Organic Residue

The plant and animal remains deposited on or in the soil contribute organic substances. Their decomposition will be described later in this chapter. In the last stages of decomposition, such material is referred to as **humus**, a dark-colored, amorphous substance composed of residual organic matter not readily decomposed by microorganisms. Indeed, the microbial population, both dead cells and living cells, is of such a large magnitude that it contributes significantly to the organic matter of soil. Certain agriculturally important properties are contributed to the soil by humus, which improves the texture and structure of the soil, contributes to its buffering capacity, and increases its water-holding capacity.

Water

The amount of water in soil depends on the amount of precipitation and other climatic conditions, drainage, soil composition, and the living population of the soil. Water is retained as free H_2O in the spaces between soil particles and adsorbed to the surfaces of particles. Various organic and inorganic components of soil are dissolved in soil water and thus are made available as nutrients for soil inhabitants.

Gases

The soil atmosphere is derived from air but differs in composition from it because of the biological processes occurring in soil. The gaseous phase of soil consists mainly of carbon dioxide, oxygen, and nitrogen. These gases exist primarily in the spaces between soil particles which are not filled with water, although a small amount of gas, especially carbon dioxide, is dissolved in water. Obviously, then, the amount of gases in the soil is related to the amount of moisture.

MICROBIAL FLORA OF SOIL

Fertile soil is inhabited by the root systems of higher plants, by many animal forms (e.g., rodents, insects and worms), and by tremendous numbers of microorganisms.

The vast differences in the composition of soils, together with differences in their physical characteristics and the agricultural practices by which they are cultivated, result in corresponding large differences in the microbial population both in total numbers and in kinds.

The conditions described earlier as influencing the growth of organisms under laboratory cultivation are equally applicable to the soil. With specific reference to soil, these conditions can be summarized as follows: (1) amount and type of nutrients, (2) available moisture, (3) degree of aeration, (4) temperature, (5) pH, (6) practices and occurrences which contribute large numbers of organisms to the soil, e.g., floods or addition of manure. The existence of roots and the extensiveness of the root system in soil also influence the numbers and kinds of microorganisms present.

Variations of climatic conditions may selectively favor certain physiological types. Interactions between and among microbial species no doubt has an important effect on the members of the population. This is an extremely complex situation. Predatory protozoa and antibiotic-producing actinomycetes may eliminate certain groups of microorganisms. Cellulolytic and proteolytic organisms, on the other hand, may provide nutrients for less versatile biochemical species.

Few environments on earth have as great a variety of microorganisms as fertile soil. Bacteria, fungi, algae, protozoa, and viruses make up this microscopic menagerie, which may reach a total of billions of organisms per gram (Table 25-1). The great diversity of the microbial flora makes it extremely difficult to determine accurately the total number of microorganisms present. Cultural methods will reveal only those physiological and nutritional types compatible with the cultural environment. Direct microscopic counts theoretically should permit enumeration of all except the viruses, but this technique also has its limitations, especially in distinguishing living from dead microorganisms. Very often the microbiological analysis of soil is concerned with the isolation and identification of specific physiological types of microorganisms. For this purpose enrichment-culture techniques are appropriate.

Bacteria

The bacterial population of the soil exceeds the population of all other groups of microorganisms in both number and variety (see Table 25-2). Direct microscopic counts as high as several billions per gram have been reported; plate counts from the same samples yield only a fraction of this number (millions). The reason for this discrepancy is that there is such a great variety of nutritional and physiological types of bacteria in soil that no single laboratory environment (i.e., composition of medium and conditions of incubation) supports the growth of every viable cell in the inoculum. The following are all likely to be found in soil: autotrophs and heterotrophs; mesophiles, thermophiles, and psychrophiles; aerobes and anaerobes; cellulose digesters and sulfur oxidizers; nitrogen fixers and protein digesters; and other kinds of bacteria. It is generally agreed that there are many species of bacteria in soil yet to be discovered.

Table 25-1. Soil Population in a Fertile Agricultural Soil

Type	Number per Gram
Bacteria:	
Direct count	2,500,000,000
Dilution plate	15,000,000
Actinomycetes	700,000
Fungi	400,000
Algae	50,000
Protozoa	30,000

SOURCE: A. Burges, *Microorganisms in the Soil*, Hutchinson, London, 1958.

Large numbers of actinomycetes, as many as millions per gram, are present in dry warm soils. The most predominant genera of this group are *Nocardia*, *Streptomyces*, and *Micromonospora*. These organisms are responsible for the characteristic musty or earthy odor of a freshly plowed field. They are capable of degrading many complex organic substances and consequently play an important role in building soil fertility. The actinomycetes are also noted for their ability to synthesize and excrete antibiotics. The presence of antibiotic substances in soil can rarely be detected, but this does not exclude the possibility that they may be present and active in the microenvironment.

Fungi

Hundreds of different species of fungi inhabit the soil. They are most abundant near the surface, where an aerobic condition is likely to prevail. They exist in both the mycelial and spore stage. Since growth can take place from either a spore or a fragment of a mycelium, it is difficult to estimate their numbers; however, counts ranging from thousands to hundreds of thousands per gram of soil have been reported. Fungi are active in decomposing the major constituents of plant tissues, namely, cellulose, lignin, and pectin. The physical structure of soil is improved by the accumulation of mold mycelium within it. One of the characteristics of soil of considerable agricultural importance is its

the binding together of fine soil particles to form water-stable aggregates. This is accomplished by the penetration of mycelium through the soil, forming a network which entangles the small particles.

Table 25-2. Physiological Groups of Bacteria in Various Types of Soil (Numbers of Bacteria per Gram of Soil)

Soil Type	Garden	Field	Meadow	Coniferous Forest	Marshland
Moisture content in percent of moist soil	17.9	18.1	17.0	21.2	37.2
Percent calcium carbonate	4.7	5.0	11.4	0	7.6
Bacteria developing on nutrient-gelatin plates	8,400,000	8,100,000	8,100,000	1,500,000	1,500,000
Bacteria developing on nutrient-agar plates	2,800,000	3,500,000	3,000,000	900,000	1,700,000
Bacteria growing in deep cultures of glucose agar (anaerobes)	280,000	137,000	620,000	345,000	2,180,000
Urea-decomposing bacteria	37,000	8,500	5,200	8,800	2,500
Denitrifying bacteria	830	400	850	380	370
Pectin-decomposing bacteria	535,000	70,000	235,000	810,000	3,700
Anaerobic butyric acid bacteria	368,000	50,300	83,500	203,000	235,000
Anaerobic protein-decomposing bacteria	35,000	22,000	36,800	17,000	2,000
Anaerobic cellulose-decomposing bacteria	367	350	367	17.7	1
Aerobic nitrogen-fixing bacteria	2,350	1,885	18	0	17
Anaerobic nitrogen-fixing bacteria	5,500	700	370,000	2,020	67
Nitrifying bacteria	880	1,701	37	0	34

SOURCE: M. Düggeli in S. A. Waksman, *Principles of Soil Microbiology*, Williams & Wilkins, Baltimore, 1932.

Yeasts are likely to be more prevalent in soils of vineyards, orchards, and apiaries, where special conditions, particularly the presence of sugars, favor their growth.

Algae

The population of algae in soil is generally smaller than that of either bacteria or fungi. The major types present are the green algae and diatoms. Their photosynthetic nature accounts for their predominance on the surface or just below the surface layer of soil. In a rich fertile soil, the biochemical activities of algae are dwarfed by those of bacteria and fungi. In some situations, however, algae perform prominent and beneficial changes. For example, on barren and eroded lands they may initiate the accumulation of organic matter because of their ability to carry out photosynthesis and other metabolic activities. This has been observed in some desert soils.

Cyanobacteria, the oxygenic photosynthetic bacteria, are known to grow on the surfaces of freshly exposed rocks where the accumulation of their cells results in simultaneous deposition of organic matter. This establishes a nutrient base that will support growth of other bacterial species. The growth and activities of the initial algae and bacteria pave the way for the growth of other bacteria and fungi. The mineral nutrients of the rock are slowly dissolved by acids resulting from microbial metabolism. This process continues with a gradual accumulation of organic matter and dissolved minerals until a condition results that supports growth of lichens, then mosses, then higher plants. The cyanobacteria play a key role in the transformation of rock to soil, a first step in rock-plant succession.

Protozoa

Most soil protozoa are flagellates or amoebas; the number per gram of soil ranges from a few hundred to several hundred thousand in moist soils rich in organic matter. From a microbiological standpoint they are of significance since their dominant mode of nutrition involves ingestion of bacteria. Of academic interest

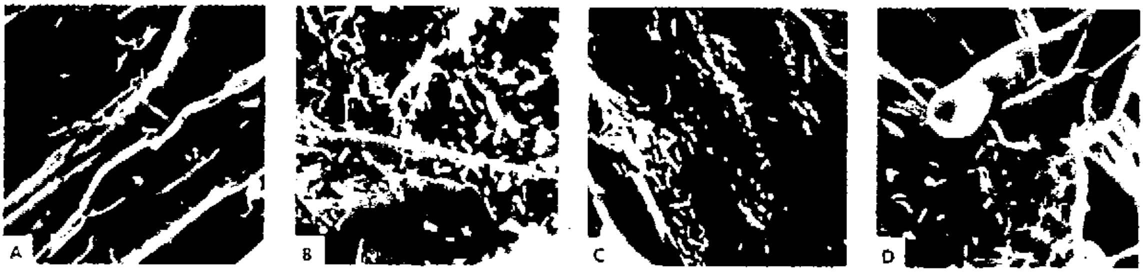


Figure 25-2. The microbial flora in the rhizosphere as seen in scanning electron micrographs of epidermal root cells. In this study plants were removed from a forest preserve, and special precautions were observed to recover root systems with as little mechanical damage as possible to the tissues. The root systems were dissected from the plants and representative specimens selected for electron microscopy. (A and B) Fungal hyphae and bacteria on the epidermal cells of *Ammophilia arenaria*. [(A) × 900; (B) × 800.] (C) Bacteria on the epidermis of a barley root, × 1,000. (D) Root hair, epidermal cell, and bacteria, × 850. (*Courtesy of K. M. Old and New Phytol*, **74**:*51, 1975*.)

is the fact that they demonstrate a preference for certain microbial species. Since not all bacteria are suitable as food for protozoa, the protozoa may be a factor in maintaining some equilibrium of microorganisms in soil.

Viruses

Bacterial viruses (bacteriophages), as well as plant and animal viruses, periodically find their way into soils through additions of plant and animal wastes. Also, soil microorganisms themselves may harbor viruses.

The Rhizosphere

The region where the soil and roots make contact is designated the **rhizosphere**. The microbial population on and around roots is considerably higher than that of root-free soil; the differences are both quantitative and qualitative. Bacteria predominate, and their growth is enhanced by nutritional substances released from the plant tissue, e.g., amino acids, vitamins, and other nutrients; the growth of the plant is influenced by the products of microbial metabolism that are released into the soil. It has been reported that amino acid–requiring bacteria exist in the rhizosphere in larger numbers than in the root-free soil. It has been demonstrated that the microbiota of the rhizosphere is more active physiologically than that of nonrhizosphere soil. The rhizosphere represents a tremendously complex biological system, and there is a great deal yet to be learned about the interactions which occur between the plant and the microorganisms intimately associated with its root system.

Electron-microscope techniques have been developed to observe microorganisms directly on the root surfaces (see Fig. 25-2).

INTERACTIONS AMONG SOIL MICROORGANISMS

The microbial **ecosystem** of soil includes the total microbial flora together with the physical composition and physical characteristics of the soil. It is the sum of the **biotic** and the **abiotic** components of soil.

The microorganisms that inhabit the soil exhibit many different types of associations or interactions. Some of the associations are indifferent or neutral; some are beneficial or positive; others are detrimental or negative. As each different type of association or interaction is discovered, it has been given a specific descriptive label. As you might presume, many of these associations do not fall neatly into discrete categories. Furthermore, and likewise not unexpected, there is the existence of some confusion and contradiction in the use of terms. The term **symbiosis**, for example, as first proposed, referred to the "living together of dissimilarly named organisms"; it was used as a general term. Later it took on a more specific meaning, namely, an association between bacteria and plants referred to as symbiotic nitrogen fixation which is described later in this chapter. Currently the trend is to use the term *symbiosis* as originally intended, that is, merely as a condition in which the individuals of a species live in close association with individuals of another species. We shall describe the following types of microbial associations:

Neutral: neutralism
Positive or beneficial: mutualism, commensalism
Negative or detrimental: antagonism, competition, parasitism, predation

Neutral Associations

Neutralism

It is conceivable that two different species of microorganisms occupy the same environment without affecting each other. For example, each could utilize different nutrients without producing metabolic end products that are inhibitory. Such a condition might be transitory; as conditions change in the environment, particularly availability of nutrients, the relationship might change.

Positive Associations

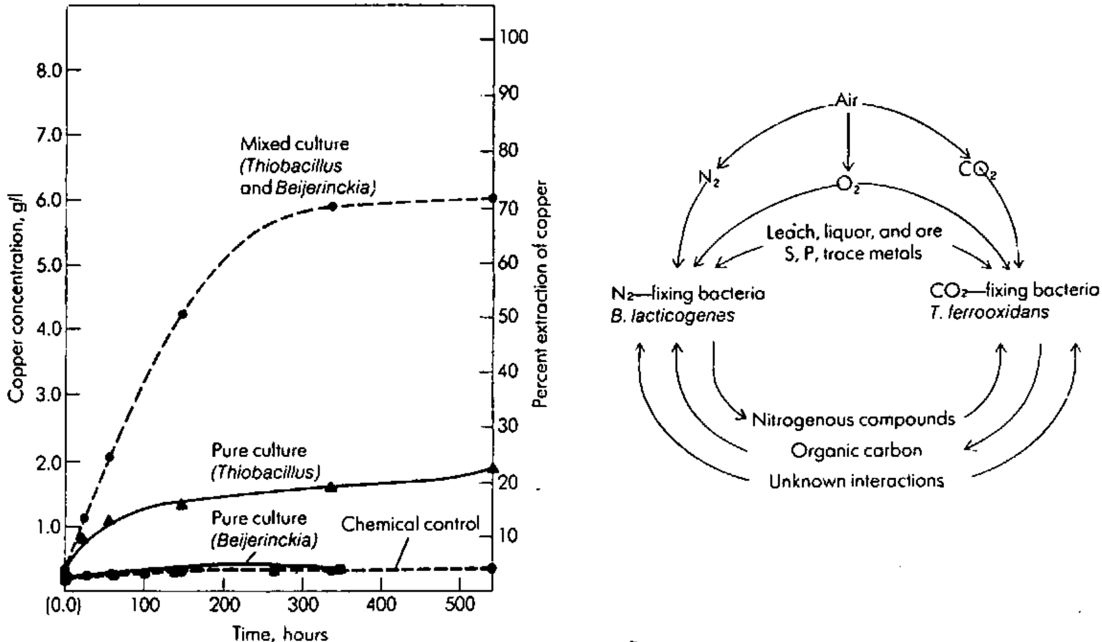
Mutualism

Mutualism is an example of a symbiotic relationship in which each organism benefits from the association. The manner in which benefit is derived varies. One type of mutualistic association is that involving the exchange of nutrients between two species, a phenomenon called **syntrophism**. Many microorganisms synthesize vitamins and amino acids in excess of their nutritional requirements. Others have a requirement for one or more of these nutrients. Still others synthesize a particular essential nutrient in suboptimal amounts. Hence, certain combinations of species will grow together but not apart when nutrient levels are very low.

Another mutalistic association is characterized by different metabolic products from the association as compared with the sum of the products of the separate species. Figure 25-3A illustrates a mutualism between *Thiobacillus ferrooxidans* and *Beijerinckia lacticogenes* in a medium which lacks carbon and nitrogen sources. The growth of the two species in association and the resulting effect on the rate and extent of leaching copper from an ore is shown. Leaching is one of the processes for recovering metals from ore; microorganisms play the important role of oxidizing insoluble metal sulfides to soluble sulfates. The interactions that occur in this mutualistic association are shown in Fig. 25-3B.

Commensalism

The phenomenon of **commensalism** refers to a relationship between organisms in which one species of a pair benefits; the other is not affected. This occurs commonly in soil with respect to degradation of complex molecules like cellulose and lignin. For example, many fungi are able to dissimilate cellulose to



A B

Figure 25-3. An example of microbial mutualism in ore leaching. *Thiobacillus ferrooxidans* and *Beijerinckia lacticogenes* were inoculated into a medium free of added carbon and nitrogen sources; sterile ore concentrate was added to the medium. (A) Results of leaching of copper with pure and mixed culture. (B) Proposed mutualistic interactions between the two species in a leaching environment devoid of fixed carbon or fixed nitrogen. (*Courtesy of N. C. Trivedi and H. M. Tsuchiya, Int J Miner Process,* **2**:*1, 1975.*)

glucose and beyond (they are cellulolytic). Many bacteria are unable to utilize cellulose, but they can and do utilize the fungal breakdown products of cellulose, e.g. glucose and organic acids. The existence of many nutritionally fastidious bacteria in soil suggests that their growth and survival is dependent upon the synthesis and excretion of vitamins and amino acids by less fastidious species.

Another example of commensalism is that of a change in the substrate produced by a combination of species and not by individual species. For example, lignin, a major constituent of wood, is generally resistant to degradation by pure cultures of microorganisms under laboratory conditions. However, the lignin in forest soil is degraded by the soil microbial flora, particularly fungi.

Negative Associations

Antagonism

When one species adversely affects the environment for another species, it is said to be antagonistic. Such organisms may be of great practical importance, since they often produce antibiotics or other inhibitory substances which affect the normal growth processes or survival of other organisms. Antagonistic relationships are quite common in nature. For instance, both *Staphylococcus aureus* and *Pseudomonas aeruginosa* are antagonistic toward *Aspergillus terreus*. Certain *Pseudomonas* pigments inhibit germination of *Aspergillus* spores. *S. aureus* produces a diffusable antifungal material that causes distortions and hyphal swellings in *A. terreous* (Fig. 25-4). Although microorganisms from a variety of natural habitats produce antibiotics, soil microorganisms are the most common producers. It is not unusual for one organism to produce five or six different antimicrobial agents. There is some question about the role of antibiotics in nature; production in the laboratory is under conditions quite different from those in nature. Production of antibiotics in soil may enable the antibiotic-producing organism to thrive successfully in a competitive environment. For example, large populations of actinomycetes have been found in the chitinaceous shells of dead crustaceans in the sea. Their existence, in the environment free of other microorganisms, is likely due to the antibiotics they produce.

Organisms that elaborate antibiotics represent the classic example of this phenomenon: however, antibiosis may result from a variety of other conditions

Figure 25-4. *Aspergillus-Staphylococcus* interaction. (A) *Aspergillus terreus* 20-h culture in nutrient broth with *Staphyloccus aureus* added after 8 h (X500). (B) *A. terreus* and *S. aureus* as in (A) in glucose-peptone broth (X600). (C) *A. fumigatus* and *S. aureus* in glucose-peptone broth (X600). (D) *A. terreus* 20-h culture in nutrient broth (X800). (Courtesy of A. *Mangan, J Gen Microbiol,* **58**:*261, 1969.*)

operative in mixed populations. Cyanide is produced by certain fungi in concentrations toxic to other microorganisms, and the algae elaborate fatty acids which exhibit a marked antibacterial activity. Other metabolic products that may result from microbial activity in the soil which are likely to be inhibitory to other species are methane, sulfides, and other volatile sulfur compounds.

Many soil microorganisms—important examples are the myxobacteria (slime bacteria) and streptomycetes—are antagonistic because they secrete potent lytic enzymes which destroy other cells by digesting their cell wall or other protective surface layers, as shown in Fig. 25-5. Presumably the degraded cellular material, as well as the released protoplasmic material, serves as nutrients. Although it might be assumed that organisms producing lytic substances have a selective advantage over sensitive microbes, microbial interactions of this type are difficult to interpret. It appears that in the natural environment producers of lytic substances are often found in close proximity with sensitive organisms and do not predominate over them.

Competition

A negative association may result from competition among species for essential nutrients. In such situations the best adapted microbial species will predominate or, in fact, eliminate other species which are dependent upon the same limited nutrient substance.

Parasitism

Parasitism is defined as a relationship between organisms in which one organism lives in or on another organism. The **parasite** feeds on the cells, tissues, or fluids of another organism, the **host**, which is commonly harmed in the process. The parasite is dependent upon the host and lives in intimate physical and metabolic contact with the host. All major groups of plants, animals, and microorganisms are susceptible to attack by microbial parasites.

An interesting example of a parasitic relationship between microbes is the bacterial parasite of Gram-negative bacteria named *Bdellovibrio bacteriovorus*, which is widespread in soil and sewage. This unusual motile bacterium attaches

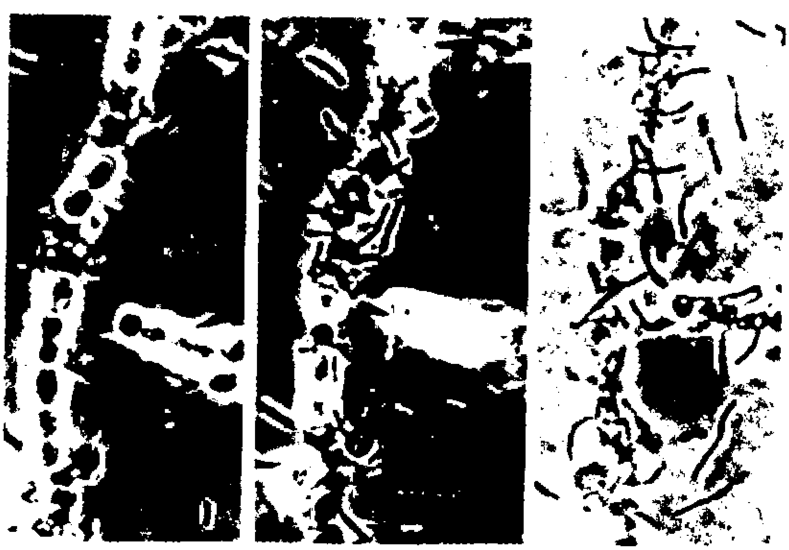


Figure 25-5. Lysis of cyanobacteria by a myxobacter. Shown in this series is a sequence of lysis of *Nostoc* filament by the myxobacter. The myxobacter culture used in this experiment was isolated from fish ponds and is capable of lysing many species of bacteria. (*Courtesy of Mirian Shilo, J Bacteriol*, **104**:*453, 1970.*)

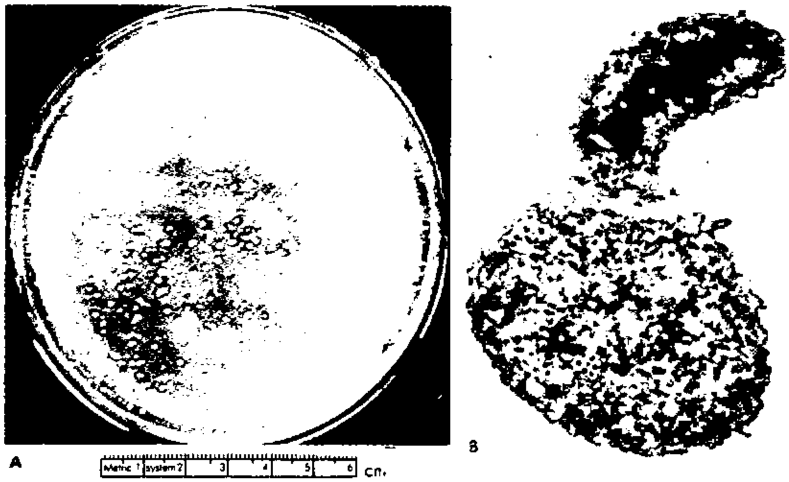


Figure 25-6. Bacteriolysis produced by *Bdellovibrio bacteriovorus.* (A) Plate culture of *B. bacteriovorus* on a lawn of *E. coli* showing whitish-gray colony surrounded by circular plaquelike clearing zone. Central colony consists of bdellovibrios and the clear zone contains a few intact *E. coli* cells and spheroplasts of the host cells (*E. coli*). (B) Electron micrograph thin section showing *B. bacteriovorus* penetration into *E. coli* cell (X48,000). (*Courtesy of J. C. Burnham, T. Hashimoto, and S. F. Conti, J Bacteriol, 96:1366, 1968.*)

to a host cell at a special region and eventually causes the lysis of that cell (see Chap. 13). As a consequence, plaquelike areas of lysis (Fig. 25-6) appear when these parasites are plated along with their host bacteria. There are also many strains of fungi which are parasitic on algae and other fungi by penetration into the host.

Viruses which attack bacteria, fungi, and algae are strict intracellular parasites since they cannot be cultivated as free-living forms. The phenomenon of lysogeny is quite important because of the possibility for genetic recombination in natural populations and the subsequent expression of new characteristics.

BIOGEOCHEMICAL ROLE OF SOIL MICROORGANISMS

Soil microorganisms serve as biogeochemical agents for the conversion of complex organic compounds into simple inorganic compounds or into their constituent elements. The overall process is called mineralization. This conversion of complex organic compounds into inorganic compounds or elements provides for the continuity of elements (or their compounds) as nutrients for plants and animals including people.

It is possible to construct a sequence of reactions to illustrate that microorganisms perform an essential role in maintaining a cyclic process for the reutilization of elements under natural conditions. In this respect we can view the planet earth as a closed system dependent upon the process of recycling for maintenance of life as we know it.

In the following paragraphs we shall discuss the role of soil microorganisms with respect to the transformations they bring about on nitrogen, carbon, sulfur, phosphorus, and their compounds.

BIOCHEMICAL TRANSFORMATIONS OF NITROGEN AND NITROGEN COMPOUNDS: THE NITROGEN CYCLE

Because of the importance of nitrogen for plant nutrition, the biochemical events that make up the nitrogen cycle have been studied in considerable detail.

The sequence of changes from free atmospheric nitrogen to fixed inorganic nitrogen, to simple organic compounds, to complex organic compounds in the tissues of plants, animals, and microorganisms, and the eventual release of this