen may be reassimilated by the organisms, either as the amino acid directly or in the form of ammonia, and changed into the protein constituents of the microbial cells. Some of the nitrogen may thus be removed again from circulation and a part of it may become quite resistant to decomposition.

The liberation of ammonia from the amino acid molecule takes place according to one of the following reactions:

$$\underset{\text{Alanine}}{CH_3\cdot CH(NH_2)\cdot COOH} + H_2O = \underset{\text{Ethyl alcohol}}{CH_3\cdot CH_2\cdot OH} + CO_2 + NH_3$$

$$\underset{\text{Amino acid}}{\begin{array}{c}R\\|\\H\text{—}C\text{—}NH_2\\|\\COOH\end{array}} + H_2O \rightarrow NH_3 + \underset{\text{Hydroxy-acid}}{\begin{array}{c}R\\|\\H\text{—}C\text{—}OH\\|\\COOH\end{array}} \rightarrow \underset{\text{Alcohol}}{\begin{array}{c}R\\|\\CH_2OH\end{array}} + CO_2$$

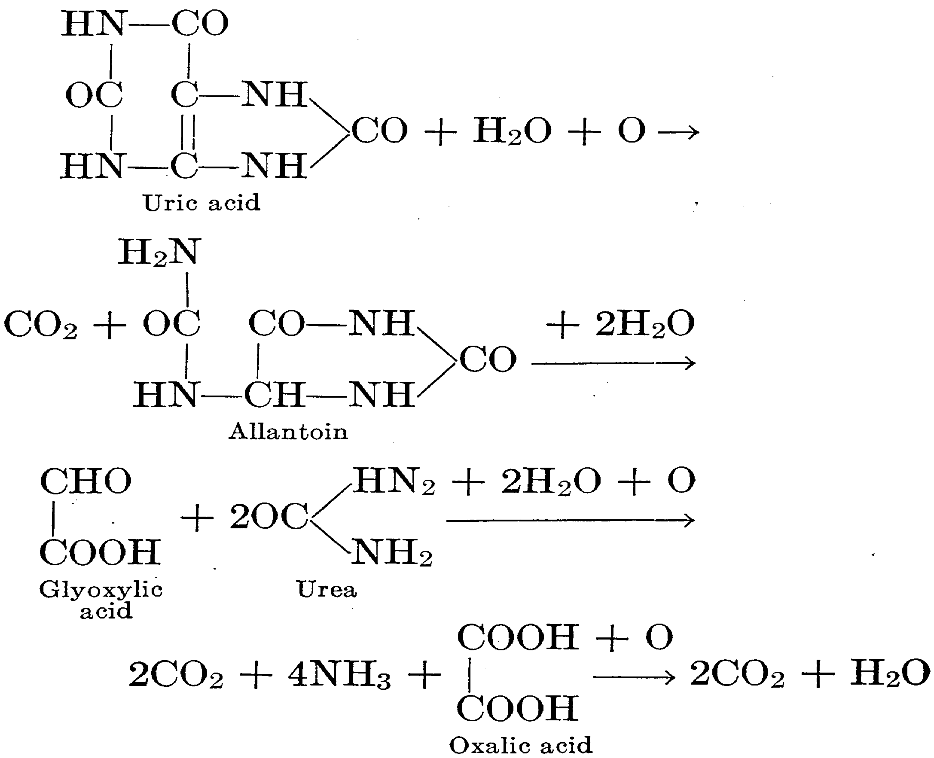
$$\underset{\text{Glutamic acid}}{HOOC\cdot CH_2\cdot CH_2\cdot CH(NH_2)\cdot COOH} + H_2$$
$$= \underset{\text{Butyric acid}}{HOOC\cdot CH_2\cdot CH_2\cdot CH_3} + CO_2 + NH_3$$

The ammonia thus produced may accumulate in the soil, it may be used by higher plants or by various microbes (in the presence of available sources of energy), or it may be changed to nitrate. In the light of what has been stated previously regarding the decomposition of such compounds as carbohydrates and the rôle of nitrogen in decomposition of plant materials, it is relatively easy to determine whether or not ammonia will be liberated in the decomposition of protein materials and about how much ammonia will appear in the complete decomposition of proteins by any one type of microorganism. The amount of nitrogen liberated as ammonia in protein decomposition may be represented as follows:

$$\begin{array}{l}\text{N of}\\ \text{protein de-}\\ \text{composed}\end{array} - \left(\begin{array}{l}\text{N assimilated by}\\ \text{microorganisms}\\ \text{in growth}\end{array} + \begin{array}{l}\text{N left as}\\ \text{intermediary}\\ \text{products}\end{array}\right) = \text{N as } NH_3$$

Since pure proteins contain 15 to 18 per cent of nitrogen, ammonia is formed in particularly large amounts in the decomposition of organic compounds rich in proteins. Ammonia is a waste product of the metabolism of microorganisms, and is of no further use to the cells concerned in its formation; it accumulates in the absence of organisms able to oxidize it. In view of the fact, however, that organic substances added to the soil do not consist of pure proteins and frequently the protein part is very small in comparison with the carbohydrates and other non-nitrogenous constituents (as in the case of rye or wheat straw which may contain only 2 per cent protein and 98 per cent of other non-nitrogenous organic substances), the liberation of the nitrogen in the form of ammonia must always be considered in the light of the composition of the organic matter as a whole.

DECOMPOSITION OF NITROGENOUS SUBSTANCES OF A NON-PROTEIN NATURE.—In addition to proteins and amino acids, the plant, animal, and microbial residues commonly added to the soil contain various other nitrogenous complexes, although in somewhat more limited amounts. These substances include nucleic acids, alkaloids, purine bases, phosphatides, various amines, glucose-amines and amid. Some of these and numerous other complexes are actually produced in the soil by the various microbes in the processes of synthesis of new cell substances. Some of these complexes decompose readily; some more slowly. The decomposition of uric acid takes place as follows:



Under favorable aerobic soil conditions, calcium cyanamid breaks down readily to urea and thence to ammonia:

$$\underset{\text{Calcium cyanamid}}{CaCN_2} + H_2O + CO_2 \rightarrow \underset{\text{Cyanamid}}{NH_2 \cdot CN} + CaCO_3$$

$$\underset{\text{Cyanamid}}{NH_2 \cdot CN} + H_2O \rightarrow \underset{\text{Urea}}{CO(NH_2)_2} \xrightarrow{+\ 2H_2O} 2NH_3 + H_2CO_3$$

Urea is one of the most important nitrogenous substances in this group. It is excreted by the animal organism in large quantities; a large part of the nitrogen in stable manure is made up of urea; it is formed by various microbes; it is produced in the decomposition of various organic nitrogenous complexes, as in the case of uric acid; it is produced in the transformation of certain synthetic nitrogenous fertilizers, such as cyanamid; finally, it is introduced into the soil as a fertilizer coming from the nitrogen-fixation industry. Urea is decomposed in the soil by various organisms according to the following reactions:

$$CO \cdot (NH_2)_2 + 2H_2O = (NH_4)_2CO_3$$

$$(NH_4)_2CO_3 = CO_2 + 2NH_3 + H_2O$$

The transformation of urea into ammonia takes place very rapidly, so much so that in the manure pile there is considerable danger of actual volatilization of this ammonia. There are certain organisms known as urea bacteria which are able to effect the hydrolysis of urea by means of an enzyme called urease. The ammonia is produced so rapidly that the reaction becomes quite alkaline and the ammonia itself is readily volatilized in the absence of any neutralizing agents. In soil, following the addition of urea, the reaction first becomes distinctly alkaline through the formation of ammonia; later the reaction becomes more acid than the original reaction of the soil through the oxidation of the ammonia to nitric acid.

Chitin is a substance formed in the cells of various microorganisms. In the tissues of these microbes it plays a rôle similar to that played by cellulose in the tissues of higher plants. It is a polymer of mono-acetyl-glucoseamine having the formula $(C_{14}H_{26}N_2O_{10})_{10}$. On hydrolysis it gives acetic acid and glucoseamine. The decomposition of the glucoseamine finally gives rise to ammonia, carbon dioxide, and water, but its transformation is quite slow compared to the decomposition of most other organic nitrogenous substances.

Formation of Ammonia by Soil Microbes.—Decomposition of various organic substances containing nitrogen is thus found to give rise to ammonia as an important end product. The organisms which form ammonia from the organic compounds are unable to cause further transformation of the ammonia to more completely oxidized inorganic compounds, such as nitrite or nitrate. Consequently ammonia may be considered as the inorganic end product of nitrogen, resulting from the activity of microbes upon organic substances.

It is apparent that organic nitrogenous compounds which may find their way into soils are very numerous and vary greatly in composition. Consequently it is not surprising that a great number of different organisms are concerned in the formation of ammonia. In fact, a large percentage of all the microbes found in soils may be able to produce ammonia from organic materials of one kind or another. The group includes large numbers of different bacteria, both aerobic and anaerobic, spore-formers and non-sporulating species, filamentous fungi and actinomyces. These organisms vary in their ability to attack different compounds, also in the speed of ammonia formation and in the environmental conditions which are favorable for their action. It is apparent from Table 29 that different compounds decompose with different rapidity, and microorganisms vary from one another in their ability to effect the transformation.

TABLE 29

FORMATION OF AMMONIA (MGM.) BY MICROORGANISMS FROM 0.5 GM. OF PROTEINS IN 40 DAYS

Protein	Organism			
	Proteolytic bacterium	*Bacillus subtilis*	*Actinomyces violaceous-ruber*	*Rhizopus* sp.
Gelatin	25.45	42.82	39.99	18.98
Casein	37.57	23.43	21.81	18.58
Gliadin	29.91	14.55	21.41	18.59
Fibrin	19.76	18.55	16.12	18.55
Albumin	15.75	14.54	15.35	11.31
Zein	25.86	7.68	8.89	2.43

The process of formation of ammonia from organic compounds of nitrogen is of such importance that, under the name *ammonifica-*

tion, it has frequently been considered together with two other processes, namely, *nitrate formation* and *fixation of nitrogen,* to be synonymous with the microbiological activities in the soil.

The amount of ammonia produced in the decomposition of organic materials varies considerably, as illustrated in Table 30. A series of different organic substances containing different amounts of nitrogen were added to 100-gram portions of soil in tumblers. The low amount of ammonia produced from rice flour, corn-meal and wheat flour is due to the original low concentration of nitrogen and comparatively high content of available energy material.

TABLE 30

AMMONIA FORMATION FROM DECOMPOSITION OF VARIOUS PLANT SUBSTANCES* (FROM LIPMAN)

Substance used	Nitrogen contained in the added organic matter, mgm.	Ammonia formed in 7 days, mgm.
Rice flour	46.4	1.30
Corn-meal	51.2	1.22
Wheat flour	94.8	5.05
Cowpea-meal	156.8	50.36
Linseed-meal	247.0	118.31
Soybean-meal	245.6	132.43
Cottonseed-meal	246.1	123.63

* 4 gm. of organic material added to 100 gm. of soil.

The addition of organic matter free from nitrogen and representing an available source of energy will depress considerably the amount of ammonia accumulating from the decomposition of the organic substance, as seen in Table 31. A small amount of nitrogen-free organic matter actually produced an accelerating effect upon ammonia formation, but larger amounts reduced the ammonia formed from the decomposition of the nitrogenous substance; the greater the amount of non-nitrogenous substance added, the less ammonia was produced. This is due to the fact that a part of the ammonia is used up by the organisms that utilize the starch and sucrose as nutrients; the presence of these also partly depresses the decomposition of nitrogenous organic matter. The mechanism of these changes is further discussed in Chapter IV.

TABLE 31

INFLUENCE OF STARCH AND SUCROSE UPON AMMONIA FORMATION FROM DRIED BLOOD* (FROM LIPMAN)

Substance used	Amount of carbohydrate used, gm.	Ammonia formed in 7 days, mgm.
Dried blood alone	....	71.01
Dried blood + starch	0.3	77.63
Dried blood + starch	1.0	56.02
Dried blood + starch	3.0	26.92
Dried blood + starch	5.0	5.05
Dried blood + sucrose	0.3	85.64
Dried blood + sucrose	1.0	83.37
Dried blood + sucrose	3.0	43.99
Dried blood + sucrose	5.0	14.03

* Dried blood containing 155 mgm. of nitrogen added to 100 gm. of soil.

The amount of ammonia formed from the decomposition of organic matter depends upon the following important factors: (1) the composition of the organic material itself, with particular regard to its nitrogen content; (2) the organisms concerned in the decomposition; (3) the prevailing environmental conditions; (4) period of decomposition.

Organic materials of different origin decompose with different degrees of rapidity. Even the decomposition of the same plant will depend upon its age. The nature and abundance of the non-nitrogenous complexes in the plant, such as sugars, cellulose, hemicelluloses, waxes, resins, lignin, tannins, and cutins, will determine to a large extent the rapidity of decomposition. The rapidity of decomposition of the readily available sources of energy, such as the sugars, starches, pentosans and other hemicelluloses, and cellulose, will influence the rapidity of decomposition of the proteins and the amount of ammonia reassimilated by the organisms or left in the soil. A young plant decomposes more rapidly than an old plant, because the young plant is richer in readily decomposable substances (sugars, amino acids, phosphatides) and poorer in slowly decomposable substances (lignin, tannins, waxes, resins). For similar reasons the leaves of some trees decompose more readily than the leaves of others.

The nitrogen content of a substance is equally important in determining the amount of ammonia liberated in a given period of time. The larger the relative nitrogen content the greater is the relative amount of nitrogen liberated as ammonia in a given period of time. If one gram of cottonseed-meal containing only 5 per cent of nitrogen and 1 gram of dried blood containing 10 per cent of nitrogen are added to a given quantity of soil, more ammonia will be produced from the dried blood in a period of 10 to 30 days. Even when 2 grams of the cottonseed-meal are added to a certain quantity of soil and 1 gram of dried blood is added to another similar quantity of soil, more nitrogen will still be produced from the dried blood than from the cottonseed-meal. The above considerations readily explain the reasons for this difference. The 2 grams of the cottonseed meal contain three to four times as much non-nitrogenous energy-yielding substance as the dried blood. Even assuming that both organic substances decompose with the same degree of rapidity, still the microorganisms decomposing the meal, which offers a greater amount of available energy, will reassimilate and store a greater amount of the nitrogen which would have otherwise been liberated as ammonia.

The amount of nitrogen liberated as ammonia will also be affected by the nature of the flora bringing about the decomposition. In the case of acid forest soils, for example, fungi are largely concerned in the decomposition processes. In the arid alkaline soils, bacteria and actinomyces are more active. Fungi synthesize larger amounts of cell substance as mycelium, and will, therefore, liberate less ammonia for the same amount oforganic matter decomposed. Actually the fungi may be more active than many bacteria and, if a short incubation period is used, they may liberate more ammonia; if a sufficiently long period is employed, the reverse may be true. The reaction of the soil, nature of the inorganic material, soil aeration, and presence of mineral nutrients all influence the type of active flora and, therefore, the nature of decomposition brought about by this flora.

So many different organisms can produce ammonia from organic materials that comparatively small differences are apparent in ammonia formation from soils varying greatly in chemical and physical conditions and in general fertility. Since anaerobic bacteria of many kinds are able to split off ammonia quite readily, there may be large amounts of ammonia formed even where oxygen is excluded. Further, while ammonia may become oxidized to nitrate under aerobic conditions, such a transformation is quite completely inhibited under anaerobic conditions, and accumulation of ammonia over a period of time may be greater in an anaerobic environment than where there is free access of oxygen. Under aerobic conditions a great variety of organisms are active. Some fungi may be as active under neutral as under acid conditions. The bacteria and actinomyces are confined to much narrower ranges of reaction.

At no time is all the nitrogen of the organic matter liberated completely as ammonia, but the longer the period of decomposition the more complete the reaction. The factors considered above are of particular importance in determining the speed of mineralization of nitrogen added to the soil in the various plant and animal residues.

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CHAPTER VI

TRANSFORMATION OF NITROGEN BY SOIL MICROBES

(Continued)

NITRATE FORMATION.—Ammonia, the end product of the reactions just considered, becomes the raw material for the process of nitrate formation. Very few species of soil organisms are concerned in this process. These organisms are conveniently divided into two groups, in each of which a limited number of bacterial species is known. One group oxidizes ammonia to nitrous acid or nitrite, and the other transforms nitrous acid to nitric acid or nitrate. The process of conversion of ammonia to the more highly oxidized inorganic compounds of nitrogen as nitrite and nitrate is frequently referred to as *nitrification.*

$$2NH_3 + 3O_2 = 2HNO_2 + 2H_2O + \text{energy (calories) liberated}$$

$$2HNO_2 + O_2 = 2HNO_3 + \text{energy (calories) liberated}$$

None of the bacteria belonging to either group is able to transform ammonia directly to nitrate, but each is confined to merely one stage of the reaction. It seems possible that quite a variety of other microorganisms besides the specific nitrifying bacteria may be concerned in the production of some nitrite or nitrate from ammonia, but information concerning the relationship of these organisms to the oxidation process is indefinite and little more than suggestive. Nitrification by the specific bacteria is proportionally rapid and considered to be of major importance in the formation of nitrate in soils.

The conditions suitable for the formation of nitrite and nitrate by nitrifying bacteria are quite simple and include merely an inorganic medium containing salts of ammonia and several nutrient elements, a neutral reaction, and aerobic conditions. If soil is introduced into such a medium, active transformation of ammonia results, first giving rise to nitrite; when a large part of the ammonia has disappeared, nitrate is formed in increasing amounts at the expense of the nitrite.

The two groups of bacteria concerned in the transformation are thus quite distinct from one another, but they are alike physiologically in that they are both autotrophic, that is, they do not use organic substances as food but can use the energy liberated in the specific oxidation processes for their growth and development. The carbon necessary for the synthesis of their cells is taken from the carbon dioxide of the atmosphere and used in a manner similar to its utilization by green plants.

$$CO_2 + H_2O = \underset{\text{Formalde-hyde}}{HCHO} + O_2$$

$$6HCHO = C_6H_{12}O_6 \text{ (sugar) or other organic compounds.}$$

The organisms forming nitrite from ammonia have been designated as *Nitrosomonas* or *Nitrosococcus*, depending upon their morphology. The organisms producing nitrate from nitrite are called *Nitrobacter*. These forms are all very similar in morphology being spherical or short oval cells, either motile or non-motile (see Figs. 13 and 14). The difficulties involved in isolating these organisms in pure culture have delayed the description of more than a few species. The fact that very few species have been obtained is of less significance than the fact that nitrifying organisms exist in practically all soils. Since Winogradsky first obtained the organisms in 1891, they have been found to be active practically everywhere, with the exception of certain acid forest and peat soils. After drainage, cultivation, and liming, even these soils can be made a favorable medium for nitrification.

The optimum reaction for the activities of the organisms is about *p*H 7.0 to 8.0, but some strains will still grow at as high an acidity as *p*H 3.5 and at an alkalinity of *p*H 10.0. Under conditions of increasing acidity or alkalinity there develop certain acid-tolerant or alkali-tolerant strains which have different reaction requirements from those strains commonly encountered in most arable soils. However, at an acidity greater than *p*H 3.5, the nitrifying bacteria practically cease functioning. Acidity is but one of the factors inhibiting development of nitrifying organisms in acid peat bogs. The limited supply of oxygen in the water-saturated environment is insufficient to satisfy the requirements of these strictly aerobic bacteria. The addition of lime to an acid soil, or the drainage of a water-logged soil, will, therefore, materially favor the development of nitrifying organisms and the process of nitrification. The organisms may be found at some depth in soils, but naturally are much less abundant and little active in the lower layers, on account of the less aerobic conditions and the lack of materials upon which to feed. In arid soils these bacteria are more abundant in deeper layers than in soils of humid regions, since plant roots penetrate to greater depths and moisture conditions are more favorable at considerable distances below the surface.

TABLE 32

Relationship between Soil Reaction and Abundance of Ammonia-Oxidizing Organisms (from Wilson)

*p*H	Abundance of organisms
6.2	Less than 1,000
6.4	3,500
6.6	6,280
6.8	25,000
7.0	35,000

The numbers of nitrifying organisms in soils vary greatly, depending upon a number of factors; in cultivated soils they have been found to range from a few hundred to more than a million cells per gram. The data in Table 32 show that with increasing acidity there is a pronounced decrease in the abundance of these organisms. In many cases the reaction of the soil affects nitrifying organisms only indirectly by modifying plant development, which, in turn, affects the soil organisms.

Because of the limited number of organisms associated with the process of nitrification and the similarity of their characteristics of growth, the soil conditions under which nitrate accumulates are much more restricted than for ammonia formation. Nitrification is affected much more by modification of soil reaction, aeration, moisture, and salt concentration than the process of ammonia formation is altered by such changes. The treatment of soils with steam or volatile antiseptics, known as processes of partial sterilization of soils, eliminates most of the nitrite- and nitrate-forming organisms, while leaving many of the ammonia-forming organisms uninjured. This results in the accumulation of ammonia in partially sterilized soils, since once the ammonia is formed, it persists as such, because of the lack of organisms capable of further transforming it to nitrate.

In acid soils, such as raw-humus forest soils and certain acid peats which have been drained and not limed liberally, the decomposition of the nitrogenous organic substances is associated with the formation and accumulation of nitrogen as ammonia, without its being changed to nitrate. In the case of forest soils this process is of greatest importance in modifying the very nature of the forest vegetation. In the "raw-humus" types of soil, only those trees develop which can utilize ammoniacal nitrogen and are capable of forming mycorrhiza with special fungi, which decompose the organic matter. However, in the case of soils favorable for nitrate formation, the "mull" types are produced. These are considered richer soils; they bring about a more rapid regeneration of the young forest, and the trees grow rapidly without having to depend upon the formation of mycorrhiza. In the case of the "raw-humus" soils, the organic matter is attacked largely by fungi that allow an abundant accumulation of organic matter which is characteristically brown; the nitrogen is liberated as ammonia. In the case of the "mull" soils, decomposition is more rapid and is brought about by a varied microbial population including bacteria, actinomyces, and fungi, which give rise to a black instead of a brown soil; nitrogen is liberated as nitrate.

Undoubtedly some plants utilize nitrogen in forms other than nitrate, but many plants use nitrate almost exclusively, and some use other forms than nitrate principally during young stages of development. The marked effect of the nitrification process on plant growth is shown in Table 33.

TABLE 33

INFLUENCE OF NITRIFICATION ON GROWTH OF BARLEY (FROM FRED)

Soil conditions	Weight of plants, gm.
Control—no nitrogen	1.5
Nitrogen as $(NH_4)_2SO_4$	66.5
Nitrogen as $(NH_4)_2SO_4$ with nitrifying bacteria	116.0

Nitrate formation is controlled or affected by numerous factors, chief among which are presence of ammonia, soil reaction, aeration, amount and kind of organic matter, temperature, moisture, and concentration of inorganic substances.

Influence of Ammonia upon Nitrate Formation.—Since formation of nitrate is dependent upon the previous formation of ammonia, all factors affecting the speed of liberation of ammonia from organic materials will also exert pronounced effects upon nitrate formation. As pointed out above, the addition of an abundance of non-nitrogenous organic materials will temporarily depress the formation of ammonia. The lower the nitrogen content of the organic matter added to the soil, the greater will be the depression and the longer will be the period during which the depression lasts. Nitrate formation follows the production of ammonia, and takes place readily either when the organic matter itself contains sufficient nitrogen (over 1.7 per cent) to permit formation of ammonia or when inorganic nitrogenous compounds are added. The lower the nitrogen content of the organic matter the longer will be the period elapsing before its complete decomposition; the greater the nitrogen content, the shorter will be the period before nitrate is formed in abundance.

In the presence of readily decomposable nitrogenous materials, such as proteins or related compounds, ammonia accumulates rapidly and nitrification follows at a slower rate. This indicates that the process of nitrification in soils may be a slower process than ammonia production, when readily decomposable nitrogenous organic materials are present. However, the organic substances generally occurring in soils in the humus complex are not rapidly transformed to ammonia. The nitrifying organisms are able to oxidize the ammonia more rapidly than it is produced from such materials in most arable soils, and consequently nitrate and not ammoniacal nitrogen appears in greater abundance. The speed of the entire series of reactions is determined by the amount and availability of the organic nitrogenous compounds which are undergoing decomposition. The nitrification of the organic constituents of stable manure illustrates the relationship. When stable manure is added to soil, only a part of the nitrogen (about one-half) is readily converted to nitrate. About one-half of the nitrogen of the manure is in the form of urea, which is rapidly hydrolyzed to ammonia. This portion of the nitrogen undergoes further rapid change to nitrate. The other nitrogen of the manure occurs in compounds which are comparatively resistant to decomposition and is consequently converted to inorganic compounds very slowly. In certain soils, after the ammonia is changed to nitrite it may persist in that form for a considerable period of time and only gradually be changed further to nitrate.

INFLUENCE OF SOIL REACTION UPON NITRATE FORMATION.—The more acid a soil is, the less nitrate is formed; when the reaction is too acid, nitrate formation may come to a standstill (Fig. 60).

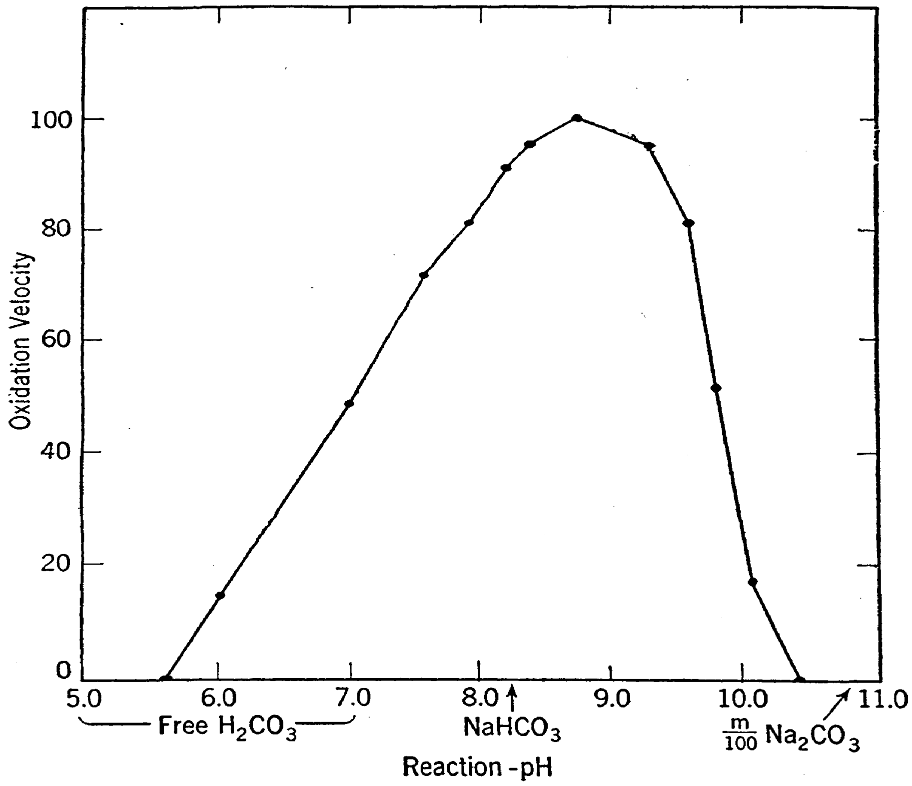

FIG. 60.—Influence of reaction upon nitrate formation (after Meyerhof).

In slightly alkaline soils, conditions appear to be particularly favorable for nitrification. The addition of lime to acid soils generally favors the formation of nitrate.

In certain arid regions of the West, particularly in Colorado and Utah, there are limited areas of basic soils so rich in nitrate salts as to prevent plant growth. Although nitrification in these soils is very active, the large quantity of nitrates probably has not been formed in these so-called "nitre spots," but has been produced principally in the soils of the vicinity and concentrated in the spots by the various movements of the soil water in these areas of deficient rainfall.

Soil reaction may be appreciably modified by active nitrification, due to the fact that the process results in the oxidation of ammonia to nitrate which leads to increases in the hydrogen-ion concentration of soils. When the nitrate is absorbed by plants or removed by drainage waters it is accompanied by certain cations (Ca, Mg, Na, etc.) which leave the soil poorer in neutralizing substances. The increase in acidity is particularly pronounced where ammonium salts are added to soils. This change is not particularly undesirable within certain limits, since it brings more of relatively insoluble plant nutrients into solution as the acid is formed, but where large amounts of ammoniacal substances gain

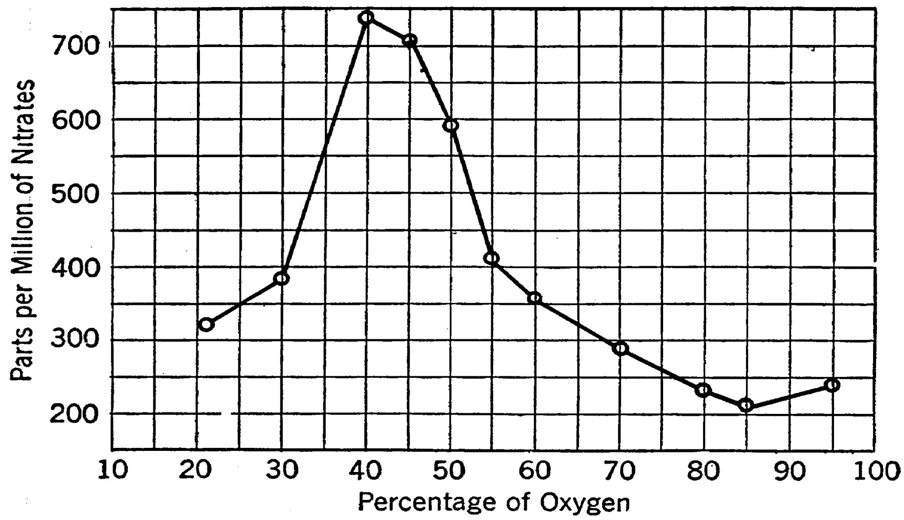

FIG. 61.—Influence of aeration upon nitrate formation (from Plummer).

entrance to soils, the increase in acidity becomes sufficiently great to justify the addition of lime to overcome the acid condition.

INFLUENCE OF SOIL AERATION UPON NITRATE FORMATION.—Since the processes of nitrification are autotrophic, considerable quantities of carbon dioxide are required. Since the transformations require large amounts of oxygen they must take place under aerobic conditions. Certain definite concentrations of these gases, higher than those that occur in the atmosphere, are most favorable to nitrification. Under soil conditions, oxygen is the only one of the two gases which may not be present in sufficient abundance. The decomposition processes of heterotrophic organisms are responsible for the liberation of large amounts of carbon dioxide, and the mixture of gases in the soil is always much richer in carbon dioxide than the atmosphere. The rate of diffusion of the gases between the soil and the atmosphere would be the factor determining the concentration of oxygen. The concentrations of oxygen most favorable to nitrification lie between 30 and 55 per cent (Fig. 61), while in soils this gas exists more commonly in concentrations between 15 and 20 per cent. In the atmosphere, oxygen comprises close to 20 per cent of the volume. Carbon dioxide occurs in the atmosphere in concentrations close to 0.03 per cent. However, this gas makes up 0.2 to 2.0 per cent of the soil air.

It is due to these relationships of the gases to nitrification that factors favoring the rapid exchange of gases between the soil air and the atmosphere lead to more rapid nitrate formation. Cultivation of soils and other treatments creating a more porous medium increase the rate of nitrification. Sandy soils nitrify more rapidly than heavy clay soils, other conditions being equal.

Organic Matter and Nitrate Formation.—The early work of Winogradsky and his colleague Omeliansky indicated that very small amounts of some organic materials may completely inhibit nitrification, as determined by studies in solution cultures. Such substances as glucose, peptone, asparagine, glycerol, and urea were distinctly injurious in concentrations of 0.2 to 1 per cent. Under most conditions, the soil solution does not contain such large quantities of these soluble organic substances. Further, the so-called humic substances which exist in the soil are not nearly as toxic as many of the organic materials given above. In fact, soil extract accelerates nitrification rather than depresses it. In the more fertile soils, which contain relatively greater amounts of organic materials, nitrification is appreciably more rapid than in the less fertile soils. Nitrification will also proceed in heaps of animal manures which contain large amounts of organic matter in solution. In the preservation of manure from losses of nitrogen one of the necessities is to keep nitrification down to a minimum, for it is subsequent to nitrate formation that large amounts of nitrogen are lost by reduction of nitrate to gaseous nitrogen.

Moisture and Nitrification.—Moisture conditions most favorable for nitrification are much the same as are favorable for many aerobic soil processes, being close to 50 per cent of the amount of water the soil holds when saturated. The influence of high moisture content is determined to a considerable degree by the retarding effect of the water on the circulation of the gases. High moisture contents are unfavorable to aerobic processes in general (Fig. 62).

A number of other factors, such as temperature, concentration of salts in the soil solution, and previous drying of soil, exert pronounced effects on the rapidity of formation of nitrate from ammonia, either produced from the decomposition of organic

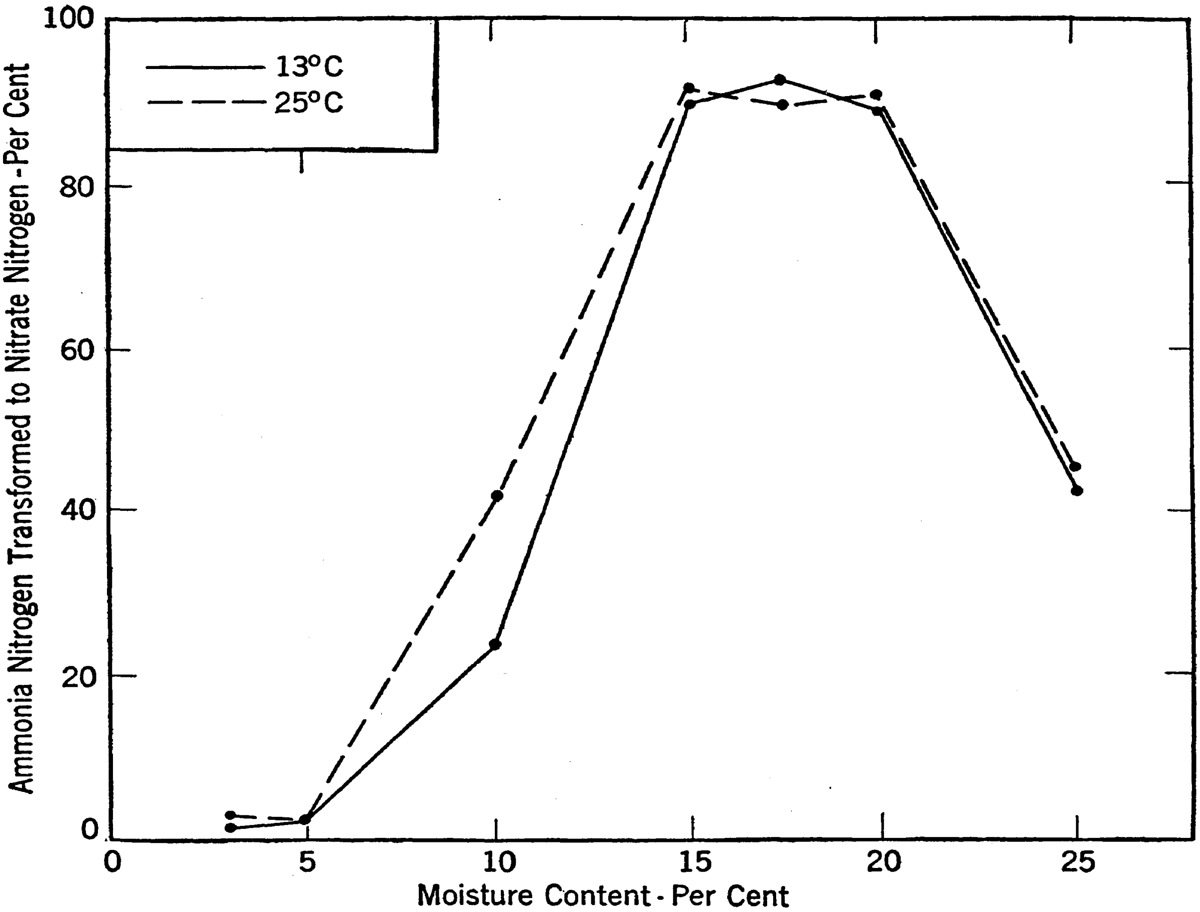

FIG. 62.—Influence of moisture upon nitrate formation (after Traaen).

matter present, or recently introduced into the soil, or added to the soil in the form of inorganic salts. It was found, for example, that drying of soil followed by remoistening causes nitrates to form more rapidly.

In general, the bacteria which bring about the processes of transformation of ammonia to nitrate are among the most sensitive of soil organisms. Conditions which can be readily withstood by other bacteria may prove injurious to these organisms. This is due to no small extent to their very specific nature.

Plant growth may greatly affect nitrate accumulation in soils but will not necessarily inhibit nitrate formation. In fact, development of plants may favor nitrification, as shown later. Through the absorption of nitrate from the soil by the plants, the amount of nitrate in the soil will decrease. It is apparent that under such conditions, the presence of small amounts of nitrate is no indication of a depression of nitrification. Consequently, because soils under plant growth contain less nitrate than soils free from vegetation, it should not be concluded that plant growth depresses nitrification. Since nitrate in soils most commonly originates from the organic nitrogen compounds, the soil containing the greatest amounts of readily decomposable organic substances will show the most rapid nitrification, other conditions being favorable for the processes (Fig. 63). Where plants are not growing on a soil no organic substances become incorporated with the soil as residues of roots and other plant parts. On the other hand, considerable amounts of organic matter gain entrance into the soil which is cropped. In an unplanted soil, the amounts of readily decomposable organic matter continuously decrease, while planted soils receive periodic additions of such substances. These considerations indicate why planted soils may be expected to show a more active nitrifying population.

Nitrate Reduction.—The nitrogen transformations already considered do not cause losses of nitrogen from soils. In fact, such reactions as nitrogen fixation even add nitrogen. With the exception of nitrogen assimilation by microorganisms, most of the reactions may be interpreted as being quite favorable to plant growth; even the assimilation processes only temporarily remove the nitrogen from availability to plants, and may prove to be desirable under some circumstances.

There is at least one set of transformations of nitrogen compounds associated with microbial activities which is generally not beneficial to plant growth, and may be decidedly undesirable from the point of view of economics of the soil. These transformations are concerned with reduction of nitrates, frequently leading to the formation of gaseous nitrogen and consequent impoverishment of the store of fixed nitrogen in the soil. Associated with the formation of free nitrogen are a number of other reactions provoked by microorganisms by which a large number of partly or completely reduced inorganic compounds of nitrogen are produced.

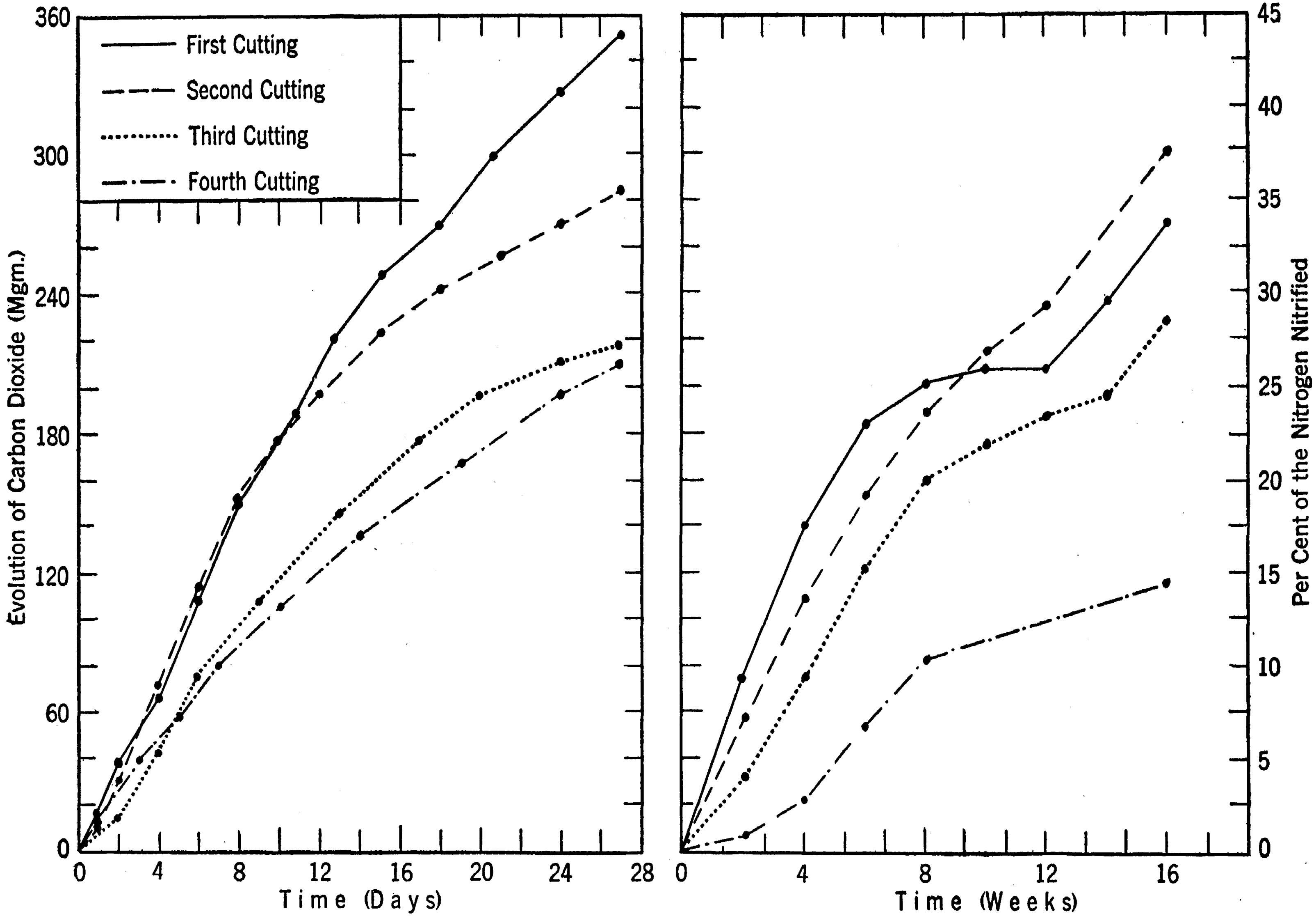

FIG. 63.—Decomposition of plants harvested at different stages of growth, showing liberation of carbon dioxide during the decomposition of a cereal plant (rye), and formation of nitrates in the decomposition of a leguminous plant (Crotalaria). (Left curves after Waksman and Tenney; right curves after Leukel, Barnette and Hester.)

Nitrate may disappear from soils through several channels:

(1) It may be assimilated by plants.

(2) It may pass into deeper regions of the soil in the movements of the water, or it may disappear from the soil dissolved in drainage waters.

(3) It may be assimilated by microorganisms in the presence of an available source of energy. In this case the nitrate is not lost but is temporarily taken from circulation and stored in the soil in complex organic forms. The reactions involved in the synthesis of cell constituents from nitrate involve reduc-

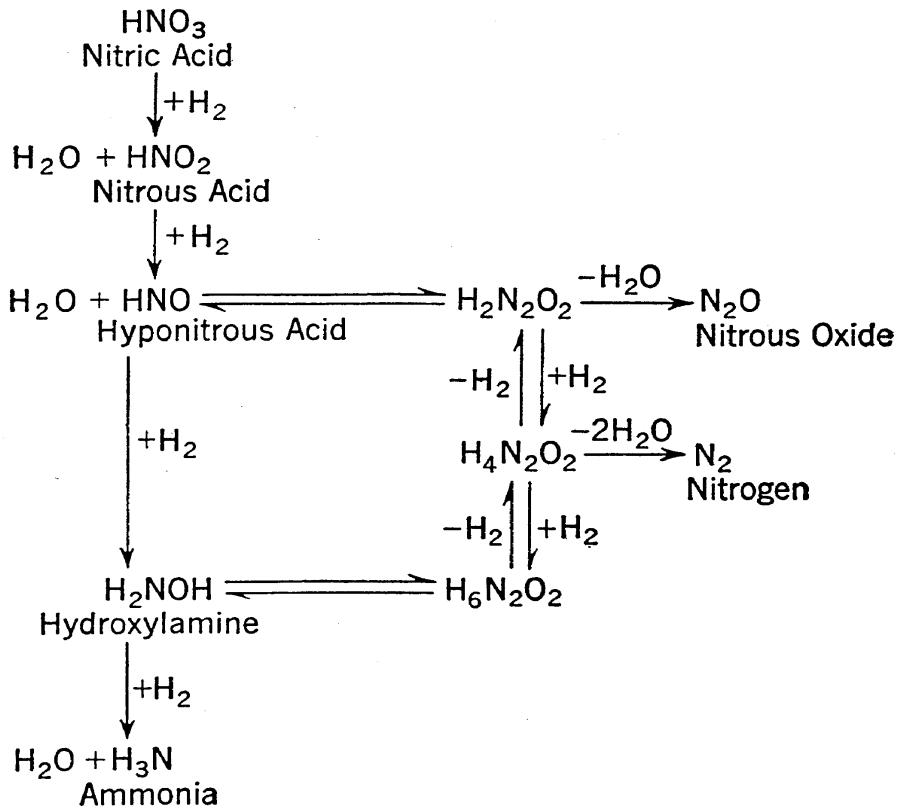

FIG. 64.—Reduction products of nitric acid.

tion processes but need not lead to the accumulation of reduced inorganic compounds of nitrogen in the medium. The general transformation may be labeled *nitrogen assimilation* to distinguish it from other changes to be considered below.

(4) It may be reduced to many compounds, such as nitrous acid, hyponitrous acid, hydroxylamine, ammonia, the gaseous nitrous oxide, and free nitrogen. The hyponitrous acid and

hydroxylamine generally do not accumulate but are formed as intermediary products, but the others may appear in appreciable amounts where active reduction occurs. The relationships of these compounds to each other are schematically represented in Fig. 64.

These reduction products are the result of either direct reduction reactions or secondary reactions. The reducing power of the organism determines the primary products which are formed (either nitrous acid, hyponitrous acid, hydroxylamine, or ammonia), these leading to the formation of the gaseous substances.

It is apparent from Fig. 65 that the reaction takes place in several stages, nitrous acid first appearing as the nitrate is reduced. In turn, the nitrous acid is reduced to other more completely reduced compounds, and eventually they also disappear coincident with the formation of elementary nitrogen.

The organisms concerned in the various reductions of nitrate are quite numerous. Bacteria play by far the most important rôle in these transformations. The formation of nitrite from nitrate is common to actinomyces as well as to numerous bacteria. The formation of ammonia, nitrous oxide, and nitrogen are reactions confined to certain species of bacteria. In the absence of uncombined oxygen, the oxygen contained in the nitrate is utilized by these organisms to perform various oxidation processes. Sulfate serves a similar purpose under anaerobic conditions, where it is reduced to sulfide.

Energy is required to effect the reductions, and the reactions do not occur in the absence of food materials from which microorganisms may obtain the necessary energy. Various soluble carbohydrates, cellulose, organic acids, and alcohols suit the requirements of the organisms. Some bacteria even use sulfur as follows:

$$6KNO_3 + 5S + 2CaCO_3 = 3K_2SO_4 + 2CaSO_4 + 2CO_2 + 3N_2$$

Table 34 indicates that the amounts of ammonia or gaseous nitrogen formed from nitrate differ with the organisms active in the reaction and the carbohydrates utilized as foods.

The formation of the gaseous products is the most undesirable change of all the nitrate reductions, since the volatilization of the

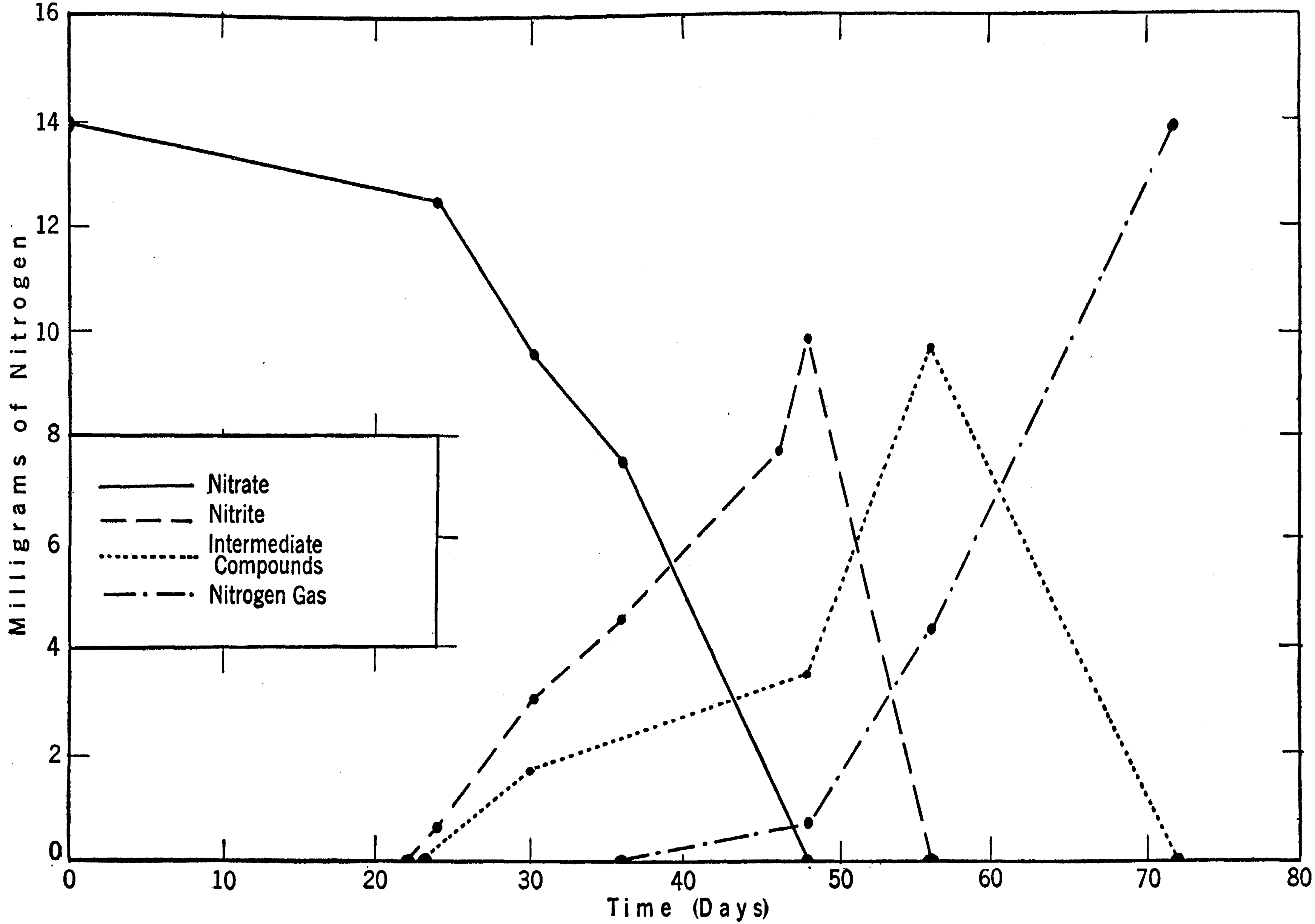

FIG. 65.—Reduction of nitrate by microbes (after Korsakowa).

TABLE 34

AMOUNTS (PERCENTAGES) OF AMMONIA OR GASEOUS NITROGEN FORMED FROM NITRATE BY BACTERIA USING DIFFERENT CARBOHYDRATES AS FOOD (FROM STOKLASA AND VITEK)

Sugar	Glucose	Levulose	Galactose	Arabinose	Xylose
Organism	Formation of Ammonia				
B. mycoides	20.69	1.9	1.72	1.91	
B. subtilis	2.41	6.55	6.22	12.24	8.11
Cl. gelatinosum	14.48	....	....	45.55	9.68
B. prodigiosus	....	....	....	4.13	2.58
Bac. mesentericus vulgatus	....	5.17	5.52	3.44	
Proteus vulgaris	2.79	2.79	2.70		
	Formation of Gaseous Nitrogen				
B. Hartlebii	93.97	87.59	74.66	66.38	83.38
B. centropunctatum	5.17	4.31			
B. nitrovorum	....	5.17	5.43		
B. fluorescens liquefaciens	84.48	57.76	37.93	7.08	
Bact. pyocyaneum	82.76	71.55	20.59		

gases represents a definite disappearance of nitrogen from the soil. This reduction of nitrate to gaseous nitrogen or nitrous oxide is the change referred to as *denitrification.* These various reactions are considered as processes of nitrate reduction. The formation of nitrite and ammonia does not necessarily result in losses of nitrogen from the soil, since, under favorable conditions, these substances may be oxidized to nitrate again. The reaction (*p*H) of the material in which the reductions are taking place will, of course, affect the loss of ammoniacal nitrogen; under alkaline conditions, considerable amounts of ammonia may be lost by volatilization.

IMPORTANCE OF DENITRIFICATION IN SOIL.—Since nitrate plays such an important part in plant nutrition, the discovery of denitrifying organisms in soils suggested that denitrification might be responsible for the limitation of plant growth by depletion of the supply of available nitrogen. However, although nitrate may disappear from the soil in numerous ways, its reduction to gaseous substances occurs only under very specific conditions, and is not apt to be of particular economic importance in most arable soils. The conditions particularly conducive to denitrification are:

(1) Presence of relatively large amounts of nitrate.

(2) Presence of an abundance of decomposable organic matter.

(3) Lack of free oxygen (anaerobic conditions).

These conditions may appear where soils become water-logged through faulty drainage or where soils are purposely flooded, as are rice fields. Conditions may also be favorable where concentrations of organic matter as composts of manures or plant residues undergo transformation in the presence of nitrate.

When nitrates are added to the soil, together with large quantities of stable manure or green manure, decomposition of the organic substances may be accompanied by denitrification. The rapid decomposition of the fresh organic substances leads to an exhaustion of the available oxygen in the soil air and the formation of equivalent amounts of carbon dioxide. Where carbon dioxide is produced more rapidly than diffusion permits access of atmospheric oxygen, anaerobic conditions are created and denitrification may temporarily become a factor of importance. The application of manures in amounts common to agricultural practice does not cause such rapid consumption of the oxygen in the soil, and no denitrification takes place.

Denitrification is responsible for a large part of the loss of nitrogen from animal manures stored in large quantities under aerobic conditions. If the manures are kept anaerobic, little loss of nitrogen in the elementary form occurs. As much as 40 to 50 per cent of the nitrogen may disappear as gaseous nitrogen under conditions favorable for denitrification. The loss is associated with the nitrification reaction. The ammonia produced from the decomposition of urea becomes changed to nitrate in aerobic regions of the manure heap. This nitrate in turn may become washed into lower depths of the heap, where it is rapidly reduced to gaseous nitrogen. This source of loss may be removed by preventing the formation of nitrate through compacting and moistening, thus creating anaerobic conditions.

In the water-logged condition, rice soils present conditions favorable for nitrate reduction. If nitrate is added to such soils as fertilizing material it undergoes reduction to nitrite and gaseous nitrogen. Either of these transformations is quite undesirable. The nitrite is toxic to plant growth, and gaseous nitrogen is no longer available to the plants. Ammonium salts are more suitable as nitrogenous fertilizers under such cultural conditions.

SUMMARY.—In soil, nitrogen undergoes a series of transformations as a result of the activities of microorganisms. The supply of combined nitrogen in the soil has been furnished to a considerable extent through the activities of certain groups of microorganisms capable of utilizing the gaseous elementary nitrogen of the atmosphere for the purpose of building up complex organic substances, namely, the constituents of the microbial cells. These organisms are either non-symbiotic in nature, leading a free existence in the soil, or are symbiotic, namely, growing in the cells of higher plants, as in the roots of legumes. When these bacteria die, the higher plants obtain the nitrogen fixed by these organisms, either directly through the action of plant enzymes, or indirectly through the activities of microbes which bring about the decomposition of the complexes built up by the nitrogen-fixing organisms.

When the nitrogenous plant residues are added to the soil, in the form of roots, leaves, twigs, green manures, and stable manures, they first undergo a series of decomposition processes carried out by various groups of microbes with the result that the nitrogen is changed from complex organic substances into a simple inorganic form (ammonia). The processes of decomposition are accompanied by processes of synthesis converting again the simple forms of nitrogen into complex organic forms of a microbial nature. The nitrogen content of the plant is apt to be very low, ranging from 0.2 per cent in certain cereal straws to 3 per cent in certain leguminous plants. The first result of the decomposition of plant residues by microbes is a narrowing of the ratio of carbon to nitrogen in the decomposed material or increasing the percentage nitrogen content.

If the amount of nitrogen in the plant residues is sufficiently large, as 1.7 per cent or more, decomposition proceeds rapidly, and the liberation of nitrogen in such an available form as ammonia takes place within 4 to 6 weeks of decomposition under favorable conditions. If the relative nitrogen content is low, considerable time may elapse before any of the nitrogen becomes available for higher plants. Under these conditions, the excess of carbohydrates, especially the cellulose, keeps the available nitrogen at a minimum until they have nearly all decomposed.

The ammonia liberated as a result of decomposition of the nitrogenous constituents of plants, animals, and microbes is either utilized directly by plants or is changed by certain specific groups of bacteria to nitrites, then to nitrates. This process, known as nitrification, is of considerable importance in soils because it is believed that the majority of higher plants obtain their nitrogen from the soil in the form of nitrate.

The nitrate thus produced, if not assimilated by growing plants, can be absorbed by microorganisms, in the presence of available carbohydrates, and changed again to microbial protein. It can be washed out from the soil by drainage waters. Under certain conditions, such as limited aeration, it can be reduced to gases of nitrogen. The nitrogen thus becomes lost from the soil and returns to the atmosphere.

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CHAPTER VII

TRANSFORMATION OF MINERAL SUBSTANCES IN SOIL THROUGH THE DIRECT OR INDIRECT ACTION OF MICROORGANISMS

Relationships of Microorganisms to the Elements Occurring in Nature.—Practically all the elements which are essential, in large or small amounts, for the growth of cultivated or uncultivated plants, as direct nutrients or as catalysts, are subject to the action of microorganisms in the soil. The transformations of carbon and nitrogen considered in the preceding pages are particularly prominent. Some of the other elements are made available for plant utilization through the direct action of microorganisms, and still others may be subject to their indirect action.

Compounds of carbon and nitrogen, available to higher plants, are apt to become very scarce in nature unless the great variety of organic materials produced by living things are constantly acted upon by microorganisms and kept in circulation. The elements hydrogen and oxygen, which are present in great excess in the atmosphere and in the lithosphere, in either combined or free forms, seldom become limiting factors in the growth of plants. However, these elements are constantly subject to the activities of microorganisms in one way or another, since they occupy an important place among the constituents of the microbial nutrients and of the microbial cells, and take an active part in the numerous oxidation and reduction processes carried on in the soil by microbes. It may be sufficient to call attention to only two processes to indicate to what extent these two elements are important in the activities of microorganisms and in the various soil transformations, and also what rôle microbes play in the changes that these two elements undergo in nature.

In the absence of sufficient uncombined oxygen, as in the case of peat bogs, where the supply of gaseous atmospheric oxygen is excluded as a result of the saturation of the material with water, complete oxidation of the organic matter is made impossible. The activities of the aerobic fungi and bacteria are thereby excluded, and the decomposition of organic matter is dependent largely upon the activities of anaerobic bacteria. Under these conditions, some of the constituents of the organic substances, such as the waxes and the lignin, are scarcely decomposed at all, and others, like the cellulose and hemicelluloses, are decomposed very slowly. The formation of peat is thus a result of the continuous accumulation of certain plant residues in habitats deprived of free oxygen by saturation with water. The peats formed in prehistoric times have finally given rise to coal, as a result of various natural phenomena, including physical and chemical processes.

When organic materials are decomposing under anaerobic conditions, considerable amounts of hydrogen are produced. When it reaches an aerobic environment, the hydrogen is utilized as food by a number of bacteria, and becomes oxidized to water.

Most of the other elements which enter into the composition of the soil are acted upon by microorganisms in one way or another. Mineral substances become transformed from one inorganic form into another, or are brought into solution, or are precipitated as a result of the direct or indirect action of microorganisms. Some elements as sulfur and phosphorus are frequently constituents of organic compounds and consequently pass through a more varied series of changes. Utilization of these organic compounds as sources of energy by microorganisms results in the mineralization of the elements. Autotrophic microorganisms are capable of using several mineral elements or their simple compounds as sources of energy. Various microorganisms produce a number of organic and inorganic acids, which increase the solubility of many mineral materials. Compounds of potassium, magnesium, calcium, and iron play important rôles in the decomposition and synthetic processes which are carried on by the soil organisms. Zinc, manganese, chlorine, sodium, etc., are required only in mere traces by the soil organisms, and their rôle in microbial processes is still imperfectly understood in most instances.

Mineral Elements and Their Inorganic Compounds as Sources of Energy.—Some of the elements or their simple inorganic compounds, which enter into the composition of the living cell and which are frequently found in the soil in considerable amounts, are used extensively as sources of energy by certain specific types of bacteria. These bacteria are usually designated as *autotrophic bacteria,* since they are able to synthesize their cell substance from mineral materials, using carbon dioxide as their only source of carbon. This process of carbon assimilation which is referred to as chemosynthesis, because the energy is obtained from chemical substances, is similar in many respects to the carbon assimilation by green plants which is spoken of as photosynthesis, wherein the energy of the rays of the sun is utilized. The utilization of hydrogen and of compounds of nitrogen (ammonia and nitrite) as sources of energy by specific bacteria has been referred to previously. In this group belong also bacteria which oxidize elementary sulfur and its incompletely oxidized compounds (sulfides and thiosulfates) to sulfate, organisms oxidizing ferrous to ferric compounds, and manganous to manganic compounds.

Use of Inorganic Salts as Sources of Oxygen.—Under conditions which favor the activities of anaerobic bacteria, as where the supply of free gaseous oxygen is excluded, many bacteria are capable of using certain inorganic salts rich in oxygen as sources of oxygen. The reduction of nitrates, sulfates, and phosphates are cases which can illustrate this group of processes. The reduction of nitrates gives rise to nitrites, ammonia, nitrous oxide, and even elementary nitrogen, as discussed previously. The reduction of sulfates gives rise to sulfites and sulfides, including hydrogen sulfide, as shown later. The reduction of phosphates gives rise to phosphites, hypophosphites, and even phosphine.

Interaction of Insoluble Inorganic Salts with Inorganic and Organic Acids Produced by Microbes.—The transformation of mineral substances as a result of their interaction in the soil with organic and inorganic acids formed by microorganisms is of common occurrence. These acids are produced in the soil by numerous bacteria and fungi. The acids interact with the insoluble carbonates, phosphates, and silicates, making them partly or completely soluble. Certain portions of the rock fragments in soil are dissolved, leaving the more resistant silicates. Minerals of the alkalies, such as sodium and potassium, are more readily acted upon than minerals of the alkaline earths, such as calcium. Magnesium is affected to much the same degree as calcium. Compounds of iron are generally somewhat more resistant than any of these.

Among the inorganic acids produced by bacteria, carbonic, nitric, and sulfuric acids are of principal importance. Nitrous acid is a precursor to the formation of nitric acid, but it is generally so rapidly converted to nitric acid that it seldom accumulates in sufficient amounts to exert pronounced effects. Carbonic acid is produced by all mircoorganisms in appreciable amounts in their growth processes; nitric acid is the end product of oxidation of ammonia by nitrifying bacteria; and sulfuric acid is the product of oxidation of sulfide and sulfur by sulfur bacteria. The group of autotrophic bacteria as a whole is particularly active in solvent activities, since strong acids are so frequently produced by their development. Chief among these are nitric and sulfuric acids.

Algae are frequently recognized as corrosive agents acting upon stone and rock surfaces. As individuals and as associated with certain fungi in the form of lichens they represent some of the first plant forms to develop upon naked rocks. They exert effects through respiration products, carbon dioxide in particular, and also indirectly by supplying organic food materials to bacteria which in turn may bring about solvent effects in different ways.

These inorganic acids vary in strength and in the amounts produced in soils, and consequently in their solvent action. Sulfuric and nitric acids are much stronger than carbonic acid, but are formed in small amounts compared to this weaker acid. Under most soil conditions nitric acid is produced in much more abundance than sulfuric acid. Exceptions to this may appear in cases where sulfur is applied to soils in considerable quantities to create acid conditions.

There may be as much as 7,300 kilograms of carbon dioxide produced each year per acre of fertile soil. This would represent about 10,300 kilograms of carbonic acid. Considering that all of the nitrogen removed by a heavy crop and lost in drainage waters had been produced as nitric acid in the soil, this would represent at a maximum close to 250 kilograms of nitric acid per acre. Similarly, it may be assumed that there would be as much as 100 kilograms of sulfuric acid produced annually in the same amount of soil. Fortunately, carbonic acid is a weak acid, exerting a mild solvent effect and readily disappearing by the volatilization of carbon dioxide under soil conditions. The amounts of carbon dioxide produced by different soils are quite unlike, as is apparent from the data shown in Table 35.

Carbon Dioxide in the Soil.—Many factors are concerned in the production of carbon dioxide, and since it is practically all of biological origin, the effects of various factors on the development of microorganisms is reflected in the amounts of the products of microbial metabolism, among which is carbon dioxide. Since

TABLE 35

Carbon Dioxide Produced by Different Soils (from Stoklasa)

Soil	Carbon dioxide produced by 1 kgm. of soil in 24 hrs. at 20° C, mgm.
Soil relatively free from organic substances	8.14
Meadow soil	10.16
Forest soil poor in organic matter	9.12
Forest soil rich in organic matter	16.26
Infertile rye and oat soil	19.25
Fertile rye and oat soil	30.36
Fertile wheat soil	30.48
Fertile clover soil	53.60
Fertile sugar-beet soil	56.68
Fertile garden mould	62.75

most of the organic residues of plants become incorporated in the superficial portions of the soil, and microorganisms find other conditions most favorable for their development in this region, the largest amounts of carbon dioxide are produced near the surface.

TABLE 36

Carbon Dioxide Evolved from Sandy Soil at Different Distances from the Surface (from Lundegardh)

Depth, cm.		CO_2 per sq. meter per hour, gm.
0–10		0.271
10–20		0.084
20–30		0.016
30–40		0.009
	Total	0.380

In the deeper layers of soil the gas is produced in smaller amounts (see Table 36). Although produced most rapidly near the surface, carbon dioxide occupies a greater portion of the pore spaces in the deeper regions of the soil (see Table 37). The air of the soil is thus considerably higher in carbon dioxide than the normal atmosphere above the soil. While the atmosphere contains close to 0.03 per cent of the gas, the soil air shows from 0.05 to 3.8 per cent, and seldom as low as the smallest figure. Table 38 gives figures for the composition of the gases in soils under aerobic and anaerobic conditions.

TABLE 37

Carbon Dioxide in the Soil Air at Different Distances below the Surface (from Lau)

Depth, cm.	Per cent carbon dioxide		
	Sandy soil	Loam soil	Moor soil
15	0.09–0.19	0.05–0.27	0.10–0.75
30	0.06–0.24	0.09–0.47	0.34–1.12
60	0.11–0.57	0.20–1.13	1.01–3.77

Since diffusion of the soil gases to the atmosphere is more rapid near the surface, the relative carbon dioxide concentration will be greater at lower depths. In the deeper layers the movement of the gases is retarded by the longer distance required to be traveled and the smaller number of channels opening to the surface. Soil water depresses the speed of movement to an important extent by

TABLE 38

Composition of Soil Gases

	Aerobic (from Russell and Appleyard)	Anerobic or rice paddy soils (from Harrison and Aiyer)
Oxygen	18.00 to 21.00 per cent ±	0 or traces
Carbon dioxide	0.06 to 2.0 per cent ±	1–20 per cent
Nitrogen	79 per cent ±	10–95 per cent
Methane	0	15–75 per cent
Hydrogen	0	0–10 per cent

filling the pore spaces. On the other hand, a relatively high temperature and moisture content favor the production of carbon dioxide, as shown in Table 39. Considering the amount of the gas produced at the lowest temperature and moisture content cited in the table as 1, increases in moisture and temperature raised the production of carbon dioxide to 40. The numerous factors which favor microbial development increase the rates of carbon dioxide production, and factors lowering rates of diffusion tend to keep the gas at a relatively high level in the soil air and consequently also at a high level in the dissolved state as carbonic acid.

TABLE 39

INFLUENCE OF TEMPERATURE AND MOISTURE ON CARBON DIOXIDE CONTENT OF SOILS (FROM WOLLNY)

Water content, per cent	Temperature		
	10° C.	20° C.	30° C.
6.8	1 vol. of CO_2	1.6 vol. of CO_2	3.4 vol. of CO_2
26.8	9.1 vol. of CO_2	26.7 vol. of CO_2	31.0 vol. of CO_2
46.8	17.2 vol. of CO_2	30.6 vol. of CO_2	40.5 vol. of CO_2

The action of carbonic acid may be exerted upon a great variety of soil materials, forming carbonates of the basic substances, and if present in sufficient amount, producing bicarbonates from these carbonates:

$$CaCO_3 + H_2CO_3 = Ca(HCO_3)_2.$$

In spite of the fact that most of the carbonic acid which is produced in the soil is lost soon after its formation by volatilization of carbon dioxide, some of the active acid reacts with the soil constituents and may dissolve considerable amounts of those mineral substances which are relatively insoluble in water alone. The reactions concerned in these changes may be represented as follows:

$$\underset{\text{Di-calcium phosphate}}{Ca_2(HPO_4)_2} + 2H_2CO_3 = \underset{\text{Mono-calcium phosphate}}{Ca(H_2PO_4)_2} + Ca(HCO_3)_2$$

$$\underset{\text{Tri-calcium phosphate}}{Ca_3(PO_4)_2} + 4H_2CO_3 = \underset{\text{Mono-calcium phosphate}}{Ca(H_2PO_4)_2} + 2Ca(HCO_3)_2$$

Stoklasa reported a case where distilled water dissolved only 0.07 gm. of phosphate (calculated as P_2O_5) from 1 gm. of di-calcium phosphate, while carbonated water dissolved 0.26 gm. The solvent action of carbonated water on various phosphates is quite pronounced, as evidenced by the data shown in Table 40. In general, its action is considerably weaker than that of the organic acids.

TABLE 40

SOLVENT ACTION OF WEAK ACIDS ON PHOSPHATES (FROM STOKLASA)

Compound	Per Cent Dissolved		
	Carbonated water	Acetic acid (0.5%)	Formic acid (0.5%)
Di-calcium phosphate	45.75	97.13	99.54
Tri-calcium phosphate	25.01	73.83	90.90
Mono-ferric phosphate	27.44	19.57	29.35
Di-ferric phosphate	14.68	13.51	27.81
Tri-ferric phosphate	7.87	8.13	16.00
Mono-aluminum phosphate	33.67	99.48	76.05
Tri-aluminum phosphate	....	22.09	93.79

In the vicinity of growing roots of plants, considerable amounts of carbonic acid appear as a product of respiration of the root tissues; this is further increased by the microorganisms in their decomposition of the organic materials originating from the roots. The solvent action of this carbonic acid in the vicinity of root absorption is an important factor in the nutrition of plants in an environment such as soils where the inorganic nutrients occur in low concentrations.

NITRIC ACID IN THE SOIL.—The amount of nitrate which occurs at any one time in soils supporting plant growth is comparatively small. This may occasionally be as much as 100 pounds, calculated as nitrogen, or 450 pounds, calculated as nitric acid, per acre. Generally the nitrate in soils would be a small fraction of this. The significance of nitrate formation lies not so much in the fact that certain amounts exist in soils at any one time combined with basic substances, but that nitric acid is formed continuously. Its absorption by plants or removal in drainage waters keeps the concentration of nitrate low in humid regions. Soon after its formation it reacts with soil constituents, such ~~as the insoluble~~ carbonates, phosphates, and ~~silicates, and, over a period of time,~~ dissolves large amounts of insoluble substances forming salts of ~~potassium, sodium,~~ magnesium, calcium, and iron. It has been stated that, in the conversion of organic nitrogen to nitrate sufficient for a hundred bushel crop of corn, there will be produced sufficient acid to convert seven times as much insoluble tri-calcium phosphate into soluble mono-calcium phosphate as would be required to supply the phosphorus for the same crop. Of course, the larger part of the nitric acid would take part in other reactions, and only a small portion of the acid would react with the phosphates. Nitrification of ammonium sulfate is responsible for greater acid formation than nitrate formation from nitrogen originally contained in organic combination. The solvent action accompanying nitrification in laboratory cultures containing tri-calcium phosphate is shown in Table 41.

TABLE 41

Solution of Phosphate and Calcium from Tri-calcium Phosphate by Nitrification of Ammonium Sulfate in Solution Cultures (from Hopkins and Whiting)

Nitrogen oxidized, mgm.	Phosphorus made soluble, mgm.	Calcium, made soluble, mgm.
2.54	4.08	3.87
3.81	5.08	5.60
4.88	10.20	18.40
5.52	9.56	14.80
6.40	12.85	22.00
6.40	10.24	23.52
6.88	16.00	31.04

The reaction concerned in this process may be expressed as follows:

$$Ca_3(PO_4)_2 + (NH_4)_2SO_4 + 4O_2 = Ca(H_2PO_4)_2 + Ca(NO_3)_2 + CaSO_4 + 2H_2O$$

This reaction can be considered as representative not only of the solvent effects following nitrification but also of the development of increased acidity through the absorption or transformation of a cation of an inorganic salt. By removing or changing the ammonium ion, the sulfate radical takes on hydrogen, which gives to it as great solvent powers as the nitric acid formed from the oxidation of the ammonia.

When urea is added to the soil, the reaction of the soil is first made alkaline, by the transformation of the urea to ammonium carbonate, then acid following the oxidation of the ammonia to nitric acid:

$$CO(NH_2)_2 + 2H_2O = (NH_4)_2CO_3$$

$$(NH_4)_2CO_3 + 4O_2 = 2HNO_3 + CO_2 + 3H_2O$$

These general considerations of the solvent action of nitric acid on soil minerals are equally applicable to sulfuric acid, with the exception that sulfuric acid generally appears in soils in smaller amounts, and consequently its effect is of less importance.

ORGANIC ACIDS IN THE SOIL.—In addition to the inorganic acids, various microbes produce in the soil organic acids, which can play the same function as the inorganic acids but are weaker than nitric or sulfuric acids although stronger than carbonic acid. Among the organic acids produced by soil bacteria, the following may be mentioned: lactic, butyric, acetic, propionic, and valerianic. Fungi produce the following acids: gluconic, citric, oxalic, fumaric, and succinic. Various acids may be formed from carbohydrates under suitable conditions:

$$C_6H_{12}O_6 = \underset{\substack{\text{Butyric}\\ \text{acid}}}{C_4H_8O_2} + 2CO_2 + 2H_2 \text{ (anaerobic bacteria)}$$

$$C_6H_{12}O_6 + 3O = \underset{\substack{\text{Citric}\\ \text{acid}}}{C_6H_8O_7} + 2H_2O \text{ (fungi)}$$

$$C_6H_{12}O_6 + 9O = \underset{\substack{\text{Oxalic}\\ \text{acid}}}{3C_2H_2O_4} + 3H_2O \text{ (fungi)}$$

$$C_6H_{12}O_6 = \underset{\text{Lactic acid}}{2C_3H_6O_3} \text{ (anaerobic bacteria)}$$

$$3C_6H_{12}O_6 = \underset{\text{Butyric acid}}{3C_4H_8O_2} + \underset{\text{Acetic acid}}{C_2H_4O_2} + 4CO_2 + 2H_2O + 2H_2$$
(anaerobic bacteria)

$$3C_6H_{12}O_6 = 2C_4H_8O_2 + 2C_2H_4O_2 + 4CO_2 + 2H_2O + 2CH_4$$
(anaerobic bacteria)

The decomposition of a carbohydrate may thus lead to the formation of butyric and acetic acids as well as certain gases, largely carbon dioxide and hydrogen or methane, or both together. This was actually demonstrated to take place in the anaerobic decomposition of cellulose.

In the decomposition of proteins, the amino acids which are formed may decompose further with the liberation of fatty acids:

$$\underset{\text{Amino acid}}{R\cdot CH(NH_2)\cdot COOH} + H_2O$$
$$= NH_3 + \underset{\text{Hydroxy-fatty acid}}{R\cdot CH(OH)\cdot COOH} = \underset{\text{Alcohol}}{R\cdot CH_2OH} + CO_2$$

$$\underset{\text{Glutamic acid}}{COOH\cdot CH_2\cdot CH_2\cdot CH\cdot NH_2\cdot COOH} + H_2$$
$$= \underset{\text{Butyric acid}}{CH_3\cdot CH_2\cdot CH_2\cdot COOH} + NH_3 + CO_2$$

The decomposition of fats and lipoids results in the formation of various fatty acids:

$$\underset{\text{fat (glyceryl tri-palmitate)}}{\begin{array}{l} CH_2\text{—}OOC\text{—}C_{15}H_{31} \\ | \\ CH\text{ —}OOC\text{—}C_{15}H_{31} \\ | \\ CH_2\text{—}OOC\text{—}C_{15}H_{31} \end{array}} + 3H_2O = \underset{\text{Glycerol}}{\begin{array}{l} CH_2\text{—}OH \\ | \\ CH\text{ —}OH \\ | \\ CH_2\text{—}OH \end{array}} + \underset{\text{Fatty acid (palmitic acid)}}{3C_{15}H_{31}\cdot COOH}$$

In addition to the organic acids, carbon dioxide is an important product of most of these reactions. The solvent action of organic acids compared to carbonic acid is suggested from the data in Table 40.

Under aerobic conditions, the organic acids do not persist for long in the soil, but are soon decomposed more completely, even to the final oxidation products, namely, carbon dioxide and water. Under anaerobic conditions, the acids may persist for extended periods of time.

With all of the acid substances, whether they be weak or strong, their effects on mineral materials are proportional to the degrees of acidity produced. Figs. 66 and 67 show this relationship. The stronger acids naturally cause solvent effects in low concentrations equal to those brought about by the weak acids in proportionately high concentrations.

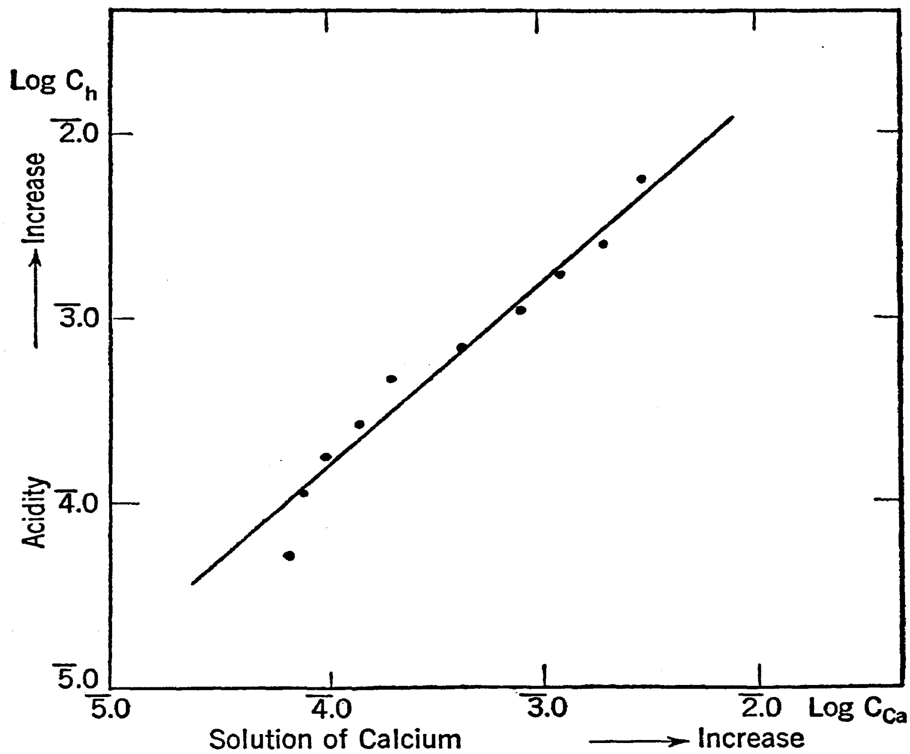

FIG. 66.—The influence of acidity created by lactic acid on solution of calcium from calcium silicate (after Wright).

CHANGE IN SOIL REACTION.—Besides being important as potential supplies of plant nutrients when acted upon by acid substances, the phosphates, carbonates, and silicates play an important rôle as agencies regulating the rapidity of change of soil reaction. In the absence of an abundance of buffering material in soils, the large amounts of acids which are produced would soon change the reaction to such acidity as to make the soil a medium unfit for the growth of most higher plants and of various important soil organisms, especially those that are sensitive to acid conditions, as Azotobacter, nitrifying bacteria, and *Bact. radicicola.* The presence of carbonates, phosphates, and silicates makes the soil strongly buffered and prevents rapid changes in reaction.

$$\underset{\text{Lactic acid}}{2CH_3 \cdot CHOH \cdot COOH} + CaCO_3 = \underset{\text{Calcium lactate}}{Ca(CH_3 \cdot CHOH \cdot COO)_2} + CO_2 + H_2O.$$

The acid may become neutralized by interacting with calcium carbonate. When the lactate is decomposed by other organisms, the calcium carbonate is regenerated:

$$Ca(CH_3 \cdot CHOH \cdot COO)_2 + 6O_2 = CaCO_3 + 5CO_2 + 5H_2O$$

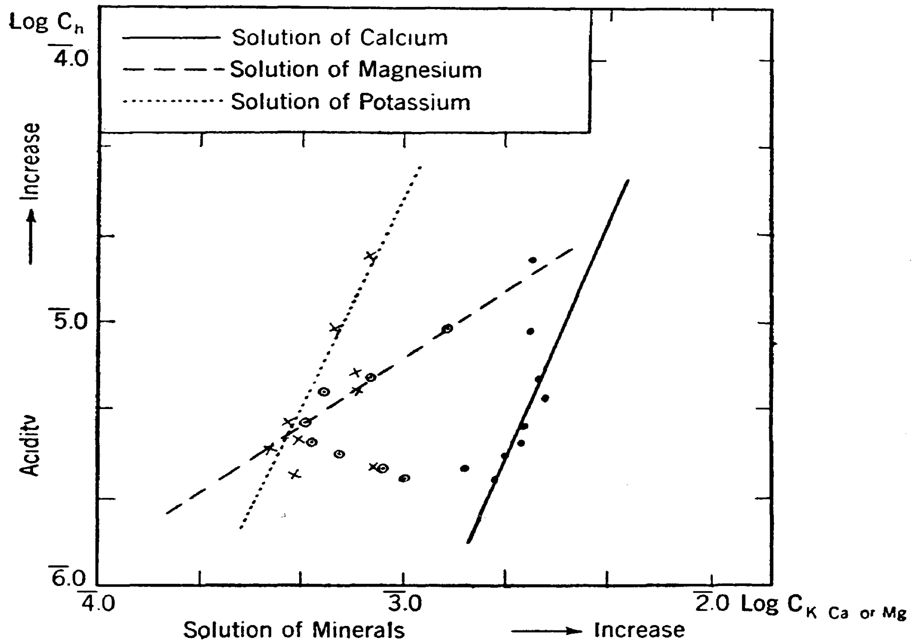

FIG. 67.—The influence of acidity created by growth of Azotobacter on solution of potassium, calcium, and magnesium from the mineral biotite (after Wright).

During the weathering of rocks, which are composed for the most part of minerals made up of strong bases (as calcium, potassium, magnesium, sodium) bound to a weak acid (silicic), there is a tendency for the development of an alkaline reaction. This is what happens in regions of deficient rainfall where the limited amount of drainage waters fails to remove the soluble salts as rapidly as they are formed. In the reclamation of alkali soils, the injurious effects of the basic reaction and high content of basic salts may be partly overcome by the addition of sulfur, which is oxidized by bacteria to sulfuric acid, as will be discussed later. In humid regions the weathering processes are accelerated by the more favorable moisture conditions and the soluble salts fail to accumulate in appreciable amounts. What amounts are not fairly rapidly absorbed by growing plants are soon removed in the percolating waters. The various acids which are so important in these weathering processes are not removed from the soil in the form of acids but are combined with various bases which arise from the compounds in the soil. It is the acceleration of the removal of basic substances in humid regions which so quickly causes the development of acidity and impoverishment in plant nutrients.

Mineral Assimilation by Microorganisms.—Minerals are of direct importance to the life of the soil organisms in that they enter the composition of the cells. Some of the mineral elements enter into organic compounds of the cell, while others play important parts as physiological agents in the cell fluids. Although required in smaller amounts than nitrogen, these minerals are nevertheless quite essential in certain amounts to the development of the living cells. In the decomposition processes carried out by microorganisms, much of the mineral content of the substances which undergo decomposition (such as plant residues) becomes liberated in forms available for subsequent development of higher plants. The phosphorus, sulfur, potassium, calcium, magnesium, and other minerals previously absorbed by the plant from the soil and stored up in its tissues again become liberated and made available for utilization by other plants. On the other hand, a part of these minerals becomes locked up in the microbial cell substance which has been synthesized during the decomposition process. This involves only a fraction of the minerals liberated in the decomposition of the plant residues, and represents the nutritional requirements of the microbial cells. The amounts liberated and not assimilated by the cells represent excess over these requirements and may be considered as waste products of the cells.

A detailed analysis is given here of the transformation of four minerals in the soil, namely, sulfur, phosphorus, iron, and potassium. From what has been said previously, one may conclude that many of the other minerals are also subject to various changes in the soil as a result of the activities of microorganisms, and that many of these minerals play important rôles in the growth of microbes and higher plants even if required only in mere traces.

Transformation of Sulfur in Soil by Microbes.—Sulfur exists in the soil and is added to it constantly in plant and animal residues, rain-water, and fertilizing materials, in several distinctly different forms, namely, as organic sulfur compounds, inorganic sulfates, sulfides, and elementary sulfur. In the organic matter of the soil, the sulfur is generally bound up in forms resistant to decomposition and only after long periods of time liberated in appreciable amounts as sulfate. The addition of elementary sulfur or sulfate in fertilizer materials may be responsible for relatively large amounts of sulfate in the soil solution from time to time, but, generally, sulfate makes up a small portion of the total sulfur content of the soil. More commonly from 80 to 90 per cent of the sulfur is present in organic combination, and only 10 to 20 per cent exists as sulfate.

The plant residues commonly added to the soil contain sulfur largely in organic combination, varying from 0.05 to 1.0 per cent. Alfalfa hay, for example, contains 0.29 per cent sulfur; turnip tops, 0.9 per cent; and wheat straw, 0.12 per cent. The sulfur in the organic compounds of such plant residues becomes liberated as sulfate more rapidly than from the so-called humic matter of the soil, just as ammonia is formed more rapidly from fresh organic matter rich in nitrogen than from the residual organic matter in the humus.

Under natural conditions, many agents, both biological and non-biological, carry the element through its course of changes. Microbial effects are largely oxidations and reductions of the inorganic sulfur compounds, and formation and decomposition of organic compounds containing sulfur. Since sulfur enters into the composition of all living cells, all forms of life become associated with its transformation. In the decomposition of the organic residues by microbes, the sulfur present in organic combination is changed, after several transformations, into inorganic forms such as hydrogen sulfide and sulfate.

Some microorganisms utilize sulfate to satisfy their requirements for the element. In time, the microbial cells become destroyed and mineralized. The assimilation of sulfate is more pronounced when the organic matter which serves as food contains little or no sulfur. Table 42 shows the rapid disappearance of sulfate when an organism (*Aspergillus niger*) is feeding on a sugar.

Sulfur is present in the organic matter largely in the protein molecule. This molecule contains an amino acid, cystine, which

TABLE 42

DISAPPEARANCE OF SULFATE DURING GROWTH OF ASPERGILLUS NIGER ON A CARBOHYDRATE (FROM RIPPEL)

Time	Sulfate-sulfur, mgm.
At start	3.19
After 5 days	1.58
After 7 days	0.64
After 12 days	0

is the sulfur carrier. When proteins are hydrolyzed by microorganisms, the cystine is first liberated. When the cystine molecule is decomposed by microorganisms in the soil, the sulfur is usually liberated in the form of hydrogen sulfide:

$$\underset{\text{Cystine}}{\begin{array}{lll} CH_2\text{—}S\text{—}S\text{—} & CH_2 & \\ | & | & \\ CH\cdot NH_2 & CH\cdot NH_2 & + 4H_2O \\ | & | & \\ COOH & COOH & \end{array}}$$

$$= \underset{\text{Acetic acid}}{2CH_3\cdot COOH} + \underset{\text{Formic acid}}{H\cdot COOH} + CO_2 + 2NH_3 + 2H_2S$$

Other organic compounds containing sulfur are frequently introduced into the soil, but generally in very small amounts. These include taurine, among the animal products, and certain glucosides, among the plant residues.

The nature of the compound as well as the environmental conditions in which it is decomposed, whether aerobic or anaerobic, will determine to a large extent the resultant form of the sulfur when it appears in the inorganic state. Under anaerobic conditions sulfides are frequently produced in considerable quantities. This is the case in water-logged soils, ditches, stagnant pools, and seas that receive appreciable contributions of organic materials and sulfates. Coloration of soils may be determined by sulfides of iron under anaerobic conditions where organic matter is not so abundant as to conceal this coloration. These sulfides are formed from the decomposition of organic compounds containing sulfur and from reduction of sulfates, sulfur, or other inorganic, incompletely oxidized sulfur compounds.

Direct evidence is still lacking to indicate that microorganisms convert sulfur, associated in organic compounds, directly to sulfate. Some sulfur may be liberated in this way, but a greater portion is first changed to sulfide and appears as sulfate only after secondary attack by organisms able to oxidize inorganic sulfur compounds. Wherever protein materials undergo decomposition by the agency of bacteria, sulfide is formed. Under anaerobic conditions it is not transformed further, but under aerobic conditions it soon disappears and is oxidized to sulfate, as may be represented by the following equation:

$$H_2S + O \rightarrow H_2O + S \xrightarrow{+\ 3O} H_2SO_4$$

Various specific bacteria (largely of the Thiobacillus group) are capable of bringing about these processes in the soil (see Fig. 15). The hydrogen sulfide is first oxidized to elementary sulfur. In the case of some bacteria, this sulfur actually accumulates within or without the cells. Other bacteria, however, immediately oxidize the sulfur to sulfuric acid. This acid interacts with the soil bases:

$$H_2SO_4 + CaCO_3 = CaSO_4 + H_2O + CO_2.$$

Sulfide may be considered as an intermediate decomposition product. It is unavailable to plants as such, is injurious rather than beneficial to the activity of most soil microorganisms, and its presence in soils in any appreciable amounts is indicative of partial anaerobic conditions unsuited for plant growth. Sulfide, like any other decomposition product such as ammonia, is a waste product left by the microorganisms as unnecessary in their further development. Its formation as a product of decomposition of organic matter indicates that the compounds from which it was produced contained more sulfur than was required by the organisms in their growth while using this organic matter as food. As hydrogen sulfide it represents a source of considerable potential energy which will become liberated during its transformation to sulfate.

After elementary sulfur is added to soil, it is oxidized directly to sulfuric acid if the proper organisms are present. The oxidation proceeds particularly rapidly where some of the specific sulfur bacteria are concerned. These organisms are similar in their nutrition to the nitrifying bacteria in that they are autotrophic, but they use the sulfide or sulfur as their specific foods, oxidizing them to sulfate. Even in the absence of these organisms, sulfates are formed, presumably in some way associated with the development of various heterotrophic organisms including both bacteria and fungi. The process of sulfur oxidation can be utilized for increasing the acidity of the soil, and in view of the fact that certain organisms producing plant diseases cannot thrive at certain acid concentrations, it may be desirable to create such acid conditions as are inhibitive to their development. This is brought about by adding sulfur to soils. In the soil, it is oxidized to sulfuric acid and brings about the desired effects as considered in more detail elsewhere. This process can also be utilized for reducing the alkalinity of black alkali soils. The sulfuric acid formed by the oxidation of sulfur interacts with the sodium carbonate, giving sodium sulfate. The latter salt of sodium is much less injurious to plants than the former; it has less undesirable effects on the physical condition of the soil and it can also be more readily removed by irrigation and drainage waters. The oxidation process is further utilized in transforming the insoluble phosphate of the rock phosphate to more soluble forms. When sulfur is mixed and composted with rock phosphate and soil, in the proper proportions, the sulfur becomes oxidized to sulfuric acid and changes the phosphate to the di-calcium and mono-calcium forms. The application of such material to soils gives much the same effects as the application of "superphosphate." Similar solution of potassium occurs when certain insoluble minerals containing potassium are substituted for the rock phosphate in these composts.

Large amounts of sulfide may be produced in nature by the reduction of sulfates as well as elementary sulfur itself. These processes consume appreciable amounts of energy which must be supplied from other sources, such as the oxidation of organic compounds. Further, the reaction proceeds only under anaerobic conditions. In such an environment the reactions take place somewhat as follows:

$$2S + 2H_2O + \underset{\text{Glucose}}{C_6H_{12}O_6} = \underset{\text{Acetic acid}}{2CH_3\cdot COOH} + \underset{\text{Formic acid}}{2H\cdot COOH} + 2H_2S$$

$$3CaSO_4 + \underset{\text{Sodium lactate}}{2(C_3H_5O_3)Na} = 3CaCO_3 + Na_2CO_3 + 2H_2O + 2CO_2 + 3H_2S$$

In these reductions the sulfur and sulfate act as oxidizing substances, and serve, under these anaerobic conditions, in much the same capacity as oxygen itself does in an aerobic environment.

In case conditions are changed, after the formation of sulfide, so as to permit the entrance of free oxygen, thus creating aerobic conditions, the sulfide does not persist but is oxidized again to sulfate. In certain lakes and seas, as well as in a number of curative muds, the two processes, namely, the oxidation of hydrogen sufide to sulfate and the reduction of sulfate to hydrogen sulfide, may go on side by side. In the lower layers of the lake or sea where free oxygen is scarce, the reduction process predominates. The hydrogen sulfide once formed moves upward in the convection currents or as bubbles of gas, and at or near the surface of the lake, on coming in contact with the oxygen of the air, it is oxidized to sulfate by specific oxidizing bacteria. The sulfate may diffuse downward again, where it is again reduced to sulfide under the anaerobic conditions.

The reduction of sulfur to sulfide may be brought about by a great number of different bacteria, but the reduction of sulfate is limited to very few organisms, although these are widely distributed. *Spirillum desulfuricans* is the name generally applied to the reducing form which is found in fresh water and soils (see Fig. 16).

As this short discussion indicates, there are striking similarities between the transformations of various compounds of sulfur and nitrogen through the agency of microorganisms. Organic compounds of nitrogen decompose to form ammonia, while the sulfur compounds lead to hydrogen sulfide. Ammonia is changed to nitrite and nitrate, while sulfide is oxidized to sulfur, sulfate, and numerous incompletely oxidized inorganic substances. These oxidations are all the result of the action of specific autotrophic bacteria. Both nitrates and sulfates may be reduced by anaerobic bacteria, leading to a variety of products including ammonia and hydrogen sulfide. Although a process like nitrogen fixation is not found in sulfur transformations, the reactions associated with oxidation and reduction of elementary sulfur are similar in certain respects. Inorganic and organic nitrogen compounds furnish different microorganisms with their requirements for this element, and similar sulfur compounds serve a like purpose in microbial metabolism. In fact, most of the nitrogen transformations have very close counterparts in changes of sulfur compounds.

Transformation of Phosphorus by Soil Microbes.—Phosphorus is usually added to the soil in the form of various plant and animal residues (in stable and green manures), in certain organic fertilizers (tankage, bone meal, guano), and in various inorganic fertilizers. Phosphorus is present in the latter either as insoluble rock phosphate, the chief constituent of which is $Ca_3(PO_4)_2$, or as superphosphate, which consists of rock phosphate previously treated with sulfuric acid to make it soluble, or as various other basic phosphates included in slag.

In the plant and animal residues, as well as in stable and green manures, phosphorus is present in the form of such compounds as phytin, phospholipids, of which lecithin is the best known representative, and nucleoproteins. Before the phosphorus can be utilized again by higher plants, these organic complexes have to be decomposed by various soil organisms. These processes of decomposition can be illustrated somewhat as follows:

$$\underset{\text{Phytic acid}}{C_6H_{24}O_{27}P_6} + 6O_2 = 6H_3PO_4 + 6CO_2 + 3H_2O$$

The phytic acid originates in the decomposition of phytin. Lecithin contains both phosphorus and nitrogen; it is first hydrolyzed to cholin (a nitrogen complex), glycerophosphoric acid, and various fatty acids. Nucleoproteins are compounds of one or more protein molecules with nucleic acid; the latter contains the phosphorus. Nucleic acids themselves are also very complex in composition, such as $C_{36}H_{48}O_{30}N_{14}P_4$. On decomposition, they give, in addition to phosphoric acid, various carbohydrates and organic bases (adenine, guanine, cytosine). The decomposition processes of some of these substances are frequently very complicated, involving a number of reactions, but they all lead to the formation of phosphoric acid.

The phosphorus in organic combination may make up from 20 to 35 per cent of the total phosphorus of many soils. Organic phosphorus may also compose a large portion of the phosphorus occurring in solution in soils. In a study of twenty-one soils of Southern and Central United States, the soluble organic phosphorus (calculated as P_2O_5) was present in 0.176 parts per million of soil solution, while the inorganic phosphorus was found in only 0.034 parts per million.

The microbial cells which are constantly formed in the soil are also very rich in phosphoric acid; the ash of the microbes frequently contains 50 per cent or more phosphate, calculated as P_2O_5. Largely as a result of this consumption of phosphorus by microbes and its use in cell synthesis, the organic matter of the soil contains a definite amount of phosphorus. The ratio of the carbon content of the soil to the organic phosphorus content is more or less constant, just as is the case in the ratio of the carbon to nitrogen. When the organic matter of the soil or that added to the soil is decomposed, there is a continuous liberation of phosphorus in an inorganic form, and the more rapidly the organic matter is decomposed the more rapidly the phosphorus is liberated. In the presence of an abundance of available organic food material containing little or no phosphorus, the microbes will reassimilate part of this phosphorus in the synthesis of their cell substance, and a deficiency of phosphorus available for plant growth may be created. The amount of food material used by microorganisms for growth, the amount of microbial cell substance synthesized from the food, and the quantities of both nitrogen and phosphorus which are assimilated in these processes are all in definite ratios to one another. In other words, a definite amount of microbial cell substance is formed per unit of organic food material consumed, and quite definite quantities of nitrogen and phosphorus are built into these cells.

Various fungi and bacteria are capable of liberating phosphorus from organic complexes in an inorganic form. Some bacteria are particularly active in this connection since they are capable of attacking the phosphorus-bearing complexes more readily.

Processes similar to those causing reduction of nitrates and sulfates may be concerned in the reduction of phosphates. Nitrates are quite readily reduced; sulfates, with somewhat more difficulty; and phosphates, least readily of all. Under anaerobic conditions, if organic food materials are available, if phosphates are present in abundance and if the necessary bacteria are active, phosphates may be reduced to phosphite (H_3PO_3), hypophosphite (H_3PO_2), and phosphine (PH_3). The reactions may be illustrated in a general way as follows, where C refers to carbon in organic combination:

$$\underset{\text{Phosphoric acid}}{2H_3PO_4} + C = \underset{\text{Phosphorous acid}}{2H_3PO_3} + CO_2$$

$$H_3PO_4 + C = \underset{\text{Hypophosphorous acid}}{H_3PO_2} + CO_2$$

$$H_3PO_4 + 2C = \underset{\text{Phosphine}}{PH_3} + 2CO_2$$

The possible significance and importance of these reactions in the movements of phosphorus in arable soils are unknown. However, since these processes are favored only by rather extreme reduction conditions, it seems likely that phosphate reduction is not responsible for very extensive changes under most soil conditions. The extent of reduction of phosphates to the various substances by bacteria under conditions favorable to the changes is shown in Table 43.

TABLE 43

REDUCTION PRODUCTS OF PHOSPHATE FORMED BY A BACTERIUM (FROM RUDAKOV)

	Mgm. P_2O_5 per Liter			
	Reduced to phosphine	Reduced to phosphite and hypophosphite	Remaining as phosphate	Biologically absorbed or changed to other compounds
Control	....	....	2500	
Inoculated	102	467	1306	625

The insoluble inorganic calcium phosphate may be present in the soil in the native rock constituents or occur there subsequent to the addition of various fertilizer mixtures. This form of phosphate is available to plants only in very limited amounts. However, as a result of interaction with the various organic and inorganic acids formed by microbes, the phosphate becomes changed into di- and mono-basic phosphates, which are more soluble and consequently more readily available to plants. In view of the fact that the formation of the inorganic and organic acids takes place in the soil constantly as a result of the decomposition and oxidation processes, insoluble phosphates tend to become gradually soluble, especially when accompanied by processes of active decomposition of organic matter and oxidation of ammonia and incompletely oxidized sulfur compounds. When the soil contains free carbonates and the reaction is neutral or alkaline, the acids will be neutralized by the carbonates in preference to the phosphates, and the latter will not go into solution so readily.

The amount of phosphoric acid made available to plants at any given time is thus found to be a result of various complex biological and chemical processes which tend either to bring the phosphoric acid into solution (when the decomposition processes are predominant) or take it out of solution (when the synthesizing microbial processes are active and the amount of available phosphate is limited). The amount of available phosphorus in any given soil can be readily and simply determined by microbiological procedures as discussed in Chapter IX.

TRANSFORMATION OF IRON BY SOIL MICROBES.—Iron is seldom added to soils in fertilizing materials and is contained in organic residues in very limited quantities, but it is one of the elements present in the soil in very large amounts. In spite of the great abundance of the element in various compounds in soils and in spite of the fact that it is required by plants in very small amounts, it is so slightly soluble in alkaline soils that the supply of plants with soluble compounds of iron is a serious problem under such conditions. Iron is of considerable importance in the physical composition of soils, and in extreme cases its solution in the upper layers and precipitation in localized deeper regions of soils may be so extensive as to play an important part in the formation of impervious layers called "hardpans."

Microorganisms are associated with the movement of iron in the soil in various ways. Among these changes may be included solution and precipitation of iron compounds; these reactions are frequently accompanied by reduction and oxidation processes.

In the precipitation of iron from soluble inorganic compounds of the element, the *iron bacteria* have been considered to play a preeminent part. These organisms are so named since iron in the reduced form serves as their source of energy for growth, and they live by the oxidation of ferrous to ferric iron and, like other autotrophic organisms, obtain carbon for their cell development from the carbon dioxide of the air. Incidentally, subsequent to oxidation of the iron during growth of the bacteria, the ferric compounds precipitate and accumulate about the cells in relatively large amounts. Organisms of this type are but one group of many that may be concerned with transformations of iron; however, they are probably little concerned with the changes of iron in the soil (Fig. 68).

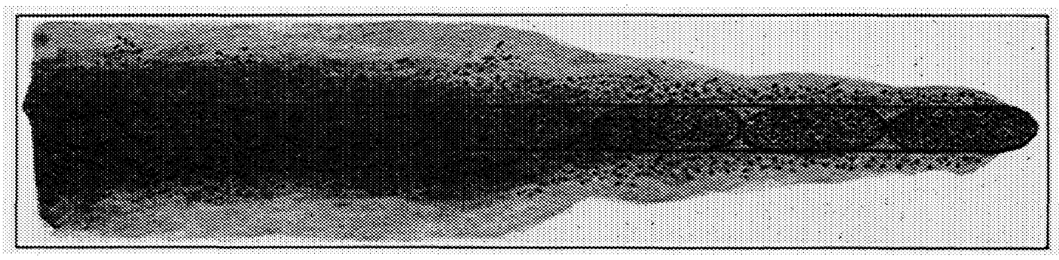

FIG. 68.—Filament of an iron bacterium, *Leptothrix crassa*, showing incrustation of ferric hydrate (after Cholodny).

Certain organic compounds of iron are quite soluble. After the utilization of the organic portion of these compounds as food by the heterotrophic microorganisms, the inorganic compounds of iron which are formed in the process are precipitated, since they are soluble in only very small amounts. As an example we may consider ferric ammonium citrate, a double salt of the organic citric acid. Upon decomposition, the citrate is changed to various products, including carbon dioxide and water. This liberates the ammonia as ammonium hydrate and the iron as ferric hydrate. The iron hydrate precipitates by reason of its slight solubility. Precipitation by iron bacteria and by the organisms decomposing organic compounds is more likely to occur under aerobic conditions. Under anaerobic conditions, iron may be precipitated as sulfide, which is formed either from organic compounds or from the reduction of inorganic compounds of sulfur.

Solution of iron may follow the formation of any of the acids produced by microorganisms in soils, among which carbonic, sulfuric, nitric, and the various organic acids are especially important. Since iron is so slightly soluble at reactions most commonly suited to growth of higher plants, the formation of certain amounts of these soluble compounds in the vicinity of the developing plant roots may be of particular importance in their nutrition.

Thus, under aerobic conditions, iron may be brought into solution provided the reaction becomes more acid. Under anaerobic conditions, the solution is greatly accelerated by reason of the fact that iron becomes reduced to the ferrous form and in this condition is much more soluble than in the ferric form at reactions close to neutrality. Solution is further favored by the fact that anaerobic conditions lead to the formation of organic acids which exert considerable dissolving effects.

Most of these changes in the amounts of iron in solution follow changes in the environmental conditions by microbial activities. In general, the development of anaerobic conditions or the formation of acids results in the solution of iron and usually its reduction. Either a change from anaerobic to aerobic conditions, or a decrease in acidity, or both, lead to oxidation of iron and may cause its precipitation. The fact that iron is readily oxidized and reduced greatly increases the capacity of microorganisms to modify its condition in the soil.

Transformation of Potassium by Soil Microbes.—Potassium is considerably less susceptible to effects of microorganisms in its relationships to plant development in soils. It belongs to a large group of elements in soil which are not affected in a great variety of ways, on account of the fact that they do not enter into organic combination to the extent of such elements as nitrogen or sulfur. Its entrance into organic compounds is more generally confined to replacing the hydrogen of the acid groups where it forms the salts of these acids. In plant residues a large portion of the potassium is present in inorganic form, occurring in the various fluids of the cells. Upon ignition of the organic substances the potassium remains in the ash residue. Another factor appreciably limiting the capacities of microorganisms to affect the element is its stable character. It is not oxidized and reduced and consequently cannot be carried through the extensive series of changes common to nitrogen, sulfur, and iron. The influence of microorganisms on such elements as potassium is confined to causing its solution from organic and inorganic substances and its assimilation in growth of the microbial cells.

Considerable potassium becomes added to soils in the form of various organic and inorganic compounds. Stable manures, green manures, plant stubble, and microbial cells all contain potassium. When these organic substances are decomposed by microorganisms in the soil, the potassium is liberated in forms available to higher plants. A small part of this potassium may be reassimilated by the microbes which bring about the decomposition processes, thus part of the potassium may be temporarily removed from circulation. The ash of bacteria and fungi usually contains between 5 to 40 per cent of potassium calculated as K_2O.

The inorganic forms of potassium added to the soil as fertilizers are generally soluble, but the minerals in the soil which contain potassium are quite insoluble. Solution of potassium from these minerals is accelerated by interaction with various acids produced by microorganisms, as shown in the case of orthoclase:

$$Al_2O_3 \cdot K_2O \cdot 6SiO_2 + 8HNO_3 = 2Al(NO_3)_3 + 2KNO_3 + 6SiO_2 + 4H_2O$$

The products of both nitrification and sulfur oxidation, as well as the carbonic acid produced by the microbial population in general, aid in such solution of potassium.

Importance of Mineral Transformation by Microbes in Soil Formation.—The various inorganic constituents of the surface of the earth are subject to a number of changes as a result of the activities of the numerous soil-inhabiting microbes, especially in the presence of organic substances. These changes cause continuous modifications in the nature of the physical and chemical composition of the surface of the earth, which for convenience is divided into layers, called the surface soil and subsoil, or horizons A, B, and C.

There is evidence which tempts speculation regarding the rôle played by microorganisms in the development of this soil layer from the rocks, which were formed subsequent to the cooling of the earth's surface. The presence of carbon dioxide in the atmosphere, the formation of small amounts of ammonia and nitrous acid by electrical discharges, the presence of sulfur and of various minerals containing phosphorus, potassium, calcium, magnesium, iron and other necessary elements in the earth's crust, was sufficient to allow the growth of autotrophic bacteria. These were capable of obtaining their required energy for growth from the ammonia, nitrous acid, and sulfur, and could satisfy their requirements for carbon from the carbon dioxide of the atmosphere. As a result of the activities of these primitive organisms, considerable amounts of organic matter might have been formed. The acids, especially nitrous, nitric, and sulfuric, would cause dissolution of the rocks and aid in the weathering processes. Some of the minerals thus brought into solution might have been appropriated by the organisms for the synthesis of their cell substance. The rest of the minerals might be washed away by streams, or accumulate, giving rise to the beginning of the inorganic part of the disintegrated surface layer of the earth. The two processes of dissolution of rock constituents and synthesis of organic matter were the beginnings of the origin of the soil.

To these reactions might be added the activities of the nitrogen-fixing bacteria, both alone and in symbiosis with algae, which could have contributed to increases in the supply of fixed nitrogen on the surface of the earth. Algae themselves are known to have a corroding effect upon stones, and, in association with various bacteria, the extraction of mineral substances from rocks is further increased.

The general phenomena considered in these pages should quite definitely indicate that microbes have played parts of major importance in rock disintegration and in soil formation. Although the climatic factors exert regulating influences on the decomposition processes brought about in the soil, an inquiry into the activities of microbes reveals a great diversity of action and the indispensable nature of their rôles in soil formation. In some cases they assist the purely chemical, physical, and mechanical transformations; in some cases they take an active part in the transformation processes; and in other cases they play the dominant rôle, as in the case of formation of forest soils and peat soils.

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CHAPTER VIII

INTERRELATIONSHIPS BETWEEN HIGHER PLANTS AND SOIL MICROORGANISMS

INTERDEPENDENCE OF HIGHER PLANTS AND MICROBES.—To casual observation, the development of higher plants may appear to be an isolated phenomenon. Each plant seems to be quite capable of developing independently of other living things, transforming sunlight into available food and drawing from the soil its requirements of mineral nutrients. However, although one cannot accurately estimate the absolute extent to which the activities of other forms of life are concerned with this apparently isolated development of the higher plants, careful observation of the conditions about plant roots indicates that there is a striking dependence of higher plants upon microorganisms. This is particularly the case in the absorption by plants of mineral nutrients from the soil, and in some cases also in the absorption of organic substances from the soil. The carbon dioxide of the atmosphere, upon which the plant depends for the synthesis of its body substance, also results from the activities of microorganisms. These, however, depend upon the plant and its residues for their energy sources and other nutrients.

Neither higher plants nor microorganisms can develop long in nature in the absence of the other without showing certain definite abnormalities. Consequently, the fact that plant growth continues in soil and that microorganisms are increasingly active in this habitat are suggestive of the associative development of roots and microorganisms in soil.

Some of these effects are only indirect, while others are associated with a very intimate development of the plant and the microbe, even to the actual penetration of the plant by the microbe. In fact, it is difficult to determine what activities of microorganisms in soils do not exert certain effects on plant growth; it is equally difficult to differentiate between those activities of plant growth which do or do not affect microbial development, directly or indirectly.

The plant withdraws from the soil through its root system a considerable amount of substances, principally inorganic; it consumes the supply of carbon dioxide which is continually evolving from the soil; it renders the soil more porous by the penetration of its roots; it exerts a solvent effect by the excretion of carbon

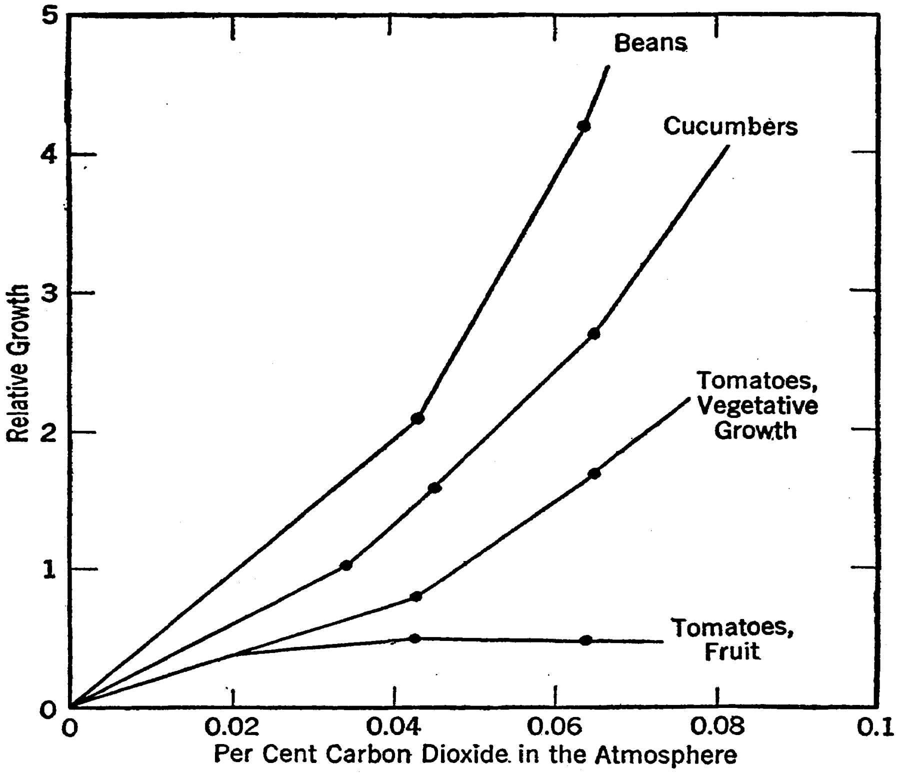

FIG. 69.—Influence of concentration of carbon dioxide in the atmosphere on growth of plants (after Lundegardh).

dioxide; eventually the plant itself returns to the soil and undergoes disintegration through the agency of the microorganisms.

The activities associated with the complex transformations of organic and inorganic substances in soils are largely regulated by microbial development. While practically all organic compounds disappear under favorable conditions in soils, the course and speed of their transformation, the agents involved in the change, and the immediate and ultimate results on plant growth vary greatly. There may be an immediate or delayed benefit to plant growth determined by the nitrogen content of the substances concerned.

Providing that the nitrogen content of the material under consideration is sufficiently great, there may be a rapid transformation of the nitrogen to forms available for plant growth.

Non-symbiotic nitrogen fixation may, under certain conditions, be sufficient to produce appreciable effects upon plant development. Microbial disintegration of plant residues and organic fertilizers

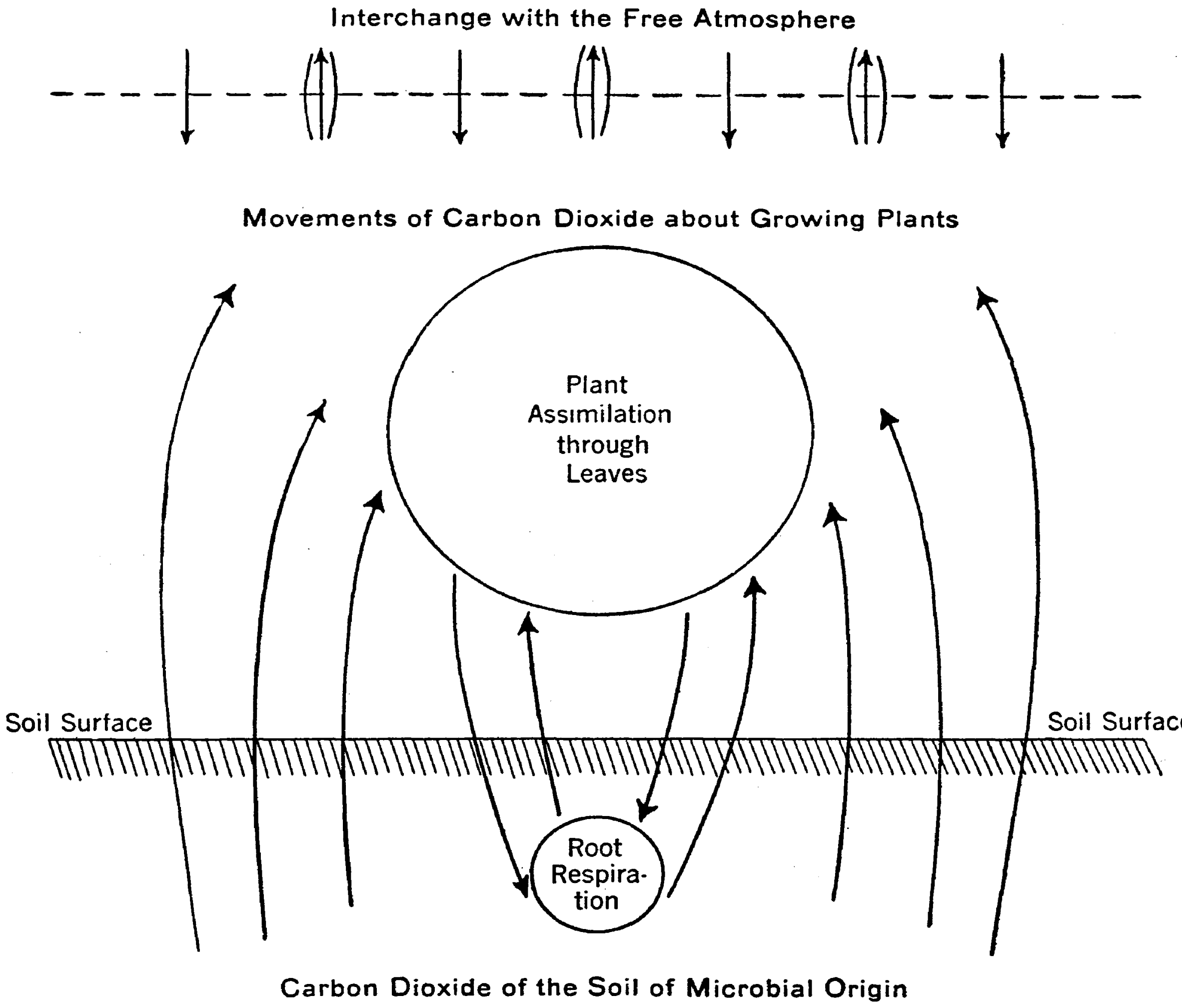

FIG. 70.—Diagrammatic representation of the movements of carbon dioxide between soil and atmosphere about plants (after Lundegardh).

may exert beneficial effects on the development of higher plants, not only through the roots but also through the aerial parts of the plant; this effect is associated with the increase in the concentration of carbon dioxide in the zone of leaf absorption. During the warm seasons and in periods of bright sunlight the absorption of carbon dioxide appears to be more rapid than its supply from the soil. This is clearly apparent from Fig. 69. Where light intensity is low, little or no increase in plant growth follows an increase in the carbon dioxide content; the assimilation processes of plants under such conditions are limited by the low light intensity. Any acceleration of decomposition processes by soil organisms tends to offset deficiencies of carbon dioxide in the atmosphere. Generally, however, the content of carbon dioxide is below the level for maximum plant development in brilliant sunlight. The movements of the gas between soil, atmosphere, and plant are shown in Fig. 70.

Evolution of Carbon Dioxide.—The carbon dioxide evolved from soils is almost entirely of biological origin, although some may arise from soil carbonates. The origin of this gas is divided between the activities of plant roots and soil microorganisms. Whether roots or microorganisms produce most of the gas is determined by the type of plant and soil conditions. Generally the roots of higher plants produce much less carbon dioxide than the microbes. In very sandy soils deficient in organic matter, the roots may give rise to much the greater amount of carbon dioxide, as shown from certain results obtained by Lundegardh:

Respiration of sandy soil and plant roots. 0.45 mgm. of carbon dioxide per hour
Respiration of sandy soil .... 0.14 mgm. of carbon dioxide per hour
Respiration of plant roots .... 0.31 mgm. of carbon dioxide per hour
Per cent of the carbon dioxide liberated
from plant roots 68.9

Under most soil conditions, however, plants produce a smaller portion of the total carbon dioxide which comes from the soil.

TABLE 44

Carbon Dioxide Produced per Square Meter per Hour in Soil Supporting Plant Growth and in Bare Soil (from Lundegardh)

Date	Bare soil, gm.	Soil growing oats, gm.	Increase due to plant development, gm.	Per cent of the gas due to plant development
June 29	0.220	0.273	0.053	19.4
July 24	....	0.250		
July 27	0.216	....	0.034	13.6
August 10	....	0.399		
August 14	0.268	....	0.131	32.8

According to the results given in Table 44, it seems likely that as much as one-third of the carbon dioxide may originate from roots at certain stages of plant development. The gas which arises from the plant roots may come from two sources: (1) from actual elimination of the gas from the root cells, as a product of cell respiration; (2) from microbial decomposition of organic exudation products of the roots. Which of these two sources may supply the greatest amounts of carbon dioxide is determined by the age of the plant and its environmental conditions. In general, the larger portion of the gas comes from root respiration, but at times as much as 45 per cent of the gas may be the product of microbial activities, as shown by Lundegardh:

Root respiration in unsterilized sand	5.57 mgm. per hour
Root respiration in sterilized sand	3.05 mgm. per hour
Difference due to microorganisms	2.52 mgm. per hour

This difference due to microorganisms was equal to 45 per cent of the total amount produced as a result of the plant growth. Consequently, although gas arising from roots as a product of plant respiration may be considerable, the amounts proceeding from microorganisms acting upon root materials and other soil substances are far greater.

It may be assumed that 30 mgm. of carbon dioxide are evolved in 24 hours per kilogram of soil to a depth of 16 inches. Assuming that there are 2,024,000 kgm. of soil per acre to a depth of 16 inches, there would be evolved 60.72 kgm. of carbon dioxide per day. Since more of the gas is produced during the warm than the cold months, it may be expected that most of the gas would be evolved during 200 days of the year in the temperate zone. Thus there would be produced about 12,144 kgm. of carbon dioxide from each acre of soil during the year.

It has also been determined that the daily evolution of carbon dioxide by a cereal plant is about 30 mgm. Assuming a growth period of 100 days and that there are 810,000 plants per acre, there would be about 2,430 kgm. of carbon dioxide produced by plant roots. From these calculations it appears that plants may be an important source of carbon dioxide which is formed within the soil. During the active growing period there may be times when more of the gas comes from the roots than from the microbial activities in certain regions of the soil, but over the entire year, considering the soil both about roots and at a distance from roots, the soil microorganisms produce a much larger amount of carbon dioxide.

The development of plants not only increases the production of carbon dioxide from soils by contributing some gas from root respiration, but also favors microbial processes to such an extent that the organic matter already in the soil is more rapidly decomposed. This is shown in Table 45.

TABLE 45

THE INFLUENCE OF PLANT DEVELOPMENT UPON FORMATION OF CARBON DIOXIDE FROM THE ORGANIC MATTER CONTAINED IN THE SOIL PREVIOUS TO PLANTING (FROM NELLER)

Plant	Carbon Dioxide from the Soil (Mgm.)			
	Planted soil	Unplanted soil	Increase due to planting	Per cent increase due to planting
Soybeans	2,093.4	1,737.0	356.4	20.5
Soybeans	2,128.2	1,281.8	846.4	66.0
Soybeans	4,795.2	2,320.7	2,474.5	106.6
Soybeans	2,403.6	1,196.8	1,302.1	108.8
Buckwheat	953.1	440.1	513.0	116.6
Field peas	751.6	440.1	311.5	70.8
Wheat	2,403.6	1,196.8	1,206.8	100.8
Barley	3,274.9	2,532.4	742.5	29.3

It is further apparent from Fig. 71 that plants increase the evolution of carbon dioxide in the soil, but the course of formation of the gas appears to be distinct for each plant and related to differences in characteristics of growth of the plants. Slight effects appear in the early stages of growth, and abundant formation of carbon dioxide occurs when the plants reach stages of extensive vegetative development. During degeneration the gas is produced in less abundance, and subsequent to death there is little excess over that produced where plants are not developing. Since biennials have a much longer growing period than the annuals, their influences upon the soil are much more prolonged.

The formation of carbon dioxide about plant roots, whatever its origin, is particularly important in plant development in that its solvent action is one of the principal agencies responsible for the solution of relatively insoluble soil minerals containing phosphorus, potassium, calcium, and magnesium. The development of

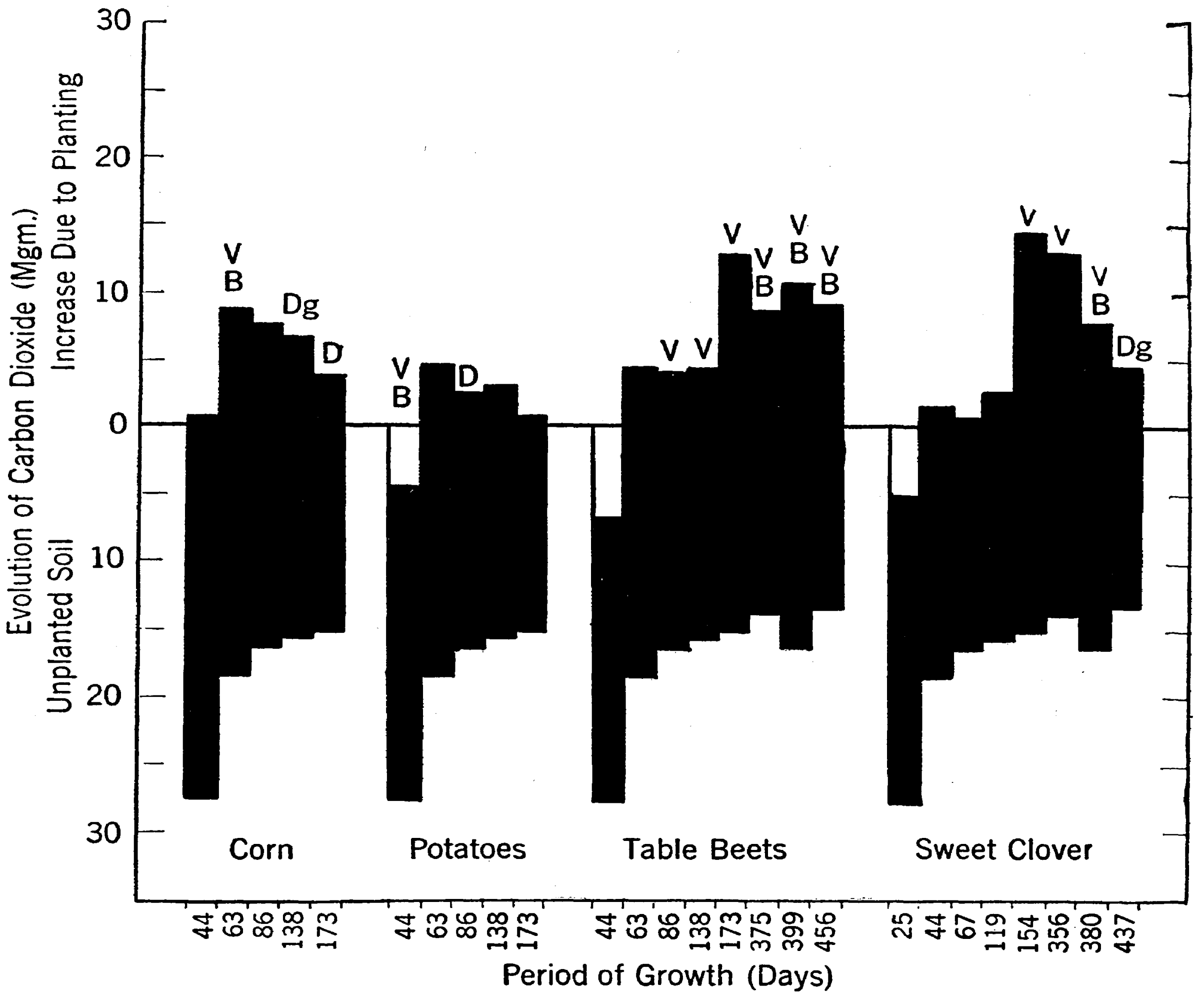

Fig. 71.—Influence of plant development upon the evolution from the soil of carbon dioxide of microbial origin; V—height of vegetative development, B—blooming, Dg—degeneration, D—death (after Starkey).

organisms on the roots greatly increases this corrosive action, and is a very important factor in determining the feeding power of plants. There appear to be certain factors, not correlated with either the extent of the root system or the amount of carbon dioxide produced, which determine the ability of plants to absorb certain proportions of the various nutrients, at least where the plants are dependent upon relatively soluble sources of inorganic nutrients. In addition to that, the production of relatively large amounts of carbon dioxide in soil is very important in determining the ability of plants to absorb nutrients from relatively insoluble compounds. Organic acids are eliminated by roots in only limited amounts under conditions favorable to plant growth and, consequently, the solvent action is almost entirely exerted by the carbon dioxide which is evolved.

The extent of the increase in solvent effects due to micro-

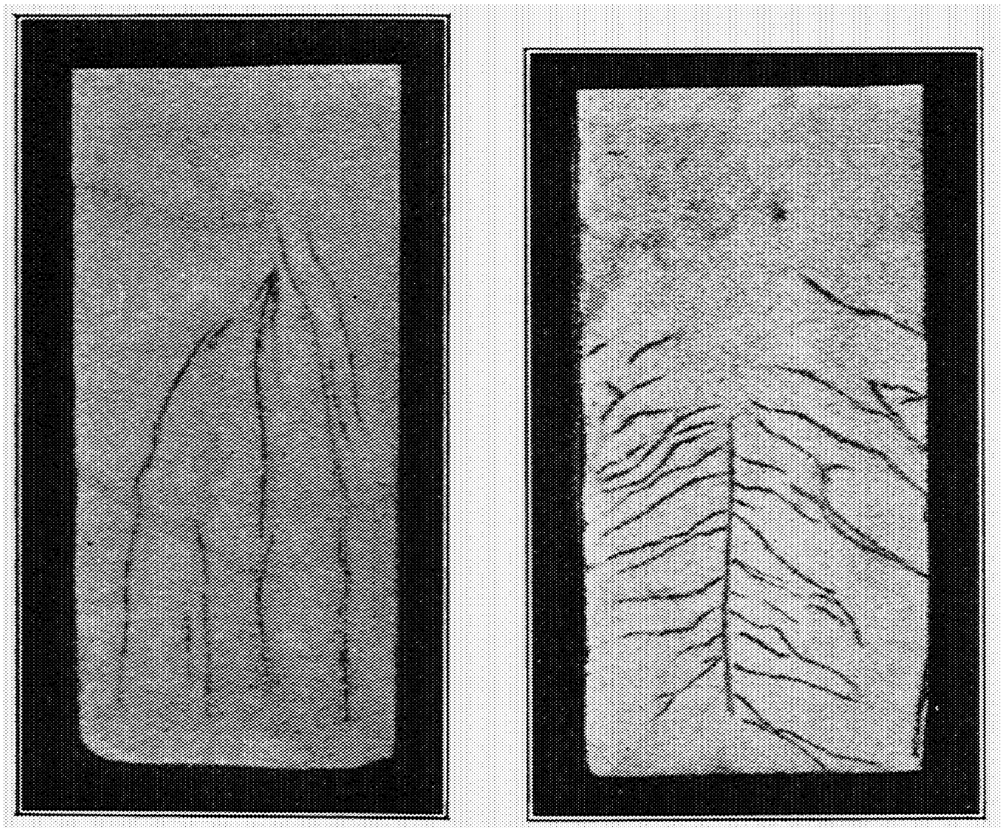

FIG. 72.—Etching of marble as a result of root development. No microbes were present on the roots which etched the marble on the left. The greater etching of the slab on the right was due to acids produced by microorganisms developing on the roots (from Fred and Haas).

organisms is suggested by Fig. 72. Plants were grown both under sterile conditions and in the presence of microorganisms, in such a manner that the roots passed over marble slabs. Where the roots were free of microbes there was much less etching of the marble than where they were present. These effects are largely associated with differences in amounts of carbonic acid produced in the absence and presence of microbes.

The factors responsible for the various changes in microbial activity about plant roots are numerous. There are physical effects produced by the addition of organic matter from plant roots, by the liberation of carbon dioxide from the plant roots and microorganisms, by root penetration opening passages in the soil, by absorption of inorganic nutrients and large quantities of water. The dominating influence of the plants on soil organisms is most commonly exerted by the addition of organic materials in the form of root excretions and degenerated root tissues. These are subsequently used by the soil microbes as food. Such effects are most apparent in the zone of root development, frequently referred to as the rhizosphere, since it is here that the root residues become introduced. Table 46 indicates that the abundance of bacteria in soils supporting different plants is different, and that, in general, the abundance of bacteria about the roots is closely correlated with the activity of the microbes, as indicated by the formation of carbon dioxide.

TABLE 46

INFLUENCE OF PLANT GROWTH UPON THE NUMBER OF BACTERIA IN SOIL AND PRODUCTION OF CO_2 (FROM STOKLASA)

Plant	Millions of bacteria per gm. soil	Mgm. CO_2 per kgm. soil in 24 hours
Alfalfa	120	86.8
Red clover	98	82.4
Beets	78	74.3
Buckwheat	63	78.7
Corn	62	70.6
Wheat	51	61.3
Barley	49	69.4
Potato	46	58.5
Oats	45	59.0
Rye	42	68.2

Not all elements of the soil population are affected alike by the development of plants. The influences of any one plant on the soil population at different stages of plant growth are characteristic, as shown by Fig. 71. Different plants affect the microbes in distinct ways. Further, the various microbes in the soil respond differently to plant growth. As shown by Fig. 73, the general bacterial population is affected to a much greater extent that either fungi or actinomyces; however, some species of bacteria develop to a greater extent than others in proximity to root systems of plants. This appears from the fact that the organisms closely related to the Radiobacter group increase many times more in response to plant development than do the microbes of the general bacterial population.

It is on the immediate root surfaces and within the superficial tissues of the roots that the greatest influences of plants are exerted, and there are progressively decreasing effects the greater the distance from the region of soil penetrated by the roots (see Table 47).

TABLE 47

INFLUENCE OF PLANT DEVELOPMENT UPON THE ABUNDANCE OF MICROBES AND THEIR ACTIVITY IN SOILS AT DIFFERENT DISTANCES FROM THE PLANT ROOTS* (FROM STARKEY)

Plant	Region of sampling	Abundance of bacteria, millions	Abundance of actinomyces, millions	Abundance of fungi, thousands	Formation of carbon dioxide, mgm.
Bean	15 inches from main roots...	18.6	7.6	24.6	9.7
Bean	9 inches from main roots...	32.8	10.0	21.6	12.0
Bean	3 inches from main roots...	36.2	8.0	20.0	12.0
Bean	Close to main roots.........	55.4	6.2	19.2	15.1
Bean	Superficial layer of the roots.	199.4	12.6	55.2	
Beet	15 inches from main root....	18.6	10.0	25.8	11.2
Beet	9 inches from main root....	27.0	11.4	25.0	13.6
Beet	3 inches from main root....	33.4	10.4	25.8	14.9
Beet	Close to main root.........	57.4	6.8	30.0	18.2
Beet	Superficial layer of the roots.	427.4	10.6	156.0	
Corn	15 inches from main root....	22.8	8.4	29.6	10.3
Corn	9 inches from main root....	26.2	11.8	23.2	15.2
Corn	3 inches from main root....	44.8	8.8	29.6	15.2
Corn	Close to main root..........	93.2	10.2	49.6	25.0
Corn	Superficial layer of the roots.	653.4	8.6	278.0	

* Age of plants—113 days.

Since all plants require some oxygen about their roots and since microbial activities and root excretions tend to lower the oxygen concentration and raise the concentration of carbon dioxide, there may be times when microbial activity becomes distinctly injurious by creating a condition of oxygen deficiency in the root environ-

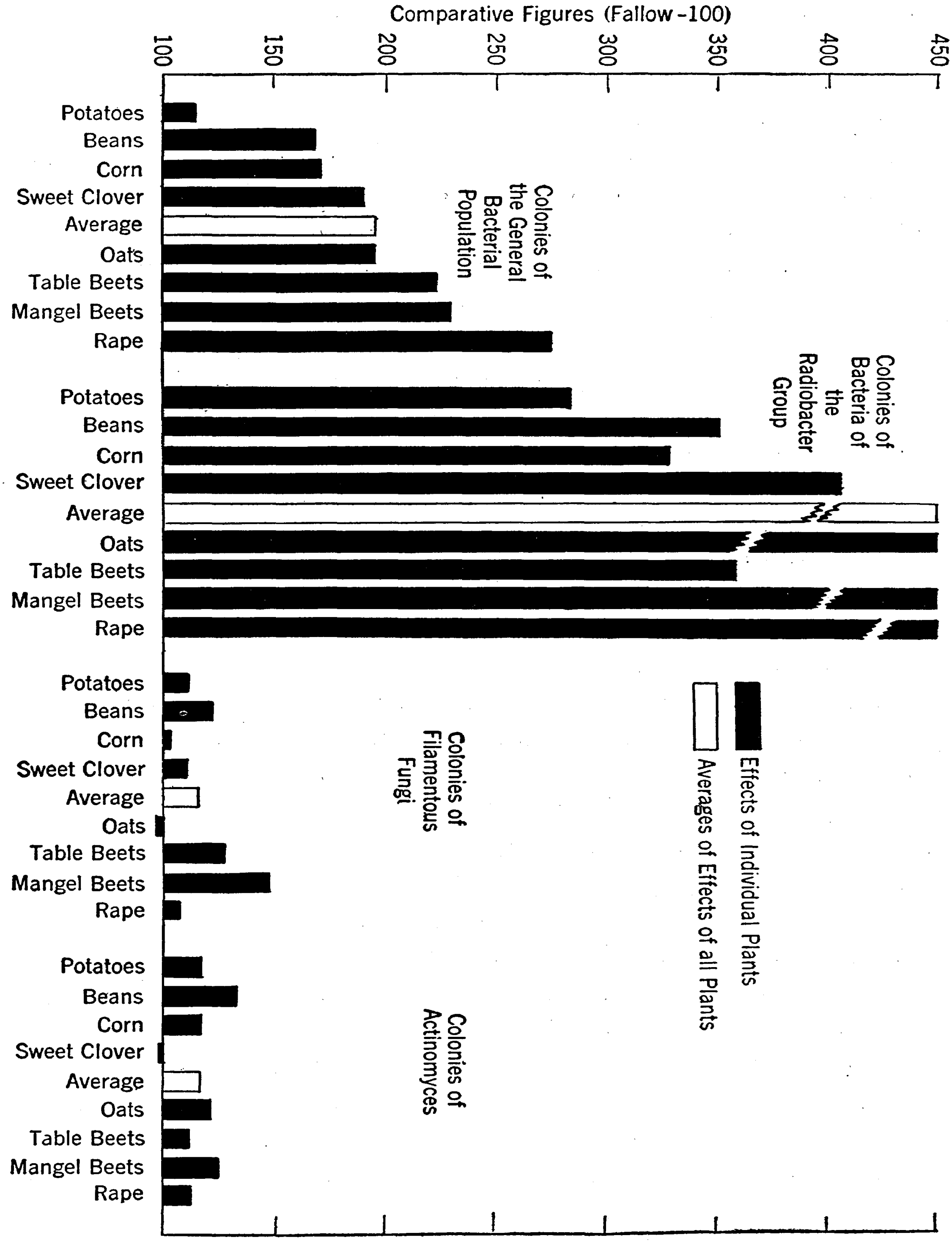

FIG. 73.—Increase in abundance of some groups of soil organisms in response to plant development; averages of effects of plant development on soil organisms during the extent of growth (after Starkey).

ment. A moderate reducing condition about roots may not be injurious to plants. In fact, it is conceivable that such a condition may be favorable to certain phases of plant nutrition. Iron is slightly soluble in a strictly aerobic environment at reactions close to neutrality, but becomes much more soluble as it is reduced. Its reduction about plant roots may be important in supplying available iron to the plants in most soils.

INFLUENCE OF ROOT EXCRETIONS.—The roots of higher plants are organs not only of absorption but also of excretion. The substances that are excreted and the amounts eliminated at any one period of growth vary with the kind of plant, its stage of development, and soil conditions. Although it is apparent that higher plants must absorb more of the inorganic substances than they excrete, still at certain stages of growth there is appreciable elimination of various substances, both organic and inorganic, nitrogenous and non-nitrogenous. In studies carried out with plants growing in sterile culture solutions, it was noted that from 1.5 to 3.0 per cent as much organic matter was present in solution (excreted by the plant) as was contained in the developed plant. About one-fifth of this organic matter in solution was nitrogenous in nature. A certain amount of the insoluble organic matter which was eliminated from the plants consisted of cellular material sloughed off from the roots.

The amounts of substances entering the soil solution during growth of certain plants may be sufficiently abundant to modify greatly the development of other plants which are growing in close proximity. This is of considerable importance in the growth of mixed stands of legumes and non-legumes. In such mixtures the non-legumes may profit from the nitrogen which is fixed through the agency of nitrogen-fixing bacteria in the roots of legumes. Certain nitrogenous substances enter the soil solution from the roots of the legumes, and probably through the action of soil microbes become modified and are then absorbed by the non-legumes. Such effects are shown in Fig. 74. The pots were prepared in the following manner. Two large pots were filled with sand. In the center of one large pot a smaller unglazed pot was placed. In the other pot a glazed, impervious pot was introduced. Both small pots contained the same kind of sand as the larger pots. The unglazed pot permitted diffusion of substances back and forth between the contents of the small pot and the larger pot, while the glazed pot prevented such movement. All nutrients required for plant development with the exception of nitrogen were added to the sand. A non-legume (oats) was planted in the small pots and a legume (peas) in the outer pots. As growth progressed, the oats in the unglazed pot developed far better than the oats in the glazed pot, showing effects of nitrogen fertilization. It is apparent that some nitrogenous substances became available to the oat plants as a result of development of the peas.

Unfavorable conditions for root development enhance the

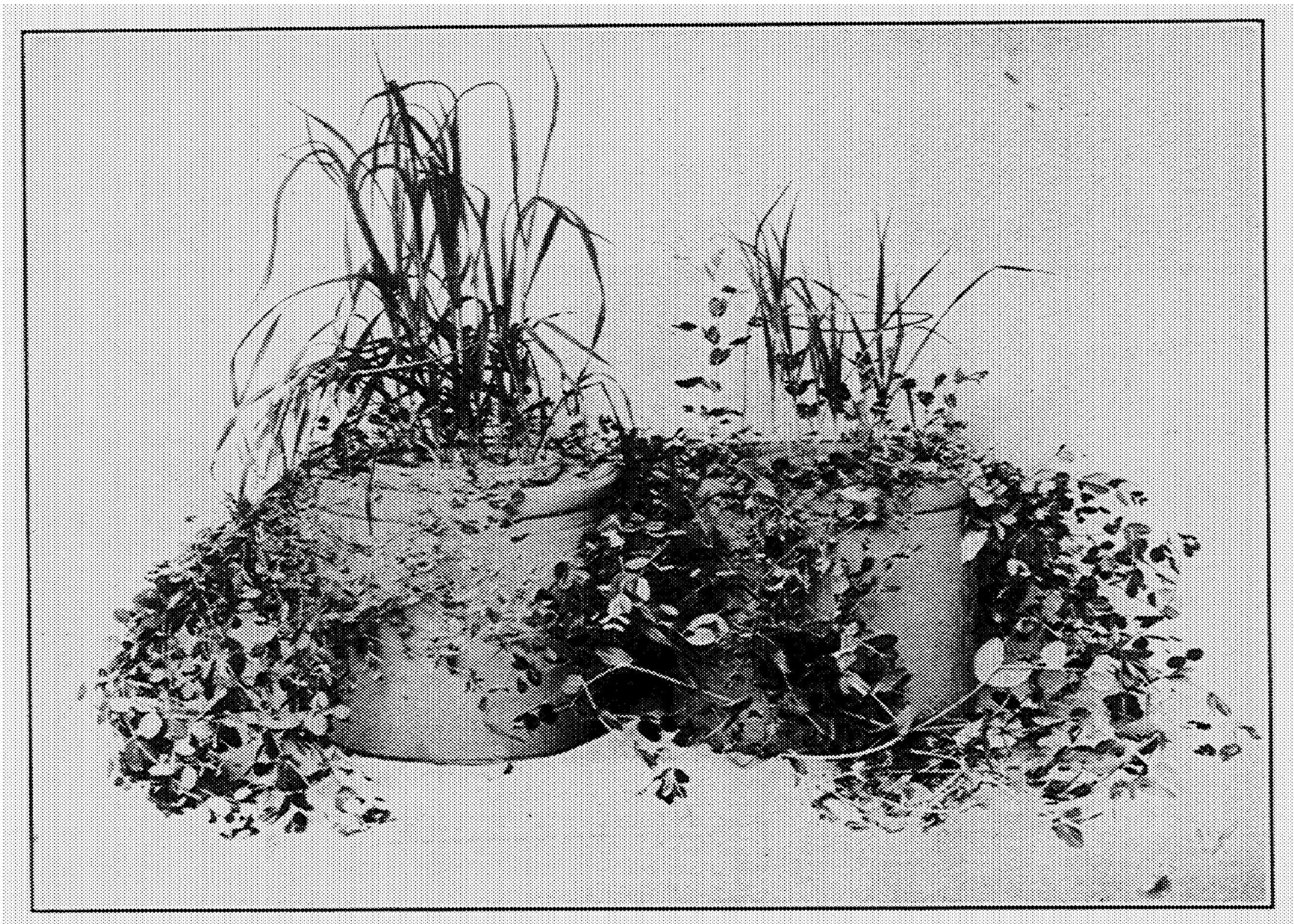

FIG. 74.—Influence of a legume (peas) on growth of a non-legume (oats). Oats in inner pot, peas in the outer. Porous inner pot on left; glazed inner pot on right (from Lipman).

activity of microorganisms about the roots, on account of death of the root parts and attack by soil saprophytes. Under conditions of deficient aeration, certain substances may accumulate which become toxic to plants and microorganisms; they generally disappear rapidly under conditions favoring thorough aeration of soils. The conditions brought about by continued cultivation of cereals and the introduction of appreciable amounts of organic matter relatively low in nitrogen appear to be more closely associated with nitrogen starvation than toxic excretions by plants.

Among the excretions of roots, phosphatides and certain mucilaginous substances appear to be of particular importance in certain cases. It has been noted that such compounds act as stimulants to the growth of certain microorganisms, increasing growth to a greater degree than would seem to be likely from the energy which the substances might furnish in the concentrations in which they occurred. Such substances may be of importance in stimulating growth of microorganisms in the rhizosphere, and may even lead to a penetration of the root cells by these organisms.

That the numerous root excretions enhance development of microorganisms is quite well defined, but the nature of the organisms affected is unknown except in a few instances such as mycorrhiza fungi. With the common cereals and root crops, there appears to be as varied an assortment about the roots as in the soil distant from the roots; this assortment includes bacteria, actinomyces and filamentous fungi of a great variety. Whether any transformations of the organisms in the rhizophere may be of particular importance beyond the ways mentioned above is not known. The limitation of our present knowledge leads to the conclusion that the general abundance of the population is greater here and the activity of the cells is greater, as indicated by formation of carbon dioxide.

Conditions Favoring Nitrogen Fixation.—The following conditions favor fixation of nitrogen: the presence of available non-nitrogenous organic matter and necessary inorganic nutrients, a favorable reaction, and the presence of nitrogen-fixing organisms. These conditions appear to exist about the roots of many developing plants, and it is quite likely that the soil becomes enriched in nitrogen, because of non-symbiotic development of nitrogen-fixing organisms about plant roots. Although such nitrogen might not be available to the crop which was responsible for its fixation, nevertheless it would become available as decomposition of these fixed nitrogen compounds progressed.

Absorption of Organic Compounds by Plants.—Not only inorganic substances but also many organic compounds may be absorbed by plant roots. Some may have pronounced beneficial effects upon root development. The organic substances which may be utilized by green plants include carbohydrates, alcohols, organic acids, peptones, amino acids, and purine bases. In fact, certain plants, as many of the Orchidaceae, cannot initiate growth from the seeds without having access to certain assimilable organic substances furnished either artificially or by means of their symbiotic fungus associates.

Long periods of field experiments argue very favorably for the use of a certain amount of organic fertilizers, or at least of organic residues in the rotation. While in the first few years crop growth may be fully as abundant with inorganic fertilizers alone, plants respond more favorably to organic manures over long periods of time. It is recognized that organic substances greatly improve the physical condition of the soil, but it is likely that certain other properties of the organic materials also contribute to the beneficial effects upon plant growth, and that these effects are not apparent with the use of inorganic substances alone. It has been suggested, for example, that animal manures when used as fertilizers result in the production of seeds which are much superior to those of plants grown on soils either receiving no fertilization at all or merely mineral fertilizers. Such seeds produced from soils receiving animal manures were found to grow plants bearing a much greater abundance of seeds. These results, of course, need further confirmation and more extensive study.

Under certain conditions, organic substances elaborated by microorganisms, formerly referred to as *auximones*, appear to have a stimulating effect on plant growth; the hypothesis has been brought forth that certain vitamines, similar to those which are so prevalent in higher plants, are formed by microorganisms and absorbed by the plants. Because of the fact that information on this subject is extremely limited this hypothesis is little more than suggestive. Organic substances other than those contained within plant seeds are apparently not essential to plant growth, but they may prove beneficial as buffering agents and may in some case even act directly as plant nutrients.

In general, microorganisms in the plant rhizosphere may exert effects similar to those produced by microbes at a distance from the roots, but their effects may become more quickly apparent by reason of the fact that the action occurs nearer to the organ affected.

Associative Growth of Green Plants and Microbes.—There are certain much more intimate associations between microorganisms and higher plants than those already mentioned. In such cases, as with the lichens where there is associative development of algae and certain higher fungi, definite dual plant formations develop which appear like single plants. The fungus derives organic nutrition from the material synthesized by the alga, while the latter makes use of inorganic nutrients absorbed by the fungus. Such an association permits the lichens to develop under conditions almost prohibitive to growth of either of the associates alone.

Under conditions where some of the single-celled algae grow in association with nitrogen-fixing bacteria, the latter develop at the expense of some of the organic matter formed by the algae and fix nitrogen during their growth.

MYCORRHIZA.—Various symbiotic and parasitic associations are known with the higher plants. Penetration by certain microorganisms of roots of most plants, including annuals and perennials, woody plants, and herbaceous plants, is probably the rule rather than the exception. Roots of all plants growing in soil are liable to invasion by any fungal hyphae which can effectively penetrate them. Some of the fungi attack the roots and destroy the vegetative tissue. The type of development depends upon the nature of the parasite, resistance of the plant, and conditions favorable or unfavorable to the parasite and host.

In some cases, however, the host plant is able to overcome the attack by the fungus and may even benefit from it. An association may thus be established, which is referred to as *mycorrhiza*, derived from the words *myces* = fungus, and *rhiza* = root. This formation is a very widespread and common phenomenon among plants. It may prove to be of considerable importance in plant growth, affecting it favorably or unfavorably. The extent of the penetration of the fungus and degree of its effect upon the growth of the plant vary with the soil conditions, which determine the plant vigor and degree of its resistance. Under favorable cultural conditions, there may be no apparent injury to the plant, but under adverse conditions of moisture, temperature, or nutrient supply, the fungus may become a parasite upon the plant and cause definite injury.

The nature of mycorrhiza produced on various plants, as well as their rôle in the nutrition of the plants, differs with different plants. In some cases, the fungus does not penetrate into the cells of the roots but forms a mass of mycelial growth around the root tips, entering the tissues largely between the cells; this type of mycorrhiza is referred to as *ectotrophic* (Fig. 75). It is very common among forest trees, especially evergreens. Other fungi invade the cells within the roots, producing *endotrophic* mycorrhiza, as in the case of the orchids (Fig. 76) and heather. Some fungi produce slight penetration into the epidermis, and others invade the cortical cells. Many of the fungi which develop upon roots of woody trees belong to the Basidiomycetes, or fleshy fungi; those that grow upon orchids are species of Rhizoctonia; and those

FIG. 75.—Ectotrophic mycorrhiza developing on roots of *Pinus sylvestris* (from Melin).

on Ericaceous plants (heaths) are species of Phoma. In many cases the specific fungi capable of bringing about mycorrhiza formations are accompanied by other fungi in their invasion.

The root fungi reach a high state of development in the case of some of the orchids. These plants have very minute seeds containing small amounts of reserve food material. The development of the mycorrhiza appears to be indispensable to the growth of orchids in nature. The fungi seem to increase the concentration of soluble organic substances about the embryo and also act as root hairs for the young plants, furnishing organic and inorganic nutrients during the early stages of growth. With the advance in plant development, the fungi do not appear to be as essential. Orchids have been cultivated from the seeds in the absence of the fungus, or endophyte, when high concentrations of sugar were furnished in the early stages of their development. Subsequent to growth of the roots, the plants appear to be able to develop

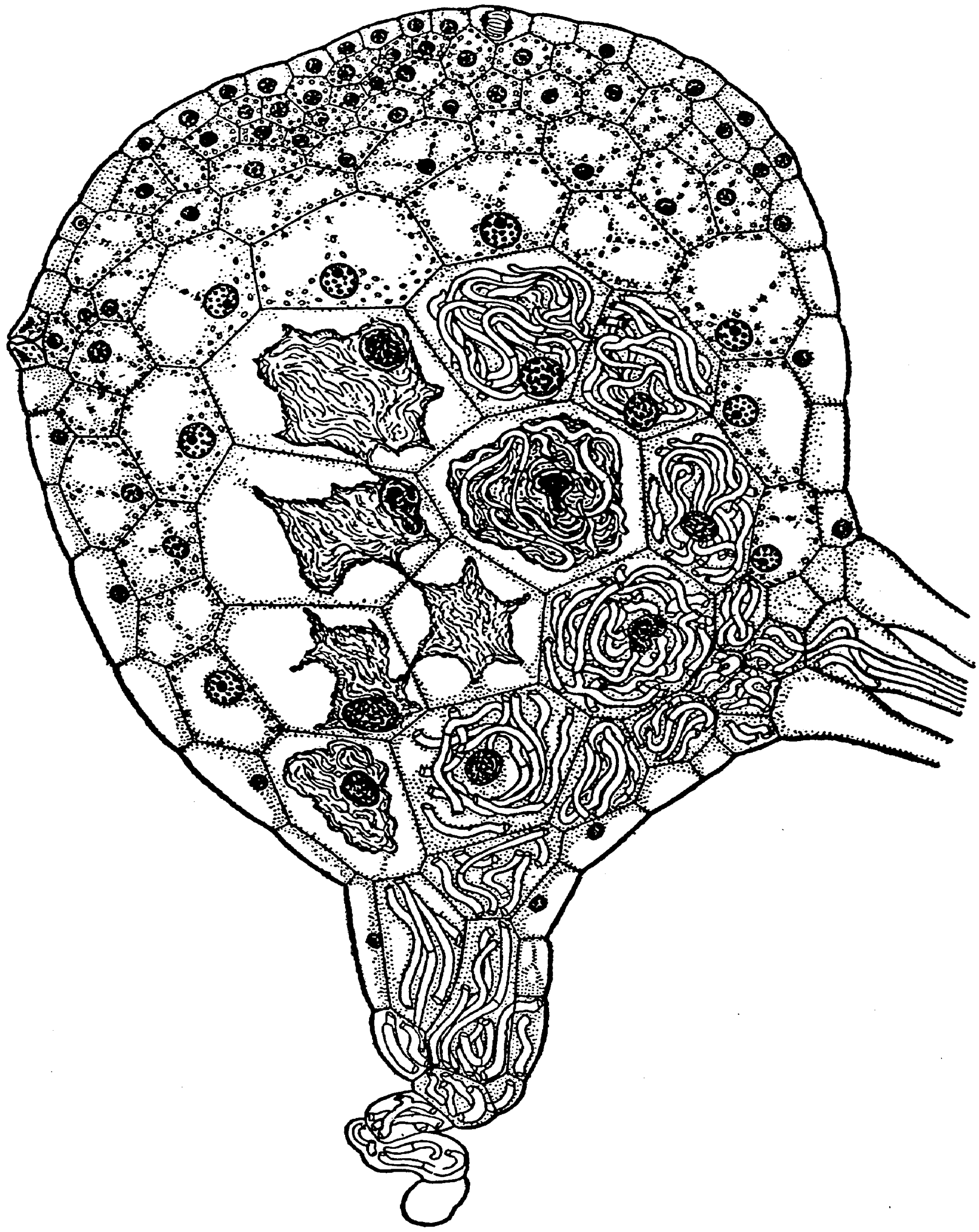

FIG. 76.—Median longitudinal section of orchid seedling one month after infection by the mycorrhiza fungus; twisted fungal hyphae appear in many of the cells (from Bernard).

as other green plants, obtaining their energy from the sunlight and mineral substances from the soil. With certain non-chlorophyllous plants, as a few species of orchids and Monotropa, or the Indian pipe, mycorrhiza fungi play an even more indispensable rôle.

Here the fungi furnish organic and inorganic nutrients throughout the growth of the higher plants, which act as parasites upon the fungi. The interrelationship is so intimate between the roots of the plant and the fungus that the latter also appears to benefit from the association; it is not known whether this is similar to the phenomenon found in chlorophyllous plants, where the fungus obtains carbohydrates and possibly other food materials from the plant cells which it penetrates.

In certain of the heaths (Ericaceae) and woody trees (pine, spruce, fir, larch, beech, maple, oak, hazel, and chestnut), the mycorrhiza develop into a somewhat less intimate association. In many cases, the roots are penetrated less deeply, and the fungi make a more abundant development on the exterior of the roots. Although mycorrhiza formation may not be indispensable in any stage of the development of such plants, the fungus associate undoubtedly favors the growth of its host under certain soil conditions, such as in organic acid soils. Under these conditions, the fungus breaks down the complex organic substances, supplying the roots of the plant with the products thus made soluble. The association is much more pronounced with these forms in the so-called "raw-humus" and is not as extensive or active in the "mull" or less acid forest soils. Under these latter conditions, absorption of plant nutrients may not be so specialized a process since bacterial decomposition is more active and nitrate is formed in abundance.

In certain species of the heaths, the fungus invades the host plant completely through the entire root system, stems, leaves, and even to the floral parts, remaining absent only from the endosperm and embryo. The seeds carry an abundant fungus growth which acts as an inoculant; the fungus invades the new plant as the seed germinates and develops. The fungi grow usually very poorly in the absence of their vascular hosts; they find food materials in the cells of these higher plants which greatly favor their development. In turn, they act as agents for the decomposition of some of the substances contained in the organic portion of the leafy detritus of the soil and render these available to the roots of the host. The fungus may in this way furnish organic and inorganic substances to the higher plant which would not be otherwise available. In a few cases where the mycorrhiza fungus is a species of Phoma there is some suggestion that the fungus may fix nitrogen and favor plant development much the same as the legume symbiont does. With most fungi, however, fixation of nitrogen does not occur.

The effects exerted by the mycorrhiza differ with different plants. In some cases there are definite parasitic effects, and the invading fungus injures the cells which it penetrates without benefiting the host in any way. In other plants the fungus is undoubtedly not parasitic, and produces no injury to the living cells although it may penetrate these cells. The fungus causes a disappearance of starch and other organic substances from the root cells but does not destroy them; on the contrary, the plant is much favored by its development. The fungus mycelium may act as root hairs absorbing water, inorganic nutrients, and organic substances, which become available either directly to the plant from the penetrating parts of the fungus or subsequent to the digestion of the mycelium, which takes place within the penetrated cells. These effects, besides possible nitrogen fixation in certain few cases and the rôle of decomposing soil organic matter, may explain some of the more important possible benefits which the higher plants derive from the fungus association. Undoubtedly in most cases the mycorrhiza may be considered as symbiotic phenomena in which both organisms, the fungus and the vascular host, derive decided benefit from the association.

Nodule Formation on Leguminous Plants.—By far the best known instance of symbiosis between vascular plants and microscopic organisms is the association between legumes and a bacterium called *Bacillus radicicola.* This type of symbiosis is apparent in most legumes and some few known non-leguminous plants. Furthermore, the legume invader is of widespread occurrence in soils wherever legumes develop naturally. Domestic cultivation of legumes frequently requires the inoculation of the organism in order to insure its presence. By reason of this specific symbiotic growth, the legumes are particularly favorably prepared to develop where many other plants could not grow; they have, therefore, found wide use as cover crops to increase the fertility of soils, particularly with reference to nitrogen. This relationship has been discussed at considerable length in preceding pages.

Bacteriorrhiza.—Bacterial invasion similar in nature to fungus invasion by mycorrhiza has been observed in some instances with cultivated annuals. These developments, called "bacteriorrhiza," have been noted in the epidermis, cortex, or even the bast, both in the interior of the cells and in the intercellular spaces. Whether they are of general or exceptional occurrence, whether parasitic to the plants or favorable to their development, is still undetermined.

Just as different fungi may penetrate plant roots to a greater or less extent, causing definite injury or exerting favorable effects on plant development, so bacteria are also not excluded entirely from entrance into the roots. It has been shown in the preceding pages that bacteria make very extensive development on the immediate root surfaces. Some cells of bacteria penetrate the tissues to greater depths, acting merely as saprophytes upon the degenerating or weak tissues. Some pathogenic organisms produce much more extensive penetration and cause pronounced injurious effects.

Summary.—Microorganisms may thus be considered to affect root systems of higher plants in numerous ways. At some distance from the roots, the microorganisms may either (1) act as general agents of decomposition of organic constituents of the soil leading to the formation of water, carbon dioxide, ammonia, sulfates, and phosphates; (2) act as transformers of such mineral constituents of the soil as ammonia and sulfur, oxidizing them to nitrate and sulfate, respectively; (3) act as agents of assimilation of nutrients which are thus removed at least temporarily from the zone of absorption of plants; (4) act as agents lowering the oxygen concentration in the soil system and thus create conditions unfavorable to root growth; (5) produce toxic substances or reduce such substances as nitrates and sulfates to gaseous nitrogen and sulfides, thus rendering them unavailable; (6) act as solvent agents through the organic and inorganic acids which are produced in various transformations; (7) act as nitrogen-fixing organisms, the nitrogen sooner or later becoming available to higher plants.

Close to the root systems many similar effects are exerted, but the changes take place more rapidly because of the accelerated activity of microbes in the rhizosphere. These influences may include: (1) decomposition of organic products of excretion from the roots; (2) solvent action of the carbon dioxide which arises both from microbial activity and root excretion; (3) fixation of nitrogen where the organic root excretions contain little or no nitrogen; (4) assimilation by plants of such organic compounds as amino acids or lower carbohydrates which may be produced by microorganisms as incompletely decomposed products or synthetic products; (5) associative development in such special cases as algae and bacteria, or fungi and algae.

There may be actual penetration of the roots in certain instances. Some invasions of the microbes result injuriously; such effects are produced by the numerous plant pathogens which inhabit the soil. The invasion of symbiotic agents, as the legume associates and certain mycorrhiza fungi, produces very evident beneficial results.

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CHAPTER IX

MODIFICATION OF THE SOIL POPULATION

THE SOIL POPULATION SUBJECT TO ALTERATION.—The total numbers as well as the relative abundance of the numerous kinds of microorganisms found in the soil can be modified in various ways by certain soil treatments. These treatments may be primarily physical, chemical, or biological. The modifications in the soil population thus brought about may either favor the development of certain groups of organisms in preference to others, or they may bring about the entire elimination of certain representative types of the soil population, permanently or temporarily.

The introduction into the soil of microbes which have not been there previously may be another means of modifying the soil population. When these organisms find in the soil a favorable habitat, they will develop readily; when, however, the soil conditions are not suited to the requirements of these microbes, they will fail to become established, irrespective of inoculation by natural or artificial means. The mere addition of microorganisms to soils provides no assurance that they will develop there, or that they will bring about the specific transformations which are expected of them, even though they are able to grow. The changes in the nature of the population brought about by any of the soil treatments are accompanied by corresponding changes in the chemical soil processes, which directly or indirectly influence the fertility of the soil, as expressed by the response in the growth of higher plants.

By reason of the fact that microorganisms do not occur in the same abundance in all soils and that they are generally favored by conditions that lead to best plant growth, there exists a close relationship between the biological activity of soils and soil fertility. Since some of the microbial tests are comparatively simple and of short duration, they are frequently preferable to vegetation experiments with higher plants to test the fertility of soils.

Influence of Soil Treatment upon the Nature and Abundance of Microbes.—Each modification of the physical and chemical condition of the soil will be accompanied by a change in the biological soil condition, as expressed by the nature and abundance of microbes in the soil. Some treatments result in profound alterations of the biological activities which persist for considerable periods of time; other treatments exert only slight effects of short duration. Among the important treatments of the soil which might be considered here are the following:

(1) The addition to the soil of organic matter in the form of stable manures, green manures, artificial manures, plant stubble, or other plant and animal residues, as well as the organic fertilizers of commerce.

(2) Physical modification of the soil by plowing, cultivation, and similar mechanical processes.

(3) The addition of different inorganic fertilizers or lime in its various forms—carbonate, oxide, and hydroxide.

(4) Cropping the soil according to various systems.

(5) Partial sterilization of soil by heat or antiseptics.

(6) Air-drying of soil.

Influence of Organic Matter upon the Soil Microbes.—The addition of organic matter to the soil brings about a number of changes in the soil population, depending upon the nature of the organic matter and upon the conditions in the soil. Green manures bring about a rapid development of various bacteria and fungi which use the water-soluble substances, proteins, cellulose, and the various hemicelluloses. These organisms are soon followed by protozoa that feed upon the bacteria and by various larvae and worms that feed upon both the residual organic matter and upon the fungi and bacteria. Finally, when the whole organic mass has become largely transformed, other bacteria and actinomyces begin to develop, attacking the more resistant residual substances. The entire mass of decomposing organic matter loses its identity as plant material and is transformed into the finely divided mass which is referred to as soil organic matter or humus.

Frequently the decomposition does not proceed very far, but stops at an intermediary stage, as in the case of the so-called *raw-humus* in certain forest soils. The acid nature of the organic matter (*p*H 4.0 and even lower), and the fact that this organic matter is not appreciably mixed with the inorganic portion of the soil but remains on its surface, are responsible for its accumulation. These conditions favor an abundant development of fungi, many of which belong to the Basidiomycetes (mushroom fungi). The absence of extensive development of bacteria and actinomyces and the relative absence of an animal population further favors the persistence of the organic matter which is known as raw humus. Under these conditions the essential plant nutrients either remain in the undecomposed plant residues or become removed from circulation by the fungi which assimilate these elements and build them up into their own cell substance. The trees can obtain some of these nutrients only through the agency of certain fungi (mycorrhiza) which grow in association with the roots and serve as root hairs. Many of these mycorrhiza fungi are able to decompose the resistant organic matter of the forest soil and make the nutrients contained therein available to the growth of the forest vegetation. This association is symbiotic in nature and appears to be of benefit to both the tree and the fungus (see Chapter VIII).

In certain other forest conditions an entirely different series of changes takes place. When the fresh plant residues which are first attacked by the fungus population are further acted upon by the numerous soil bacteria and invertebrate animals, the so-called *mull* type of soil develops. This is considered to be a richer soil, since the nutrients are liberated from it much more readily by processes of decomposition. This type of organic residue or humus is formed only when the soil is well supplied with lime, and when the organic substances are well mixed with the inorganic particles of the soil. Under these conditions the organic matter has a reaction of *p*H 5.0 to 7.0, and its thorough mixture with the soil is brought about largely through the agency of invertebrate animals which find these more nearly neutral soils a much more favorable habitat for their development than the acid raw-humus soils. In the latter, the nitrifying bacteria are absent and the nitrogen does not become changed beyond the form of ammonia, but in the mull type of soil, conditions are favorable to active nitrification and the ammonia is rapidly changed to nitrate. One need hardly emphasize the advantage of the conditions existing in such soils as compared with conditions in raw-humus soils. Cultural treatments and application of fertilizers are seldom practical in forests, but, where the forest land is cleared for other cropping systems, more elaborate practices are justified. To bring about the decomposition of the organic matter in the raw-humus soils, the addition of $CaCO_3$ and cultivation are often recommended.

INFLUENCE OF GREEN MANURES.—The addition of green manures to soil brings about a considerable modification in the soil population. Table 48 shows that organic matter exerts a

TABLE 48

INFLUENCE OF ORGANIC MATERIALS, WITH AND WITHOUT $NaNO_3$, ON THE DEVELOPMENT OF MICROORGANISMS IN THE SOIL (FROM WAKSMAN AND STARKEY)

Treatment of soil	Number of Organisms 14 Days After Adding the Organic Matter	
	Fungi	Bacteria and actinomyces
Untreated	87,300	8,875,000
0.5 per cent straw	136,000	34,200,000
0.5 per cent straw + 0.025 per cent $NaNO_3$	233,000	35,700,000
0.5 per cent alfalfa	297,000	74,600,000
0.5 per cent alfalfa + 0.025 per cent $NaNO_3$	247,000	73,400,000

marked effect not only upon the bacteria and actinomyces but also upon the fungi. When fresh, undecomposed plant material is introduced into the soil, the Mucorales generally develop first, and are later followed by various species of Penicillium, Aspergillus, Fusarium, Trichoderma, and others. The increase in bacterial growth is closely followed by a rapid development of numerous protozoa. Some of the members of this diverse population live on the organic substances originally contained in the plant material which is added to the soil; some exist on intermediary products of decomposition, while others feed upon the microbial inhabitants themselves.

The degree of the effects thus exerted is related to a great variety of factors, among which may be included the soil structure and its reaction, moisture, temperature, and supply of nutrient salts. Of major importance also are the composition and the amount of the plant material which is added to the soil, as shown in Table 49. The development of microbes upon the organic materials added to the soils follows a changing course (Fig. 77). The initial growth of the bacteria and fungi upon the readily available plant constituents is apparent from the rapid formation of carbon

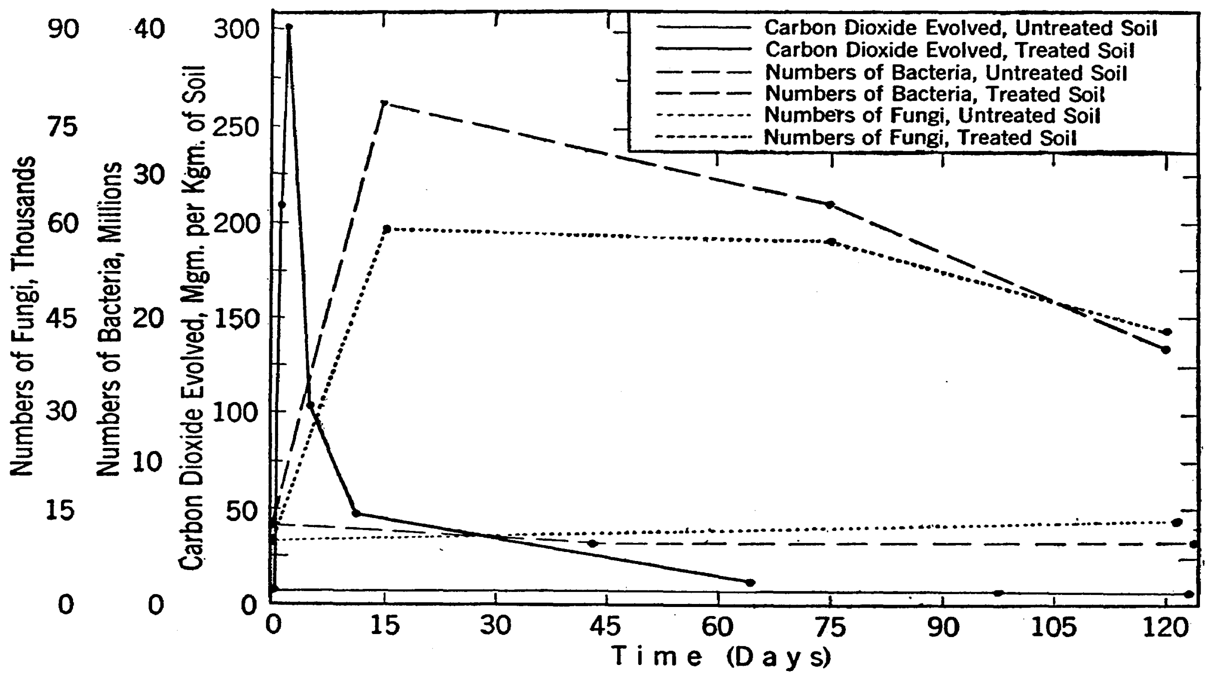

FIG. 77.—The influence of organic matter on biological activities in soil. Changes following the addition of 2 grams of ground dry alfalfa meal to 1 kilogram of soil (after Waksman and Starkey).

dioxide and from the increase in numbers of bacteria and fungi during the period immediately following the addition of the organic matter. This period of rapid development is followed by a stage of reduced speed of decomposition of the unattacked portions of the organic substance and of the incompletely decomposed products. During this stage, there is a decrease in the abundance and activities of the microorganisms, but the drop is slower than the initial rise. The available food material continuously decreases in amount, and the biological conditions slowly approach the more stabilized state which existed previous to the treatment of the soil.

The time elapsing before this stage is reached is determined by a variety of factors. In most cases it is an extended period and may consume many months.

TABLE 49

INFLUENCE OF DIFFERENT AMOUNTS OF STRAW ON DEVELOPMENT OF BACTERIA IN SOIL—AVERAGE NUMBERS OVER A PERIOD OF 71 WEEKS AFTER TREATMENT (FROM MURRAY)

Amount of straw added to the soil, per cent	Number of bacteria (counted on nutrient agar)	Amount of straw added to the soil, per cent	Number of bacteria (counted on nutrient agar)
0.0	1,677,000	0.8	11,467,000
0.1	2,163,000	0.9	15,726,000
0.2	3,399,000	1.0	16,157,000
0.3	4,180,000	2.0	28,242,000
0.4	5,650,000	3.0	60,377,000
0.5	8,953,000	4.0	91,040,000
0.6	9,034,000	5.0	94,623,000
0.7	8,497,000		

Green manure, whether it consists of leguminous plants, of young cereal plants, or others, is comparatively rich in water-soluble substances (sugars, amino acids, etc.), as shown in Table 18. Such material serves as a readily available source of energy, and may be quite completely changed within a period of a few weeks in the soil. After a series of transformations, the nitrogen of the green manure is liberated as ammonia, then soon oxidized to nitrite, and further oxidized to nitrate. The amount of nitrogen liberated from the decomposition of the green manure depends largely upon the nature and type of plant. The younger the plant, the more rapidly it decomposes, and the more rapidly its nitrogen is liberated in an available form. Consequently, less of it remains to increase the organic matter of the soil over any appreciable period of time.

The addition of green manure thus brings about a series of changes in the microbial population of the soil, affecting practically every group of microorganisms, either directly or indirectly. Organic acids may be formed in the initial stages of decomposition, but they do not persist for long under aerobic conditions.

INFLUENCE OF STABLE MANURE.—The addition of stable manures to the soil results in modifications of the soil population in three distinctly different ways: (1) The various constituents of the manure, namely, the straw, faeces and urine, offer favorable and readily available sources of energy, nitrogen and minerals (especially phosphates and potassium salts) to many different microorganisms. (2) Manure contains large numbers of a variety of microorganisms that have originated in the digestive system of the animal; the addition of considerable quantities of manure to the soil may thus considerably modify the soil population through actual mass inoculation. (3) The addition of manure leads to modifications of the physical condition of the soil. The general results of all these effects is the creation of an environment more suitable for the development of higher plants.

An understanding of the nature and composition of animal manures makes possible an explanation of the changes taking place during their decomposition and suggests an interpretation of the fertilizing values of the manures in comparison with ordinary plant residues; the latter are quite different in composition from the stable manures. This is the result of at least three factors: (1) in many cases the chemical nature of the food of animals is different from the organic matter entering the soil with the plant roots and stubble; (2) the organic matter consumed as food by animals is greatly altered during its passage through the digestive systems of the animals; (3) a large part of the nitrogen in the manure is in a readily assimilable form, and another part is in the form of microbial cells.

During animal digestion, the more readily decomposable substances of the feeds are removed from the organic matter and, consequently, the excreted material represents substances more resistant to decomposition than the original food. The animals consume practically all of the sugars and a large portion (70 to 80 per cent) of the fats, starch, hemicelluloses, and cellulose. The lignin is removed to a slighter degree than any other group of the organic substances in the foods. The manure consequently contains a much higher percentage of lignin than the food which was consumed. It is apparent that the composition of the food material has an effect on the composition of the excreted material; the foods containing the most lignin will be least digested.

During the passage of the food material through the digestive system of the animals, many bacteria develop and contribute to a change in the nature of the organic matter. So extensive is this bacterial development that the cells of the microorganisms compose a considerable part of the total mass of the excreted material. In the case of man, it has been calculated that as much as 20 per cent of the faeces consists of bacterial cells. The same is probably true in the case of other omnivorous and carnivorous animals. With herbivorous animals, especially those feeding on less concentrated foods, the bacterial mass is smaller in amount, due to the fact that a larger portion of the excreta consists of undigested constituents of the roughage. The numbers of bacteria per gram of faeces is calculated in billions. Since such large numbers of bacteria develop as a result of their feeding on the material that passes through the animal digestive tract, these organisms must play an important part in decomposing various organic substances contained in the food.

The nature of the nitrogenous compounds in animal manure is distinctly different from that of the compounds in plant manures which have been considered previously. As a result of the digestive processes, practically half of the nitrogen is excreted as urea which may be quickly transformed in the soil or in stored manure into compounds which are available to plants. In fact the transformation is so rapid that, unless particular care is taken to preserve this nitrogen, it may soon be lost either by volatilization or by leaching.

The decomposition of animal manures is slow in comparison with the transformation of certain other plant residues. This is apparent from the curves of Fig. 78. Decomposition, as measured by the formation of carbon dioxide, indicates that within a period of about two months considerably less material was decomposed in ten tons of manure in soil than in one ton of either oats or clover, these being representative of green manuring crops. During this period of 53 days, 60.8 per cent of the clover was decomposed, 49.0 per cent of the oats, and only 4.23 per cent of the animal manure. The slow decomposition of the manure is explained by the chemical composition of the material. As shown in Table 50, the manure contains relatively large amounts of certain chemical complexes which do not decompose readily. The amount of water-soluble material in the sheep manure is particularly high, since both the solid and liquid excreta were mixed together. It is apparent that, in the transformation of the manures, the hemicelluloses and cellulose disappear most rapidly. The crude protein increases in proportion to the rest of the materials, because of the growth of microorganisms. The relative amount of lignin increases as a result of its greater resistance to decomposition and as a result

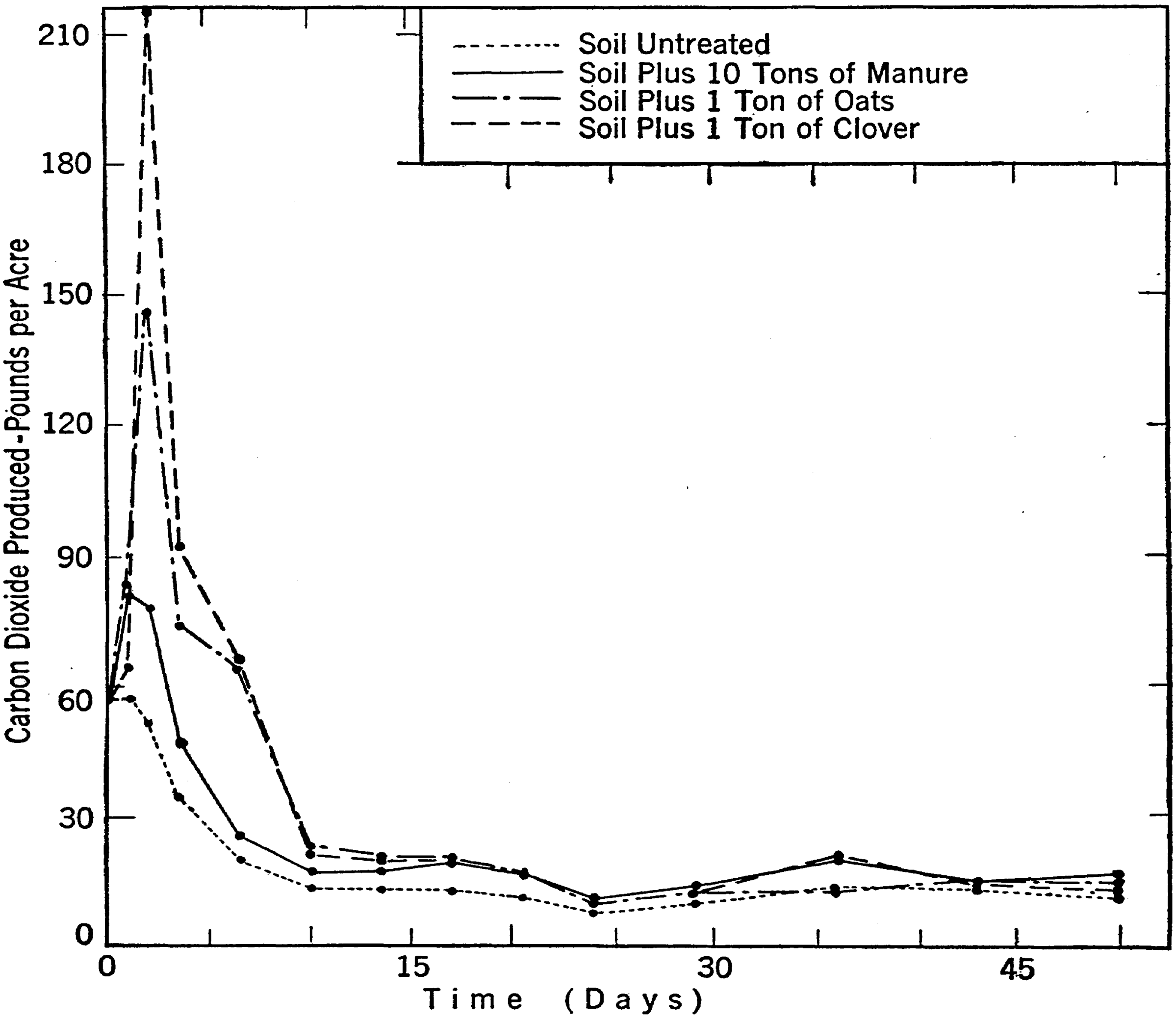

FIG. 78.—Amount of carbon dioxide evolved from soils treated with organic matter (after Potter and Snyder).

of the microbial synthesis of materials included in the lignin complex. Some of the lignin actually decomposes, but so slowly as compared to the rest of the constituents that its relative abundance is greatly increased.

Decomposition of the sugars, hemicelluloses, and cellulose, of the urea and proteins, and of the fats and lignin, represents a definite series of changes brought about by microorganisms. On

TABLE 50

CHANGE IN COMPOSITION OF ANIMAL MANURES DURING DECOMPOSITION (FROM WAKSMAN AND DIEHM)

Chemical constituents	Percentage of Dry Material			
	Sheep Manure*		Horse Manure†	
	Original manure	Manure decomposed for 192 days	Original manure	Manure decomposed for 290 days
Ether-soluble substances	2.83	2.58	1.89	0.95
Water-soluble substances	24.92	17.89	5.58	5.71
Hemicelluloses	18.46	7.31	23.52	12.67
Cellulose	18.72	12.79	27.46	5.97
Lignin	20.68	27.31	14.23	28.43
Crude protein	....	....	6.81	16.38
Ash	17.21	19.23	9.11	19.32

* Includes the mixed solid and liuqid excreta.
† Includes solid excreta only.

the other hand, the synthesis of microbial cells is always associated with these changes, whether the decomposition of the manure takes place in composts or in the soil.

In the decomposition processes, the liquid and solid excreta should be considered separately, since the transformations associated with the two materials are distinctly different. This applies particularly to the nitrogenous constituents. Urea, which makes up a large part of the organic material in the liquid manure, is very rapidly transformed to ammonium carbonate. The solid excreta may contain about one per cent of nitrogen on the basis of the dry material. On the other hand, the liquid manure contains about 4.50 per cent of nitrogen on the dry basis. Not only do the solid excreta contain a low percentage of nitrogen, but a considerable portion of the organic compounds containing this nitrogen are only slowly decomposed. There is still another factor which lowers the rate of liberation of ammonia from the solid manure. The latter contains appreciable amounts of non-nitrogenous organic matter, which is further increased by straw and similar forms of bedding material. In the decomposition of these organic substances, considerable nitrogen becomes stored away in microbial cells. These factors preclude the rapid formation of ammonia from solid excreta and result in the persistence of the organic matter in soils for extended periods of time, during which the nitrogen slowly but continuously becomes liberated in forms available to higher plants—a very important factor in soil fertility.

The losses of nitrogen from composting manure are associated with transformations of the urea which is contained in the liquid excreta. So much ammonia is rapidly formed that the reaction becomes sufficiently alkaline to permit the volatilization of some of the ammonia. The ammonia which remains in the compost may be further transformed to nitrate, and, as the nitrate may pass to certain regions of the mass of organic matter where conditions are favorable for reduction, the nitrate may be broken down to gaseous nitrogen or oxides of nitrogen and become dissipated into the atmosphere.

The control of such losses of nitrogen is based on several different principles. The losses of ammonia can be prevented by the addition of acid-reacting substances such as superphosphate, sulfuric acid, or sulfur. The fact that under these conditions superphosphate reverts to the more insoluble phosphates somewhat decreases the desirability of such treatments. In order to eliminate the losses of nitrogen through denitrification the manure is so handled as to prevent the formation of nitrate. Since anaerobic conditions prevent nitrate formation, manures are frequently packed tightly, kept in tight pits and otherwise handled to prevent appreciable penetration of air. The addition of disinfectants prevents excessive microbial activity but is not generally practical.

By hastening the processes of decomposition of the carbohydrates in the manure, the conversion of the ammoniacal nitrogen into insoluble forms is accelerated by microorganisms, as they transform the nitrogen into their cell substance. These processes are frequently based upon the acceleration of the decomposition by high temperatures. Preliminary aerobic conditions favor rapid decomposition. Subsequent tight packing of the manure results in heating. Under these conditions the temperature rises to about 65° C., and the transformation of the carbohydrates is sufficiently rapid to result in the conservation of most of the original nitrogen which is present.

Green manure added to the soil is comparatively free from microorganisms, except those which are carried down by the dust and may be adhering to the plants, or those organisms which may actually be pathogenic upon the living plant. Also, some microbes may have begun an attack of those parts of the plants which have died off. Leguminous plants are, however, very rich in the specific nodule-forming bacteria, which become liberated on the disintegration of the nodules; the soil thus becomes heavily inoculated.

Stable manure is very rich in bacteria. It has been suggested that the favorable effect of manure upon soil fertility is due not so much to the nutrients in the manure (about 10 pounds of nitrogen, 2 pounds of phosphorus, and 8 pounds of potassium in one ton of fresh manure containing 80 per cent of mositure), as to the presence of large numbers of bacteria. The introduction of these bacteria into the soil was believed to bring about a modification in the soil population of such magnitude as appreciably to influence the soil processes. If that were the case, the frequent additions of small quantities of manure might suffice, since even a hundred pounds of manure would be more than sufficient to properly inoculate the soil with the various bacteria. However, the effects of the manure do not appear to be the result of such modifications of the soil population. In the first place, the kinds of bacteria in the manure are of a distinctly different nature from those in the soil. The numerous cells of *Bacterium coli* and of other intestinal bacteria present in such abundance in the manure soon die off, after the manure is plowed into the soil. On the other hand, the extent of microbial development in the soil is associated with the amount of available food and with the environmental conditions. If the food supply is increased, or if the environmental conditions are made more favorable, the numbers of microorganisms capable of using this energy will also increase. No matter how many bacteria are added to the soil (the same is true of fungi as well), the nature of the population will not be changed, unless additional food is added or unless the soil is treated in such a manner as to render the organic matter already present there more available. There are certain exceptions to this rule, as in the case when microorganisms are added to soils in which they do not exist, finding in the soil a suitable habitat, and capable of using the soil organic complexes as sources of energy or bringing about other reactions more effectively than the organisms already present in the soil. However, such microbes are only seldom present in animal manures.

The changes in the abundance of bacteria in soil as a result of the addition of animal manure is shown in Table 51. There is an appreciable increase in numbers following the application of fresh manure; however, there is even a much greater effect as a result of the treatment with sterile manure. This action of the sterile manure results from the greater availability of the organic matter, on account of the killing of the large numbers of bacteria in the manure, as well as the modification of various organic constituents during the sterilization process.

TABLE 51

INFLUENCE OF MANURE (APPLIED AT RATE OF TEN TONS PER ACRE) ON NUMBER OF BACTERIA IN A LOAM SOIL (FROM TEMPLE)

Period of treatment	Untreated soil	Soil plus manure	Soil plus sterile manure
At start	5,190,000	5,190,000	5,190,000
7 days	6,477,000	8,590,000	23,600,000
14 days	5,160,000	6,280,000	13,850,000
21 days	5,650,000	6,600,000	11,740,000
28 days	4,880,000	6,070,000	10,280,000
42 days	4,730,000	7,010,000	8,560,000
49 days	4,820,000	6,580,000	7,860,000

The increase in the number of microbes following the addition of stable manure to the soil may be considered to consist of several steps. At first there is a decided increase in the abundance of bacteria in the soil, due to the actual introduction of the bacteria in the manure. This is soon followed by a drop in the number of these organisms and by a rapid increase of the bacteria and fungi of the soil itself, especially those organisms that use the constituents of the manure as sources of energy. Frequently there is a sequence of organisms depending upon the nature of the manure and the soil conditions. This sequence can be illustrated by consideration of a single constituent of the manure, namely, the urea. Half of the nitrogen in the manure is in this form, while the other half is in the form of proteins and protein derivatives. The urea is immediately attacked by a number of specific groups of bacteria and is changed to ammonium carbonate. The latter is soon acted upon by the nitrite- and nitrate-forming bacteria. The nitrate may be finally reduced by denitrifying bacteria and changed to nitrogen gas and oxides of nitrogen, or is assimilated by fungi and various heterotrophic bacteria (which use the constituents of the straw in the manure as sources of energy) and changed to microbial proteins. Thus the addition of one compound to the soil leads to a number of different reactions which are accompanied by numerous changes in the microbial population.

ARTIFICIAL MANURES.—Because of the ever-decreasing quantities of stable manure, methods have been developed for the composting of straw and other farm residues with inorganic fertilizer, yielding a product similar in its chemical composition and action upon soils and crops, and referred to as *artificial manure*. The principles upon which the process is based are the decomposition of the cellulose and other carbohydrates in the plant residues by microorganisms; accompanying this process is the synthesis of large quantities of microbial cell substance for which the inorganic nitrogen is required. As a result of this action, there is a narrowing in the carbon/nitrogen ratio from 80 : 1, in the straw, to 20 : 1, in the compost. Although this compost contains an abundance of various microorganisms, its beneficial effect upon the soil and the crop is due largely to the nature of the organic and inorganic complexes introduced.

INFLUENCE OF SOIL CULTIVATION.—The frequent cultivation of the soil considerably modifies the numbers and activities of soil microbes in various ways. The processes of plowing, harrowing, and cultivating the soil accomplish several distinct purposes: (1) They mix the plant residues with the soil itself, thus leading to better conditions for decomposition of the various residues by microorganisms. (2) They favor aeration of the soil, thus accelerating the exchange of carbon dioxide of the soil air for the oxygen of the atmosphere; the oxygen is required for the various oxidation processes in the soil, especially nitrate formation, the decomposition of organic compounds, and the oxidation of the reduced inorganic substances. (3) Finally, soil cultivation brings about a more uniform distribution of the moisture necessary for the activities of the soil microbes.

The effect of stirring soil upon the development of bacteria is shown in Table 52. In this investigation the soil was kept at a uniform moisture content and thoroughly stirred at frequent intervals. The influence of this treatment was very pronounced, as shown by an increase in numbers of bacteria from about two millions per gram present in the soil initially to over twelve millions, after a period of 24 days. The effect of such mechanical alteration of soil is more pronounced with the deeper layers of soil than with the surface soil, since the deeper layers are more imperfectly aerated in an undisturbed condition.

TABLE 52

INFLUENCE OF TILLAGE UPON ABUNDANCE OF BACTERIA IN SOIL (FROM CHESTER)

Period of time	Numbers of bacteria per gram
At start	2,040,000
After 7 days	5,495,000
After 9 days	6,171,000
After 14 days	11,326,000
After 24 days	12,600,000

EFFECT OF LIMING.—There is no better way of demonstrating the effects of simple soil treatment upon the nature and activities of the microbial population than by examining the effects of lime on an acid soil. Following the addition of lime, the hydrogen-ion concentration of the soil diminishes (the pH increases). The extent of the decrease in acidity depends upon the amount of lime added and upon the nature of the soil. The more lime is added to the soil, the greater is the change in reaction, up to a certain point which is distinctly alkaline, namely, at a pH of 8.2. Above this point any further addition of lime will exert no effect upon the reaction, as expressed in terms of hydrogen-ion concentration.

Soils differ in their buffering properties, that is, in their ability to react with considerable amounts of acids or bases without appreciably changing in reaction. To effect a definite change in reaction, a clay soil may require two times as much lime as a loam soil. The greater the buffering action of the soil (combining power with base or acid), the greater is the amount of lime required before a definite change in reaction is produced. Since this buffering capacity resides principally in the colloidal material, the finer-textured soils require more neutralizing agent to cause a certain change. Consequently, one would not expect all soils to react equally to the same applications of lime, even though the reactions of both were alike previous to the treatment. The effects of lime are further complicated by the fact that soils differ so greatly in reaction. Acid soils will generally respond to lime, while soils which are alkaline in reaction may show no appreciable change in biological activities or plant growth following the same treatment. Lime causes its effects principally through its influences on the soil reaction, but it may produce other changes in the soil micropopulation, both directly and indirectly. Lime is particularly effective in improving the physical structure of the soil, by causing flocculation of the colloidal particles with the development of granular structure. Plant growth may be favored by the creation of a more suitable soil reaction and physical condition. As a result of this enhanced growth, the plant residues furnish the soil organisms with larger amounts of food material.

Thus, the influences of lime on soils cannot be analyzed in simple terms. The effects are numerous and diverse, and may be exerted upon plants and microorganisms both directly and indirectly over a period of years. The improved plant growth following liming is a suggestion of the extent to which the concealed microbes are affected. The accelerated activity of the soil population contributes to the improvement in plant development, and, over an extended period of time, one of the factors which causes the pronounced increases in the abundance of soil organisms is the modified plant growth itself. Referring again to Table 11, one can readily observe that liming practices may modify the distribution of soil organisms to a marked degree. The fertilizer and lime treatments created marked differences in fertility of these soils, and the microbial population changed with the growth of plants. When such soils are compared with respect to the nature and abundance of their soil population, it is found that, at reactions below *p*H 6.0, the more acid the soil the less favorable it is to the development of bacteria and actinomyces and the more favorable it is to the growth of fungi, provided that other conditions are the same. Table 53 indicates that organic materials added to soils of different fertility also lead to different responses in the develop-

TABLE 53

INFLUENCE OF ORGANIC MATTER UPON THE NUMBER OF BACTERIA, ACTINOMYCES, AND FUNGI IN A FERTILE SOIL AND IN AN INFERTILE SOIL (FROM WAKSMAN AND STARKEY)

Nature of soil	Organic substance added	Bacteria		Actinomyces		Fungi	
		At start	After 2–17 days	At start	After 2–17 days	At start	After 2–17 days
Fertile (mineral fertilizer and manure)	Glucose, 0.5 per cent	4,700,000	40,000,000	1,800,000	3,000,000	87,300	109,000
	Cellulose, 1 per cent	4,700,000	15,000,000	1,800,000	6,400,000	87,300	320,000
	Cellulose, 1 per cent + 0.1 per cent $NaNO_3$	4,700,000	36,000,000	1,800,000	4,600,000	87,300	3,100,000
	Rye straw, 1 per cent	4,700,000	19,000,000	1,800,000	2,800,000	87,300	750,000
	Dried blood, 1 per cent	4,700,000	190,700,000	1,800,000	190,900,000	87,300	2,079,000
Infertile (no fertilizer)	Glucose, 0.5 per cent	2,600,000	20,260,000	1,260,000	1,940,000	115,700	82,000
	Cellulose, 1 per cent	2,600,000	3,000,000	1,260,000	600,000	115,700	160,000
	Cellulose, 1 per cent + 0.1 per cent $NaNO_3$	2,600,000	4,400,000	1,260,000	400,000	115,700	4,800,000
	Rye straw, 1 per cent	2,600,000	25,000,000	1,260,000	200,000	115,700	600,000
	Dried blood, 1 per cent	2,600,000	471,700,000	1,260,000	2,200,000	115,700	1,438,000

ment of the microbial population. The greater abundance of organisms in the limed soils is also reflected in crop yields, as will be considered later.

Many of the common cultural treatments of soils bring about changes in the soil population in as varied a manner as lime. Seldom can the influence of the treatment be ascribed to a single effect or a single soil change. Even a slight modification of soil causes a series of changes both in plant growth and microbial development, but some treatments have more pronounced and extended effects than others. Cultivation and the application of fertilizer salts affect biological activity directly to some extent, but the indirect effects of such treatments and the direct effects of the incorporation of organic substances in soils cause a greater degree of modification.

INFLUENCE OF REACTION UPON SOIL MICROBES.—Not all microorganisms are affected alike by acid and alkaline conditions in soils. Some microbes will develop over a narrow range of soil reaction with an optimum zone at *p*H 6.0 to 7.5. Others are tolerant to as extreme acid or alkaline conditions as ever appear in soils; many of the soil fungi, for example, will grow well at a series of reactions ranging from *p*H 2.0 to *p*H 9.0. In general, soil microbes will tolerate greater concentrations of hydrogen-ions than hydroxyl-ions, that is, greater degrees of acidity than alkalinity. In humid regions, soils as acid as *p*H 5.0 are not uncommon, and such soils may support good growth of a number of cultivated crops and a large and varied microbial population. The concentration of hydroxyl-ions in soils as alkaline as *p*H 9.0 to 10.0 is the same as the concentration of hydrogen-ions in soils of *p*H 5.0 to 4.0. Such alkaline soils exist only in regions of deficient rainfall. They are fairly unproductive and harbor relatively smaller numbers of organisms.

Many mycorrhiza fungi grow well at *p*H 5.0 and make little growth at neutrality (*p*H 7.0). Nitrifying bacteria have an acid minimum at *p*H 4.0 and an alkaline maximum at *p*H 9.4. Most species of Azotobacter have an acid minimum at *p*H 6.0, so that in soils which are more acid than *p*H 6.0 these organisms are either inactive or absent. However, the anaerobic nitrogen-fixing organism, *Clostridium pastorianum,* will grow at a reaction as acid as *p*H 5.2, and is, therefore, much more widely distributed in acid soils than Azotobacter.

In most soils, practically all actinomyces fail to grow if the acidity is greater than *p*H 4.0. Some of these organisms, such as *Actinomyces scabies*, the causative agent of potato scab, will not grow at a *p*H less than 4.8. Control of potato scab and of sweet potato pox by treatment of soil with sulfur and other acid-reacting fertilizers is based upon this sensitivity of actinomyces to high acidity. Sulfur becomes oxidized by biological agents to sulfuric acid, which increases the acidity of the soil. If sufficient sulfur is added to change the reaction of the soil to *p*H 4.6–4.8 (the amount to be used depending upon the initial reaction and the buffer content of the soil), the development of *Actinomyces scabies* will be checked, but the potato plants will still make sufficient growth to produce a good crop. When the soil is to be used the following year for another crop, it should be properly limed before that crop is planted, so as to make the reaction less acid. Certain other microbes which cause plant diseases are inhibited in their development by an alkaline reaction of the soil. Such an organism is *Plasmodiophora brassicae*, which produces club-root on cabbage. For the control of this disease, soils are liberally treated with lime. Thus, a thorough knowledge of the characteristics of the pathogenic organisms and their response to differences in reaction sometimes enables one to control plant diseases by judicious soil management.

Different cells of microorganisms of a certain species are not all alike in their tolerance to different degrees of acidity and alkalinity. The variability among the different individuals results in the selective development of the forms which are best adapted to the environment in which they exist. Consequently strains of an organism obtained from one soil may show certain characteristics which are different from those of another strain obtained from a different soil. Some organisms natural to acid soils may show greater tolerance to acid reactions than strains of the same organisms present in alkaline soils. Even under laboratory conditions, by growth in certain media, cultures may be obtained which are more tolerant to acid or alkaline reactions than the mother culture from which the modified strains were obtained. By gradual successive growth of nitrifying bacteria on alkaline media, strains were obtained which grew at a reaction more alkaline than *p*H 10.0.

Influence of Artificial Fertilizers upon Soil Microbes.—Although not as pronounced as many other effects on soil organisms, the direct effects of inorganic artificial fertilizing materials on the abundance and activities of microbes are nevertheless quite evident. This is apparent from the data presented in Tables 54 and 55. Of the various salts mentioned, phosphates exerted a somewhat more marked influence than sulfates. Since most of the inorganic compounds are present in the soil solution, the greatest stimulating effects might be anticipated when the salts are added to soils which contain the materials only in small amounts. Under certain conditions, as in alkali soils, they may occur in such abundance as to be quite toxic to microbial development.

TABLE 54

INFLUENCE OF PHOSPHATES AND SULFATES ON THE NUMBER OF BACTERIA IN SOIL (FROM FRED AND HART)

Treatment	Bacteria per gram of soil (Average during 12 days of treatment)
Untreated	12,330,000
0.5% $CaSO_4 \cdot 2H_2O$	14,212,000
0.5% K_2HPO_4	23,680,000
1.0% $Ca_3(PO_4)_2$	12,702,000
1.0% $CaH_4(PO_4)_2$	16,922,000

The effects of inorganic materials are exerted in a variety of ways: (1) Some constituents of the fertilizing materials may furnish a supply of elements which are deficient in the soil and may be consumed by the microorganisms in their nutrition. In the presence of an abundance of undecomposed plant residues, artificial fertilizers increase the activities of the various fungi and bacteria to a much greater extent than would be the case where only the organic materials were present. The nutritional demands of the organisms for inorganic substances become multiplied proportionally with the increases in organic food supply. This applies particularly to available forms of nitrogen, phosphorus, and potassium. (2) The inorganic materials may affect the physical properties of the soil, making it a more or less favorable environment for development of the organisms. (3) The inorganic compounds may affect the composition of the soil solution by either exerting solvent effects on the insoluble soil minerals, or by precipitating substances from the soil solution, or by increasing the

TABLE 55

INFLUENCE OF PHOSPHATES AND SULFATES ON FORMATION OF CARBON DIOXIDE IN SOIL (AFTER FRED AND HART)

Treatment	Carbon dioxide produced during 6 days (comparative figures)
Untreated	100
0.1% K_2HPO_4	194
0.1% $(NH_4)_2SO_4$	152
0.1% $Ca_3(PO_4)_2$	123
0.1% $CaSO_4 \cdot 2H_2O$	105
0.1% $MgSO_4$	107
0.1% K_2SO_4	101

salt concentration as a result of their addition to the solution. (4) The salts may change the reaction of the soil solution, making it either more basic or acid as a result of physical-chemical action, selective adsorption, or transformation of certain portions of the compounds by soil organisms. (5) Certain catalytic effects may be exerted, as in the stimulation produced by compounds containing such elements as manganese or radioactive compounds. (6) Probably the most pronounced effects upon microbes exerted by the addition of inorganic fertilizing substances are brought about indirectly, namely, through higher plants, particularly where fertilizing practices have been in operation for extended periods of time. The response of plants to the fertilizers is transferred to the soil population, as the plant residues are incorporated in the soil. The resultant effects of these interwoven reactions is apparent in the data given in Table 11. Changes in the physical conditions of the soil accompany the various soil treatments.

Certain fertilizers may affect specific groups of soil organisms to a far greater extent than the soil population as a whole. This is particularly the case where certain constituents of the fertilizers may serve directly as foods for the organisms. The ammonia in ammonium salts is used by the nitrifying bacteria as a source of energy. Urea favors the development of specific urea-decomposing bacteria which transform the urea into ammonia. Dicyanodiamid, frequently an impurity of cyanamid, may even prove injurious to various important soil bacteria. The numerous organic compounds of nitrogen which are contained in fertilizers influence a variety of soil organisms, the kinds of organisms affected and the degree of the effects being determined by the nature of the fertilizers.

Influence of Plant Growth.—The type of plant grown in a soil will also considerably influence the microbial population, as shown in detail in the previous chapter. The influence of the plant is due to several factors. Considerable material becomes added to soils from plant roots, either as excretions or as parts of the roots themselves. The influence of plants on soil moisture, salt concentration, and physical conditions is largely responsible for the changes in the population. Subsequent to the death of the plant, appreciable amounts of organic residues may find their way into the soil and serve as food for the microbial inhabitants.

Influence of Partial Sterilization of Soil upon the Soil Population and its Activities.—Soils may be treated in numerous ways for the control or elimination of certain insects and soil-harbored plant diseases; they may be steamed, treated with volatile antiseptics, as toluene and carbon bisulfide, and also with some non-volatile substances. These treatments are referred to as partial sterilization, since they destroy certain members of the soil population without killing all of the organisms. Some species or even groups of organisms might be entirely eliminated during the process, while most of the other microbes become affected to some extent at least. As a result of studies of the diverse effects of partial sterilization treatments upon the soil and its population, it has been shown that plants respond to these treatments in ways which cannot be explained completely by merely assuming the elimination of plant parasites. A brief survey of some of the factors involved will aid in understanding the phenomenon.

Partial sterilization of soil not only eliminates certain organisms but greatly modifies the soil as regards its capacity to support plant growth. The soil acts much as if it had been fertilized with nitrogenous substances. The extent of the response of plant growth is apparent from Fig. 79. The effects vary greatly with different soils as well as with different treatments. Fertile soils rich in a great variety of organic constituents appear to be most susceptible to changes.

The changes in the soil population are numerous and pronounced; these changes are reflected in the transformations with which the microbes are associated. There is at first a depression in the total number of bacteria. Following this temporary depression there is a pronounced increase in the number of organisms over that found in the soil before treatment. After a period of time, the numbers decrease slowly, and many weeks or months may elapse before they drop to the original level. The actinomyces appear to be affected somewhat less than the bacteria. The increase in these organisms is slower than that of the bacteria, consequently they comprise a proportionately smaller part of the soil population subsequent to the treatment.

The protozoa and fungi are considerably reduced in numbers,

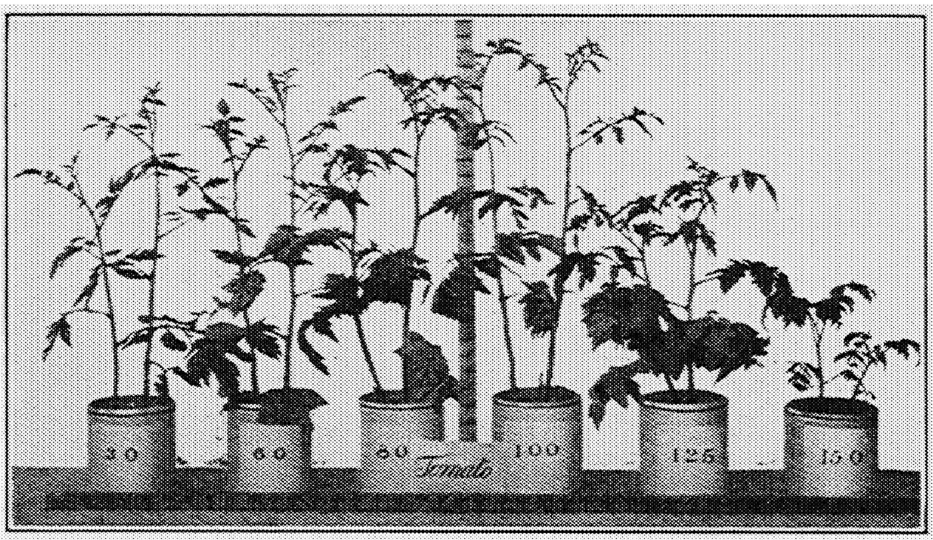

Fig. 79.—Influence of partial sterilization by heat upon growth of tomatoes. The soils in the pots from left to right were heated as follows before planting: 30° C., 60° C., 80° C., 100° C., 125° C., 150° C., for two hours (from Pickering).

and are frequently almost all destroyed. These organisms do not become active in the soil again before an extended interval of time has elapsed.

A considerable rise in the evolution of carbon dioxide from the soil accompanies the rise in bacterial numbers, as shown in Fig. 80. The initial rapid rise in carbon dioxide is followed by a rapid decline to a certain level; the decrease then becomes comparatively slow, the rate of carbon dioxide production of the untreated soil not being reached for a long time. The initial rapid increase generally precedes the increase of bacterial numbers, and the decrease also occurs more rapidly than the decline in the numbers.

This manifestation of increased biological activity is accompanied by a rapid formation of ammonia, as shown in Fig. 81. This nitrogen originates principally from the organic matter of the soil, and it accumulates as ammonia, since the nitrifying organisms are destroyed by the partial sterilization treatments. When the nitrifying bacteria again become established in the soil, the ammonia is converted to nitrate and only small quantities of ammonia are subsequently found in the soil at any one time. The increased rate of production of both carbon dioxide and ammonia clearly indicates that the soil organic matter is undergoing more rapid decomposition as a result of the treatment.

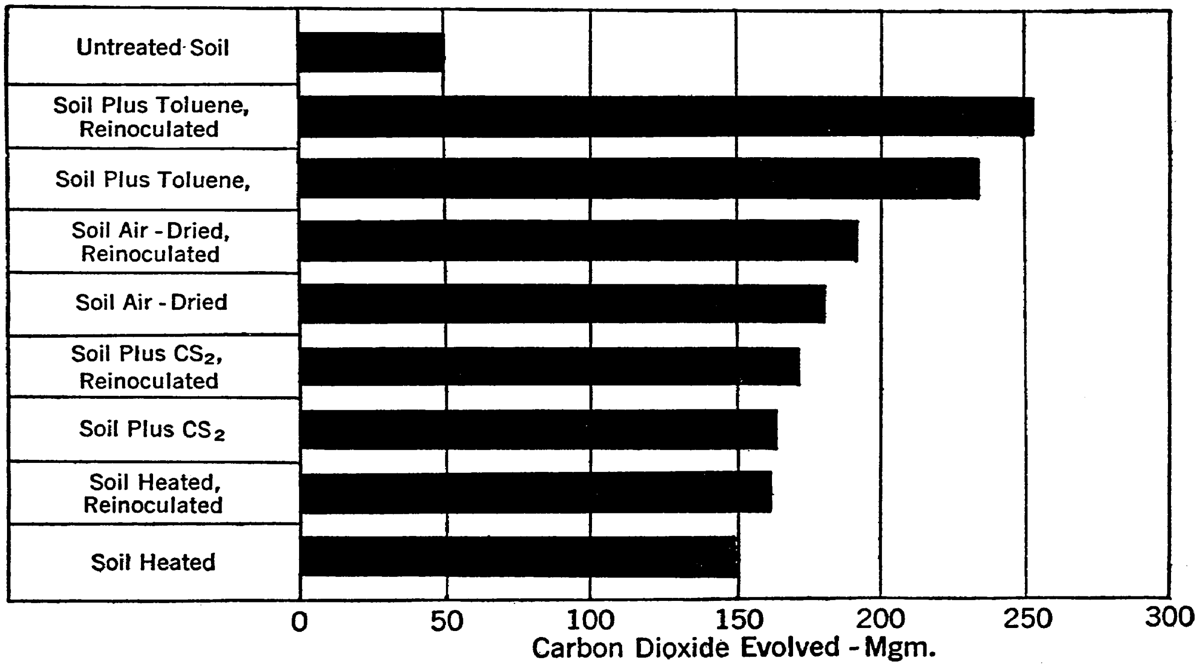

FIG. 80.—The influence of partial sterilization treatments on biological activities in soil as reflected in the formation of carbon dioxide during the first week after treatment (mgm. CO_2 per kgm. of soil). In some cases the soils were reinoculated with infusion of fresh soil after treatments (after Waksman and Starkey).

Many compounds in the soil are modified by heat and antiseptics, as evidenced by the greater solubility of both the organic and inorganic soil constituents. Besides the modification of the inanimate material, there is to be taken into consideration also the destruction of large numbers of living soil organisms. The soil may thus be altered in many ways by partial sterilization, these alterations leading to or being responsible for an increase in productivity.

To explain some of these phenomena, Russell and Hutchinson suggested the following ingenious hypothesis. It is known that the soil harbors, in addition to bacteria, various other groups of microbes, among which are the protozoa. It is quite generally agreed that protozoa exist in soils in the trophic state and, since many of them feed largely upon bacteria, they limit bacterial development to a certain extent. One may assume that some of the most important soil processes bearing upon soil fertility are carried out by bacteria. If it were possible to destroy the protozoa, the bacteria would begin to develop rapidly, reaching much larger numbers than in the original soil. If bacteria are the causative

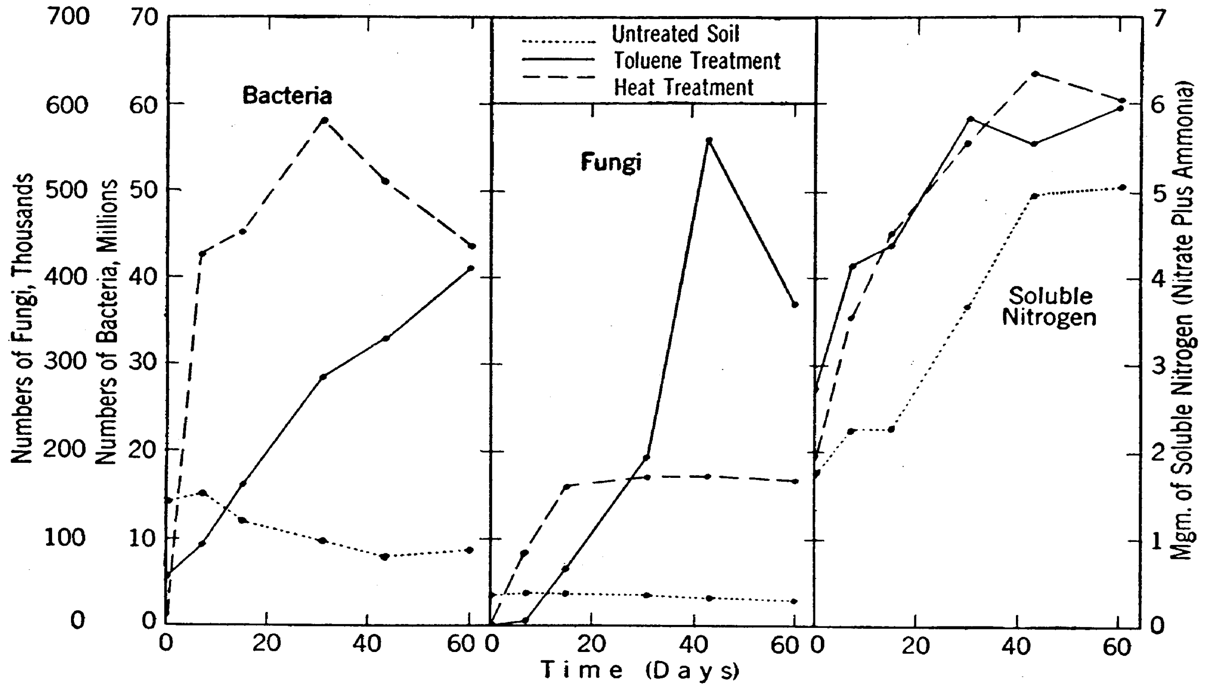

FIG. 81.—Influence of partial sterilization treatments upon numbers of bacteria, numbers of fungi, and accumulation of soluble nitrogen (ammonia plus nitrate) in an acid soil rich in organic matter (after Waksman and Starkey).

agents of the liberation of plant nutrients, the removal of such organisms as the protozoa would favor bacterial development, and also the processes which are of the greatest importance to soil fertility. The treatment of soil with antiseptics and heat brings about the destruction of protozoa, and this change has as its direct result the favorable effect upon soil fertility.

From such considerations it was concluded that protozoa comprise the limiting factor to the development of bacteria in all soils under various conditions; by their characteristic habits of feeding they hold the bacteria in restraint and consequently limit soil fertility. The removal of protozoa, particularly of the amoebae and ciliates, permits a much enhanced development of the rest of the soil micro-flora. Irrespective of whether soils are dried, heated, treated with lime, acids, volatile antiseptics, or non-volatile poisons, according to this theory it is the greater susceptibility of the protozoa, which are injured and partially or completely eliminated, which results in the accelerated development of bacteria.

The value of this explanation is greatly decreased by reason of the fact that it does not explain all of the numerous changes associated with partial sterilization. The increase in the number of bacteria and in the decomposition of organic materials need not be, and frequently is not, correlated with the presence or absence of protozoa; identical changes may occur whether protozoa are or are not eliminated. In addition to bacteria, other microbes are capable of decomposing organic matter in the soil and of liberating the constituents in forms available for plant consumption. Soil fungi, actinomyces, and algae were left out of consideration in this hypothesis. A reasonable consideration of the relationship of fungi to certain of the soil reactions indicates that they are responsible for some of the effects ascribed to protozoa. It has recently become apparent that the destruction of some of the bacteria in soils does not necessarily result in an injury to soil processes carried out by these bacteria. One need not deny, however, that protozoa play a part of some importance in soil processes. Their great abundance seems adequate evidence to indicate that they are an important element of the microscopic population of the soil.

The favorable effects of partial sterilization treatments on soil fertility are probably due to a number of factors, including (1) changes in the physical and chemical characteristics of the inorganic and organic soil material; (2) destruction of numerous organisms, including fungi, protozoa and other invertebrate animals, bacteria, actinomyces, and algae; (3) direct stimulating effects of disinfectants, in some instances. Different treatments differ in their effects because they act upon different soil constituents, both qualitatively and quantitatively. The relative abundance of the different organisms in soil is not determined by one group of organisms destroying another group, although this may take place under exceptional circumstances, but it is chiefly a question of competition for food materials. Under most soil conditions small amounts of available nutrient salts and energy sources are present at any one time. The partial sterilization treatments so modify the soil constituents that the supply of available foods is increased and the soil organisms show a sudden and pronounced response.

Inoculation of Soil with Microorganisms.—Although many of the soil organisms appear to be widely distributed and are quite generally present in most soils, conditions appear where certain desirable types are absent. This suggests the possibility of actual modification of the soil population by inoculation of the organisms which are lacking. An investigation into the causes for the absence of these organisms may suggest whether inoculation may be expected to be of any value.

The organisms may be absent because conditions are unfavorable. This may be due to the fact that the soil reaction or aeration is unfavorable. It may also be due to the absence of available food materials. Certain organisms are limited in their nutrition to few sources of energy; some sulfur bacteria are able to utilize only inorganic incompletely oxidized compounds of sulfur, or elementary sulfur itself. The occurrence of the organisms which develop in association with leguminous plants within the nodules on their roots is frequently limited to soils which have recently grown this particular legume, or representatives of the cross-inoculation group to which this legume belongs. In the presence of the legume host the organisms find food to satisfy their requirements, but in the absence of the plant the organisms may disappear in course of time, especially in acid soils.

On the other hand, certain bacteria may never have become established in soil even though conditions are favorable to their development. This may be due to the fact that conditions have been altered recently by agricultural practices; the new conditions may be favorable to organisms which could not develop previous to the alteration.

The possibility also exists that certain strains of organisms may be developed which are capable of performing important soil processes more efficiently than those already resident in the soil. Their addition to soils may improve conditions for plant development.

It is known that forest soils, peat soils, and ordinary field and garden soils possess characteristic floras. In the forest soils, especially the raw-humus soils, fungi predominate both in the amount of active cell substance present and in the transformation of the soil constituents. In peat bogs under natural conditions, both fungi and aerobic bacteria are absent, especially a few inches below the surface. The conditions are favorable only for the growth of anaerobic bacteria. When peat bogs are drained, limed in the case of acid peat bogs, and cultivated, and thus converted into peat soils, they may profit considerably from inoculation with fresh garden or field soil. One of the most important groups of organisms thereby introduced is the group of nitrifying bacteria.

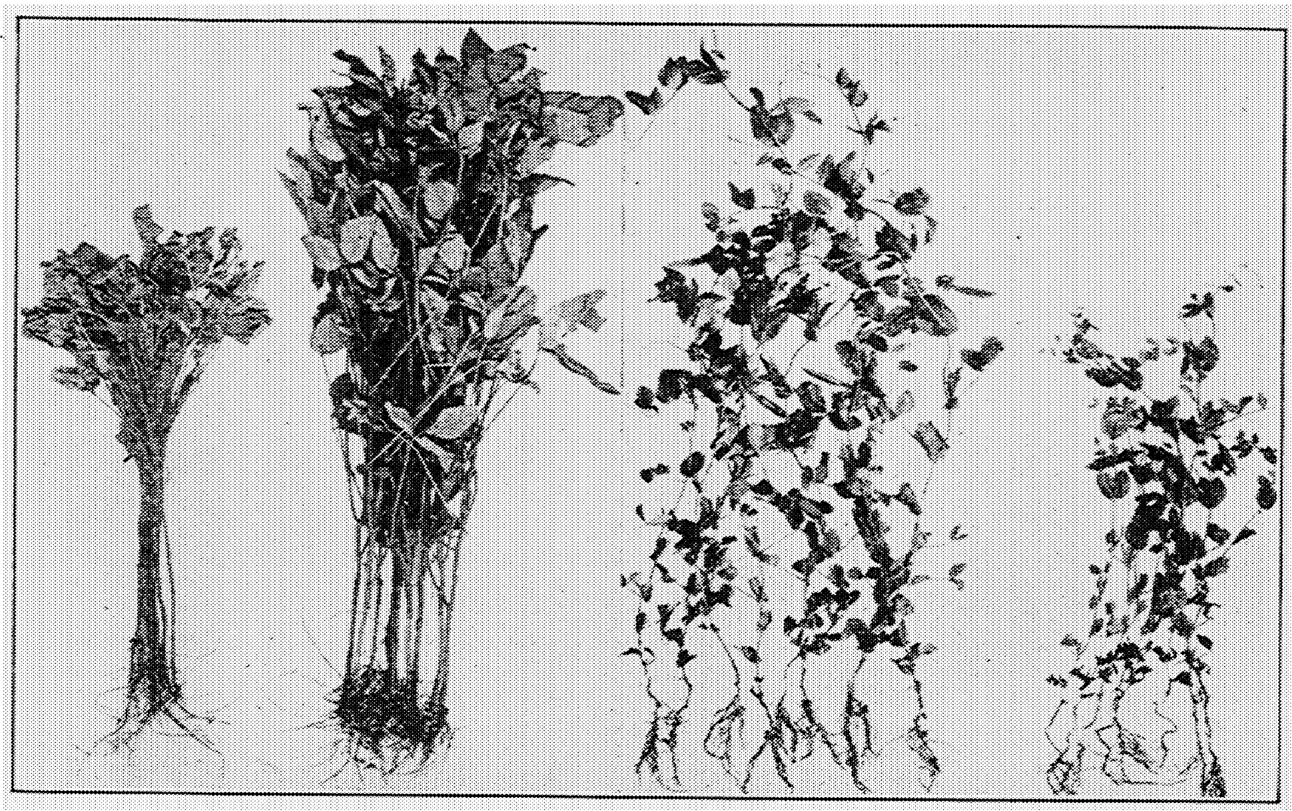

FIG. 82.—Effect of inoculation upon growth of legumes. *Left to right*—Soybeans; ten plants from an uninoculated plot, ten plants from an inoculated plot. Pea vines; eight plants from an inoculated plot; eight plants from an uninoculated plot (from Fred, Whiting and Hastings).

When various leguminous plants are grown upon soil in which the particular plants have never grown before, it is essential to inoculate the soil with the specific types of the nodule-forming bacteria, if a proper crop is to be secured and the best use made of the particular plant on the given soil (Fig. 82). This is especially true in the case of plants like alfalfa, soybeans, and cowpeas, when introduced into regions in which these plants have never grown before. Although the bacteria in general are quite widespread, they are by no means ubiquitous. Artificial cultivation of the legumes over considerable areas widely separated from regions where legumes have been previously grown necessitates special treatment to insure the presence of the organisms.

For the purpose of inoculation, some soil in which the particular crop has been grown before may be used. One disadvantage of this practice is the likelihood of also introducing plant parasites and weed seeds at the same time that the bacteria are added. The practice of inoculating with specially prepared cultures of the specific legume bacteria has found most satisfactory use. Pure cultures of the specific organisms grown in liquid or solid media have proved to be effective. Other useful inoculating material is prepared by mixing pure cultures with ground peat or similar substances. Either the seeds themselves or the soils are inoculated with these special cultures of the specific legume bacteria or with soil which is known to carry the organisms; this inoculation insures the presence of the proper bacteria at the time and place where the seeds develop. Inoculation of the seed is most effective and requires the least amount of effort. A definite quantity of the culture is mixed with the seed in a place not exposed to the sun.

Once a soil has been inoculated with the specific organism and has had a crop of the specific plant grown on it, it need not be reinoculated. However, in view of the fact that different strains of the same organism vary in their vitality, in inoculating power and in the amounts of nitrogen that they are able to fix, it has been suggested that it is fully worthwhile to reinoculate the soil with fresh cultures when a new crop is grown. Even a slight increase in crop yield and in nitrogen content may more than cover the small additional cost of the culture. This is especially true of acid soils, in which the nodule-forming bacteria deteriorate more rapidly than in neutral and alkaline soils, so that within a very few years they may completely disappear in the acid soils.

Among the other processes which necessitate the artificial introduction of microbes, it may be sufficient to mention the bacterial oxidation of sulfur to sulfuric acid. Although there is no doubt that most soils harbor organisms capable of oxidizing sulfur to some extent, few soils contain organisms capable of performing the oxidation very rapidly. The introduction of such an organism as *Thiobacillus thiooxidans* into the soil with the sulfur may considerably hasten the formation of acid. The use of sulfur for the purpose of making the soil more acid becomes essential in a system of soil management devised to control such plant diseases as potato scab, sweet potato pox, and potato wart (Fig. 83). Other instances where soil inoculation appears to be quite beneficial have been observed; the inoculation of seed beds

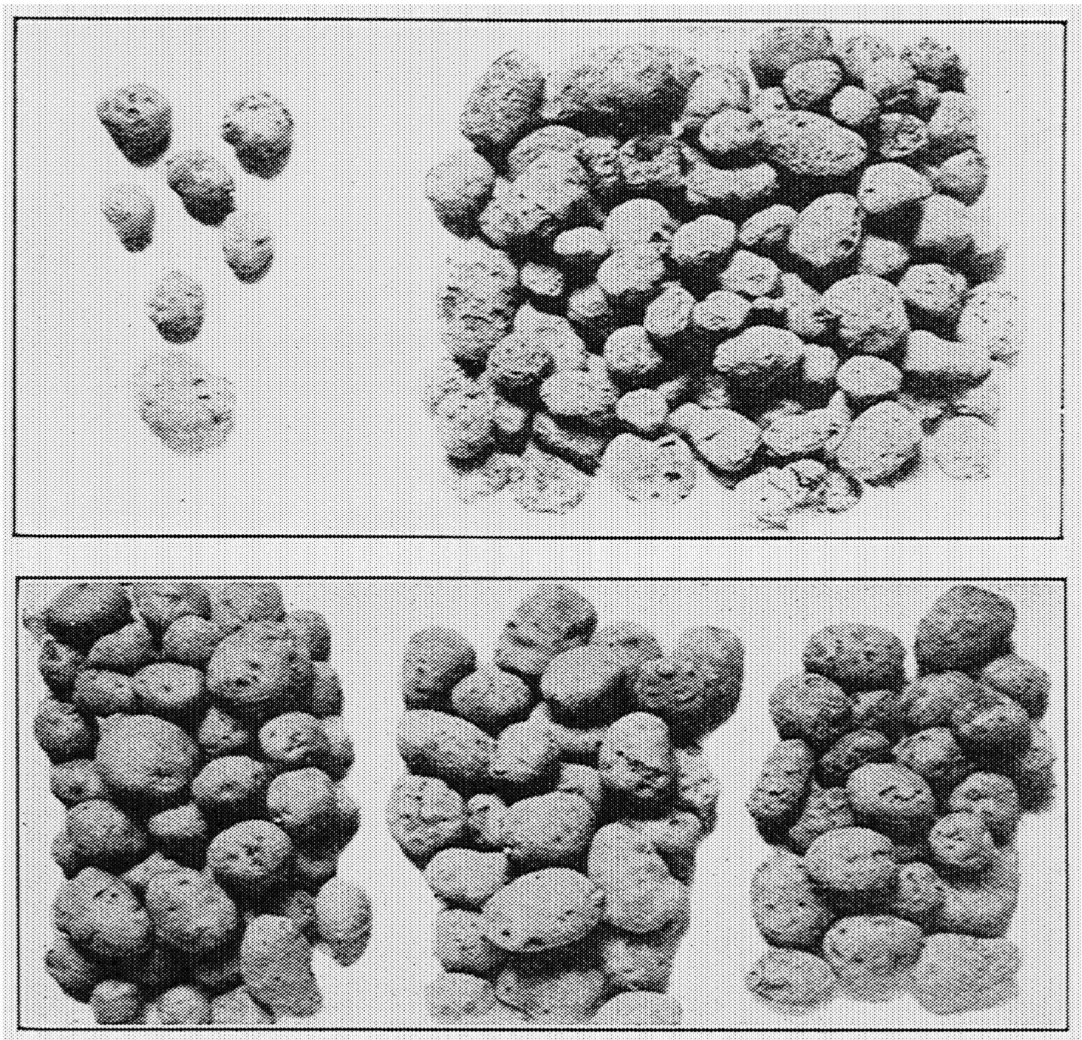

Fig. 83.—Influence of sulfur and inoculation on potato scab. Upper series, tubers from check plot. Left to right: salable scabby, unsalable scabby. Lower series, tubers from plot treated with 600 pounds of inoculated sulfur. Left to right: clean, salable scabby, unsalable scabby (from Martin).

of forest seedlings with mycorrhiza fungi and the inoculation of soils with certain cannibalistic nematodes which are capable of destroying the nematodes which cause certain galls on plants have proved useful. Such treatments have value only in very specific cases, and their use is still of uncertain practical application.

Although the soil is a good medium for practically all of the desired soil organisms so far known, and although one can isolate from the soil most of the known saprophytic microbes if one looks for them long enough, only those microbes are found abundantly in a particular soil in which the necessary nutrients are available and where environmental conditions are suitable. Numerous organisms continually compete with one another for these nutrients. Those microbes become predominant which are best adapted to the soil and the particular set of conditions and are able to utilize the nutrients found in that particular soil. A new microbe can become established only after a change in the conditions of the soil, or in the presence of a specific host plant or a selective nutrient. When the host plant is removed or the selective nutrient is exhausted, the specific organisms may die out, unless the microbe has become adapted to the soil in such a manner as to find other available nutrients; the microbe may also be resistant to unfavorable soil conditions and capable of persisting until the specific conditions again become established. The creation of conditions favorable for the development of specific organisms results in the appearance of the desired organisms, without recourse to inoculation. Conversely, the inoculation of an organism into the soil which is unsuited to its development cannot be expected to be of value.

The introduction into the soil of various other organisms than those mentioned above has been recommended from time to time. Most of these, on careful investigation, proved to have their value based upon insufficient evidence. It has been claimed that certain cultures were composed of numerous organisms capable of bringing about a more active decomposition of the organic matter of the soil than the ordinary microbes. Other preparations were claimed to contain microbes capable of inducing nitrogen fixation in non-leguminous plants. Still others were claimed to favor plant growth in general or the growth of some particular plants, in some unexplained fashion. The interest of the layman is often aroused by the important activities of the microbes. This interest coupled with incomplete knowledge of the nature of soil microbes and their relationships to soil processes unfortunately presents an opportunity for dishonest exploitation by the charlatan.

In conclusion, it may be restated that, with very few exceptions, all soil inoculants, other than those for legume bacteria, have so far proven to be worthless, at best not better than a mere infusion of some stable manure. Repeated, critical, controlled tests of the effects of an inoculant under a variety of conditions are necessary to establish justification for its use in agricultural practice.

THE ESTIMATION OF SOIL FERTILITY BY MICROBIOLOGICAL METHODS.—Even as the numerous and varied soil conditions determine the intensity of plant development and the kinds of plants which can be economically grown, so also the environmental conditions are responsible for the microbiological activity. Growth of both higher plants and the less conspicuous microbes are reflections of the soil conditions. The inanimate portion of the soil itself is the product of diverse activities operating through long periods of time. Different organisms are contained in some soils than in others, but the difference in quantitative distribution of organisms in various soils is more pronounced. It is this difference in the relative abundance of various species of microorganisms which gives the soils some of their characteristic properties.

The type and abundance of plant growth are important factors determining the microbiological soil conditions. Also, the nature of the activities of the soil microbes is important in determining the kind and degree of development of the higher plants. Since the two are so closely related, it is obvious that certain correlations should exist between the biological activity of a soil and its fertility. Based upon this assumption, numerous studies have been made to determine what methods could be devised to estimate conveniently the productive capacity of soils. Some of these methods have been developed to further determine the desirability of adding fertilizers or lime to soils.

THE ACTIVITY OF PHYSIOLOGICAL GROUPS OF SOIL MICROBES.—Some of the earliest methods which were used involved attempts to determine the potential activity of such groups of the soil population as could produce ammonia from organic compounds of nitrogen. The following procedure is typical of these studies. A nutrient solution containing peptone, urea, protein, or other nitrogenous organic substance is inoculated with soil or soil infusion and then the speed of ammonia formation is determined. On the assumption that the soils which contained the most organisms or the most active organisms decompose the nitrogenous compounds most rapidly, it was suggested that the rapidity of ammonia formation be used to determine the microbial condition of the soils. Such studies are not as valuable as had been anticipated for the reason that bacteria develop so rapidly in the nutrient solutions that the microbial conditions of the soils which are used as inocula are quickly obscured.

Somewhat more suggestive results are obtained when the soils themselves are used as the media in place of nutrient solutions; the organic nitrogenous materials are incorporated with the various soils which are under investigation. Even these studies prove to be of limited value, since so many and diverse types of organisms have the capacity of forming ammonia from organic substances that great differences in soils do not appreciably affect the rate of transformation of the added nitrogenous compounds.

There are certain other tests which have proved more useful than the ammonification tests. Principal among these are determinations of the rate of nitrate accumulation, which may be studied in various ways: in solution media containing ammoniacal nitrogen and inoculated with soil material; in the soil itself without alteration other than creating proper moisture relationships; in soil to which is added some source of nitrogen such as an ammonium salt or some organic nitrogenous material. Since nitrification is brought about by a limited number of organisms whose activity is affected in a similar way to many cultivated plants by similar degrees of reaction, temperature, moisture, and aeration, it can be readily understood why the nitrifying capacity of fertile soils may be greater than the nitrifying capacity of infertile soils (Fig. 84).

A study of the decomposition of cellulose may also be of use in an interpretation of certain soil characteristics. For such studies the cellulose is generally added to the soil in a finely divided condition and the rate of its disappearance is followed. Since large numbers of various organisms may be associated with the decomposition, and since different members of the group develop under different environmental conditions, the rate of decomposition in soils is not decreased by all of the conditions which limit development of higher plants; the transformation is of more value for determining the readily available nitrogen in the soil. The decomposition of cellulose is accompanied by the assimilation of considerable quantities of nitrogen, which are used by the organisms that are effecting the decomposition in order to satisfy their nutritional requirements. If appreciable amounts of cellulose are added to soils, nitrogen is generally present in insufficient quantities to permit most rapid decomposition. Consequently, the rate of decomposition is regulated by the rate of liberation of nitrogen from the soil organic matter.

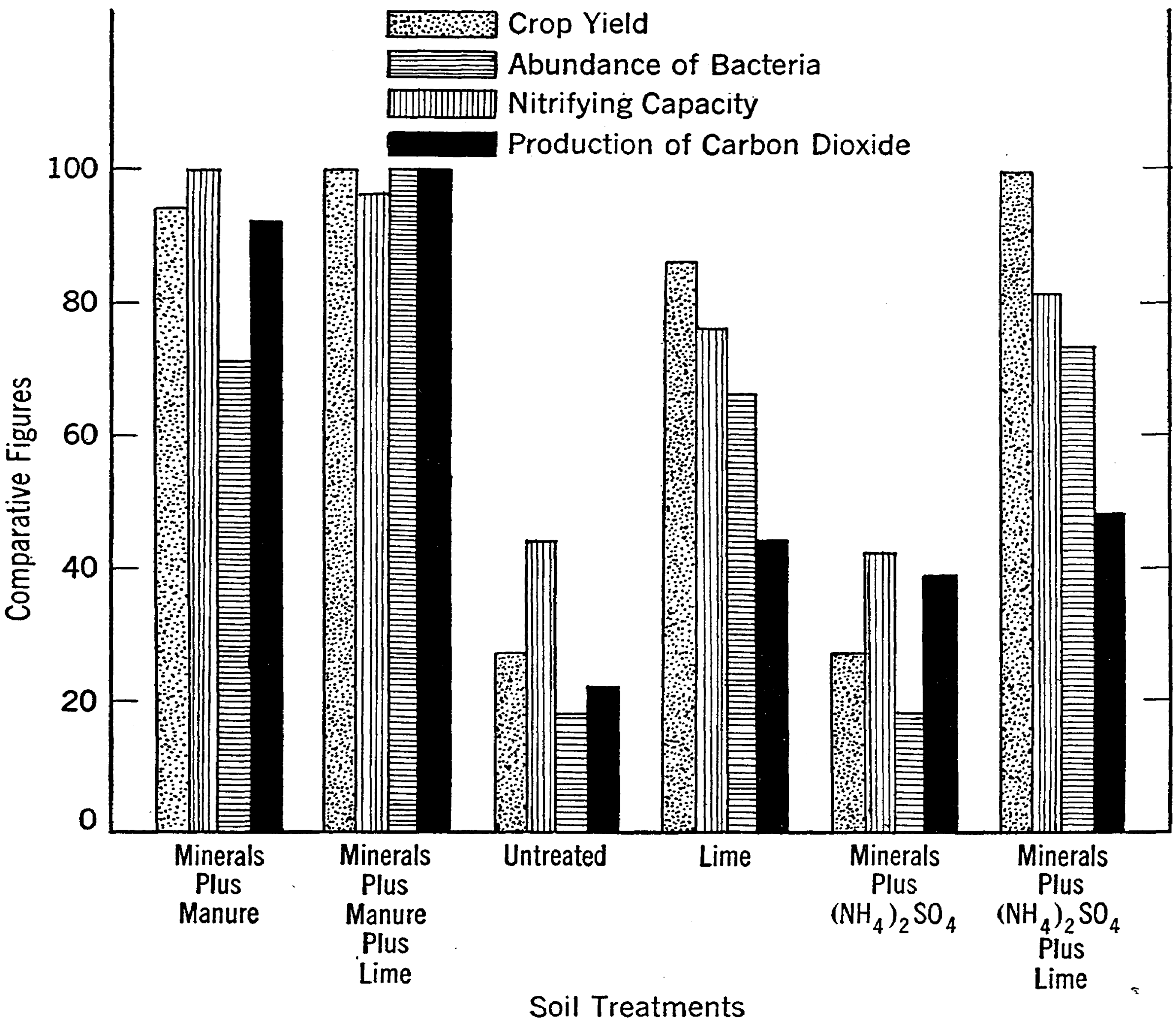

FIG. 84.—Relationships between crop yields, abundance of bacteria, and microbial activities in soils of similar origin but differing in fertility due to different fertilizer treatments extended over fifteen years (after Waksman and Starkey).

ABUNDANCE OF MICROBIAL INHABITANTS OF SOILS.—One of the methods first used to obtain information concerning the biological condition of soils was the plate technique for determining the abundance of bacteria in the soil. Various other methods have since been utilized to obtain additional information concerning the abundance of certain specific groups of soil microorganisms. Some methods give information concerning the bacteria as a whole; others deal with individual physiological groups of organisms such as those that decompose cellulose, fix nitrogen, or oxidize ammonia to nitrite or nitrite to nitrate. Actinomyces, fungi, algae, and protozoa are somewhat less frequently the object of such investigations (see Table 11).

There are many striking correlations between the abundance of organisms, as determined by these methods, and the productive capacity of soils (Fig. 84). The relationships are not absolute, however, since not all microbial cells even of a single species work with the same speed in the soil. A small number of organisms in one soil may be more active than a larger number in another soil, because of the fact that many individuals of the soil population are resting forms during a large portion of the time.

BIOLOGICAL ACTIVITY OF THE SOIL POPULATION AS A WHOLE.—A large number of the soil microbes are concerned with the decomposition of the organic materials in the soil. Consequently, knowledge of their activity is of considerable value in estimating the biological condition of the soil. In order to obtain such information, measurements have been made of the rate of formation of carbon dioxide in the soil. Since this gas is one of the principal products resulting from the decomposition of all organic substances, and since most of the gas is of biological origin, information concerning its formation is of exceptional value as an index of microbial activity in soils (Fig. 84).

One of the most accurate measurements of biological activity is the determination of the amount of heat produced during the development of the organisms. The potential energy contained in the various substances which are used by microbes as foods undergoes a change, a part becoming transformed to other forms of energy as heat and a part remaining in the new compounds which have been synthesized by the cells of the organisms that grow at the expense of the food material. Microorganisms are frequently very inefficient in utilizing the energy contained in food materials, and a very large portion of the energy is eliminated as heat. Such heat is formed irrespective of the source of energy that furnishes the organisms with the power to develop.

DETERMINATION OF THE AVAILABILITY OF SPECIFIC NUTRIENTS IN SOILS.—Utilizing the available knowledge of the nutrition of soil organisms, several methods have been developed which may be used to obtain information concerning the relative amounts of available phosphorus, potassium, and calcium in soils. Cultures of Azotobacter have been quite generally used in such studies. The following procedures are typical of these methods.

For determining deficiencies of phosphate in soil, a solution medium is prepared to contain all nutrients required by Azotobacter, with the exception of phosphorus. To this medium is added a certain amount of the soil to be tested. The material is sterilized and then inoculated with Azotobacter. Since the only source of phosphorus is the soil which is added, and since considerable phosphorus is required by the bacterium to grow, the relative growth of Azotobacter or the amount of nitrogen fixed is an index of the amount of available phosphorus contained in the soil. By adding graded quantities of available phosphate to a series of flasks containing the same medium treated in the manner mentioned above, still further information can be obtained regrading the response of the soil to phosphate treatment. By following a similar technique but with regard to calcium carbonate instead of phosphate, the studies yield information concerning reaction and lime requirement of soil.

Such methods have been modified so that the soil itself is used in place of solution media. A series of samples of soil are mixed with starch and with graded quantities of phosphate, calcium carbonate, or potassium salt depending upon whether phosphate, lime, or potash deficiencies are to be studied. The soils are then made pasty with water and put into dishes for incubation. If conditions are favorable, Azotobacter grows well and forms raised colonies on the surface which have the appearance of tiny pearls. If conditions are unfavorable, no colony development occurs or there is very limited growth (Fig. 85).

Growth of certain fungi has also been utilized to give information of the same nature. For phosphorus determinations, nutrient solutions are prepared containing an abundance of all nutrients with the exception of phosphate, which is added in graded amounts to a series of flasks containing the medium. A certain amount of the soil under investigation is added to each flask, and the media are all inoculated with a fungus such as *Aspergillus niger*. After the incubation period, the fungus growth is removed from the flasks, washed, dried, and weighed. The extent of growth of the fungus is limited by the amount of available phosphate in the soil. For potash determinations the method is the same, with the exception that the media contain ample phosphate but graded amounts of some potassium salt (Table 56).

There are thus numerous methods which have been used for attempting to determine the productive capacity of soils. Some of these methods are devised to give quite specific information, while the object of others is to provide more general facts about the soil. Use of several methods of study is preferred to use of indi-

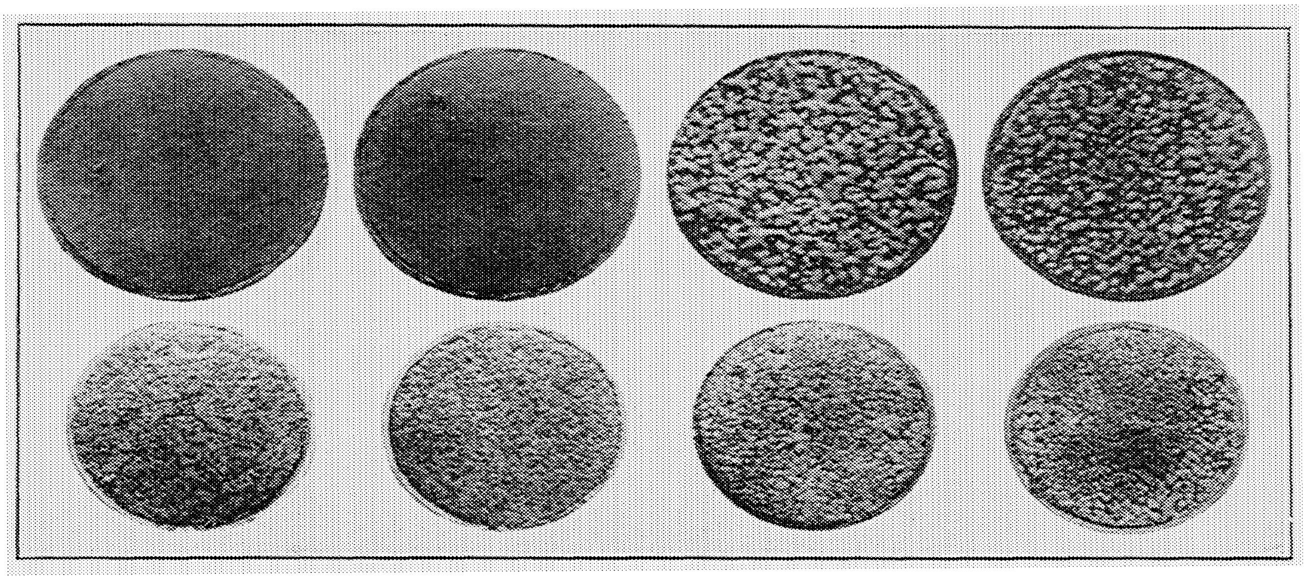

FIG. 85.—Growth of Azotobacter in soils treated with starch to test for deficiencies in available nutrient elements. *Upper row:* soil deficient in phosphate. *Reading left to right:* check, nothing added; potash added; phosphate added; phosphate and potash added.
Lower row: soil not deficient in either potash or phosphate. *Reading left to right:* check, nothing added; potash added; phosphate added; phosphate and potash added (from Sackett).

vidual procedures. Even such information is incomplete, and is most valuable when it is used in conjunction with chemical and physical determinations in estimating soil fertility. It is because of the fact that the abundance of the soil population, the types of organisms composing it and their activity are products of the environment and are susceptible to change with modifications in any of numerous conditions, that a certain amount of information concerning the fertility of a soil can be obtained from a knowledge of its microbiological condition. One of the principal difficulties is to obtain a true conception of the biological status of the soil.

TABLE 56

DETERMINATION OF POTASH REQUIREMENTS OF SOILS USING GROWTH OF THE FUNGUS *Aspergillus niger* AS THE INDEX (FROM NIKLAS)

Soil number	Amount of available potash as tested by vegetation experiments, (mgm.)	Weight of growth of the fungus, (gm.)
1	7.77	0.82
2	11.98	0.94
3	12.27	1.19
4	10.13	1.21
5	18.00	1.42
6	18.00	1.46
7	21.73	1.61
8	25.99	1.80
9	31.73	2.34
10	35.89	2.52
11	44.89	3.30
12	78.12	5.10

SUMMARY.—The invisible soil inhabitants are the victims of the circumstances in the regions where they are located. They have no means of migrating long distances except by occasional fortuitous transportation by wind, water, or similar agencies. They must be able to sustain themselves from the food materials in their vicinity or else die. They must be able to tolerate sudden changes in the environmental conditions, since they have little or no means of regulating the conditions under which they live. They must be able to tolerate high and low temperatures, periods of deficient and excess moisture, change in reaction, and a variety of other factors related to their capacity to absorb and utilize the nutrients contained in the soil.

Organisms cannot all tolerate the same conditions, and consequently there is a selective change following any modification in their habitat. If the conditions are not too severe, numerous organisms may persist even though they are relatively inactive. With a change to more favorable conditions they often quickly respond with renewed growth and activity. These relationships are responsible for the occurrence of a greater variety of organisms and more numerous individuals where considerable amounts of organic substances reach the soil in such forms as plant residues, animal manures, or commerical fertilizers. Certain inorganic substances also accelerate microbial development. It is thus possible to modify the population and consequently its activity by systematically regulating the cultural treatments. In some special cases it is desirable to modify the population to an extreme degree by the addition of certain antiseptics or by heating the soil. Likewise, soil inoculation is effective where certain leguminous plants are to be grown.

It is by reason of the fact that the soil population is more active in fertile soil that microbiological methods are available for estimating soil fertility. Information as to whether or not the organisms are relatively numerous or scant, as well as information concerning what treatments improve their development, is very useful in judging soils and determining requirements for their improvement.

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CHAPTER X

IMPORTANCE OF MICROBES IN SOIL FERTILITY

RELATIONSHIPS OF MICROBES TO SOIL PROCESSES.—In conclusion, it may not be amiss to summarize the results presented in the previous chapters dealing with the occurrence, abundance, and activities of various microbes in the soil, and point out their importance for plant growth. The very existence of higher plants and, therefore, also of animals, including man, depends upon the activities of the soil microbes. Consequently there is little likelihood of overestimating the importance of microbes in soil processes; the growth of soil microbes is intimately concerned with the availability of nutrients which determine the fertility of a soil. Anything that hastens the decomposition of the soil organic matter also favors an increase in soil fertility. A fertile soil is distinguished from an infertile soil not by the fact that it contains more nitrogen, phosphorus, and potassium, but by the fact that the nutrients present in the soil are liberated with greater rapidity in the fertile than in the unfertile soil.

Liming and cultivation of soil improve the physical condition; they provide a more favorable reaction for the activities of the numerous soil bacteria and admit larger quantities of oxygen which are necessary for the growth of the aerobic organisms. These treatments are of great importance in soil fertility, not only because they produce in the soil a favorable physical and chemical condition for plant growth. They also create more favorable conditions for the activities of the microbes which effect more rapid liberation of the soil nutrients.

Also part of the response in plant growth following the use of artificial fertilizers is brought about indirectly. The fertilizers increase microbial activities which lead to more rapid transformation of the soil constituents.

Even a superficial examination indicates that there are numerous important processes in which microorganisms take an active part: *rock disintegration and soil formation, decomposition of organic matter in soil, liberation of nitrogen in an available form as ammonia, liberation of carbon as carbon dioxide, formation of nitrate, fixation of nitrogen, disappearance of available nitrogen from soil, synthesis of microbial cell substance, oxidation and reduction of soil constituents, transformations in composted stable manure, production of artificial manure, disintegration of green manures, liberation of mineral elements such as phosphorus from relatively insoluble substances, transformation of sulfur, the improvement of certain plant growth following soil inoculation, changes following partial sterilization treatments, injury to plants and animals by bacterial and fungus parasites.* Some of these processes may be considered at somewhat greater length.

RÔLE OF MICROORGANISMS IN THE CYCLE OF ELEMENTS IN NATURE.—The activities of microorganisms in the soil are responsible for completing in nature the cycles of various elements, especially those that enter into the composition of organic substances. Here may be included first of all the element carbon which is the basis of all organic compounds. The element nitrogen which, in combination with carbon, hydrogen, and oxygen, forms the most important group of constituents of all living protoplasm, is of no less importance. The mineral elements sulfur, phosphorus, and potassium are likewise absolutely essential for the growth of green plants and are frequently the controlling factors in development of plants in soil. A number of other elements, such as iron, calcium, and magnesium, are directly or indirectly acted upon by microorganisms in their cycle in nature.

Chief among the activities of microorganisms is the mineralization of organic matter in nature. The abundant life of green plants in and on the surface of the earth, from the microscopic algae to the thousand-year-old evergreen and deciduous trees, tends to build up organic matter from inorganic elements or from simple inorganic compounds. From the carbon dioxide in the atmosphere as the only source of carbon, from the nitrogen present in the soil in the form of simple compounds (ammonium salts and nitrates), out of the phosphorus, potassium, sulfur, iron, calcium, magnesium, and traces of other elements in the form of simple inorganic compounds, in addition to water and gaseous oxygen, the green plants are capable of manufacturing every year several tons of organic matter per acre of soil. A large part of this organic matter is returned to the soil in the form of plant stubble, leaves, branches, green manures, stable manures, and various waste products of plant utilization by man, while another part is used as the source of human and animal food.

Animals, being unable to manufacture their own organic substances from simple inorganic materials, depend upon the plants for the organic matter. The synthesized products resulting from the development of higher plants furnish the sole source of energy which keeps the other living organisms active in the world. The animal, using the energy derived from the disintegration of some of these compounds, changes the composition of the materials manufactured by the plant, and forms, from these, substances which are needed for the synthesis of its own tissues and for its numerous functions. A large part of this animal organic matter returns to the soil sooner or later in the form of animal excreta and other waste materials. Finally, as the animals themselves die, their own bodies, from those of man to the lowest insects or microscopic worms, are returned to the soil. Consequently, whatever is taken from the soil and from the atmosphere is sooner or later returned, but generally in an entirely different form. The plant has originally taken its nutrients from the soil and from the air in the form of simple inorganic salts; these are returned in the form of numerous complex compounds of plant and animal origin.

Since the amount of carbon dioxide in the atmosphere is quite limited, being not more than three-hundredths of one per cent of the atmospheric gases, and since nitrogen is present in the soil in mere traces in the inorganic forms of nitrates and ammonium salts, it would take but a short time before growth of plants and animals would stop, were it not for the activities of the microorganisms in the soil. The microbes of the soil must constantly replenish the supply of available nitrogen and carbon for the plants and keep these two most important elements in constant circulation. As soon as this stops, which rarely, if ever happens under natural conditions, the soil is unable to support further life of plants and animals. The elements hydrogen and oxygen are present in such great abundance in available forms in the water and as gases that they rarely become limiting factors in the cycle of plant and animal life.

When fresh organic substances, whether of plant or of animal origin, are introduced into the soil, the numerous bacteria, fungi, protozoa and other invertebrates immediately become active and rapidly attack the various constituents, changing them back to the simple compounds from which the plant started to manufacture its tissues. This process or group of processes may be carried out by one or more than one group of organisms, through one reaction or through a series of very complicated reactions, usually one organism following another or one competing with another, the nature of the organisms and the mechanism of transformation depending upon the nature of the organic matter and environmental soil conditions. The sum total of the activities of the various organisms in bringing about the transformation of the complex plant and animal organic materials into simple inorganic elements or compounds is known as the *mineralization of organic matter,* frequently spoken of in the older literature as decay and putrefaction.

In the process of the mineralization of organic matter, many of the elements are liberated in forms which cannot be assimilated directly by higher plants. The mineralized inorganic elements or simple inorganic compounds may have to be transformed first into compounds which the plants can utilize and assimilate more readily. For example, in the decomposition of organic matter, the nitrogen is liberated as ammonia, while most plants prefer and many even require the nitrogen in the form of nitrate; the sulfur may be liberated as hydrogen sulfide, while the plant needs its sulfur as sulfate. A large part of the carbon may be left in the form of various organic acids, aldehydes, and alcohols, while the plant uses its carbon as carbon dioxide. The final transformation of the materials into forms available for higher plants depends again upon the activities of various groups of microbes, some oxidizing the ammonia to nitrous acid then to nitric acid which combines with the soil bases to give nitrates; others oxidize the sulfides to sulfates; still others oxidize the various organic acids, aldehydes, and alcohols, liberating carbon dioxide.

SYNTHETIC ACTIVITIES OF MICROORGANISMS.—As a result of their various activities, the microorganisms in the soil build up considerable organic matter of their own. Microorganisms are not like catalysts, which do not increase or decrease in amount in bringing about a certain reaction; on the contrary, the reactions brought about by a microbe result in synthesis of microbial cell substance, with the result that certain of the simple products formed in the reaction are once more built up into complex substances. The microbe may be likened in this respect to any other living organism which is large enough to be seen with the naked eye. Food is consumed by an animal in the form of organic compounds which furnish energy for its development. Some of the organic materials are broken down with the liberation of energy which is utilized for synthesizing cell substance and conducting other vital processes. During active growth, an appreciable part of the consumed elements remains in the body as tissues of the animal. The remainder is eliminated as waste products such as carbon dioxide, water, urea, and a variety of organic substances. Similarly, in the development of the microbe, the transformations are associated with cell growth and liberation of waste products.

Although the results of this synthesis may or may not be desirable from the point of view of soil fertility, this is merely a result of the nutritional development of microorganisms under the environmental conditions existing in the specific habitat. The organism develops in competition with its associates to make the best growth possible under the circumstances. In the absence of growing plants for a period of time, there is a continuous depletion of sources of energy for cell development, and the waste products of the cells become the simple inorganic substances from which further plant growth develops. The renewal of growth of higher plants again starts the supply of food for the microbe.

Each reaction brought about by microbes is accompanied by a definite amount of growth as indicated by the amount of cell substance synthesized, depending upon the nature of the organism, amount of energy and nutrients available, and environmental conditions. The larger organisms of the soil, namely, those of an animal nature, feed on the smaller microbes. These numerous changes thus result in the manufacture of a considerable amount of organic matter of microbial origin in the soil. Frequently large quantities of nitrate nitrogen, phosphates, and other nutrient elements which are so essential to the growth of higher plants are temporarily stored away in the bodies of the microbes. In carrying out these processes, the microbes compete with the green plants for the available soil nutrients. Fortunately, even the microbes are not invulnerable against the attack of their own kind, and the process of mineralization continues even in the case of microbial cell constituents. A part of the nutrients may remain for a more or less extended period of time, thus locked up in the bodies of the microbes, or in the form of disintegration products of their cells.

Disappearance of Nitrogen from the Soil.—There are other ways whereby microorganisms may bring about processes which tend to injure the growth of the green plants, as by the reduction of nitrates to atmospheric nitrogen carried out under certain conditions and by certain organisms. Once the nitrogen is changed into its elementary or gaseous form, it is lost from the soil as far as the green plants are concerned. Notwithstanding the fact that nearly 80 per cent of the gases of the atmosphere is made up of nitrogen, which amounts to many thousands of tons (31,250) over each acre of soil, nitrogen is usually the limiting element in the growth of green plants. This is simply due to the fact that this gaseous nitrogen is inert as far as green plants are concerned. Any activities of microorganisms which tend to change even a small fraction of the limited supply of combined nitrogen into the gaseous form are distinctly injurious to plant growth. As a result of continued cropping, certain farm practices, and natural environmental conditions, these losses of nitrogen may be far from negligible.

Nitrogen may be removed in crop plants, later fed to animals and a small portion returned to the soil as excretion products of these animals. It may be lost as volatilized ammonia from manure heaps. This may later become precipitated upon the soil and become available to plants, but, from a practical standpoint, the nitrogen volatilized is of little value. Nitrogen may become stored away in the cells of many microorganisms at least temporarily. Nitrates may be removed from soils in the natural leaching waters in humid climates and in the irrigation waters in arid regions. Nitrogen may also disappear in some cases in the gaseous form through the reduction of nitrates.

Nitrogen Fixation in Soil.—To counterbalance such losses of the combined nitrogen from the soil, nature has provided certain groups of organisms with the capacity of fixing atmospheric nitrogen, *i.e.*, using the nitrogen in its gaseous elemental form and building from it compounds of nitrogen which sooner or later become available to green plants. These microbes are known as nitrogen-fixing bacteria. Some, as the non-symbiotic forms, live in a free state in the soil and require sources of energy (in the form of various carbohydrates) to enable them to fix the nitrogen. Others live in the roots of certain plants (largely legumes), or even in the leaves, the bacteria fixing the nitrogen and making it available to the plants, and the latter manufacturing sugars and other carbohydrates which they supply to the bacteria as sources of energy. This mutually beneficial growth of green plants and bacteria is known as *symbiosis*, and the bacteria are known as symbiotic nitrogen-fixing bacteria.

Subsequent to the elimination of nitrogenous compounds or the death of the bacteria, the complex proteins produced by fixation processes are sooner or later mineralized by other bacteria, fungi, or actinomyces, and the nitrogen made available to free-growing green plants. These nitrogen-fixing bacteria may be consumed by protozoa, but no loss of nitrogen is involved in this process. The protozoa use the organic nitrogen in their nutrition and in turn die and themselves pass through the mineralization process. Certain nitrogen-fixing bacteria may live symbiotically not only with higher plants such as the Leguminosae, but also with certain microscopic green plants as the algae. This associative development appears to favor both the algae, which synthesize carbohydrates from the carbon dioxide of the air, and the bacteria, that build up complex organic proteins and other nitrogen compounds from the gaseous nitrogen of the atmosphere; each of the two groups of organisms is able to use the products synthesized by the other, thus bringing about processes which lead both to an increase of soil organic matter and of combined soil nitrogen.

RÔLE OF MICROBIAL METABOLIC PRODUCTS IN SOIL TRANSFORMATIONS.—The metabolic processes of the microorganisms become very involved because of the fact that the medium, the soil, is so complex, containing an almost limitless variety of organic and inorganic substances. The environment may be very different in one locality from that in another, on account of the inherent soil characteristics and the influence of climate and vegetation. The conditions favor the development of a microscopic population composed of an abundance of different forms which further complicate the mechanism of the transformations by their varied food requirements and metabolic products. Many of the transformations of the soil constituents are affected by the microbes only indirectly, as the metabolic products exert their effects as agents of solution, precipitation, oxidation, and reduction. A few illustrations may indicate the nature of these effects.

Phosphorus is frequently introduced into the soil in the form of rock phosphate or as insoluble tri-calcium phosphate. In this form it can be used by green plants to only a very limited extent. When the phosphate is acted upon by the various organic acids which are formed in the soil by fungi and bacteria (gluconic, citric, oxalic, fumaric, lactic, butyric, formic, acetic, valerianic, or by the carbonic acid, which is always present in abundance), it is changed from an insoluble to a soluble form, which is more readily available to plants.

Of the various acids produced by the microorganisms, the carbonic and the organic acids exert much weaker effects as solvent agents than some of the inorganic acids, because of their relatively low ionization. Much more pronounced effects are exerted by the inorganic acids formed by microbes, such as nitric, resulting from the oxidation of ammonia, and sulfuric, formed from elementary sulfur. Under natural soil conditions, relatively small amounts of organic and strong mineral acids are produced by microorganisms. On the other hand, carbon dioxide is produced in a continuous flow of greater or less intensity wherever living organisms are present. Its effects upon more resistant soil minerals would not be pronounced except over comparatively long periods. The strong mineral acids, even if formed in small amounts, can liberate some potassium from its combination with the zeolitic portions of the soil and even from the more resistant soil minerals.

As a result of the decomposition processes which take place in the soil, the reaction changes either to more acid or to more alkaline, and frequently one way, then another. The decomposition of urea, for example, leads to the formation of ammonium carbonate, which tends to make the reaction of the soil more alkaline; the ammonium carbonate is then oxidized to nitric acid, which tends to make the reaction more acid than it was initially.

Reduction and Oxidation Processes.—Numerous other reactions take place in the soil as a result of the activities of microorganisms, especially the processes of reduction and oxidation. The following may be mentioned as suggestions of these reactions: the reduction of nitrates to nitrites, to ammonia, to oxides of nitrogen, or elementary nitrogen; the reduction of sulfates to sulfides, of phosphates to phosphites and even phosphene; the reduction of selenium and tellurium compounds; the reduction of amino acids and various organic compounds; the numerous oxidation processes, some of which have been mentioned previously; the various hydrolytic and other transformations of organic and inorganic compounds in the soil.

SUMMARY.—By comparing, in a very general way, the activities of the microorganisms in the soil with those of a population of a large modern country, certain interesting similarities are evident. One gram of soil possesses as many micro-inhabitants as the total human population of the United States. These inhabitants also take part in as great a variety of activities as the human population. Some are occupied with obtaining raw materials from the earth; a part of these materials is consumed by those that labor for them, while a part is turned over to the industries for further exploitation and processing. The industrial workers consume a part of the materials either in a raw or in an industrialized form and turn back a part of the manufactured products to the original cultivators of the soil. Influences responsible for the concentration of raw materials in a favorable environment lead to an abnormally abundant population in the region and an accelerated consumption of these materials. Similarly, limitations of the food supply or lack of favorable environmental conditions cause a quick depletion of the population. Even the parasites of human society have their equivalent in the society of the microscopic population below our feet—these are the numerous fungi, nematodes, and bacteria that can cause diseases of plants, or are so cannibalistic as to feed on their own kind or on other members of the soil population of an equal or lower social stratum.

Methods used in an approach to the study of the soil organisms are all quite similar to those used in a study of the human population. Certain observations are confined to an enumeration of the total population, to those of different races or physical types, or those concerned in specific activities. An entirely different type of examination might be applied to studies of the activities of the population in an attempt to estimate the amounts of raw materials transformed, the rates of transformation, the end products formed and their fate or further utilization.

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