INTRODUCTION TO SOIL MICROBIOLOGY

SECOND EDITION

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PREFACE

In the last few years, there have been many significant contributions in and from soil microbiology; for example, in the realm of applied science, the studies showing the crucial role of the soil microflora in modifying or destroying environmental pollutants, the finding that the subterranean populations themselves may form toxic products, the large-scale utilization of legume inoculation, and the investigations of the influence of the microflora on the degradation and persistence of agricultural pesticides. There has also been considerable emphasis on the interrelationships between saprophytes and pathogens in the soil as related to plant disease. In the realm of pure science, information on the ecology, function, and biochemistry of the microflora has grown considerably so that a clear picture of soil biology is beginning to emerge.

This second edition is not a definitive monograph but an introduction to soil microbiology. The innumerable developments in recent years make a complete review impossible within the scope of a single volume. Some of the more detailed points have been omitted for the sake of brevity, yet, where conflicts still exist, the contrasting viewpoints are presented. In certain problematic areas, however, I have weighed the evidence and presented the stronger case. Time may change these views, but it is in the very nature of science to be in a constant state of flux and for the errors of one generation to be mended by the next.

Soil microbiology is not a pure discipline. Its origins may be traced through bacteriology, mycology, and soil science; biochemistry and plant pathology have also made their mark, especially in recent years. The last few years have also witnessed the contribution of many facets of environmental science and technology. Any approach to soil microbiology consequently must consider the variety of individuals and disciplines that have created the mold. The approach here is to use microbiology, soil science, and biochemistry as partners. Where possible, each transformation is viewed as a reaction of importance to soil and to crop production, as a biological process brought about by specific microorganisms whose habitat is the earth's crust, and as a sequence of enzymatic steps.

Because these three disciplines are woven together into the fabric of soil microbiology, one should be familiar to some extent with basic principles of soil science, microbiology, and the chemistry of biological systems. In the framework of agriculture and the environmental sciences, the microflora is significant because it has both a beneficial and a detrimental influence on people's ability to feed themselves and on the quality of their environment. For the microbial inhabitant, the soil functions as a unique ecosystem to which the organism must become adapted and from which it must obtain sustenance. But, in the last analysis, the microbiologist can find definitive answers as to how these processes are brought about only through biochemical inquiries.

The book is divided into three general areas. First is a discussion of the major groups of microorganisms, particularly their description, taxonomy, abundance, and their significance and function. Then the major transformations carried out by the microflora are reviewed, including the reactions centered on carbon, nitrogen, phosphorus, sulfur, iron, and other elements. These are presented in terms of agronomic importance, the specific organisms concerned, the possible ecological consequences, and the biochemical pathways involved. Finally, ecological interrelationships affecting the microflora and higher plants are discussed.

References are of great value not only to the research worker but to the advanced student as well. The blind acceptance of secondary sources when the primary material is readily accessible is not the hallmark of the serious student. Where available, reviews are cited at the end of each chapter so that the finer points of each topic may be sought out. Pertinent original citations are similarly included since these permit student and researcher alike to examine the original source, observe the techniques utilized, and draw their own conclusions. Emphasis is given to the more recent papers, but certain of the classical works are included, particularly where the studies have indicated a unique approach. Absence of citations reflects not the lack of quality of an investigation but only the lack of adequate space between the covers of any single book.

As an introduction to soil microbiology, many things are left unsaid. I hope that a groundwork is laid here for a fuller inquiry on the reader's part. If this goal is reached even in a small way, the book will have served its purpose.

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Ithaca, New York, 1976 Martin Alexander

CONTENTS

MICROBIAL ECOLOGY

1
The Soil Environment

In an introduction to the microbiology of soil, it is essential to consider carefully the nature of the environment in which the microorganisms find themselves. The forces that play a role in the dynamics of soil populations and the effects of these populations on their environment are governed to a very great extent by the physical and chemical properties of the soil. It is to these, therefore, that attention must first be drawn.

The term *soil* refers to the outer, loose material of the earth's surface, a layer distinctly different from the underlying bedrock. A number of features characterize this region of the earth's crust. Agriculturally, it is the region supporting plant life and from which plants obtain their mechanical support and many of their nutrients. Chemically, the soil contains a multitude of organic substances not found in the underlying strata. For the microbiologist, the soil environment is unique in several ways: it contains a vast array of bacteria, actinomycetes, fungi, algae, and protozoa; it is one of the most dynamic sites of biological interactions in nature; and it is the region in which occur many of the biochemical reactions concerned in the destruction of organic matter, in the weathering of rocks, and in the nutrition of agricultural crops.

GENERAL DESCRIPTION OF SOIL

The soil is composed of five major components: mineral matter, water, air, organic matter, and living organisms. The quantity of these constituents is not the same in all soils but varies with the locality. Of the inanimate portion, the amount of mineral and organic matter is relatively fixed at a single site; the proportion of air and water, however, fluctuates. Air and water together account for approximately half the soil's volume, the volume so occupied representing the *pore space*. The mineral fraction, contributing generally slightly less than half the volume, originates from the disintegration and decomposition of rocks, but the fraction has become, during the course of time, modified from the rocks from which it was derived. Organic matter usually contributes some 3 to 6 percent of the total. The living portion of the soil body—including various small animals and microorganisms—makes up appreciably less than 1 percent of the total volume, yet it is undoubtedly essential for crop production and soil fertility.

The inorganic portion of soil, because of its influence on nutrient availability, aeration, and water retention, has a marked effect on the microbial inhabitants. In the mineral fraction are found particles of a variety of sizes, ranging from those visible to the unaided eye to clay particles seen only under a microscope. The various structural units are classified on the basis of their dimensions. At the largest extreme are stones and gravel, materials whose diameter exceeds 2.0 mm. Somewhat smaller are the sand particles that have a diameter of 0.05 to 2.0 mm. Structures whose diameter falls between 0.002 and 0.05 mm are classified as silt, and those with a diameter less than 0.002 mm (2 μm) are considered to be clay particles.

The individual particle types differ from one another in other ways in addition to their dimensions. Thus, many more individual structural units are present in one gram of pure clay than in a gram of silt, and more particles are found in a gram of silt than in a like quantity of sand. More important, however, is the far greater surface area exposed per unit of mass of clay than for the larger particles (Table 1.1). Because the chemical properties and activities of the particles are directly related to their surface area, the status of clay as a reactive constituent in the soil body assumes prominence. In turn, the clay fraction is the most influential in terms of microbiological effects. The clay minerals contain silicon, oxygen, and aluminum; also, iron, magnesium, potas-

TABLE 1.1

Size and Surface Area of Soil Particles (Foth and Turk, 1972)

Particle Type	Diameter mm	No. of Particles/g[a]	Surface Area sq cm/g
Very coarse sand	2.00–1.00	90	11
Coarse sand	1.00–0.50	720	23
Medium sand	0.50–0.25	5,700	45
Fine sand	0.25–0.10	46,000	91
Very fine sand	0.10–0.05	722,000	227
Silt	0.05–0.002	5,780,000	454
Clay	<0.002	90,300,000,000	8,000,000

[a] Assumed to have spherical shapes. Calculated on the basis of maximum diameter of the particle type.

sium, calcium, sodium, and other elements may be found to varying degrees. Three of the major clay minerals in soils of the United States are kaolinite, montmorillonite, and illite. Subsequent discussion will reveal, in part, the unique biological role of these and other clay minerals.

By comparison, silts exert a lesser influence on the physical, chemical, and biological properties of soil. The sand particle, a comparatively large unit exposing a small surface area, is of still lesser consequence. Sand, however, does affect the movement of water and air.

For the purposes of description, textural classes have been established. *Texture* is determined on the basis of the soil's content of sand, silt, and clay, and the name of the textural class is ascertained from the triangle shown in Figure 1.1. To obtain the class name, a line originating at the point corresponding to the percentage of silt is drawn inward and parallel to the left side of the triangle. From the point corresponding to the percentage of clay, a second line is drawn parallel to the base of the triangle. The class name is given by the segment in which the two lines intersect.

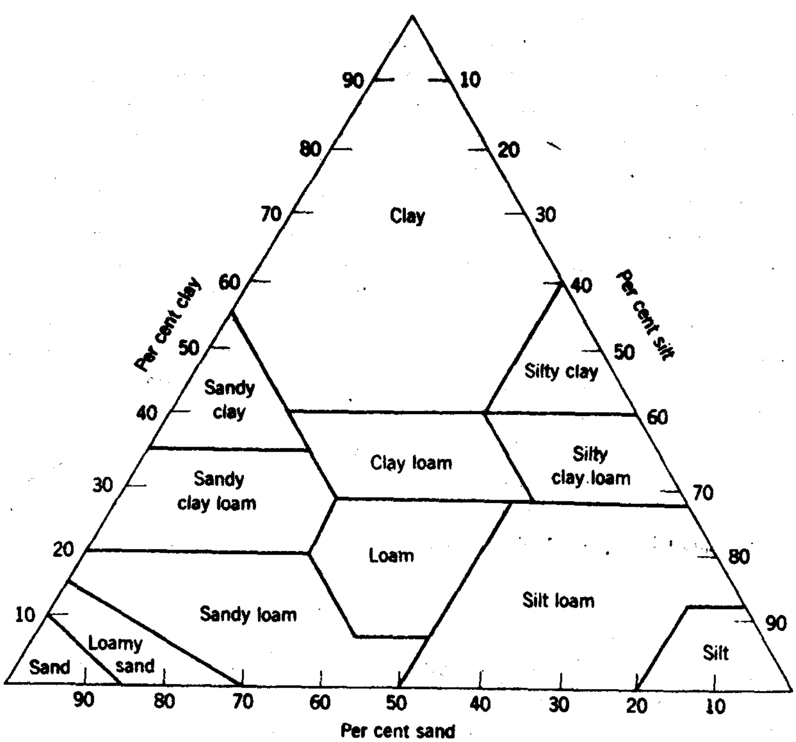

Figure 1.1. The textural triangle from which the names of textural classes are obtained.

The diagram, it will be noted, introduces a new term, *loam*. A loam is a soil not dominated by any of the particle sizes. It may be further noted that considerable emphasis in the textural triangle is placed upon clay content; thus, a soil with less than 40 percent clay may be classified as a sandy clay. This emphasis is a necessary outcome of the great reactivity of clays. Often, the adjectives light and heavy are used in technical or common parlance. Soils dominated by large particles exhibit a coarse texture and are termed light. Heavy soils, by contrast, have a fine texture and are dominated by small particles. It should be borne in mind that textural designations serve a far greater purpose than simple nomenclatural subdivisions because the ease with which a soil is worked, its aeration, and moisture relationships—hence its biological activity—are governed to a great extent by texture.

A vertical section cut into the soil reveals that it possesses a distinct *profile*. In the profile are several horizontal layers known as *horizons*. Even the most casual examination of the layers reveals appreciable differences in structure, texture, and color. These horizons are used in the classification of soils.

As a rule, three major layers make up the profile, the A, B, and C horizons. In addition, an organic horizon may be present, especially in forests. A typical profile may contain (*a*) a shallow or thick surface zone of decaying organic debris, (*b*) an underlying horizon from which certain inorganic constituents have been removed during the long period of soil formation, (*c*) a horizon at greater depth in which is deposited some of the constituents from the upper layers, and (*d*) a bottom layer similar to the original material from which the soil had developed. The organic debris layer is the O horizon. The A horizon, the surface soil, designates the stratum subjected to marked leaching. It is also the layer of greatest biological concern as roots, small animals, and microorganisms are here most dense. In this zone, the concentration of organic matter is highest; hence it is the dominant reservoir of microbial food. The B horizon, the subsoil underlying the A horizon, usually has little organic matter, few plant roots, and a sparse microflora. In it, iron and aluminum compounds often accumulate. At the very bottom of the profile is the C horizon, the layer containing the parent material of the soil proper. In this stratum, organic matter is present in very small quantities, and little life is noted.

No single description adequately characterizes the nature of soil profiles as individual profiles and horizons differ from one another in their thickness, chemical constitution, aeration, color, texture, and water relations. It is not surprising, therefore, that they support microbial communities differing in size and in activity. The attention of the microbiologist is usually drawn to the surface soil because here the population is most dense and the nutrient supply greatest; likewise, the beneficial or detrimental effects of the microflora on higher plants are most pronounced in the A horizon. On the other hand, the subsoil modifies the characteristics of the surface layer as a habitat for both macro- and microorganisms.

DIFFERENCES AMONG SOILS

There are several broad soil belts on the earth's surface. Large areas of the Northern Hemisphere contain *Spodosols*, a group of soils developed in temperate, humid climates in forest areas. These soils usually are poor in organic matter and tend to be acidic. The formation of Spodosols is of considerable interest to the microbiologist since the process is associated with the decomposition of organic matter accumulated at the soil surface and with the downward movement of organic substances formed or released by the subterranean microinhabitants. Soils that are termed *Mollisols* occupy vast areas of land in temperate regions and smaller areas in the tropics. They commonly have a thick A horizon that often is particularly rich in organic matter. In tropical or semitropical zones are found *Oxisols* and *Ultisols*; in these soils, the horizons are usually not distinct. In desert regions are found *Aridisols*, which contain little organic matter, a result of the sparse vegetation in the arid areas. Several representative profiles are shown in Figure 1.2.

The broad soil belts delineated above have been investigated intensively and a classification system established. A review of the classification scheme currently used in soil science is beyond the scope of the present discussion, but the reader can obtain further information from the references cited at the end of the chapter.

The subdivisions within the profile and the common classification schemes are applied to *mineral soils*, whose dominant solid matter is inorganic. *Organic soils* (or *Histosols*)—including the mucks and peats—are widespread and frequently are highly fertile in terms of crop-producing capacity. Organic soils generally have 60 to 95 percent organic matter and, therefore, only a small proportion of mineral constituents. As a result, their chemical and physical properties do not resemble those of the mineral soils. Mucks and peats are formed in bogs and marshes where conditions for the microbiological decomposition of organic matter are poor, and large quantities of carbonaceous substances accumulate. With time, the accumulated residues assume the brown or black coloration typical of organic soils. Because of the way in which mucks and peats are formed, they do not have the usual type of profile. Unfortunately, organic soils have not received their deserved attention so that much of the chemical and microbiological literature is concerned specifically with mineral soils.

Local differences are found among soils. In moving from one area to the next, the depth, color, pH, and chemical composition of the various horizons are found to be dissimilar. The variations may often be traced to the nature of the rock material from which the soil developed, climatic factors, the type of vegetation, and topography. Indeed, it is common to find a single farm situated on several soil types. The differences may be small or they may be appreciable. Physical, chemical, and biological variations need not be measured in kilome-

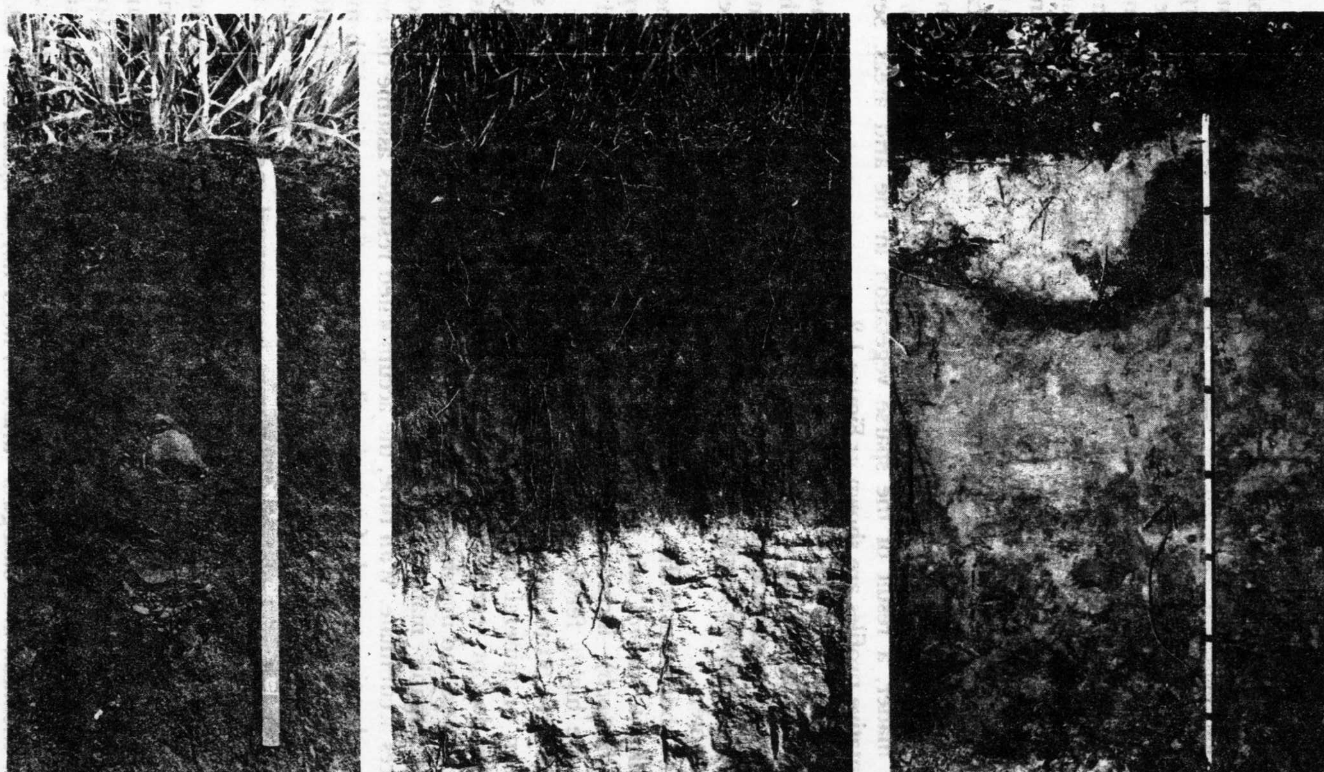

Figure 1.2. Profiles of three great soil groups. Left: Oxisol; center: Mollisol; right: Spodosol. (Courtesy of W. M. Johnson.)

ters, however, as many differences can be found within a small area. Thus, a poorly drained area a short distance from a well-drained site possesses a somewhat altered community of microorganisms. In later chapters, it will be demonstrated that these differences can be measured even in centimeters; for example, the microorganisms immediately surrounding the root surface are not the same as those just one centimeter away from the plant tissue. For the microbiologist, therefore, the soil type is of great consequence as is the microscopic environment within any one soil.

SOME PHYSICAL CONSIDERATIONS

Solid materials occupy only about half the soil's volume. The remainder is composed of pores filled with air and water, both essential for life. The amount of pore space is dependent on the texture, structure, and organic matter content. In clay soils, the pores are generally small. In sandy areas, on the other hand, the pores are large, but the total quantity of pore space is less than in soils rich in fine particles. The size of individual pores and the total pore space affect the movement and retention of water. In sandy soils, water moves rapidly through the large pores, but little is retained. The numerous micropores of heavier soils, on the other hand, contribute to the greater water retention.

The porosity of heavy soils is affected by the state of aggregation. *Aggregates* are large structural units composed of clay and silt particles. In contrast with sand, silt, and clay, aggregates are temporary structures whose stability varies with land management practices, meteorological conditions, microbial activity, and other factors. They range in size from large bodies that are easily broken apart to small granules of firm consistency. In addition to their effects on water and air movement—which in turn regulate the activities of the microflora—aggregates are of interest microbiologically since the cell material and excretions of bacteria, fungi, and actinomycetes are factors affecting the formation and stability of the granules.

The water relationships of soils and the biological effects of moisture have received much study. In certain regions or during certain parts of the year, the soil is quite wet, and too much water is present for optimum biological action. At other times, the moisture level is low, and microorganisms suffer. Because soil water is derived from atmospheric precipitation, the supply is quite variable, and marked fluctuations in the soil water content are the rule in nature.

Part of the water moves with the pull of gravity, this being called *free* or *gravitational water*. Such water is situated within the larger soil pores that are often filled with air; as a result, gravitational water directly affects aeration. In addition, some water is retained against the gravitational pull; the retention against gravity results from the attraction between the water and other soil constituents. Not all of the water in soil is biologically available, and only part of that portion held against gravitational attraction can be used by living systems. Apparently, the nonbiological constituents of soil compete well with microorganisms for water, an indication of the great binding power of the inanimate materials.

The soil solution contains a number of inorganic salts, but except in arid regions, the solution is quite dilute. The liquid phase is of importance to the subterranean flora because it contains several required nutrients. As needed food materials are found in the soil solution, the downward movement of water removes from the zone of microbial accessibility substances essential for proliferation. Nitrogen, potassium, magnesium, sulfur, and calcium but little phosphorus or organic matter are lost through leaching in this way. The rate and magnitude of such losses are regulated by the quantity of precipitation, the presence and type of vegetation, and the soil texture.

Aeration and moisture are directly related because that portion of the pore space not containing water is filled with gas. Air moves into those pores that are free of water; water in turn displaces the air. The gas found in the profile may be said to constitute the soil atmosphere. This subterranean atmosphere is not identical with that in the air above the earth or that at a point several centimeters from the soil surface. Commonly, the CO_2 concentration exceeds the atmospheric level by a factor of tenfold to one-hundredfold, but O_2 is less plentiful. The difference in the composition of the above-ground and below-ground atmospheres arises from the respiration of microorganisms and plant roots, living organisms consuming O_2 and releasing CO_2. Diffusion of the gases tends to right somewhat the concentration gradient so that the content of O_2 and CO_2 is governed by both the diffusion rate and by the rate of respiration. As a rule, the O_2 content declines and the CO_2 level in the gas phase increases with depth.

Changes in the soil atmosphere alter the size and functions of the microflora as both CO_2 and O_2 are necessary for growth. It is of interest, therefore, to speculate on the possibility of attaining a well-aerated (or more appropriately, oxygenated) soil. A well-aerated soil, from the microbiological viewpoint, is one in which microbial processes requiring O_2 proceed at a rapid rate. However, it is unlikely that soil ever becomes sufficiently aerated to satisfy all of its inhabitants because of the problems of gas movement into the small pores and microenvironments in which the organisms are situated. Hence, a soil that is sufficiently well aerated for the growth of higher plants does not necessarily contain an optimum concentration of O_2 for the microflora.

Improper aeration, the opposite extreme, is associated with poor drainage and waterlogging. Since small pores have a greater tenacity for water than the larger pores, the aeration status of heavy soils, which are dominated by micropores, is often poor; that is, a large part of the volume is occupied by liquid rather than by gas. In conditions where the O_2 supply is inadequate, the rates of many microbial transformations are reduced, and some processes may be eliminated. In O_2-deficient habitats, new microbiological processes may come into play, some of which may be deleterious to plant development; for example,