Soil Microbiology,
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Soil microbiology traditionally has been the study of microorganisms and their processes in soil. The interaction of organisms with each other and their environments involves soil ecology. Soil biochemistry includes microbial processes, soil enzymes, and the formation and turnover of soil organic matter. Soil, in the nonengineering definition, is usually defined as the surface of the earth affected by plant roots, even though life, especially that of microorganisms, occurs at great depths in geological deposits, caves, and sediments. Although the organisms involved are often different, their ecological and abiotic controls and the products of their metabolism have great similarities in all locations. Thus, there is now a recognized similarity and interaction with soil and biogeochemical studies in marine and fresh water systems, sediments, and the atmosphere. What we know from these processes on earth will also guide future extraterrestrial investigations and, as a result, the number of people interested in this field has greatly increased.

The textbook “Soil Microbiology and Biochemistry” by Paul and Clark (1989, 1996) is available in Chinese and Korean translations. It has been incorporated into the teaching of engineering, biogeochemistry, ecology, and general biology in a variety of university departments, including those of private, undergraduate, and teaching universities, and is widely used in many research applications.

The biological processes that occur in soil are intertwined with and inseparable from activities of the soil fauna, which feed on plants, soil microorganisms, and litter. Their larger forms act as environmental engineers through their soil-mixing functions. They also contain microbial endophytes that carry out much of their decomposition function. The name of this edited volume has been changed to reflect its broader applicability and has been expanded to include both more basic and applied approaches. Soil microbiology, ecology, and biochemistry are being
used in a broad range of applications from agronomy, plant pathology, general ecology, microbial ecology, engineering, organic agriculture, forestry, range management, and global change. We have thus included chapters on invertebrate–microbial interactions, basic physiology, and ecological interpretations. Information on the management of microorganisms and their reactions has been expanded while we have strived to retain readability, conciseness, and a reasonable cost.

The definition of microbiology is usually associated with organisms not seen without the use of a microscope, although this does not apply to many fungal lichen and algal growth forms. The communal structure of the Armillaria associated with tree roots in a number of areas is hectares in size, although it is still a fungus by definition. The soil fauna also range in size and diversity. This book reflects the great advances in molecular techniques, the broader use of tracers, and the maturation of modeling in interpretation of data and development of new concepts. We finally know enough about our field to be able to impact management of such modern problems as biodiversity, biological invasions, global change, ecosystem services, sustainable agriculture, and urban ecosystems. This textbook has been designed to provide access to necessary knowledge for those working in these diverse fields. The authors of the individual chapters hope that the readers will find this a readable, accessible introduction to both the concepts involved and the background literature.
PART

I

BACKGROUND
GENERAL HISTORY AND SCOPE

The processes that occur within soil are closely related to those in sediments and aquatic environments. They are also associated with the beginning of life on this planet. Biochemical and biological changes were associated in the earth’s early stages. Molecular biomarkers, isotope modification (such as differences in $^{34}$S and $^{13}$C), and identifiable fossils are important in the study of the earth’s history. The primordial soup theory of Oparin and Haldane assumed that organic compounds in water underwent polymerization and condensation reactions similar to those that describe modern soil organic matter formation. The formation of macromolecules that catalyze their own replication is known to be assisted by clays, metals, imidazole derivatives, and selective adsorption onto mineral surfaces that promote concentration and polymerization (Bada and Lazcano, 2003). Carbon and associated N substrates may have arrived on meteorites in association with minerals.

The first written history of soil and soil biota originated in the East, where scholars were recognized in the early Chinese royal courts. Coleman et al. (2004)
stated that soils were classified during the Yao Chinese dynasty from 2357 to 2261 BCE. This dynasty should be recognized for both basic and applied studies of soils as they used a soil classification for taxation purposes. The ancient Chinese regarded earthworms “as angels of the earth.” Romans, such as Aristotle, considered earthworms as “intestines of the earth” (Coleman et al., 2004). Further evidence for the early recognition of soil is that the Hebrew word for soil is “adama,” from which is derived Adam, the first man in Semitic religions (see Hillel, 1991). The ancient Vedic literature of India classified soils by color (and thus organic matter content) and recognized the importance of land forms, erosion, vegetation, land use, and human health implications.

Fungi were known for their fermentation reactions in wine, beer, and bread making and also as a food source that could at times be toxic. Inscriptions on Egyptian walls from 2400 BCE show the production of beer and bread involved the use of a starter and required an incubation time. Eastern, and later Roman, scholars recognized the soil-improving qualities of legumes and crop residue additions. Roman literature on agriculture and soil management was extensive. This was updated and condensed into a single volume by Petrus Crescentius in 1240 CE and for many years was copied, even into the time of the printing press (e.g., Ruralium Commodorium libri duodecin Augsburg, 1471).

Knowledge stagnated in Europe for the one and a half thousand years prior to the Renaissance at the end of the 15th century; not from a lack of intelligence, but from the firmly held belief that the world was governed from the outside and was not an object to be questioned (i.e., intelligent design). The end of the 15th century marked the end of the Western medieval world with the emergence of the perspective that laws that govern the world are subject to study. The concept of biological and abiotic controls that can be studied and influenced by humans marked the beginning of our present knowledge of the soil biota and their processes. The ability to transmit this knowledge by the printed word after the invention of the printing press also greatly aided scientific discovery and discussion.

We are getting further away from our historical roots, an understanding of which is so important to our thinking and ability to formulate scientific questions. The advent of the computer with its easy access to recent literature seems to delay visits to the library to look at not only the original thinking in our field during the early 20th century, but also important literature from 1950 to 1980. I have tried to summarize briefly some of the important early discoveries. In doing so, I have not referred to the original literature, but to reviews often found in textbooks that should be available in many libraries. The history of our science is not merely a listing of the important discoveries, but an important example of scientific thought processes and the relation between methodology, ideas, and concepts.

Our field is still methodology-driven as shown by the great increase in knowledge being derived from molecular techniques and tracers. Another methodology breakthrough was nearly driven to excess, as shown by the fact that the three most cited papers from the Soil Biology and Biochemistry Journal from 1975 to 2000
involved the application of the fumigation technique (earlier used by Schloesing and Müntz for nitrification studies) for the measurement of microbial biomass. Today we are benefiting greatly from the availability of automated techniques, the use of computers in data transformation, modeling and knowledge dissemination, and the presence of active scientists in many new parts of the globe.

A look at our history shows how ideas were generated. It also shows that we should look at some of the misconceptions of the past to help us clearly define our thoughts and concepts. I realize that my biases show and that I have concentrated on the positive. The literature is full of examples showing that many of our founders also developed some “doozies.” It would also be rewarding to look at what did not pass the test of time so that our own ideas do not end up in the same dustbin. A brief survey of citations in some search engines, such as the U.S. National Agricultural Library, Commonwealth Agricultural Bureau, ISI Science Citations, and Biological Abstracts, shows that the words “soil ecology” elicit more responses than “soil microbiology,” which is followed in interest by “soil biochemistry” and “microbial ecology.” There are differences in relative rankings dependent on the search engines, but processes generally involve more citations than microorganisms. Soil N is most popular, followed by soil C, N2 fixation, and the rhizosphere. The citation survey shows that new methods of analysis are being applied to continuing problems with pollutants and pesticides and their effects on the soil population. These topics are continuing to receive a great deal of attention, as is soil biodegradation. If you really want to gain a further appreciation of our field, try general search engines, such as Google, which lists 9,050,000 items for “soil microbiology,” 25,100,000 for “soil ecology,” and 7,800,000 for “soil biochemistry.” An understanding of the interest in the word “humus” would require the perusal of 4,760,000 items. This, however, includes recipes for a common Mediterranean prepared food, hummus, so maybe a better search would be for “soil organic matter,” with 14,600,000 items.

**SOIL MICROBIOLOGY**

Fungi in certain forms can be readily seen without a microscope; thus, they received early study. The first book solely about fungi (“Theatrum Fungorum”) published in 1675 by J. F. van Starbeck drew heavily on the drawings of Charles de’Egeluse prepared as early as 1601 (see Atlas, 1984). In 1665, Hooke published a work on the fruiting bodies of fungi, and by 1724, spores were known as fungal reproductive agents. Fungus–root associations were noted by earlier authors, but in 1877, Pfeffer recognized their symbiotic nature, and in 1885, Franck coined the word “mycorrhiza.” Franck later distinguished between ecto and endo associations; a classification that is still applicable in present, extensive literature on this subject. In 1886, Adametz isolated fungi from soil and gave them names. The first detailed classification of soil fungi was conducted by Oedemans and Koning in 1902 (see Waksman, 1932). In the 1920s, Charles Thom made a detailed study of
soil fungi, especially *Penicillium* and *Aspergillus*, the dominant soil fungi on most agar plates. Waksman also published extensively on soil fungi and actinomycetes.

Leeuwenhoek (1632–1723) is recognized as being the first to see bacteria in his self-designed microscopes. He observed the small animalcules in natural water and in water amended with a substrate (pepper or meat broth). The comprehensive classification system produced by Linnaeus in 1743 perhaps foretold the modern difficulties in bacterial classification when he placed all the organisms seen by Leeuwenhoek in infusions of vegetable matter and meat broth into the genus *Chaos*. In 1776, Nagelli (see Atlas, 1984) suggested that bacteria be placed into their own class entitled Schizomycetes. The work of Warington, Lawes, and Gilbert established the biological nature of many of the processes involved in N transformations, especially those involved with the growth of leguminous crops. Pasteur (1830–1890), in discrediting the theory of spontaneous generation, laid the foundation for microbiology. Although trained as a chemist, he developed vaccines for rabies and investigated many food microbiology problems. Pasteur and Liebig had both postulated that the process of nitrification was bacterial in nature. While studying sewage purification by land filters, Schloesing and Müntz found that the ammonia content of sewage passed through a sand filter did not alter for 20 days. After this period, ammonia was changed to nitrate, but the process could be stopped by a small amount of chloroform. The process could be restarted by soil extract, thus proving that this process was due to microorganisms or, as they said, “organized ferments.”

S. Winogradsky (1856–1953) is recognized as the founder of soil microbiology for his contributions to nitrification, anaerobic N₂ fixation, sulfur oxidation, and microbial autotrophy (Winogradsky, 1949). He succeeded in isolating two bacterial types involved in nitrification with the keen insight that they obtained their C from CO₂. He thus also established autotrophy in microorganisms. In the period 1872–1876, Cohn published the first comprehensive study of the bacterial content of soil. Hellriegel and Wilfarth, in 1888, grew peas in the absence of a fixed N supply, showing that legumes obtained their N from the atmosphere, whereas oats did not have this capability. They knew that the peas had nodules, but could not isolate the bacteria within. Beijerinck, in 1888, isolated the bacteria that he called “*Bacillus radicicola*” (now usually called “*Rhizobium*”). This showed the dependence of the N cycle on bacteria. The N cycle was completed when Goppelsröder observed that nitrates were reduced to nitrites in the presence of soil organic matter. In 1868, Schoenbein ascribed the reaction to bacteria and Gayon and Dupetit further developed the knowledge that led to denitrification studies.

The latter half of the 19th century saw more details on microbial processes including symbiotic and asymbiotic N₂ fixation, denitrification, and sulfate reduction and oxidation. The research on fermentation led to the delineation of anaerobic metabolism. Waksman, in his 1952 textbook “Soil Microbiology,” gives a detailed account of the early contributions and also published photographs of many of our academic forefathers in soil microbiology. His 1932 book gives detailed historical references in each of the chapters, as well as a listing of the textbooks on the various topics to that date. He gives credit (together with Winogradsky) for the foundation of soil
microbiology as a discipline to Martinis Beijerinck (1851–1931), who not only extracted the first viruses from plants, but also isolated many N$_2$-fixing organisms and developed enrichment techniques. Basic and applied sciences were as intertwined in the beginning of our science as they are now. Winogradsky and Beijerinck are also recognized as founding members of microbial physiology and microbial ecology.

The first textbook to include soil microbiology was that of Löhnis, “Vorlesungen über Landwirtschaftliche Bäkteriologi,” published in 1910 and 1913. English readers can gain an insight into its contents in the English version he published together with E. B. Fred in 1923, entitled “Textbook of Agricultural Bacteriology.” That text contains very readable accounts of bacteria, fungi, and protozoa and a good discussion of relationships of microorganisms to their environment. J. G. Lipman (1874–1939), who established the Department of Soil Chemistry and Bacteriology at Rutgers University in 1901, was especially interested in the effects of soil organisms on soil fertility and plant growth. His 1911 book entitled “Bacteria in Relation to Country Life” was the first American treatise in this field. Waksman (1952) named the period from 1890 to 1910 as the Golden Age of soil microbiology when representatives of the soil biota carrying out the major soil and biogeochemical processes were identified. The identification of at least representative members of the microorganisms mediating soil fertility and nutrient transformations led to the belief that this knowledge could do for agriculture what the identification of major disease organisms did for medical treatment.

Successes in legume inoculation led to several premature attempts to alter soil C and N transformations by inoculation and to relate microbial numbers to soil fertility. This discussion continues to this day in the many questions concerning biodiversity and ecosystem functioning addressed later in this volume. The attempts to inoculate bacteria, other than symbionts, and control microbial pathogens of plants were seldom successful because of the lack of knowledge of microbial ecology and the other controls involved. These studies did, however, help transfer attention from pure cultures and laboratory investigations to field experiments and the need for replication to account for soil heterogeneity. This period also contained the interesting conclusion that if an organism did not grow on a gelatin or agar plate, it could not be important and thus was not worth studying.

The years from 1910 to the Second World War witnessed the employment of soil microbiologists in numerous new institutions in many parts of the world. This led to a better knowledge of the global distribution of, and management effects on, organisms capable of growth in the laboratory medium. The development and use of direct microscopy led to the realization that approximately only 1% of the soil population could be grown on laboratory media. The failure of inoculants, except in the case of symbiotic N$_2$ fixation, to create meaningful management effects was a worry at that time. It is only now that we realize the huge number of unidentified organisms and that the unknown interactions between them and their environment (ecology) explain the often observed lack of impact of introduced organisms.

It was at first assumed that bacteria were the major players in soil fertility and decomposition as typified by the books of Löhnis in 1910 and Löhnis and Fred in
1923. In 1886, Adametz showed that fungi are abundant in soil. Additionally, Hiltner and Störmer had studied actinomycetes, which at that time were thought to be different from the bacteria. Cutler had studied the protozoa, and Russell and Hutchinson developed the theory that by consuming bacteria, protozoa could control the soil population and, thus, soil fertility. The early textbooks took as much license with their titles as modern ones. The Löhnis and Fred publication on agricultural bacteriology included extensive sections on the protozoa and fungi discussed under sections such as “Bacteria and related microorganisms.” Waksman’s “Soil Microbiology” included sections we would today call biochemistry. The effects of environmental factors on the rate of soil organic matter decomposition were described by Waksman in his 1932 book entitled “Principles of Soil Microbiology” and the Waksman and Starkey 1931 book entitled “The Soil and the Microbe.”

The period between the two world wars saw work on microbial interactions and nutrient transformations. Fred, Baldwin, and McCoy’s 1932 comprehensive volume on “Root Nodule Bacteria and Leguminous Plants” set the stage for the continued success in symbiotic N2 fixation. The C:N ratio required for plant-residue decomposition without N immobilization was determined as approximately 25:1, a number that is still appropriate unless large amounts of poorly degradable residues are involved, as in forest litter. Attempts to measure many of the microbial processes in soil were frustrated by the inaccuracy of the measurement techniques relative to the large stock of nutrients in soil. Waksman (1932) commented that it was difficult to measure N2 fixation by free-living organisms at levels less than 40 lb per acre, which was (and still is) the inherent error in the Kjeldahl or other methods of measuring total N. The Finnish scientist A. I. Virtanen received the 1945 Nobel Prize in Chemistry for his major contributions to legume nutrition, especially the role of rhizobia in symbiotic N2 fixation. Lie and Mulder (1971), in “Biological Nitrogen Fixation in Natural and Agricultural Habitats,” provide a record of the many advances made in that field.

The Second World War led to a concentration on the war effort. This was, however, not without its success as witnessed by the use of the fungal antibiotic, penicillin, and the development of streptomycin, for which Waksman received the Nobel Prize in Medicine in 1952. The war also resulted in studies to overcome food spoilage and rotting of clothes, as well as the beginnings of biological warfare in both preventive and causative formats. Alexander’s 1961 and 1977 “Introduction to Soil Microbiology” continued the general organization utilized by Waksman in his earlier volumes. He organized the section on the soil environment and bacteria, actinomycetes, fungi, algae, protozoa, and viruses into a section entitled “Microbial Ecology” and recognized the multitude of microbial and microbial–plant interactions. The 1960s saw an influx of new scientists that worked on symbiotic and asymbiotic N2 fixation, S cycling, the rhizosphere, mycorrhizas, and the effects of herbicides, pesticides, and pollutants on the microbial population. The mycorrhizal history to 1969 can be found in Harley (1969). The use of 15N and alternate substrates and inhibitors for specific enzyme interactions made possible for the first time the quantification of the processes in the N cycle at the levels that they occur in soil. However, method availability still hindered testing of concepts regarding
microbial populations and diversity, and it was not until the advent of nucleic acid methodology, automated biochemical measurements, such as phospholipid fatty analysis (PLFA), computers, and modeling that the great thrust of knowledge covered in the subsequent chapters of this volume could come to fruition.


The advances in molecular techniques utilizing culture-independent direct retrieval of 16S rRNA genes have allowed an examination of the occurrence and biodiversity of microorganisms. A survey conducted by Morris et al. (2002) examined the primary scientific literature from 1975 to 1999 in 525 journals. Figure 1.1 shows data for six soil-associated habitats.

**FIGURE 1.1** Publications per year from 1975 to 1999 in microbial diversity: (○) fungal–plant pathosystems, (▲) rhizosphere and mycorrhiza, (△) microbial habitats in soil, (◆) aquatic systems, (—) bacterium plant systems, and (■) food microbiology (Morris et al., 2002).