Chapter 4

Earth Environments

Ian L. Pepper and Terry J. Gentry

4.1 EARTH'S LIVING SKIN

Soil is the thin veneer of material that covers much of Earth's surface. This fragile part of Earth's skin is frequently less than a meter thick, yet is absolutely vital for human life. It has a rich texture and fragrance and teems with plants, insects and microorganisms. Young and Crawford (2004) described it as "the most complicated biomaterial on the planet." The complexity of soil is driven by two components: the abiotic soil architecture and biotic diversity which is driven and supported by large amounts of energy from the sun through photosynthesis. Integrated together these components result in amazing physical, chemical and biological heterogeneity among soils globally.

The abiotic portion of all soils consists of inorganic particles of different size ranges, notably sands, silts and clays. Not only are the size ranges different, but the shapes and morphology of these particles also differ. This results in different specific surface areas of the particulates, with the smaller clays having larger surface areas per unit of mass than the silts and sands. Surface area in turn impacts the surface chemistry of the soil in question, as well as the rates of chemical reactions and transformations. Under the influence of the soil biota, the different sized inorganic particles combine to form secondary aggregates. Pore spaces within the aggregate structure (intraaggregate pore space) and between the aggregates (interaggregate pore space) are crucial to the overall soil architecture (Figure 4.1). The soil architecture in turn is critical for the regulation of water movement and retention, gas exchange and microsite redox potentials within the soil. Totally enclosed pores within aggregates can have much lower redox potentials than open pores between aggregates. The resulting heterogeneity that develops means that both aerobic and anaerobic microorganisms can exist in very close proximity to one another.

Soils also contain biotic components (e.g., plant vegetation, decaying residues, stable soil humus and soil organisms), which add to the soil matrix complexity and architecture. Plant vegetative growth originates in soils, and following the death of plants, senesced organic vegetation is returned to the soil where it is degraded by heterotrophic soil microorganisms. Nutrients released during degradation are utilized by soil microbes and by new vegetation. Inorganic substrates such as ammonium, nitrate or sulfate are subject to autotrophic microbial

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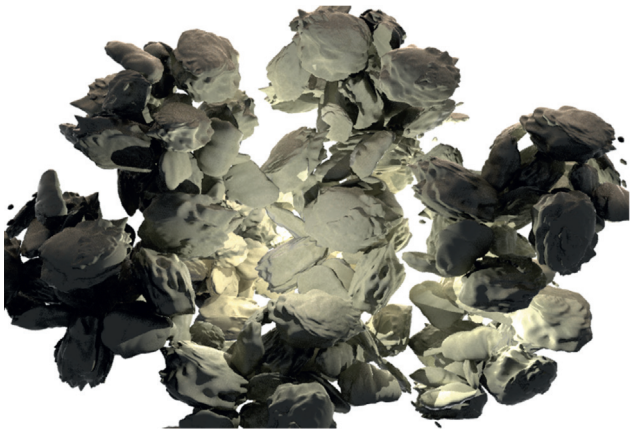

FIGURE 4.1 Soil architecture resulting from secondary aggregate formation with intraaggregate and interaggregate pore space. *Source:* Pepper (2014).

transformations. Some organic residues are incorporated into the organic backbone of soils known as humus. Degradation of organic substrates also results in microbial gums and slimes, which together with fungal hyphae enhance the process of binding primary inorganic particles into secondary aggregates. Microbial populations proliferate in soil, with billions of bacteria and fungi coexisting in close proximity. Other biological entities include phage and protozoa which are important for the control of bacterial populations. The diversity of these microbes with respect to substrate utilization (organic versus inorganic) and redox requirements (aerobic versus anaerobic) results in diverse microbial communities capable of coexisting in microsite niches within the heterogeneous soil matrix. The microbial populations mediate innumerable biochemical transformations within soils. Despite their very large numbers, microbes occupy less than 1% of the total soil surface area, about the same land area on Earth occupied by humans (Young and Crawford, 2004).

The soil colloidal matrix, which consists of micron-sized particles including inorganic, organic and biological entities, dominates soil architecture. Soil architecture, in turn, controls soil chemical and biochemical transformations and soil diversity. The diversity of soil is characterized by physical and temporal heterogeneities across all measured scales from nm to km, and is probably the driving force for the microbial diversity that we see in soil (Young and Crawford, 2004). Diversity estimates of the number of bacterial species in soil range from 2000 to 8.3 million per gram of soil depending on the methodologies utilized (Roesch *et al*., 2007). Regardless of the true estimate, the microbial diversity within soil is clearly enormous and greatly impacts soil health and ultimately human health (Pepper, 2014).

Information Box 4.1 The Five Soil Forming Factors

Parent material. The rock and mineral base from which soil is formed through weathering.

Climate. Precipitation and temperature are particularly important in weathering of parent material.

Organisms. Plants, animals, and microbes add organic matter and aid in decomposition and nutrient cycling that are part of the weathering process.

Topography. In particular the site slope angle and length.

Time. Essential for the soil weathering process; soils generally form more rapidly in warm environments than in cold ones.

4.2 PHYSICOCHEMICAL CHARACTERISTICS OF THE EARTH ENVIRONMENT

4.2.1 Earth Environments

4.2.1.1 Soil

Soil is the weathered end product of the action of climate and living organisms on soil parent material with a particular topography over time. We refer to these factors as the five soil-forming factors (Information Box 4.1). The soil weathering process can take decades to millions of years depending on the soil-forming factors involved. The physical and chemical characteristics of soils are discussed in detail in Section 4.2.2. The major difference between a surface soil and the subsurface is that in the subsurface, the parent material has generally not been weathered by climate. In addition, microbial numbers are much lower in subsurface environments than in soil because of reduced inputs of plant residues that function as substrate for heterotrophic microbes.

4.2.1.2 Vadose Zone

The vadose zone is defined as the subsurface unsaturated oligotrophic environment that lies between the surface soil and the saturated zone. The vadose zone contains mostly unweathered parent materials and has a very low organic carbon content (generally $<0.1\%$). Thus, the availability of carbon and micronutrients is very limited compared with that in surface soils. The thickness of the vadose zone varies considerably. When the saturated zone is shallow or near the surface, the unsaturated zone is narrow or sometimes even nonexistent, as in a wetland area. In contrast, there are many arid or semiarid areas of the world where the unsaturated zone can be hundreds of meters thick. These unsaturated regions, especially deep

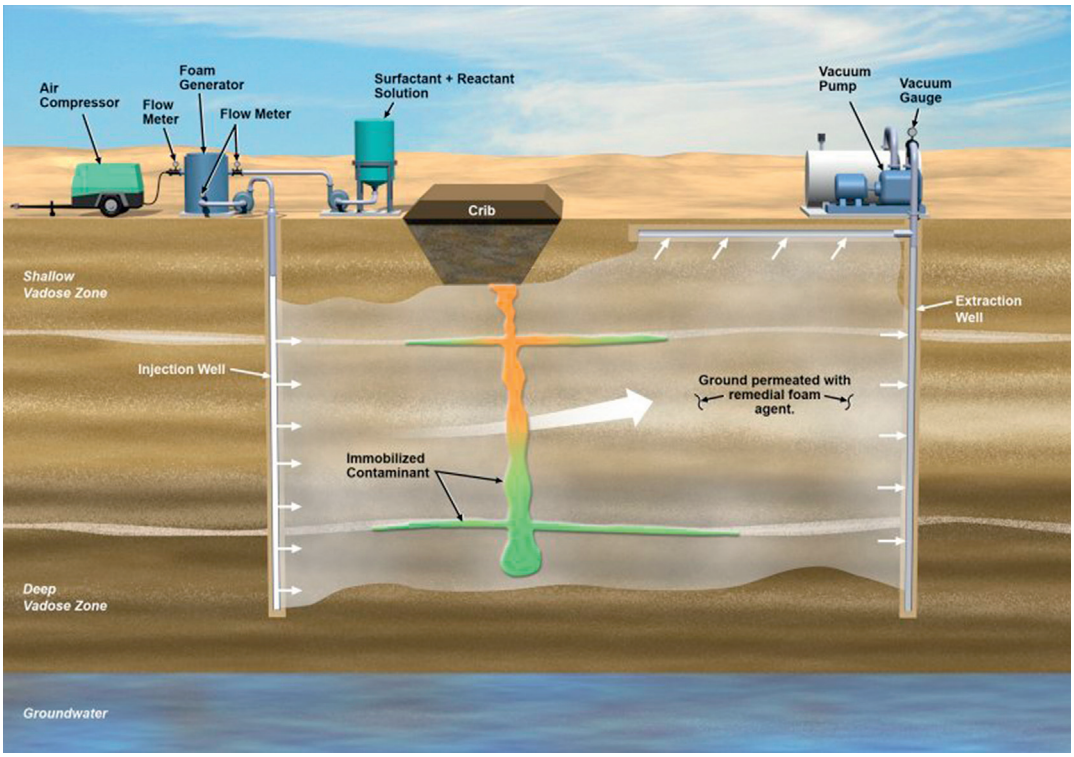

FIGURE 4.2 Delivery of remedial solutions through a heterogeneous deep vadose zone to remove contaminants. *Source:* Pacific Northwest National Laboratory.

unsaturated regions, may receive little or no moisture recharge from the surface, and normally have limited microbial activity because of low nutrient and/or moisture status. However, these regions are receiving more attention from a microbiological perspective, because pollutants that are present from surface contamination must pass through the vadose zone before they can reach groundwater (Figure 4.2).

4.2.1.3 Saturated Zone—Aquifers

The saturated zones that lie directly beneath the vadose zone are commonly called aquifers and are composed of porous parent materials that are saturated with water. Like the vadose zone, aquifers are generally oligotrophic environments. The boundary between the vadose zone and the saturated zone is not a uniformly distinct one, because the water table can rise or fall depending on rainfall events. The area that makes up this somewhat diffuse boundary is called the capillary fringe (Figure 4.3). Aquifers serve as a major source of potable water for much of the world. For example, in the United States, approximately 50% of the potable water supply currently comes from aquifers.

There are several types of aquifers, including shallow table aquifers and intermediate and deep aquifers that are

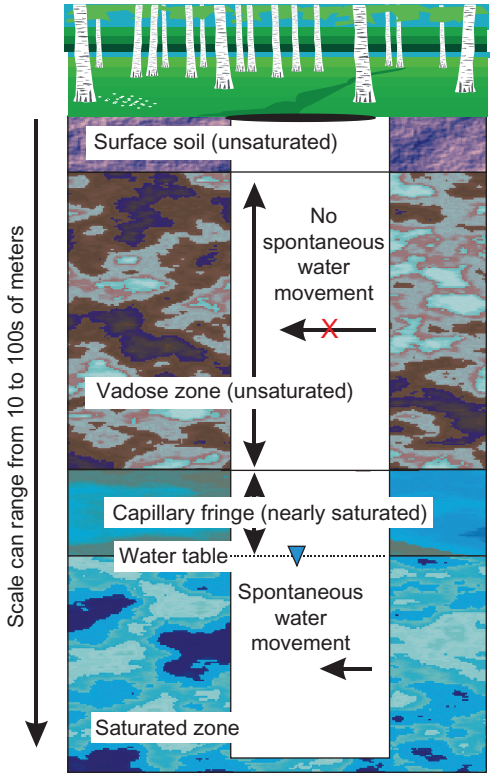

FIGURE 4.3 Cross-section of the subsurface showing surface soil, vadose zone, capillary fringe and saturated zone.

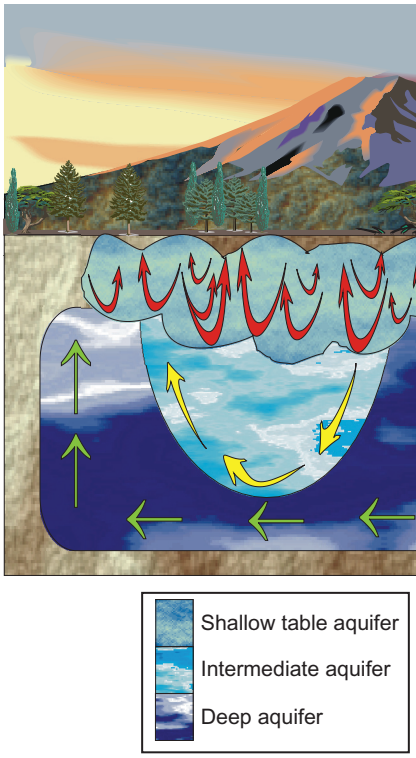

FIGURE 4.4 Shallow, intermediate and deep aquifer systems. Arrow thickness indicates the relative flow rates (the thicker the arrow, the faster the flow rate) in the different aquifer systems. Adapted from Chapelle (1993) and reproduced by permission of Wiley, New York.

separated from shallow aquifers by confining layers (Figure 4.4). Confining layers are composed of materials such as clay that have very low porosity. Such layers allow little water movement between shallow and deeper aquifers. Of these different types of aquifers, shallow aquifers are most closely connected to the Earth's surface and have the highest organic carbon content. They receive water from rainfall events and provide recharge to adjacent streams or rivers. In addition, shallow aquifer systems are very active with rapid groundwater flows (meters per day), and hence usually remain aerobic. Confined aquifers within 300 m of the surface soil are termed intermediate aquifers. These have much slower flow rates, on the order of meters per year. It is this aquifer system that supplies a major portion of drinking and irrigation water. Deep aquifers, those more than 300 m in depth, are characterized by extremely slow flow rates (meters per century). Because so little water flow occurs, these aquifers are usually anaerobic. Deep aquifers are not directly recharged or affected by surface rainfall events.

4.2.1.4 Saturated Zone—Wetlands

Wetlands are important ecosystems throughout the world in areas that have a temperate climate and include swamps, marshes and bogs. Such areas are saturated for most or all of the year because the water table is at or above Earth's surface. These ecosystems are of increasing interest to environmental microbiologists for their potential to treat polluted waste streams such as sewage effluent (Chapter 25) and acid mine drainage.

One important example of wetlands are bogs, which are extensive worldwide, covering 5 to 8% of the terrestrial surface. Bogs are composed of deep layers of waterlogged peat and a surface layer of living vegetation. The peat layers are composed of the dead remains of plants that have accumulated over thousands of years. Thus, in these extensive areas, the production of plant material has consistently exceeded the rate of decomposition of plant material. There are several reasons for this. Because these areas are completely submerged, the limited dissolved oxygen in the water is quickly used up, resulting in extensive anaerobic regions. Under anaerobic conditions, the rate and extent of decomposition of organic material is much lower. A second factor is that many bogs become highly acidic (pH 3.2 to 4.2) as a result of the growth of sphagnum mosses that are an integral part of these areas. The combination of anaerobic and acidic conditions suppresses the growth of most microorganisms that are essential for plant or animal decomposition (Information Box 4.2).

Canada has the most extensive bog system in the world, with 129,500,000 hectares or 18.4% of its land area composed of bogs. Harvesting of peat is an important industry in Canada (peat moss for gardening) and in Ireland and Finland (for production of energy). However, the mining and use of peat sources globally returns fixed and sequestered carbon back to the atmosphere as carbon dioxide. In fact, it is estimated that peat bogs hold three times the amount of fixed carbon than rainforests do and so their use and destruction is likely one factor that is contributing to global warming.

Information Box 4.2 Lindow Man

"Lindow Man" was buried for 2000 years in an English peat bog and his body was discovered in August, 1984. This was an exciting find because his body was so well preserved that scientists could tell that his last meal consisted primarily of cereal grains. Scientists also discovered that "Lindow Man " was murdered (he was hit on the head, strangled, and his throat was cut for good measure). It is thought that a number of factors were important for the preservation of " Lindow Man's " body: the acidic pH, the absence of oxygen and the presence of antimicrobials in the peat, along with the presence of peat components such as sphagnan that reacted with collagen tissue in the body and basically tanned it (Painter, 1991). All of these factors aided in suppressing microbial activity that normally acts to degrade dead tissue.

4.2.2 The Solid Phase

All soils and subsurface environments are three-phase systems consisting of: (1) a solid or mineral inorganic phase that is often associated with organic matter; (2) a liquid or solution phase; and (3) a gas phase or atmosphere. Soil properties are dependent on the specific composition of each of these phases, which are discussed in the following sections.

4.2.2.1 Primary Particles and Texture

Typically, a soil contains 45 to 50% solids on a volume basis (Figure 4.5). Of this solid fraction, 95 to > 99.9% is the mineral fraction. Silicon (47%) and oxygen (27%) are the two most abundant elements found within the mineral fraction of Earth's crust. These two elements, along with lesser amounts of other elements, combine in a number of ways to form a large variety of minerals. For example, quartz is SiO_2 and mica is $K_2Al_2O_5[Si_2O_5]_3Al_4(OH)_4$. These are primary minerals that are derived from the weathering of parent rock. Weathering results in mineral particles that are classified on the basis of three different sizes: sand, silt and clay (Information Box 4.3). The distribution (on a percent by weight basis) of sand, silt and clay within a porous medium defines its texture. Soils predominated by sand are considered coarse textured while those with higher proportions of silt and clay are known as fine textured.

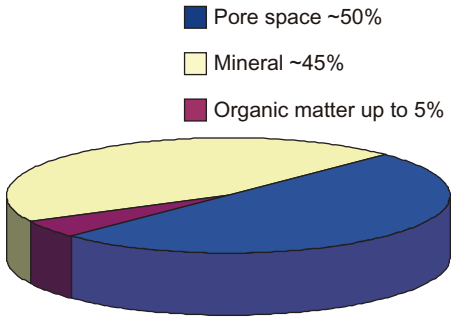

FIGURE 4.5 Three basic components of a porous medium, such as a typical surface soil, on a volume basis.

Information Box 4.3 Primary Mineral Size Classifications

Sand	0.05 to 2 mm
Silt	0.002 to 0.05 mm
Clay	<0.002 mm (2 μm)

4.2.2.2 Soil Architecture

Soil particles do not normally remain as individual entities. Rather, they aggregate to form secondary structures or soil architecture (Information Box 4.4). These structures occur because microbial gums, polysaccharides and other microbial metabolites bind the primary particles together. In addition, particles can be held together physically by fungal hyphae and plant roots. These secondary aggregates, which are known as peds, can be of different sizes and shapes, depending on the particular soil (Figure 4.6). Soils with even modest amounts of clay usually have well-defined peds, and hence a well-defined soil structure. These aggregates of primary particles usually remain intact as long as the soil is not disturbed, for example, by plowing. In contrast, soils that are primarily sand with low amounts of clay have less well-defined soil structure.

In between the component mineral particles of a porous medium are voids known as pore space. These pores allow movement of air, water and microorganisms through the porous medium. Pores that exist between aggregates are called interaggregate pores, whereas those within the aggregates are termed intraaggregate pores (Figure 4.7). Pore space may be increased by plant roots, worms and small mammals, whose root channels, worm holes and burrows create macro openings. These larger openings can result in significant aeration of soils as well as preferential flow of water through these large pores where flow is the easiest.

Texture and structure are important factors that govern the movement of water, contaminants and microbial populations in porous media. Of the three size fractions that make up a porous medium, clay particles are particularly dominant in determining the physical and chemical characteristics. For example, clays, which are often composed of aluminum silicates, add both surface area and charge to a soil. As shown in Table 4.1, the surface area of a fine clay particle can be five orders of magnitude larger than the surface area of a 2-mm sand particle. To put this into a microbial perspective, the size of a clay particle is similar to that of a bacterial cell. Clays affect not only the surface area of a porous medium but also the average pore size (Figure 4.8). Although the average pore size is smaller in a clay soil, there are many more pores than in a sandy soil, and as a result the total amount of pore space is larger in a fine-textured (clay) soil than in a coarse-textured (sandy) soil. However, because small pores do not transmit water as fast as larger pores, soils with higher clay content will slow the movement of any material moving through it, including air, water and microorganisms (Figure 4.9). Often, fine-textured regions or layers of materials, e.g., clay lenses, can be found in sites composed primarily of coarser materials, creating very heterogeneous environments. In this case, water will prefer to travel through the coarse material and flow

Information Box 4.4 The Importance of Aggregation – Cryptobiotic Crusts a Special Case

Aggregation is an extremely important factor for soil sustainability because aggregated soils resist water and wind erosion. One can find a special case of aggregate formation in semiarid and arid areas of the world called cryptobiotic crusts. Cryptobiotic crusts are highly specialized communities of cyanobacteria, mosses, and lichens that form a surface crust of soil particles bound together by organic materials. These crusts are important for soil stability and protect against erosion processes. Unfortunately, these crusts are slow growing and fragile and easily destroyed by hikers and large animals. The pictures show (right) cryptobiotic crusts growing on the Colorado Plateau (United States) and (below) a close-up of a piece of crust showing the aggregate architecture.

From United States Geological Survey, 2006.

FIGURE 4.6 Soil structure results from secondary aggregates known as peds.

around the fine-textured lens. However, once water moves into a clay lens it will be retained more tenaciously than within sandy materials because of the smaller pore spaces.

For microorganisms, which are much larger than individual water molecules, a fine-textured horizon or lens will inhibit bacterial movement either into or out of the region. Such heterogeneity poses great difficulties when trying to remove contaminants because some finely textured regions are relatively inaccessible to water flow or to microorganisms—a process also known as micropore exclusion (Section 15.1). Thus, contaminants trapped within very small pores may remain there for long periods of time, acting as a long-term "sink" of contaminant that diffuses out of the pores very slowly with time.

4.2.2.3 Soil Profiles

The process of soil formation generates different horizontal layers, or soil horizons, that are characteristic of that particular soil. It is the number, nature and extent of these horizons that give a particular soil its unique character. A typical soil profile is illustrated in Figure 4.10. Generally, soils contain a dark organic-rich layer, designated as the O horizon, then a lighter colored layer, designated as the

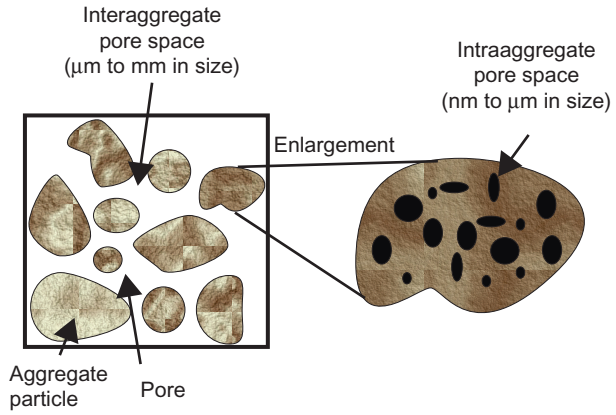

FIGURE 4.7 Pore space. In surface soils, mineral particles are tightly packed together and even cemented in some cases with microbial polymers forming soil aggregates. The pore spaces between individual aggregates are called interaggregate pores and vary in size from micrometers to millimeters. Aggregates also contain pores that are smaller in size, ranging from nanometers to micrometers. These are called intraaggregate pores.

A horizon, where some humified organic matter accumulates. The layer that underlies the A horizon is called the E horizon because it is characterized by eluviation, which is the process of removal or transport of nutrients and inorganics out of the A horizon. Beneath the E horizon is the B horizon, which is characterized by illuviation. Illuviation is the deposition of the substances from the E horizon into the B horizon. Beneath the B horizon is the C horizon, which contains the parent material from which the soil was derived. The C horizon is generally unweathered parent material and marks the transition between a soil and the vadose zone. Although certain diagnostic horizons are common to most soils, not all soils contain each of these horizons.

4.2.2.4 *Cation-Exchange Capacity*

The parameter known as cation-exchange capacity (CEC) arises because of the negative charge associated with clay

TABLE 4.1 Size Fractionation of Soil Constituents

Specific Surface Area Using a Cubic Model	Soil		
	Mineral Constituents	**Size**	**Organic and Biologic Constituents**
0.0003 m^2/g	**Sand** Primary minerals: quartz, silicates, carbonates	2 mm	**Organic debris**
0.12 m^2/g	**Silt** Primary minerals: quartz, silicates, carbonates	50 μm	**Organic debris, large microorganisms** Fungi Actinomycetes Bacterial colonies
3 m^2/g	**Granulometric clay** Microcrystals of primary minerals Phyllosilicate Inherited: illite, mica Transformed: vermiculite, high-charge smectite Neoformed: kaolinite, smectite Oxides and hydroxides	2 μm	**Amorphous organic matter** Humic substances Biopolymers **Small microorganisms** Bacteria Fungal spores Large viruses
30 m^2/g	**Fine clay** Swelling clay minerals Interstratified clay minerals Low range order crystalline compounds	0.2 μm	Small viruses

Adapted from Robert and Chenu (1992).

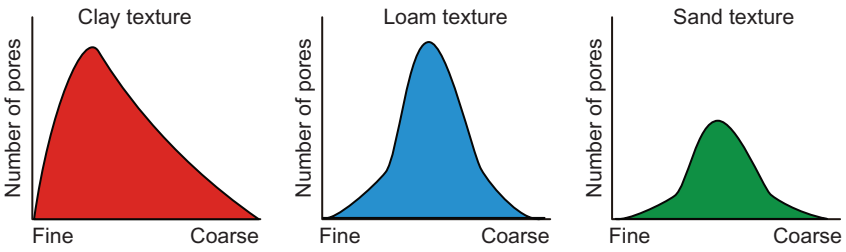

FIGURE 4.8 Typical pore size distributions for clay-, loam- and sand-textured horizons. Note that the clay-textured material has the smallest average pore size, but the greatest total volume of pore space.

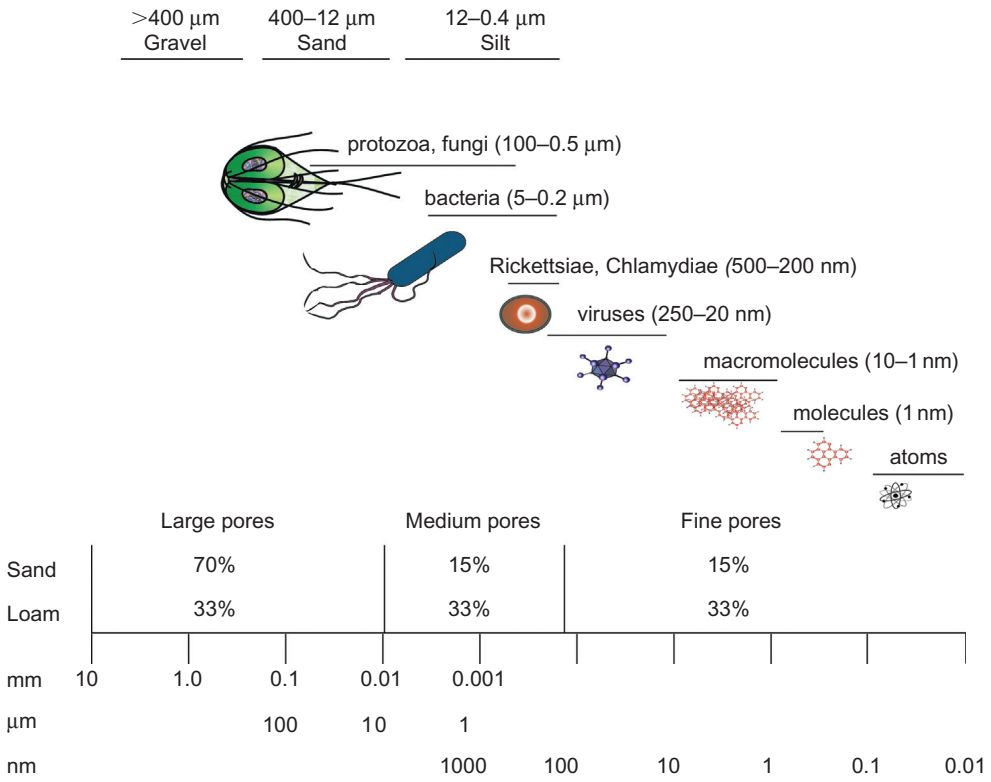

FIGURE 4.9 Comparison of sizes of bacteria, viruses and molecules with hydraulic equivalent diameters of pore canals. Adapted from Matthess *et al.* (1988).

particles and organic matter (Information Box 4.5). Clays are negatively charged for one of two reasons:

1. **Isomorphic substitution:** Clay particles exist as inorganic lattices composed of silicon and aluminum oxides. Substitution of a divalent magnesium cation (Mg^{2+}) for a trivalent aluminum cation (Al^{3+}) can result in the loss of one positive charge, which is equivalent to a gain of one negative charge. Other substitutions can also lead to increases in negative charge.
2. **Ionization:** Hydroxyl groups (OH) at the edge of the lattice can ionize, resulting in the formation of negative charge:

$$Al - OH = Al - O^- + H^+$$

These are also known as broken-edge bonds. Ionizations such as these usually increase as the pH increases, and are therefore known as pH-dependent charge.

The many functional groups of organic matter, such as carboxyl moieties, are also subject to ionization, and can contribute to the total pH-dependent charge. The clay and organic particles that participate in creating CEC are generally very small, <1 µm in diameter, and due to their small size are referred to as soil colloids. Because of their small size, these colloids offer extensive surface area for CEC to occur.

How does the process of cation exchange work? Common soil cations such as Ca^{2+}, Mg^{2+}, K^+, Na^+ and H^+, which exist in the soil solution, are in equilibrium with cations on exchange sites. If the concentration of a cation in the soil solution is changed, for example, increased, then that cation is likely to occupy more exchange sites, replacing existing cations within the site (Figure 4.11). Thus, a monovalent cation such as K^+ can replace another monovalent cation such as Na^+, or two K^+ can replace one Mg^{2+}. Note, however, that when working with charge equivalents, one milliequivalent of K^+ replaces one milliequivalent of Mg^{2+}. Cation exchange ultimately depends on the concentration of the cation in soil solution and the adsorption affinity of the cation for the exchange site. The adsorption affinity of a cation is a function of its charge density, which in turn depends on its total charge and the size of the hydrated cation. The adsorption affinities of several common cations are given in the following series in decreasing order:

$$Al^{3+} > Ca^{2+} = Mg^{2+} > K^+ = NH_4^+ > Na^+$$

Highly charged small cations such as Al^{3+} have high adsorption affinities. In contrast, monovalent ions have lower affinities, particularly if they are highly hydrated such as Na^+, which increases the effective size of the cation. The extensive surface area and charge of soil colloids (clays + organic material) are critical to microbial activity since they affect both binding or sorption of solutes and microbial attachment to the colloids.

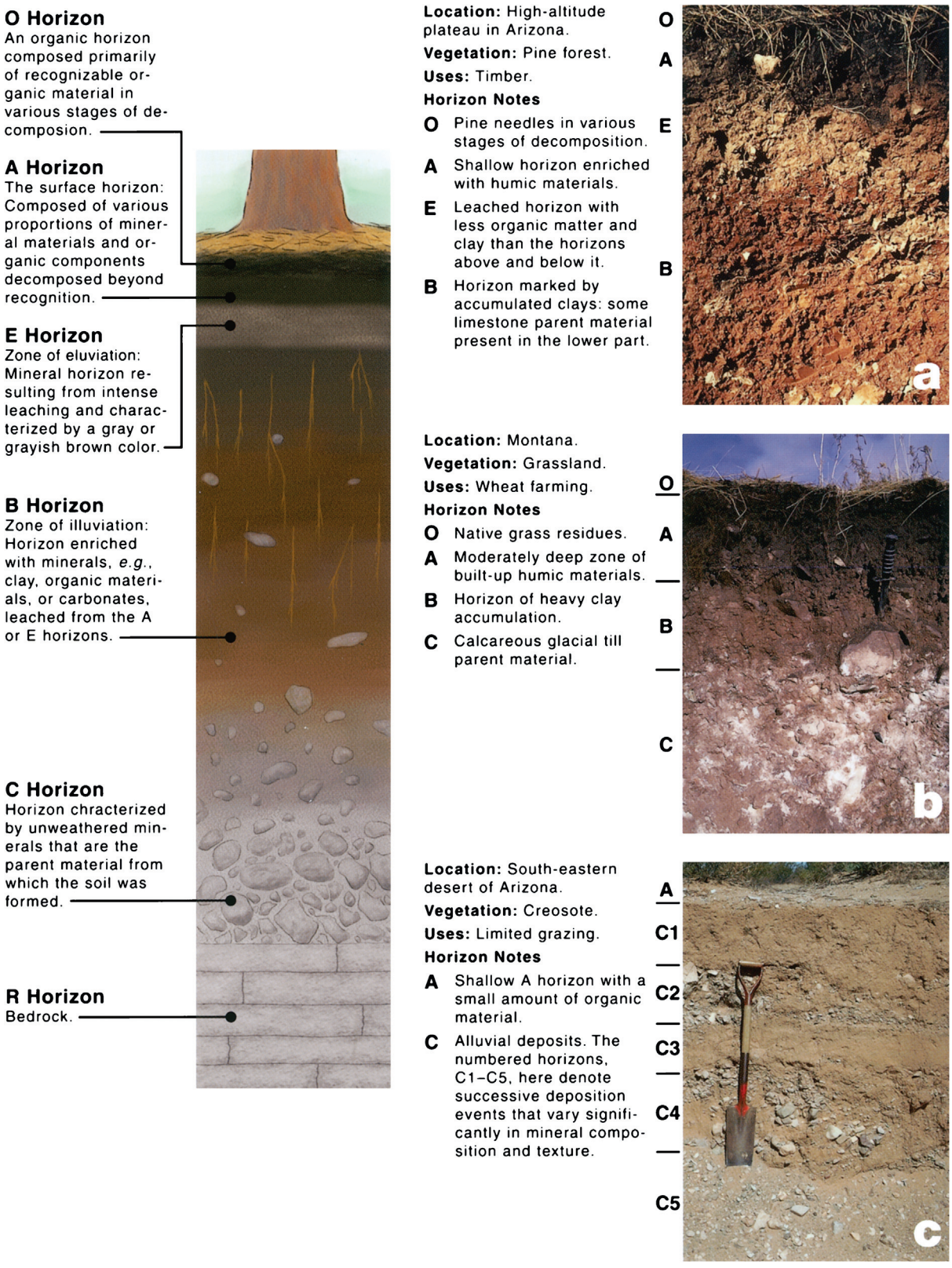

FIGURE 4.10 Typical soil profiles illustrating different soil horizons. These horizons develop under the influence of the five soil-forming factors and result in unique soils. From Pepper *et al.* (2006).

Information Box 4.5 Cation Exchange Capacity in Soil

The total amount of negative charge in soil is usually measured in terms of equivalents of negative charge per 100 g of soil and is a measure of the potential CEC. A milliequivalent (meq) is one-thousandth of an equivalent weight. Equivalents of chemicals are related to hydrogen, which has a defined equivalent weight of 1. The equivalent weight of a chemical is the atomic weight divided by its valence. For example, the equivalent weight of calcium ion is 40/2 = 20 g. The five most common exchangeable cations in soil are Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}, and Al^{3+}.

In soil, a CEC of 15–20 meq per 100 g is considered to be average, whereas a CEC above 30 is considered high. Soils with low CEC (sandy, low organic matter) often have limited nutrient content because they cannot hold cations tightly and therefore these nutrients are leached during precipitation events. Thus, soils with low CEC generally do not support plant growth as well as those with higher CEC.

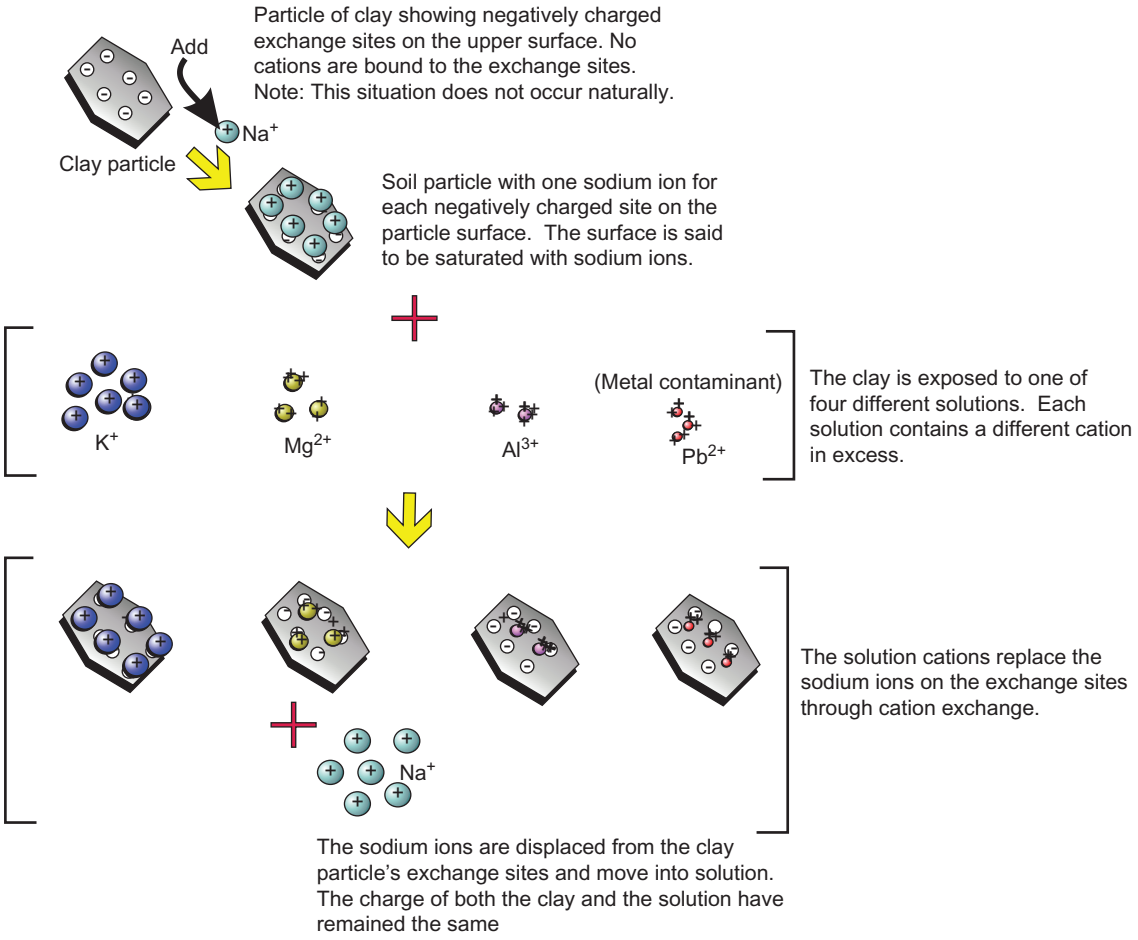

FIGURE 4.11 Cation exchange on clay particles. Adapted from Pepper *et al*. (2006).

Sorption is a major process influencing the movement and bioavailability of essential compounds and pollutants in soil. The broadest definition of sorption is the association of organic or inorganic molecules with the solid phase of the soil. For inorganic charged molecules, cation exchange is one of the primary mechanisms of sorption (Figure 4.11). Generally, positively charged ions, for example, calcium (Ca^{2+}) or lead (Pb^{2+}), participate in cation exchange. Since sorbed forms of these metals are in equilibrium with the soil solution, they can serve as a long-term source of essential nutrients (Ca^{2+}) or pollutants (Pb^{2+}) that are slowly released back into the soil solution as the soil solution concentration of the cation decreases with time.

Attachment of microorganisms can also be mediated by the numerous functional groups on clays (Figure 4.12). Although the clay surface and microbial cell surface both have net negative charges, clay surfaces are neutralized

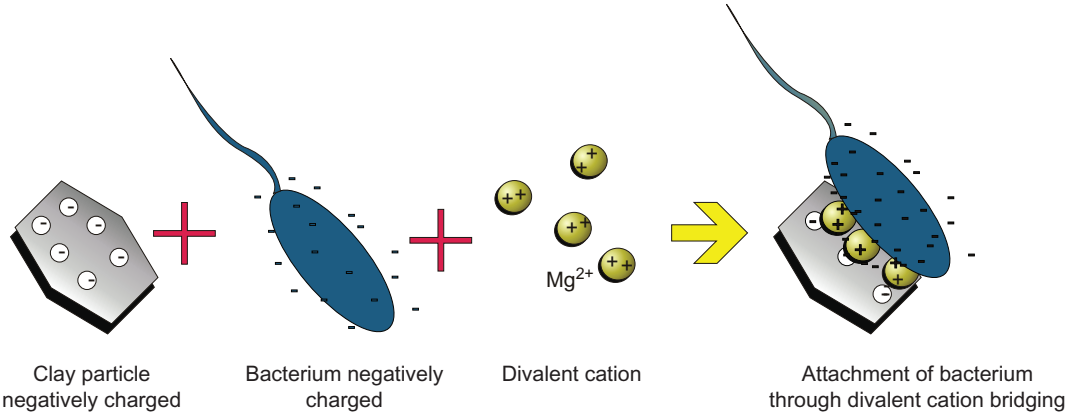

FIGURE 4.12 Attachment of a bacterial cell to a clay particle via cation bridging.

by the accumulation of positively charged counterions such as K^+, Na^+, Ca^{2+}, Mg^{2+}, Fe^{3+} and Al^{3+}. Together, these negative and positive surface charges form what is called the electrical double layer. Similarly, microbes have an electrical double layer. The thickness of the clay double layer depends on the valence and concentration of the cations in solution. Higher valence and increased cation concentrations will shrink the electrical double layer. Because the double layers of the clay particles and microbial cells repel each other, the thinner these layers are, the less the repulsion between the clay and cell surfaces. As these repulsive forces are minimized, attractive forces such as electrostatic and van der Waals forces allow the attachment of microbial cells to the surface (Gammack *et al.*, 1992). As a result, most microbes in terrestrial environments exist attached to soil colloids, rather than existing freely in the soil solution (see Section 15.1.3).

4.2.2.5 Soil pH

Soil pH affects the solubility of chemicals in soils by influencing the degree of ionization of compounds and their subsequent overall charge (Information Box 4.6). The extent of ionization is a function of the pH of the environment and the dissociation constant (p*K*) of the compound. Thus, soil pH may be critical in affecting transport of potential pollutants through the soil and vadose zone.

In areas with high rainfall, basic cations tend to leach out of the soil profile; moreover, soils developed in these areas have higher concentrations of organic matter, which contain acidic components and residues. Thus, such soils tend to have decreased pH values (<5.5) and are acidic in nature. Soils in arid areas do not undergo such extensive leaching, and the concentrations of organic matter are lower. In addition, water tends to evaporate in such areas, allowing salts to accumulate. These soils are therefore alkaline, with higher pH values (>8.5). Neutral soils range from pH 6 to 8.

Information Box 4.6 pH

pH is defined as the negative logarithm of the hydrogen ion concentration:

$$\mathrm{pH} = -\log[H^+]$$

Usually, water ionizes to H^+ and OH^-:

$$\mathrm{HOH} \rightleftharpoons H^+ + OH^-$$

The dissociation constant (K_{eq}) is defined as

$$K_{eq} = \frac{[H^+][OH^-]}{[HOH]} = 10^{-14}\ \mathrm{mol/L}$$

Since the concentration of HOH is large relative to that of H^+ or OH^-, it is normally given the value of 1. Therefore, [H] $[OH^-] = 10^{-14}$ mol/L. For a neutral solution, $[H^+] = [OH^-] = 1 \times 10^{-7}$ mol/L and

$$\mathrm{pH} = -\log[H^+] = -(-7) = 7$$

A pH value of less than 7 indicates an acid environment while a pH value greater than 7 indicates an alkaline environment.

4.2.2.6 Organic Matter

Organic matter in soil is defined as a combination of: (1) live biomass, including animals, microbes and plant roots; (2) recognizable dead and decaying biological matter; and (3) humic substances, which are heterogeneous polymers formed during the process of decay and degradation of plant, animal and microbial biomass (Figure 4.13). Soil organic matter contents range from less than 1% in hot arid climates that have low plant residue inputs, to 5% in cooler more humid areas with large plant inputs. In contrast, subsurface environments usually contain only very small amounts of organic matter, <0.1%. This is due to an absence of plant residues and other macroorganisms as well as a smaller numbers of microorganisms.

The humic fraction of organic matter is a stable nutrient base that serves as a slow release source of carbon and energy for the autochthonous (indigenous), slow-growing microorganisms in soil (see Section 3.3). The turnover rate is 2–5% per year. Humic substances have extremely complex structures that reflect the complexity and diversity of organic materials produced in a typical soil. They range in molecular weight from 700 to 300,000. An example of a humic acid polymer is shown in Figure 4.14.

Overall, humus has a three-dimensional, spongelike structure that contains both hydrophobic (water-hating) and hydrophilic (water-loving) regions. Thus, the humus molecule folds so that the hydrophilic portions face the charged exterior water or mineral phases and the hydrophobic portions are attracted toward the interior of the molecule. This hydrophobic interior provides a favorable environment for solutes that are less polar than water. This means that humus can sorb nonpolar solutes from the general soil solution through a sorption process called hydrophobic binding (Figure 4.15). Humic substances also contain numerous hydrophilic functional groups, the most important of which are the carboxyl group and the phenolic hydroxyl group, both of which can become negatively charged in the soil solution. As noted earlier, these functional groups are similar to those found on clays, and can contribute to the pH-dependent CEC of soil and participate in sorption of solutes and attachment of microorganisms by cation exchange, as shown in Figures 4.11 and 4.12.

4.2.3 The Liquid Phase

4.2.3.1 Soil Solution Chemistry

The soil solution is a constantly changing matrix composed of both organic and inorganic solutes in aqueous solution. The composition of the liquid phase is extremely important for biological activity in a porous medium,

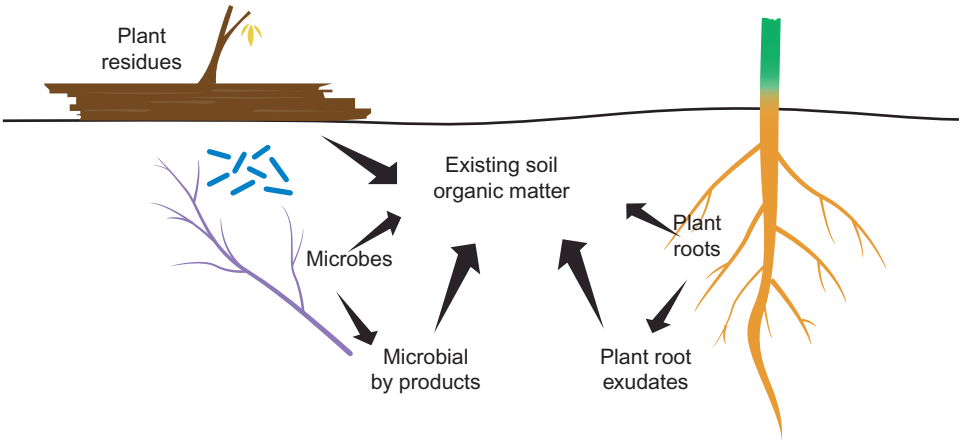

FIGURE 4.13 Schematic representation of the formation of soil organic matter.

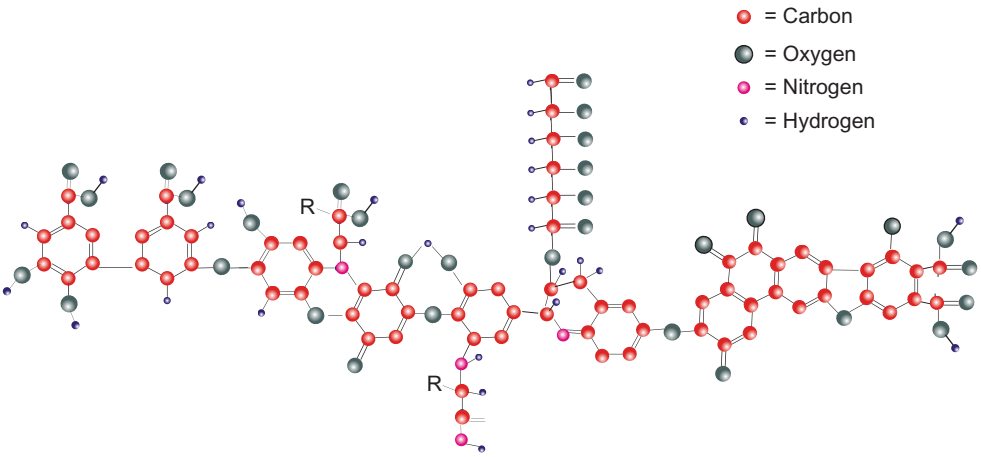

FIGURE 4.14 Humus polymer. R can represent various functional groups.

because microorganisms are approximately 70% water and most require high levels of water activity (>0.95) for active metabolism. Indeed, all microorganisms, even attached ones, are surrounded by a water film from which they obtain nutrients and into which they excrete wastes. Thus, the amount and composition of the liquid phase ultimately controls both microbial and plant growth.

The soil solution composition reflects the chemistry of the soil as well as the dynamic influx and efflux of solutes in response to water movement. Water movement results from rainfall or irrigation and affects mineral weathering, organic matter formation and decomposition (Figure 4.16). This composition is also altered by anthropogenic activities, such as irrigation, fertilizer and pesticide addition, and chemical spills. The chemistry of the soil affects not only the composition of the soil solution, but also the form and bioavailability of nutrients which are those in the soil solution (Figure 4.16). For example, as illustrated in Table 4.2, the form of the common cations found in a soil varies as a function of soil pH. As shown in this table, most cations are found in a more soluble form in acidic environments. In some cases, as for magnesium and calcium, this results in extensive leaching of the soluble form of the cation, leading to decreased concentrations of the nutrient. For many metallic cations, this can lead to increased metal toxicity (see Section 18.6). In other cases, as for iron and phosphate, a slightly acidic pH provides optimal availability of the element. Overall, the pH range that supports maximum microbial and plant activity is between 6.0 and 6.5.

4.2.3.2 *Soil Water Potential*

Water is the primary solvent in porous medium systems, and water movement is generally the major mechanism responsible for the transport of chemicals and

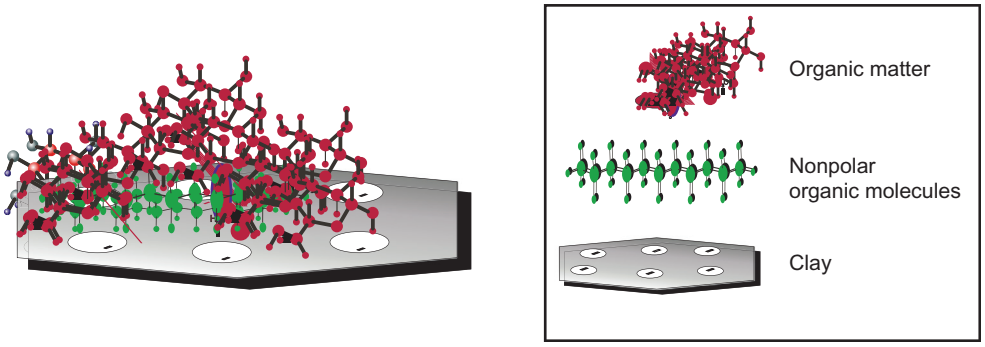

FIGURE 4.15 Hydrophobic sorption mechanisms. Nonpolar organic molecules tend to sorb to organic matter that is associated with solid mineral surfaces by diffusing into the sponge-like interior of organic matter molecules. Adapted from Schwarzenbach *et al.* (1993); reproduced by permission of John Wiley and Sons, Inc.

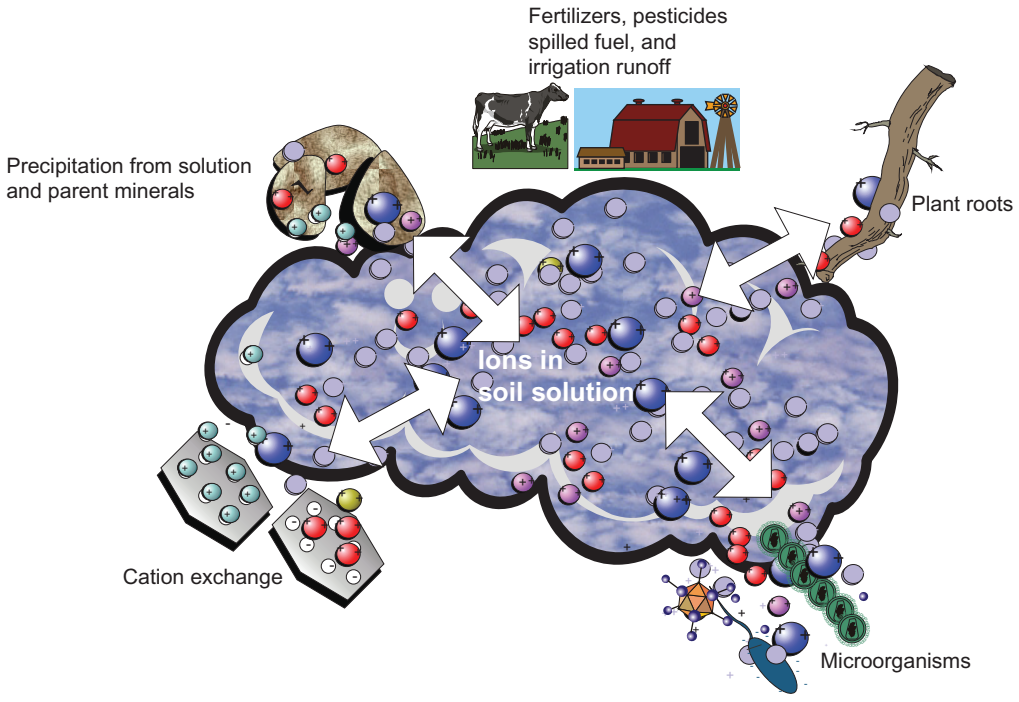

FIGURE 4.16 Paths of dissolution and uptake of minerals in the soil.

TABLE 4.2 The Form of Common Cations Found in Acid and Alkaline Soils

Cation	Acid Soils (Low pH)	Alkaline Soils (High pH)
Na^{+}	Na^{+}	Na^{+}, $NaHCO_3^0$, $NaSO_4^0$
Mg^{2+}	Mg^{2+}, $MgSO_4^0$, organic complexes	Mg^{2+}, $MgSO_4^0$, $MgCO_3^0$
Al^{3+}	organic complexes, AlF^{2+}, $AlOH^{2+}$	$Al(OH)_4^-$, organic complexes
Si^{4+}	$Si(OH)_4^0$	$Si(OH)_4^0$
K^{+}	K^{+}	K^{+}, KSO_4^-
Ca^{2+}	Ca^{2+}, $CaSO_4^0$, organic complexes	Ca^{2+}, $CaSO_4^0$, $CaHCO_3^+$
Mn^{2+}	Mn^{2+}, $MnSO_4^0$, organic complexes	Mn^{2+}, $MnSO_4^0$, $MnCO_3^0$, $MnHCO_3^+$, $MnB(OH)_4^+$
Fe^{2+}	Fe^{2+}, $FeSO_4^0$, $FeH_2PO_4^+$	$FeCO_3^+$, Fe^{2+}, $FeHCO_3^+$, $FeSO_4^0$
Fe^{3+}	$FeOH^{2+}$, $Fe(OH)_3^0$, organic complexes	$Fe(OH)_3^0$, organic complexes
Cu^{2+}	Organic complexes, Cu^{2+}	$CuCO_3^0$, organic complexes, $CuB(OH)_4^+$, $Cu[B(OH)_4]_4^0$
Zn^{2+}	Zn^{2+}, $ZnSO_4^0$, organic complexes	$ZnHCO_3^+$, $ZnCO_3^0$, organic complexes, Zn^{2+}, $ZnSO_4^0$, $ZnB(OH)_4^+$
Mo^{5+}	$H_2MoO_4^0$, $HMoO_4^-$	$HMoO_4^-$, MoO_4^{2-}

Adapted from Sposito (1989).

microorganisms. Water movement in a porous medium depends on the soil water potential, which is the work per unit quantity necessary to transfer an infinitesimal amount of water from a specified elevation and pressure to another point somewhere else in the porous medium. The soil water potential is a function of several forces acting on water, including matric and gravitational forces. Soil water potential is usually expressed in units of pressure (pascals, atmospheres or bars). Values of the matric contribution are negative because the reference is generally free water, which is defined to have a soil water potential of zero. Since the matric force decreases the free energy of water in the soil solution, the soil water potential becomes increasingly negative as this force increases.

In the saturated zone, the presence of a regional hydraulic gradient usually results in a general horizontal flow of water. In contrast, flow is generally downward in the unsaturated zone. In an unsaturated zone, where the soil pores are not completely filled with water, there are several incremental forces that affect water movement. These are related to the amount of water present (Figure 4.17). In very dry soils, there is an increment of adsorbed water that exists as an extremely thin film on the order of angstroms (Å) in width. This thin film is held very tightly to particle surfaces by surface forces with soil water potentials ranging from −31 to −10,000 atm. As a result, this water is essentially immobile. As water is added to this soil, a second increment of water forms as a result of matric or capillary forces. This water exists as bridges between particle surfaces in close proximity and can actually fill small soil pores. This results in soil water

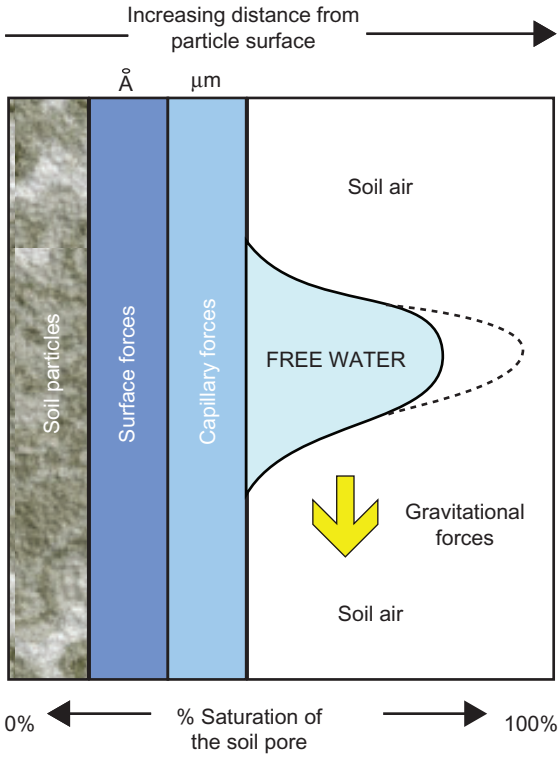

FIGURE 4.17 The continuum of soil water. Adapted from Dragun (1988). Reproduced with permission of ASP.

potentials ranging from −0.1 to −31 atm. This water moves slowly from larger to smaller pores in any direction, and is held against gravitational forces. The next increment of water added, free water, can be removed by

gravitational forces. The soil water potential for free water ranges from 0 to −0.5 atm. Although we have classified these different types of water in categories, in porous media they actually occur as a continuum rather than with sharply defined boundaries.

The amount of water present in the pore space of a medium is another important parameter in understanding the level and type of microbial activity in an environment. The optimal environment for active aerobic microbial growth in a porous medium is one in which water is easily available but the medium is not completely saturated. Why is this? As stated earlier, microorganisms obtain nutrients from the water phase surrounding them, so water is absolutely necessary for active microbial growth. Generally, microbial activity in the soil is greatest at −0.1 atm. As the water potential becomes less negative, the soil becomes saturated. In a completely saturated environment, oxygen, which governs aerobic microbial activity, may become limiting because of its limited solubility (9.3 mg/L at 20°C and 1 atm pressure) in water. Because the diffusion of oxygen through water is slow, once the available dissolved oxygen is used up, it is not replenished rapidly. As the water potential becomes more negative than −0.1 atm, water becomes less available because it is held tightly by matric and capillary forces.

4.2.4 Soil Atmosphere

4.2.4.1 Constituents of Soil Atmosphere

Soil and the atmosphere are in direct contact; therefore, the soil atmosphere has the same basic composition as air: nitrogen, oxygen and carbon dioxide. As shown in Table 4.3, there is little difference between the atmospheres in a well-aerated surface soil and in the air. However, plant and microbial activity can greatly affect the relative proportions of oxygen and carbon dioxide in soils that are not well aerated, that are far removed from the soil surface or that have undergone a recent flooding event due to heavy rains or irrigation. For example, in a fine clay or a saturated soil, oxygen can be completely removed by the aerobic activity of respiring organisms. During this respiration process, carbon dioxide (CO_2) is evolved, eventually resulting in elevated CO_2 levels. These changes affect the redox potential of the porous medium, which affects the availability of terminal electron acceptors for aerobic and anaerobic microbes.

TABLE 4.3 Soil Atmosphere

Location	Composition (% volume basis)		
	Nitrogen (N_2)	Oxygen (O_2)	Carbon Dioxide (CO_2)
Atmosphere	78.1	20.9	0.03
Well-aerated surface soil	78.1	18–20.5	0.3–3
Fine clay or saturated soil	>79	≈0–10	Up to 10

4.2.4.2 Availability of Oxygen and Soil Respiration

The amount of oxygen in the atmosphere (21%) allows aerobic degradation of the overwhelming proportion of the organic matter produced annually. In the absence of oxygen, organic substrates can be mineralized to carbon dioxide by fermentation or by anaerobic respiration, although these are less efficient processes than aerobic respiration. Thus, the oxygen content of soil is vital for aerobic activity, which depends on oxygen as a terminal electron acceptor during degradation of organic compounds.

Soil moisture content controls the amount of available oxygen in a soil (Information Box 4.7). In soils saturated with water, all pores are full of water and the oxygen content is very low. In dry soils, all pores are essentially full of air, so the soil moisture content is very low. Since aerobic microorganisms require both oxygen and water, soils at field capacity (moist but drained soils), which have moderate soil moisture and optimize both air (oxygen) and moisture, will maximize aerobic microbial metabolism. It is important to note, however, that even in field capacity soils, low-oxygen concentrations may exist in certain isolated pore regions (Figure 4.18). These wet but unsaturated regions of soil can quickly go anaerobic due to microbial activity since the rate of oxygen diffusion through water is roughly 10,000 times slower than

Information Box 4.7 Oxygen in the Gas and Water Phases of Soil

Oxygen can be found either in the soil atmosphere or dissolved in the soil solution, but the relative solubility of oxygen in water is low:

Compare 9.3 mg O_2 per liter water to approximately 1300 mg O_2 per liter air.

Microorganisms obtain oxygen from the water phase. Therefore as the dissolved oxygen is utilized by aerobic respiration, oxygen will be driven from the soil atmosphere into the water phase until it is used up, finally creating an anaerobic environment. In the environment, saturated soils will hold less oxygen (due to its limited solubility) than unsaturated ones will and, therefore, are more prone to developing anaerobic conditions.

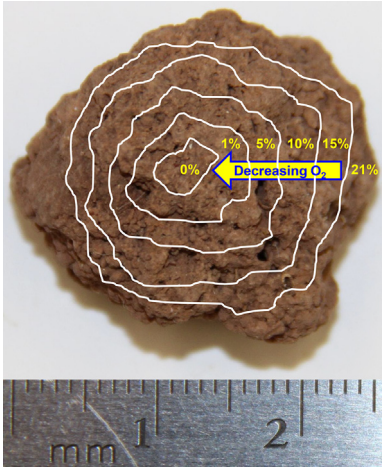

FIGURE 4.18 Gradient of oxygen concentrations in a typical soil aggregate. Adapted from Sextone *et al.* (1985).

FIGURE 4.19 The harsh soil environment found at the White Sands National Monument still supports plant and microbial life. *Source:* Pepper (2014).

through air and much of the oxygen will be used by other microorganisms as it slowly diffuses into the interior portions of soil aggregates. Thus, anaerobic microsites will exist even in aerobic soils that support transformation processes carried out by facultative anaerobes and strict anaerobes. This is an excellent example of how soil can function as a discontinuous environment of great diversity.

4.2.4.3 Oxygen and Respiration in the Subsurface

Overall activity levels in subsurface environments are lower than in soils. This is primarily because there are low amounts of organic carbon to serve as substrate in subsurface environments. Thus, the vadose zone, because it has low organic carbon and is unsaturated, is generally aerobic. However, due to the heterogeneous nature of the subsurface, anaerobic zones can occur particularly in clay lenses. In contrast, oxygen availability in saturated zones of the subsurface varies considerably, depending on availability of organic carbon.

4.3 SOIL AS A MICROBIAL ENVIRONMENT

Given the physical and chemical characteristics just described, just what kind of environment is soil for its microbial inhabitants? The short answer is that the soil environment, like most, is a competitive one. Those microorganisms that are best adapted to the stresses of the soil environment are most successful. The stresses found are both biotic, including competition from other microbes, and abiotic, including the physical and chemical characteristics of the environment. However, despite such stresses, even the harshest soil environments will support plant and microbial life (Figure 4.19).

4.3.1 Biotic Stresses

Since indigenous soil microbes are in competition with one another, the presence of large numbers of organisms results in biotic stress factors. Competition can be for substrate, water or growth factors. In addition, microbes can secrete allelopathic substances (inhibitory or toxic), including antibiotics, that harm neighboring organisms. Finally, many organisms are predatory or parasitic on neighboring microbes. For example, protozoa graze on bacteria, and viruses infect both bacteria and fungi. Because of biotic stress, nonindigenous organisms that are introduced into a soil environment often survive for very short periods of time (days to several weeks) unless there is a specific selective niche. This effect has important consequences for survival of pathogens and for other organisms introduced to aid biodegradation or for biological control.

4.3.2 Abiotic Stresses

4.3.2.1 Light

Sunlight does not penetrate beyond the top few centimeters of the soil surface. Phototrophic microorganisms are therefore limited to the top few centimeters of soil. At the surface of the soil, however, such physical parameters as temperature and moisture fluctuate significantly throughout the day and also seasonally. Hence, most soils provide a somewhat harsh environment for photosynthesizing microorganisms. Some phototrophic organisms, including algae, have the ability to switch to a heterotrophic respiratory mode of nutrition in the absence of light. Such "switch-hitters" can be found at significant depths within soils. Lichens, a mutualistic association between

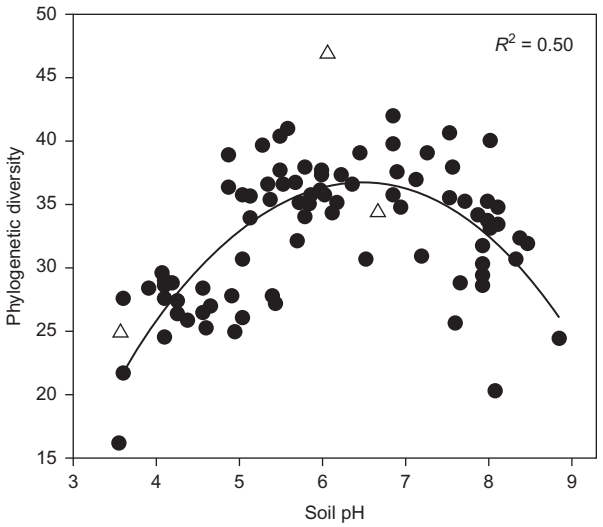

FIGURE 4.20 Soil bacterial diversity in soil samples from North and South America is strongly correlated with soil pH. From Lauber *et al.* (2009).

algae or cyanobacteria and fungi, are common in extremely harsh environments. In this association the fungus provides protection from desiccation and the phototroph provides energy from photosynthesis.

4.3.2.2 Soil Moisture

The availability of water is critical for microbial activity. Typically, optimal microbial activity occurs at −0.1 atm, which is the transition between capillary water and free water (Figure 4.17). As a group, the fungi are most desiccation resistant, followed by actinomycetes and finally the Bacteria.

4.3.2.3 Soil Temperature

Soil temperatures vary widely, particularly near the soil surface. Most soil populations are resistant to wide fluctuations in soil temperature although soil communities can be psychrophilic (prefer < 20°C), mesophilic (prefer 20−45°C), thermophilic (45−90°C) or hyperthermophilic (> 90°C) depending on the geographic location of the soil. Most soil organisms are mesophilic because of the buffering effect of soil on soil temperature, particularly at depths beneath the soil surface.

4.3.2.4 Soil pH

Undisturbed soils usually have soil pH values within the range of 6−8, and most soil organisms have pH optima within this range. There are, of course, exceptions to this rule, as exemplified by *Thiobacillus thiooxidans*, an organism that oxidizes sulfur to sulfuric acid and has a pH optimum of 2−3. Interestingly, soil pH appears to be a major determinant of bacterial diversity (Figure 4.20; Case Study 4.1).

Case Study 4.1 Soil pH is a Major Determinant of Bacterial Diversity

Fierer and Jackson (2006) used terminal restriction fragment length polymorphism (T-RFLP) analysis to determine which environmental factors were related to bacterial diversity in 98 soils from across North and South America. Interestingly, bacterial diversity was not correlated with diversity of vegetation, site temperature or latitude. The best predictor of bacterial diversity was soil pH ($r^2 = 0.70$) with the highest diversity occurring in soils with a near-neutral pH. Similarly, Lauber *et al.* (2009) later used bar-coded pyrosequencing to investigate the bacterial communities in 88 soils from North and South America. They also found that overall diversity was also correlated with pH ($r^2 = 0.50$) and was highest in soils with a near-neutral pH. Furthermore, they found that bacterial community composition was strongly correlated with soil pH ($r = 0.79$), largely due to changes in populations of *Acidobacteria*, *Actinobacteria* and *Bacteroidetes*. Although these results suggest that soil pH is one of the main drivers of bacterial diversity at a continental scale, other factors such as vegetation type and soil C may be more important at regional or local scales.

4.3.2.5 Soil Texture

All soils contain microbial communities regardless of the soil texture. However, soils with a mixture of sand, silt and clay particles offer a more favorable habitat for organisms because they hold more nutrients (Section 4.2.2.4) and provide for better water (Section 4.2.3) and air flow (Section 4.2.4) than do pure sands or clays. Microbial communities found in pure sands or clays are lower in numbers and activity.

4.3.2.6 Soil Nutrients

Carbon and nitrogen are generally the most important limiting nutrients that are found in soils although any limiting nutrient will reduce microbial activity. Since both carbon and nitrogen are usually present in low concentrations, growth and activity of soil organisms are slow. In fact, many organisms exist in soil under semi-starvation conditions and hence are dormant. The major exception to this is the plant rhizosphere, where root exudates maintain much higher microbial numbers and activity.

4.3.2.7 Redox Potential

Redox potential (E_h) is the measurement of the tendency of an environment to oxidize or reduce substrates. An

aerobic soil, which is an oxidizing environment, has an E_h of + 800 mV; an anaerobic soil, which is a reducing environment, has a negative E_h which can reach −300 mV. Oxygen is found in soils at a redox potential of about + 800 mV. When soil is placed in a closed container, oxygen is used by aerobic organisms as a terminal electron acceptor until all of it is depleted. As this process occurs, the redox potential of the soil decreases, and other compounds can be used as terminal electron acceptors. Table 4.4 illustrates the redox potential at which various substrates are reduced, and the activity of different types of organisms in a soil.

TABLE 4.4 Redox Potential at which Soil Substrates are Reduced

Redox Potential (mV)	Reaction	Type of Organism and Metabolism
+800	$O_2 \rightarrow H_2O$	Aerobes, aerobic respiration
+740	$NO_3^- \rightarrow N_2$, N_2O	Facultative anaerobes, nitrate reduction
−220	$SO_4^{2-} \rightarrow S^{2-}$	Anaerobes, sulfate reduction
−300	$CO_2 \rightarrow CH_4$	Anaerobes, methanogenesis

4.4 MICROORGANISMS IN SURFACE SOILS

Surface soils are occupied by indigenous populations of bacteria (including actinomycetes), archaeans, fungi, algae and protozoa. In general, as the size of these organisms increases from bacteria to protozoa, the number present decreases. It is also known that there may be phages or viruses present that can infect each class of organism, but information on the extent of these infectious agents in surface soils is limited (see also Chapter 2). In addition to these indigenous populations, specific microbes can be introduced into soil by human or animal activity. Human examples include the deliberate direct introduction of bacteria as biological control agents or as biodegradative agents. Microbes are also introduced indirectly as a result of application of animal manures or sewage sludge to agricultural fields (see Chapter 26). Animals introduce microbes through bird droppings and animal excrement. Regardless of the source, introduced

TABLE 4.5 Characteristics of Bacteria, Actinomycetes, and Fungi

Characteristic	Bacteria	Actinomycetes	Fungi
Numbers	Most numerous	Intermediate	Least numerous
Biomass	Bacteria and actinomycetes have similar biomass		Largest biomass
Degree of branching	Slight	Filamentous, but some fragment to individual cells	Extensive filamentous forms
Aerial mycelium	Absent	Present	Present
Growth in liquid culture	Yes—turbidity	Yes—pellets	Yes—pellets
Growth rate	Exponential	Cubic	Cubic
Cell wall	Murein, teichoic acid, and lipopolysaccharide	Murein, teichoic acid, and lipopolysaccharide	Chitin or cellulose
Complex fruiting bodies	Absent	Simple	Complex
Competitiveness for simple organics	Most competitive	Least competitive	Intermediate
Fix N	Yes	Yes	No
Aerobic	Aerobic, anaerobic	Mostly aerobic	Aerobic except yeast
Moisture stress	Least tolerant	Intermediate	Most tolerant
Optimum pH	6–8	6–8	6–8
Competitive pH	6–8	>8	<5
Competitiveness in soil	All soils	Dominate dry, high-pH soils	Dominate low-pH soils

organisms rarely significantly affect the abundance and distribution of indigenous populations.

The following discussion is an overview of the dominant types of microbes found in surface soils, including their occurrence, distribution and function. Overall soil microorganisms are critical in a variety of areas including surface soil formation, nutrient cycling (Chapter 16), bioremediation (Chapter 17) and land application of municipal wastes (Chapter 26).

4.4.1 Bacteria

Bacteria are almost always the most abundant organisms found in surface soils in terms of numbers (Table 4.5). Culturable numbers vary depending on specific environmental conditions, particularly soil moisture and temperature. Culturable bacteria can be as numerous as 10^7 to 10^8 cells per gram of soil, whereas total populations (including viable but nonculturable organisms) can exceed 10^{10} cells per gram. In unsaturated soils, aerobic bacteria usually outnumber anaerobes by two or three orders of magnitude. Anaerobic populations increase with increasing soil depth but rarely predominate unless soils are saturated and/or clogged.

Indigenous soil bacteria can be classified on the basis of their growth characteristics and affinity for carbon substrates. As explained in Section 3.3, two broad categories of bacteria are found in the environment, those that are K-selected or autochthonous and those that are r-selected or zymogenous. The former metabolize slowly in soil, utilizing slowly released soil organic matter as a substrate. The latter are adapted to intervals of dormancy and rapid growth, depending on substrate availability, following the addition of fresh substrate or amendment to the soil.

Bacteria are also classified according to diversity or the different types present. An intriguing question that has not yet been completely answered is: "How many different bacteria are there in soils?" Traditionally, this has been determined using culture techniques, but most recently, estimates of diversity have been made based on DNA sequencing in combination with statistical approaches. In this case, diversity is indicated by the number of operational taxonomic units (OTU) where each OTU theoretically represents a different bacterial population in the community (Chapter 19). These approaches are providing estimates of diversity at $>10{,}000$ species (OTUs) of bacteria per gram of soil (Roesch *et al.*, 2007). It is important to remember that most of these populations are not easily cultured and we are just beginning to develop methods to study the viable but difficult-to-culture

TABLE 4.6 Dominant Culturable Soil Bacteria

Organism	Characteristics	Function
Arthrobacter	Heterotrophic, aerobic, Gram variable. Up to 40% of culturable soil bacteria.	Nutrient cycling and biodegradation.
Streptomyces	Gram-positive, heterotrophic, aerobic actinomycete. 5–20% of culturable bacteria.	Nutrient cycling and biodegradation. Antibiotic production, e.g., *Streptomyces scabies*.
Pseudomonas	Gram-negative heterotroph. Aerobic or facultatively anaerobic. Possess wide array of enzyme systems. 10–20% of culturable bacteria.	Nutrient cycling and biodegradation, including recalcitrant organics. Biocontrol agent.
Bacillus	Gram-positive aerobic heterotroph. Produce endospores. 2–10% of culturable soil bacteria.	Nutrient cycling and biodegradation. Biocontrol agent, e.g., *Bacillus thuringiensis*.

TABLE 4.7 Examples of Important Autotrophic Soil Bacteria

Organism	Characteristics	Function
Nitrosomonas	Gram negative, aerobe	Converts $NH_4^+ \rightarrow NO_2^-$ (first step of nitrification)
Nitrobacter	Gram negative, aerobe	Converts $NO_2^- \rightarrow NO_3^-$ (second step of nitrification)
Thiobacillus	Gram negative, aerobe	Oxidizes $S \rightarrow SO_4^{2-}$ (sulfur oxidation)
Thiobacillus denitrificans	Gram negative, facultative anaerobe	Oxidizes $S \rightarrow SO_4^{2-}$; functions as a denitrifier
Thiobacillus ferrooxidans	Gram negative, aerobe	Oxidizes $Fe^{2+} \rightarrow Fe^{3+}$

TABLE 4.8 Examples of Important Heterotrophic Soil Bacteria

Organism	Characteristics	Function
Actinomycetes, e.g., *Streptomyces*	Gram positive, aerobic, filamentous	Produce geosmins "earthy odor," and antibiotics
Bacillus	Gram positive, aerobic, spore former	Carbon cycling, production of insecticides and antibiotics
Clostridium	Gram positive, anaerobic, spore former	Carbon cycling (fermentation), toxin production
Methanotrophs, e.g., *Methylosinus*	Aerobic	Methane oxidizers that can cometabolize trichloroethene (TCE) using methane monooxygenase
Cuprivadus necator	Gram negative, aerobic	2,4-D degradation via plasmid pJP4
Rhizobium	Gram negative, aerobic	Fixes nitrogen symbiotically with legumes
Frankia	Gram positive, aerobic	Fixes nitrogen symbiotically with nonlegumes
Agrobacterium	Gram negative, aerobic	Important plant pathogen, causes crown gall disease

microbes in soil (Chapter 8). Tables 4.6–4.8 identify some of the culturable bacterial genera that are known to dominate typical surface soils and other bacterial genera that are critical to environmental microbiology. Of course, these lists are by no means all inclusive. A very important point that follows is that any methodology that relies on characterizing environmental organisms via a procedure involving culture may in fact obtain a very small subsection of the total population that may not be representative of the majority of the community (Figure 4.21; Case Study 4.2).

4.4.2 Actinomycetes

Actinomycetes are prokaryotic organisms that are classified as bacteria, but are unique enough to be discussed as an individual group. Actinomycete numbers are generally one to two orders of magnitude smaller than the total bacterial population (Table 4.5). They are an important component of the bacterial community, especially under conditions of high pH, high temperature or water stress. Morphologically, actinomycetes resemble fungi because of their elongated cells that branch into filaments or hyphae. These hyphae can be distinguished from fungal hyphae on the basis of size with actinomycete hyphae much smaller than fungal hyphae (Figure 4.22). Characteristics and unique functions of actinomycetes are shown in Information Box 4.8. One distinguishing feature of this group of bacteria is that they are able to utilize a great variety of substrates found in soil, especially some of the less degradable insect and plant polymers such as chitin, cellulose and hemicellulose. Although originally recognized as soil microorganisms, it is now being recognized that marine actinomycetes are also important. Specifically, marine actinomycetes have been shown to possess novel secondary metabolites that add a new dimension to microbial natural products (Jensen *et al.*, 2005) that have been discovered within soil actinomycetes (Chapter 19).

4.4.3 Archaea

Once thought to occur primarily in extreme environments such as thermal springs or hypersaline soils, culture-independent techniques have revealed that archaeans are actually widespread in nature. Although archaeal populations can be very large ($>10^8$ per gram of soil), they are typically two or more orders of magnitude less numerous than bacteria. The Archaea contribute to multiple soil processes including the biogeochemical cycling of C, N and S. For example, they have major roles in nitrification (ammonia oxidation) and methanogenesis (Chapter 16). Numerous studies have documented large populations of ammonia-oxidizing archaeans (AOA) in a variety of ecosystems. In general, AOA appear to be more important to ammonia oxidation in environments that have lower levels of N, such as natural ecosystems and pastures. In contrast, although there are also often high levels of AOA in managed ecosystems such as agricultural fields, nitrification in these environments having higher levels of N actually appears to be dominated by ammonia-oxidizing bacteria (AOB) (Taylor *et al.*, 2010; Verhamme *et al.*, 2011). However, even in these managed systems, AOA may be of greater relative importance in the subsoil and subsurface environments due to lower nutrient levels and pHs. In contrast to nitrification, which is performed by both Archaea and Bacteria, methanogenesis is solely an archaeal process and is critically important to the global C cycle with some researchers estimating that >40% of global methane emissions originate from soils and associated wetlands.

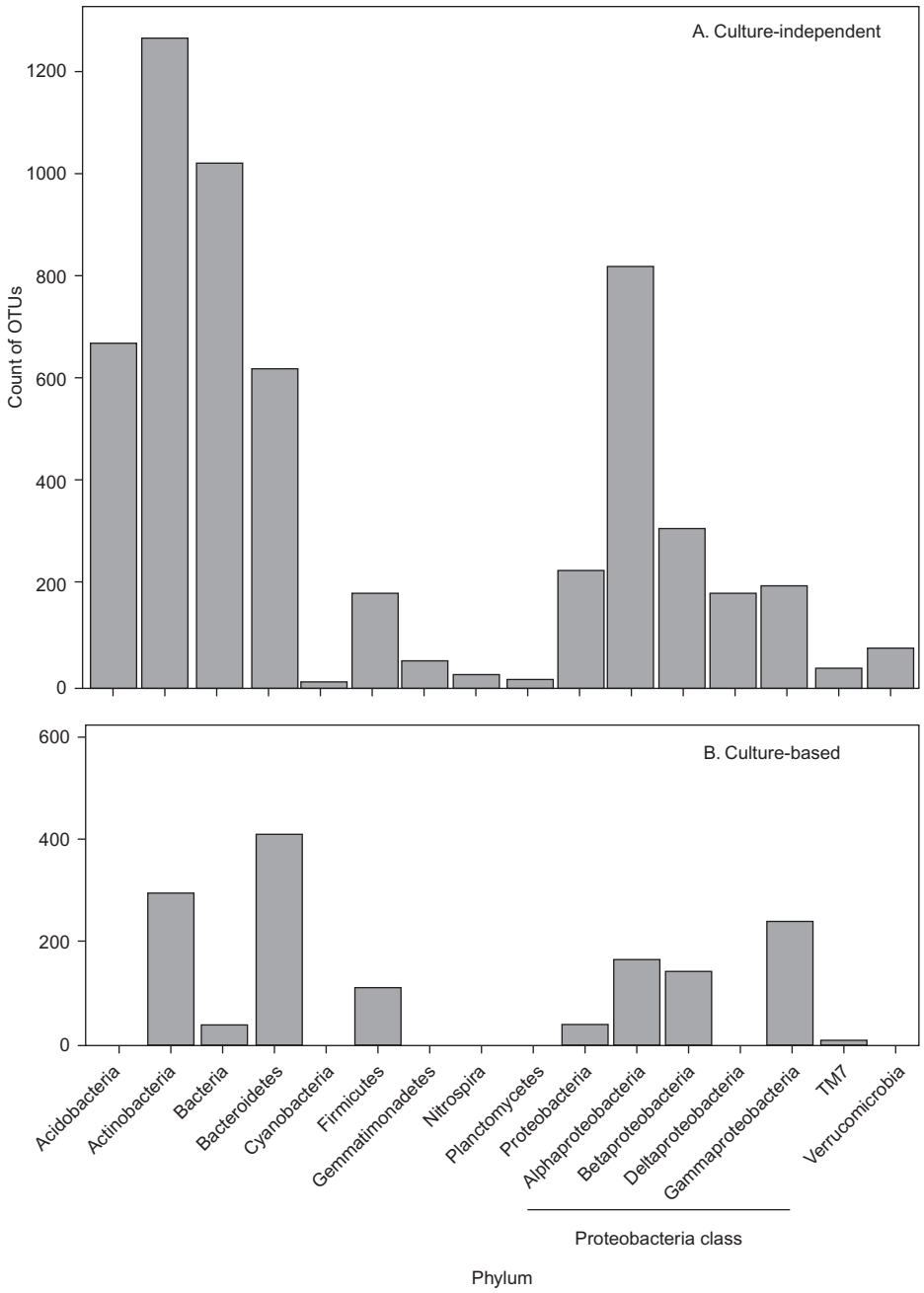

FIGURE 4.21 Impact of culture-based and -independent methods on characterization of a soil bacterial community. Note the greater number of phyla and operational taxonomic units (OTUs) detected using the culture-independent approach. From Shade *et al.* (2012).

4.4.4 Fungi

Fungi other than yeasts are aerobic and are abundant in most surface soils. Numbers of fungi usually range from 10^5 to 10^6 per gram of soil. Despite their lower numbers compared with bacteria, fungi usually contribute a higher proportion of the total soil microbial biomass (Tables 4.5 and 4.9). This is due to their comparatively large size; a fungal hypha can range from 2 to 10 μm in diameter. Because of their large size, fungi are more or less restricted to the interaggregate regions of the soil matrix. Yeasts can metabolize anaerobically (fermentation) and are less numerous than aerobic mycelium-forming fungi. Generally, yeasts are found at populations of up to 10^3 per gram of soil. Because of their reliance on organic sources for substrate, fungal populations are greatest in the surface O and A horizons, and numbers decrease rapidly with increasing soil depth. As with bacteria, soil fungi are normally found associated with soil particles or within plant rhizospheres.

Fungi are important components of the soil with respect to nutrient cycling and especially decomposition of organic matter, both simple (sugars) and complex (polymers such as cellulose and lignin). The role of fungi

Case Study 4.2 Comparison of Culture-Based and Independent Approaches for Characterizing a Soil Bacterial Community

Shade *et al.* (2012) used both culture-based and -independent methods to characterize the bacterial community in an orchard soil from Wisconsin, U.S.A. For culture-independent analysis, DNA was extracted from soil and sequenced using a 16S rRNA gene pyrosequencing approach (Chapters 13 and 21). For culture-based analysis, soil bacteria were first grown on rhizosphere isolation medium and then characterized by 16S rRNA gene pyrosequencing. Over 37,000 sequences were obtained with each approach. The results for the two methods were strikingly different, with a much greater number of bacterial phyla and operational taxonomic units (OTUs) detected using the culture-independent approach (Figure 4.21). The culture-based approach indicated that the community was dominated by *Bacteroidetes*, *Proteobacteria* and *Actinobacteria*. In contrast, the culture-independent approach indicated that the community was dominated by *Actinobacteria*, *Proteobacteria* (with increased levels of *Alphaproteobacteria* and *Deltaproteobacteria* and decreased levels of *Gammaproteobacteria*), *Acidobacteria* and *Bacteroidetes*. Approximately 90% of the bacteria detected using the culture-independent approach were not detected using the culture-based approach. For example, no *Acidobacteria* were detected using the culture-based method, but they comprised >10% of the bacterial community based upon the culture-independent results and represented the third most abundant phylum. Numerous other studies using culture-independent methods have also found that *Acidobacteria* may comprise ≥ 30% of many environmental bacterial communities. Prior to use of culture-independent methods, the abundance and distribution of *Acidobacteria* in the environment were unknown due to the inability or difficulty to grow these organisms on traditional media. Current evidence based upon a limited number of isolated representatives and genome sequence data indicates that *Acidobacteria* can metabolize a wide variety of substrates and they have a competitive advantage in low C and pH environments.

What is perhaps most surprising about the Shade *et al.* (2012) study is that approximately 60% of the bacteria detected using the culture-based approach were not detected using the culture-independent approach. This indicates that the culture-based method captured rare members of the soil community that were missed with the sequencing approach and highlights an important limitation of the scale of sequencing currently used in most studies for characterizing bacterial communities. Due to costs associated with sequencing, many studies only sequence ≤ 10,000 bacteria per sample, thus only detecting the most dominant organisms (unless a targeted sequencing approach is used). For illustration, even if the 40,000 most abundant bacteria in a soil sample were characterized out of a total community of 1 million bacteria (a very conservative estimate), this would represent only the top 4% of the community. In other words, 96% of the bacterial community would be missed! Fortunately, rapid advances in sequencing technologies are allowing more thorough sequencing of these communities and thus increasing our ability to detect and characterize these rare members of the soil biosphere using culture-independent methods.

in decomposition is increasingly important as the soil pH declines because fungi tend to be more acid tolerant than bacteria (Table 4.5) Some of the common genera of soil fungi involved in nutrient cycling are *Penicillium* and *Aspergillus*. These organisms are also important in the development of soil structure because they physically entrap soil particles with fungal hyphae (Figure 4.23). As well as being critical in the degradation of complex plant polymers such as cellulose and lignin, some fungi can also degrade a variety of pollutant molecules. The best-known example of such a fungus is the white rot fungus *Phanerochaete chrysosporium* (Information Box 2.6). Other fungi, such as *Fusarium* spp., *Pythium* spp. and *Rhizoctonia* spp., are important plant pathogens. Still others cause disease; for example, *Coccidioides immitis* causes a chronic human pulmonary disease known as "valley fever" in the southwestern deserts of the United States. Finally, note that mycorrhizal fungi are critical for establishing plant−fungal interactions that act as an extension of the root system of almost all higher plants. Without these mycorrhizal associations, plant growth as we know it would be impossible.

4.4.5 Algae

Algae are typically phototrophic and thus would be expected to survive and metabolize in the presence of a light-energy source and CO_2 for carbon. Therefore, one would expect to find algal cells predominantly in areas where sunlight can penetrate, the very surface of the soil. However, one can actually find algae to a depth of 1 m because some algae, including the green algae and diatoms, can grow heterotrophically as well as photoautotrophically. In general, though, algal populations are highest in the surface 10 cm of soil (Curl and Truelove, 1986). Typical algal populations close to the soil surface can range from 5000 to 10,000 per gram of soil, but where a visible algal bloom has developed there can be millions of algal cells per gram of soil.

Algae are often the first to colonize surfaces in a soil that are devoid of preformed organic matter. Colonization by this group of microbes is important in establishing soil formation processes, especially in barren volcanic areas, desert soils and rock faces. Algal metabolism is critical to soil formation in two ways: algae provide a carbon input

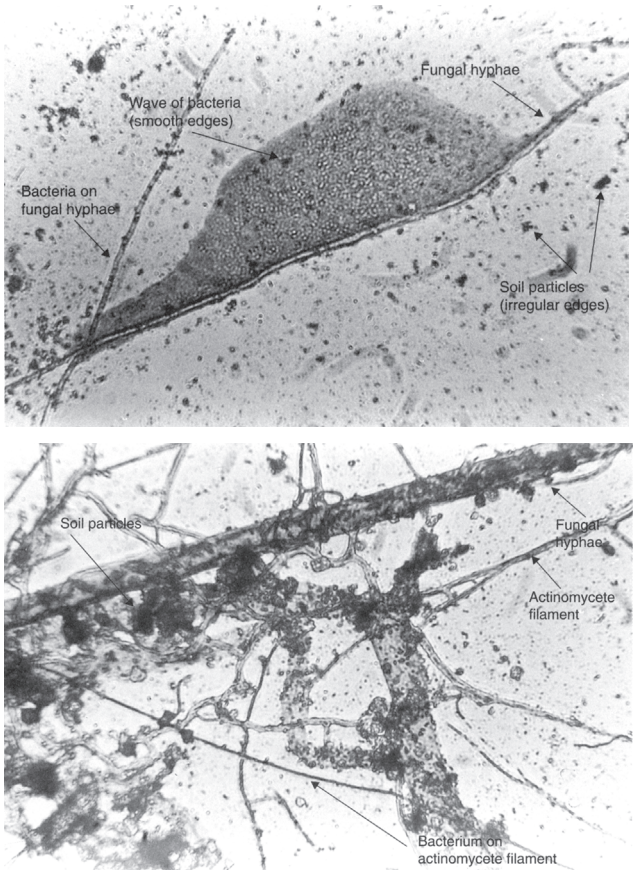

FIGURE 4.22 Comparison of soil bacteria, actinomycetes and fungi viewed under a light microscope. From Pepper *et al*. (2006).

TABLE 4.9 Approximate Range of Biomass of Each Major Component of the Biota in a Typical Temperate Grassland Soil

Component of Soil Biota	Biomass (tons/ha)
Plant roots	Up to 90 but generally about 20
Bacteria	1–2
Actinomycetes	0–2
Fungi	2–5
Protozoa	0–0.5
Nematodes	0–0.2
Earthworms	0–2.5
Other soil animals	0–0.5
Viruses	Negligible

From Killham (1994).

through photosynthesis and as they metabolize, they produce and release carbonic acid, which aids in weathering the surrounding mineral particles. Further, algae produce large amounts of extracellular polysaccharides, which also aid in soil formation by causing aggregation of soil particles (Killham, 1994).

Populations of soil algae generally exhibit seasonal variations with numbers being highest in the spring and fall. This is because desiccation caused by water stress tends to suppress growth in the summer and cold stress affects growth in the winter. Four major groups of algae are found in soil. The green algae or the Chlorophyta, for example, *Chlamydomonas*, are the most common algae found in acidic soils. Also widely distributed are diatoms such as *Navicula*, which are members of the Chrysophycophyta. Diatoms are found primarily in neutral and alkaline soils. Less numerous are the yellow–green algae such as *Botrydiopsis*, which are also members of the Chrysophycophyta, and the red algae (Rhodophycophyta, e.g., *Prophyridium*). In addition to these algal groups, there are the cyanobacteria (e.g., *Nostoc* and *Anabaena*), which are actually classified as bacteria but have many characteristics in common with algae. The cyanobacteria participate in the soil-forming process discussed in the previous paragraph, and some cyanobacteria also have the capacity to fix nitrogen, a nutrient that is usually limiting in a barren environment. In temperate soils the relative abundance of the major algal groups follows the order green algae > diatoms > cyanobacteria > yellow–green algae. In tropical soils the cyanobacteria predominate.

Information Box 4.8 Characteristics and Functions of Actinomycetes

Characteristics

Structure	Prokaryotic
Size	1–2 μm diameter
Morphology	Filamentous lengths of cocci
Gram stain	Gram positive
Respiration	Mostly aerobic, can be anaerobic
Habitat	Soil or marine
Abundance, marine isolates	5–40 CFU/ml
Abundance, soils	10^6–10^8/g

Functions

- Source of natural products and antibiotics, e.g., streptomycin
- Produce geosmin, the compound which gives soil and water a characteristic earthy odor
- Capable of degradation of complex organic molecules
- Capable of biological nitrogen fixation with species of the non-legume-associated *Frankia*

TABLE 4.10 Average Length and Volume of Soil Protozoa Compared with Bacteria

Group	Length (μm)	Volume (μm^3)	Shape
Bacteria	<1–5	2.5	Spherical to rod shaped
Flagellates	2–50	50	Spherical, pear shaped, banana shaped
Amoebae			
Naked	2–600	400	Protoplasmic streaming, pseudopodia
Testate	45–200	1000	Build oval tests or shells made of soil
Giant	6000	4×10^9	Enormous naked amoebae
Ciliates	50–1500	3000	Oval, kidney shaped, elongated and flattened

From Ingham (1998).

4.4.6 Protozoa

Protozoa are unicellular, eukaryotic organisms that range up to 5.5 mm in length, although most are much smaller (Table 4.10). Most protozoa are heterotrophic and survive by consuming bacteria, yeast, fungi and algae. There is evidence that they may also be involved, to some extent, in the decomposition of soil organic matter. Because of their large size and requirement for large numbers of smaller microbes as a food source, protozoa are found mainly in the top 15 to 20 cm of the soil. Protozoa are usually concentrated near root surfaces that have high densities of bacteria or other prey. Soil protozoa are flatter and more flexible than aquatic protozoa, which makes it easier to move around in the thin films of water that surround soil particle surfaces as well as to move into small soil pores.

There are three major categories of protozoa: the flagellates, the amoebae and the ciliates (Chapter 2). The flagellates are the smallest of the protozoa and move by means of one to several flagella. Some flagellates (e.g., *Euglena*) contain chlorophyll, although most (e.g., *Oicomonas*) do not. The amoebae, also called rhizopods, move by protoplasmic flow, either with extensions called pseudopodia or by whole body flow. Amoebae are usually the most numerous type of protozoan found in a given soil environment. Ciliates are protozoa that move by beating short cilia that cover the surface of the cell. The protozoan population of a soil is often correlated with the bacterial population, which is the major food source present. For example, increases in protozoan populations often occur shortly after a proliferation of soil bacteria, as would result during the bacterial degradation of organic pollutants like 2,4-D. Numbers of protozoa reported range from 30,000 per gram of soil from a nonagricultural temperate soil to 350,000 per gram of soil from a maize field to 1.6×10^6 per gram of soil from a subtropical area.

4.5 DISTRIBUTION OF MICROORGANISMS IN SOIL

In surface soils, culturable microorganism concentrations can reach 10^8 per gram of dry soil, although direct counts are generally one to two orders of magnitude larger. These diverse microorganisms have been estimated to represent >10,000 species of bacteria alone (Roesch *et al.*, 2007). In addition, there are substantial populations of fungi, algae and protozoa. In general, microbial colonies are found in a nonuniform "patchlike" distribution on soil particle surfaces. This "patchlike" distribution of microorganisms in unsaturated soils results in increased microbial diversity as compared to saturated soils which are more highly connected and allow for more competitive interactions between microorganisms to occur (Treves *et al.*, 2003). Despite the large number of microorganisms found, they make up only a small fraction of the total organic carbon and a very small proportion of the soil volume (0.001%) in most soils.

In surface soils, microbial distribution is also dependent on soil texture and structure. As soils form, microbes attach to a site that is favorable for replication. As growth and colony formation take place, exopolysaccharides are formed, creating a "pseudoglue" that helps in orienting adjacent clay particles and cementing them together to form a microaggregate (Figure 4.23). Although the factors that govern whether a given site is favorable for colonization are not completely understood, several possible factors have been identified that may play a role including nutrient availability and surface properties. In addition, in surface soils, pore space seems to be an important factor. Pore spaces in microaggregates with neck diameters less than 6 μm have more activity than pore spaces with larger diameters, because the small pore necks protect resident bacteria from protozoal predation. Pore space also controls water content to some extent. Larger pores drain more quickly than smaller pores, and therefore the interior of a small pore is generally wetter and more conducive to microbial activity. It has further been suggested that Gram-negative bacteria prefer the interior of microaggregate pore space because of the increased moisture, whereas Gram-positive bacteria, which are better adapted to withstand dry conditions, tend to occupy the microaggregate exteriors.

Most microorganisms in Earth environments are attached. It has been estimated that approximately 80 to 90% of the cells are sorbed to solid surfaces and the remainder are free-living. As stated earlier, attached microbes are found in patches or colonies on particle

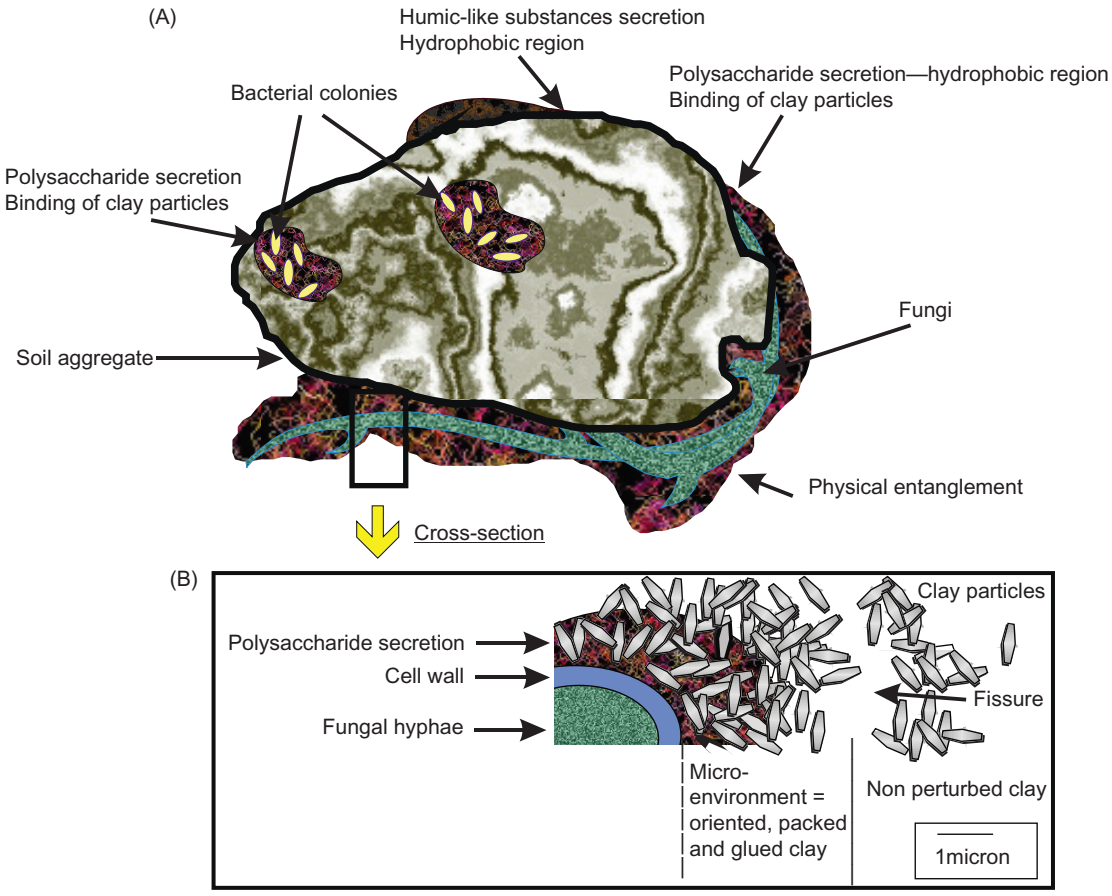

FIGURE 4.23 Microbially mediated aggregation: (A) schematic representation of the binding and stabilization of a soil aggregate by microorganisms; (B) detail of the microenvironment in the vicinity of a fungus. Adapted with permission from Robert and Chenu (1992).

surfaces. Attachment and growth into colonies confer several advantages for microorganisms, bacteria in particular (Gilbert *et al.*, 1993). Attachment can help protect bacteria from protozoal predation. Attachment and colony formation can also help provide localized concentrations of nutrients that are contained in and recycled among the attached cells within the colony rather than being diluted into the general environment. This is especially important in oligotrophic environments. Another advantage of colony formation is that a microbial colony can alter the immediate microsite environment surrounding the cell, such as pH, to optimize growth conditions. Finally, genetic exchange can occur much more frequently within a colony than between isolated cells in a soil environment.

Although free-living cells are less common, they are an important mechanism for the dispersion of microorganisms. As nutrient supplies at a particular surface site are consumed, microorganisms need a mechanism by which cells can disperse to new sites that may have additional food supplies. Fungi spread via spores released from fruiting bodies or via hyphal extension. Bacteria, which undergo only simple cell division, need a different mechanism of dispersal, namely, release of free-living daughter cells. In fact, there is evidence that bacterial cells at the surface of a colony undergo changes in their surface properties that cause the release of a newly formed daughter cell after cell division. As these free-living daughter cells grow, their surfaces undergo a chemical change that makes attachment at a new site more favorable.

4.6 MICROORGANISMS IN SUBSURFACE ENVIRONMENTS

In subsurface environments, the same patchlike distribution of microbes exists that is found in surface soils. Culturable counts range from essentially zero to 10^7 per gram of dry soil depending on the depth and type of porous medium. Direct counts generally range from 10^5 to $> 10^7$ cells per gram of porous medium. Thus, the difference between culturable and direct counts is often much larger in the subsurface than in surface soils. This is most likely due to the presence of viable but

nonculturable microbes (VBNC) (Chapter 3). These microbes exist as a result of the nutrient-poor status of subsurface environments, which is directly reflected in their low organic matter content. When subsurface cells are examined, they are rarely dividing and contain few ribosomes or inclusion bodies. This is not surprising considering the nutrient-limited conditions in which subsurface microbes live. Recall that environmental bacteria have diverse, specific, nutritional needs and thus may be difficult to culture on traditional media. Further, they often exist under adverse conditions and as a result may be sublethally injured. Such injured bacteria cannot be cultured by conventional methods. It has been estimated that 99% of all soil organisms may be VBNC. Likewise, most culture-based methods only enumerate heterotrophic microorganisms. As C concentrations decrease in the subsurface, the relative number of autotrophic microorganisms typically increases and these organisms may be missed when using typical culture-based methods.

The weathered component minerals (which serve as a source of micronutrients) and organic matter (which serves as a carbon and nitrogen source) are two of the primary differences between surface soils and subsurface materials as environments for microorganisms. These differences in nutrient content are reflected in a higher and more uniform distribution of microbial numbers and activity in surface soil environments. The other major factor impacting microbial density and activity in surface and subsurface environments is water content. Areas that have high recharge from rainfall and water flow tend to have both higher microbial numbers and activity.

4.6.1 Microorganisms in Shallow Subsurface Environments

Although the microorganisms of surface soils have been studied extensively, the study of subsurface microorganisms is relatively new, beginning in earnest in the 1980s. Complicating the study of subsurface life are the facts that sterile sampling is problematic and many subsurface microorganisms are difficult to culture. Some of the initial studies evaluating subsurface populations were invalidated by contamination with surface microbes. As a result, study of subsurface organisms has required the development of new tools and approaches for sterile sampling (Chapter 8) and for microbial enumeration and identification. For example, it has been demonstrated that rich media are not suitable for culturing subsurface organisms that are adapted to highly oligotrophic conditions and that viable counts from these environments on less-rich media often produce microbial counts one order of magnitude or more higher than those produced on richer media (Chapter 8).

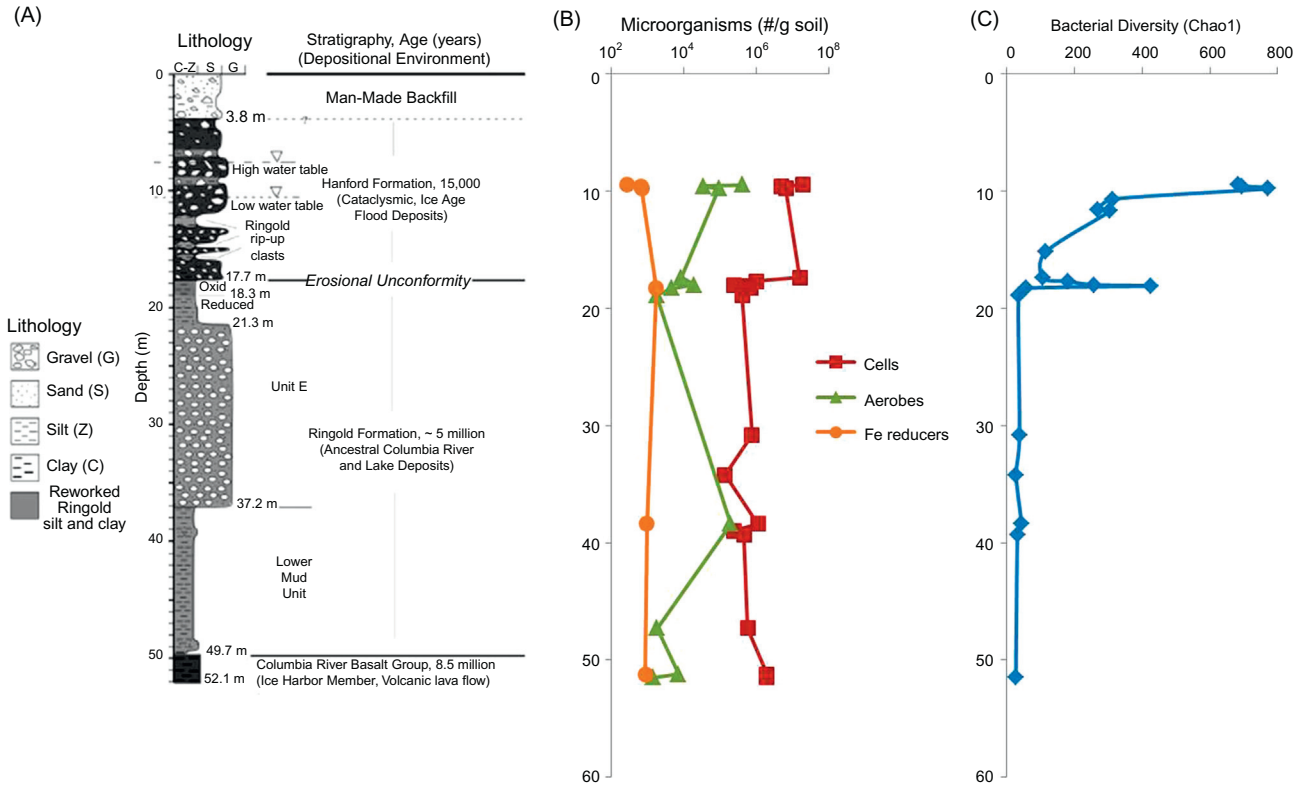

FIGURE 4.24 Distribution and diversity of microorganisms in a shallow subsurface environment. Adapted from Lin *et al*. (2012a,b).

Case Study 4.3 Microbial Counts in the Shallow Subsurface

One of the most thorough characterizations of microorganisms in the shallow subsurface environment was conducted by Lin *et al.* (2012a,b) on samples from the Hanford Site 300 Area near Richland, WA, USA. This area represents a geologically and hydrologically complex system, and is of interest due to the extensive amount of nuclear-related research (much in response to environmental contamination) that has been conducted in the area. The authors collected cores from a 52-m deep borehole at the site and used a large variety of microbial methods including DNA sequencing, quantitative PCR (qPCR), phospholipid fatty acid analysis (PLFA) and culture-based methods to characterize the microbial communities.

The water table at the site fluctuated from 7.5 to 10.5 m below the surface and became a reduced (anaerobic) environment around 18.3 m, shortly after transitioning from coarse- to fine-textured sediment (Figure 4.24). Microbial counts and diversity were greatest in the upper, aerobic region of the saturated zone and decreased dramatically in the anaerobic zone. In general, culturable counts were about two orders of magnitude lower than those obtained with direct methods (qPCR or PLFA). The region surrounding the aerobic/anaerobic interface appeared to be a zone of active biogeochemical redox cycling. Although the levels of metal-reducing bacteria were low overall, there was an increase in the proportion of the bacterial community comprised by organisms using alternate electron acceptors such as iron and sulfate in anaerobic samples with some reaching maximum levels around the aerobic/anaerobic interface. Populations of archaea also varied with depth, but likewise reached maximum levels ($\approx$8% of community) at the aerobic/anaerobic interface. Additionally, the authors detected 13 novel orders of *Deltaproteobacteria* in the samples. This group contains many members known to be involved in transformations of metals, and these novel organisms may prove to be important to the remediation of metal and radionuclide contaminants at Hanford and other contaminated sites (Chapter 18).

Because subsurface microbiology is still a developing field, information is limited in comparison with that for surface microorganisms. Yet there is still enough information available to know that many subsurface environments, once thought to contain very few if any microorganisms, actually have significant and diverse populations of microorganisms. In particular, shallow subsurface zones, specifically those with a relatively rapid rate of water recharge, have high numbers of microorganisms. The majority of these organisms are bacteria, but protozoa and fungi are also present. In general, both microbial numbers and diversity decrease with depth in shallow subsurface systems, especially once the environment becomes anaerobic (Figure 4.24; Case Study 4.3). Total numbers of bacteria, as measured by direct counts, tend to remain fairly constant, ranging between 10^5 and 10^7 cells per gram throughout the profile. For comparison, numbers in surface soils range from 10^9 to 10^{10} cells per gram. This decrease in numbers is directly correlated with the low amounts of inorganic nutrients and organic matter in subsurface materials. Subsurface eukaryotic counts are also lower than surface counts by several orders of magnitude. Low eukaryotic counts are a result of low organic matter content but, perhaps more importantly, result from removal by physical straining by small soil pores as they move downward (Chapter 15). A final point to be made is that both prokaryotic and eukaryotic counts are highest in portions of the subsurface containing sandy sediments. This does not mean that clayey regions are not populated, but that the numbers tend to be lower. This may also be due to exclusion and physical straining of microorganisms by small pores in clay-rich media.

Numbers of culturable bacteria in subsurface environments generally show more variability than those from direct counts (molecular-based or microscopic methods). Thus, the difference between direct and cultural counts in the subsurface is often greater than the difference in surface soils (one to two orders of magnitude). Several factors may explain the larger difference between direct and cultural counts in the subsurface. First, because nutrients are much more limiting in the subsurface, a greater proportion of the population may be in a nonculturable state. Second, the physiological and nutritional requirements of subsurface organisms are not well understood. Therefore, even though we know that a dilute nutrient medium is better than a rich medium, the type of dilute nutrient medium used may still not be appropriate for many environmental microorganisms (Chapter 8).

4.6.2 Microorganisms in Deep Subsurface Environments

Until relatively recently, it was thought that the deep subsurface environments contain few if any microorganisms because of the extreme oligotrophic conditions found there. However, recent research has shown that microorganisms can be found to a depth of >3 km below Earth's surface! Interest in this area began in the 1920s, when increased consumption of oil led to increased oil exploration and production. Upon examination of water extracted from deep within oil fields, Edward Bastin, a geologist at the University of Chicago, found that significant levels of hydrogen sulfide and bicarbonate were present. The presence of these materials could not be explained on a chemical basis alone, and Bastin suggested that sulfate-reducing bacteria were responsible for the hydrogen sulfide and bicarbonate found in the drilling water. Subsequently, Frank Greer, a microbiologist at the University of Chicago, was able to culture sulfate-reducing bacteria from water extracted from an oil deposit

that was hundreds of meters below Earth's surface. Bastin and Greer suggested that these microorganisms were descendants of organisms buried more than 300 million years ago during formation of the oil reservoir. However, their suggestions were largely ignored because the sampling techniques and microbial analysis techniques available at the time could not ensure that the bacteria were not simply contaminants from the drilling process.

Other research hinted at the existence of subsurface microorganisms, most notably the work of Claude Zobell. But not until the 1980s, with the growing concern over groundwater quality, did several new efforts address the questions of whether subsurface microorganisms exist and what range and level of microbial activity occur in the subsurface. Agencies involved in these new studies included the U.S. Department of Energy, the U.S. Geological Survey, the U.S. Environmental Protection Agency, the German Federal Ministry of the Interior (Umbeltbundesamt), the Institute for Geological Sciences (Wallingford, England) and the Water Research Center (Medmanham, England). A number of new techniques were developed to facilitate the collection of sterile samples from deep cores in both the saturated and the unsaturated zone (Chapter 8), and a great deal of information has been generated concerning the presence and function of microorganisms in deep subsurface environments (Fredrickson and Onstott, 1996).

4.6.2.1 Microorganisms in the Deep Vadose Zone

Several studies have looked at deep cores in the unsaturated zone. In one of the first such studies, Frederick Colwell (1989) collected a 70-m core from the eastern Snake River Plain, which is a semiarid, high desert area in southeastern Idaho. Table 4.11 shows a comparison of bacterial numbers in the surface and subsurface samples from this site. Following the pattern described in Section 4.6.1 for shallow subsurface environments, the direct counts from deep subsurface samples remained high, declining by only one order of magnitude in comparison with the surface samples. In contrast, culturable counts declined by four orders of magnitude to less than 100 colony-forming units per gram of sediment. The majority of the isolates from the subsurface in this study were Gram positive and strictly aerobic. In contrast, in surface soils Gram-negative bacteria are more numerous. The subsurface atmosphere was found to be similar to ambient surface air in most samples, suggesting that the subsurface was aerobic.

TABLE 4.11 A Comparison of Microbial Counts in Surface and 70-m Unsaturated Subsurface Environments

Sample Site	Direct Counts (counts/g)	Viable Counts (CFU/g)[a]
Surface (10 cm)	2.6×10^6	3.5×10^5
Subsurface basalt-sediment interface (70.1 m)	4.8×10^5	50
Subsurface sediment layer (70.4 m)	1.4×10^5	21

[a]*CFU, colony-forming units.*

Subsequent studies have largely confirmed these findings and have added some new information. In general, microbial numbers and activities are higher in paleosols (buried sediments) that have had exposure to Earth's surface and plant production. These materials tend to have associated microorganisms and nutrient reserves, albeit at low concentrations, that can maintain very slow-growing populations for thousands of years. These later studies also suggested that there are some vadose zone materials, most notably massive basalt samples collected by the Idaho National Engineering Laboratory, that lack viable microorganisms and do not show any detectable metabolic activity. In summary, our present understanding of the deep vadose zone is limited. However, it appears that there are areas of the vadose zone that contain microbes that may be stimulated to interact with environmental contaminants, whereas other areas of the vadose zone simply act as a conduit for the downward transport of contaminants.

It must be emphasized that although microbes are present in deep vadose zones, rates of metabolic activity are much lower than rates in surface soils. This is illustrated in Figure 4.25, which depicts metabolic activity in a range of surface and subsurface environments. Metabolic activity is expressed as the rate of CO_2 production and was estimated by groundwater chemical analysis and geochemical modeling. As can be seen in this figure, the difference between the

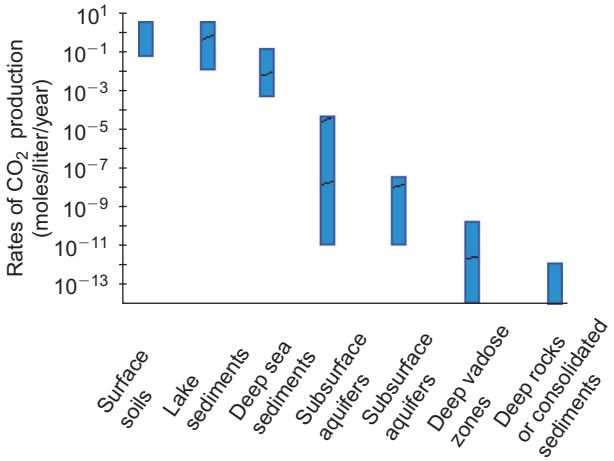

FIGURE 4.25 Ranges of rates of *in situ* CO_2 production for various surface and subsurface environments, as estimated by groundwater chemical analyses and geochemical modeling. Adapted from Kieft and Phelps (1997).

Case Study 4.4 Deep Probe—How Low Can Life Go?

In 1987, the US Department of Energy sponsored the drilling of several deep boreholes (0.5 km) in Cretaceous sediments (70 million to 135 million years old) in South Carolina, near the Savannah River nuclear materials processing facility. A team of scientists used a sophisticated sampling device to ensure that the core samples taken from the boreholes were not contaminated with microorganisms from other parts of the borehole or the surface. The samples were collected from depths of almost 0.5 km and then shipped to several laboratories, where microbial analyses were initiated immediately. Microbial analysis of core materials showed diverse and numerous populations of microbes, with total counts ranging from 10^6 to 10^7 cells per gram of sediment. Culturable counts were much lower, ranging from 10^3 to 10^6 colony-forming units per gram of sediment from samples taken from 350 to 413 m in a permeable, saturated Middendorf sediment, to nondetectable to 10^4 colony-forming units per gram of sediment from a low-permeability Cape Fear sediment (450 to 470 m) (Fredrickson *et al.*, 1991). The most abundant culturable forms in these samples were aerobic or facultatively anaerobic chemoheterotrophs. Results of these analyses have helped confirm the theory that subsurface bacteria are ubiquitous, although their abundance varies considerably, depending on the site characteristics.

More recently, between 2001 and 2006, a group of collaborating scientists took a series of water samples from depths of 0.72 to > 3 km below Earth's surface in the Witwatersrand Basin in central South Africa (Gihring *et al.*, 2006). Total microbial numbers in the samples were estimated to be as low as 10^3 cells/ml. Diversity was also low as shown by analysis of the 16S rRNA gene, which generated only an average of 11 bacterial OTUs from all the samples. Compare this to surface soils that have up to 6300 OTUs! Interestingly, these researchers found growth substrates in the samples such as methane, ethane, propane, butane, and acetate and H_2 that were clearly not being used by the microbial community (Kieft *et al.*, 2005). They speculate that there must be factors other than these electron donors that ultimately limit growth, such as the limitation of an inorganic nutrient like iron or phosphate. Growth limitations are also evidenced by the small average cell diameter of 0.3 μm.

What types of microorganisms were present in these samples and how do they compare to what are found in surface soils? The bacteria identified in the Savannah River study were dominated by the Gram-negative divisions *gamma-Proteobacteria, beta-Proteobacteria*, and *alpha-Proteobacteria*, and the Gram-positive division *Actinobacteria* (Balkwill and Boone, 1997). The Witwatersrand samples were dominated by *beta-*, *gamma-*, and *alpha-Proteobacteria*, followed by the Gram-positive spore-forming division *Firmicutes*. Each of these sample sites also had bacteria that did not match closely to any bacteria identified to date. The pattern of bacteria found in these deep subsurface samples when compared to that found in surface soils is surprisingly similar. Most uncultured soil bacterial libraries are dominated by *Proteobacteria* (predominantly *alpha-Proteobacteria*), *Acidobacteria* (known to be difficult to culture), and *Actinobacteria* (Janssen, 2006).

rates of CO_2 production in a surface soil and in the deep vadose zone is at least nine orders of magnitude.

4.6.2.2 *Microorganisms in the Deep Saturated Zone*

Intermediate and deep aquifers are characterized by low rates of recharge and groundwater flow that create a habitat for microorganisms different from that in shallow aquifers. Samples taken from deep cores (Case Study 4.4) have generally shown that there are lower numbers and a more limited diversity of microorganisms in deep saturated zones than in surface soils. However, the types of organisms detected included a wide range of aerobic and facultatively anaerobic chemoheterotrophs; denitrifiers; methanogens; sulfate-reducers; sulfur-oxidizers; nitrifiers; and nitrogen-fixing bacteria. Low numbers of unicellular cyanobacteria, fungi, and protozoa have also been detected in some samples from 0.5 km depth (Balkwill and Boone, 1997). Culture-based analysis of some of these samples has shown that the bacteria are able to metabolize simple sugars, organic acids and even complex polymers such as the storage product β-hydroxybutyric acid. Thus, subsurface microbes exhibit diverse metabolic capabilities. What is interesting about these data is that they suggest that subsurface microbes reflect in large part what is found in surface soils.

Scientists have also discovered microbial life at even greater depths (0.72 to > 3 km below Earth's surface) in water samples from deep gold mines in South Africa (Kieft *et al.*, 2005; Gihring *et al.*, 2006). Interestingly, microbial numbers in the samples were estimated to be quite low ($\approx 10^3$ cells/mL) despite the presence of relatively high concentrations of growth substrates such as methane, ethane, acetate and H_2. The researchers speculated that other factors, such as the lack of inorganic nutrients or alternate electron acceptors, may ultimately be limiting microbial growth in these deep environments. However, our knowledge of microbial communities in these environments is likely biased by the methodologies used to characterize them—based largely on our greater relative understanding of microbial communities in surface soils. Additional work on these deep subsurface environments will undoubtedly yield exciting discoveries that will only deepen our astonishment at the complexity of microbial life on Earth.

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