Chapter 4

Earth Environments

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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

I.L. Pepper, C.P. Gerba, T.J. Gentry: Environmental Microbiology, Third edition. DOI: http://dx.doi.org/10.1016/B978-0-12-394626-3.00004-1

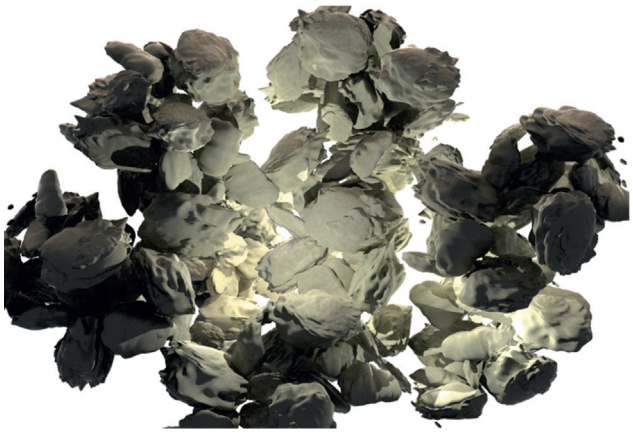

FIGURE 4.1 Soil architecture resulting from secondary aggregate formation with intraaggregate and interaggregate pore space. *Source:* Pepper (2014).

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

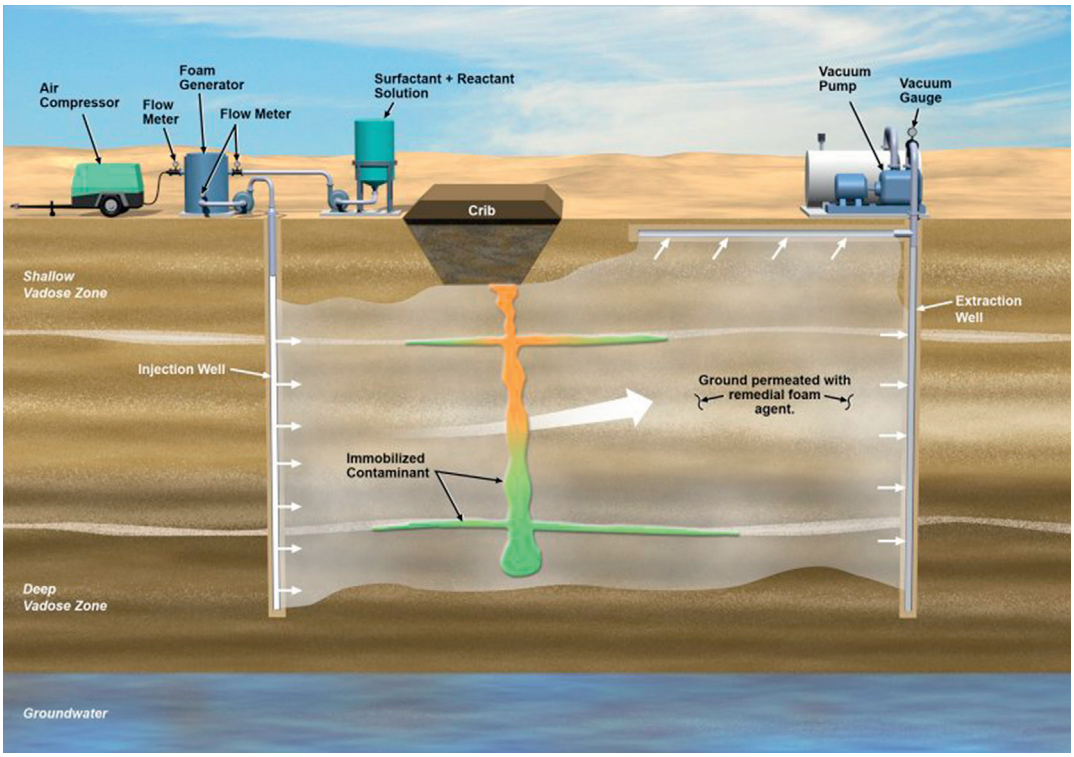

FIGURE 4.2 Delivery of remedial solutions through a heterogeneous deep vadose zone to remove contaminants. *Source:* Pacific Northwest National Laboratory.

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

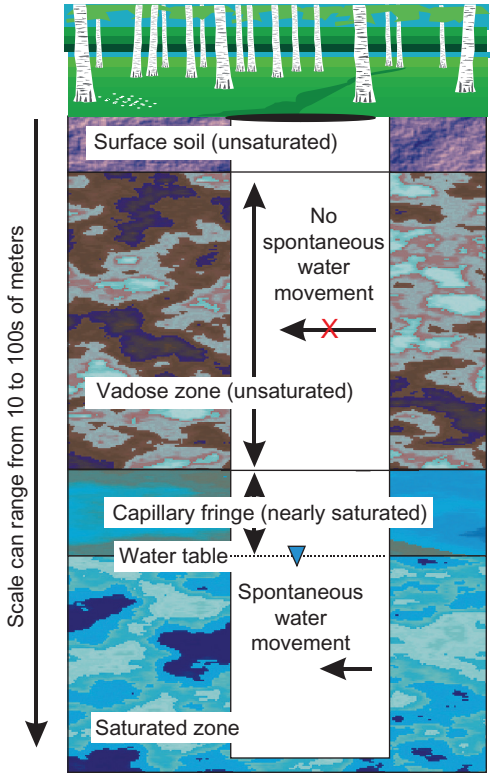

FIGURE 4.3 Cross-section of the subsurface showing surface soil, vadose zone, capillary fringe and saturated zone.

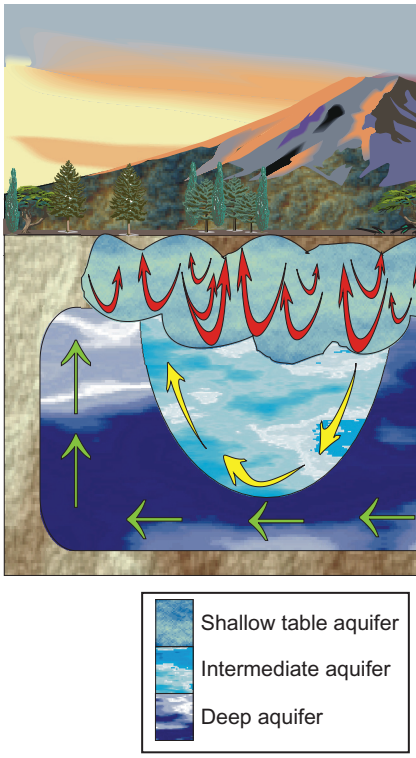

FIGURE 4.4 Shallow, intermediate and deep aquifer systems. Arrow thickness indicates the relative flow rates (the thicker the arrow, the faster the flow rate) in the different aquifer systems. Adapted from Chapelle (1993) and reproduced by permission of Wiley, New York.

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.

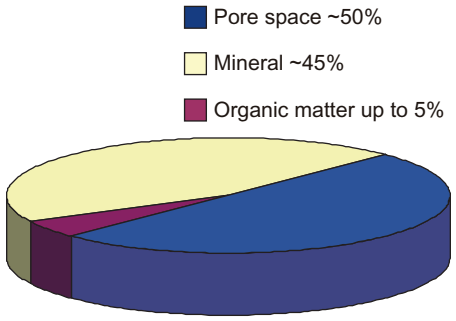

FIGURE 4.5 Three basic components of a porous medium, such as a typical surface soil, on a volume basis.

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

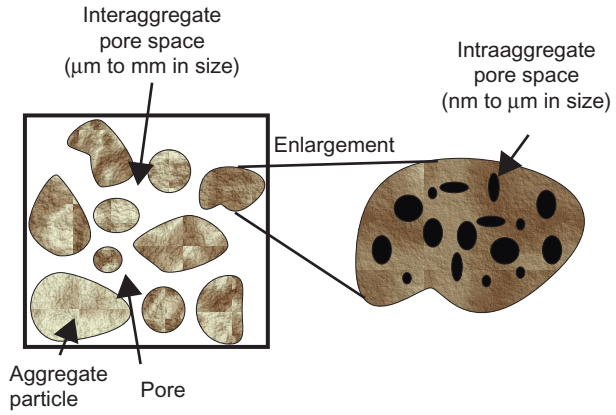

FIGURE 4.7 Pore space. In surface soils, mineral particles are tightly packed together and even cemented in some cases with microbial polymers forming soil aggregates. The pore spaces between individual aggregates are called interaggregate pores and vary in size from micrometers to millimeters. Aggregates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

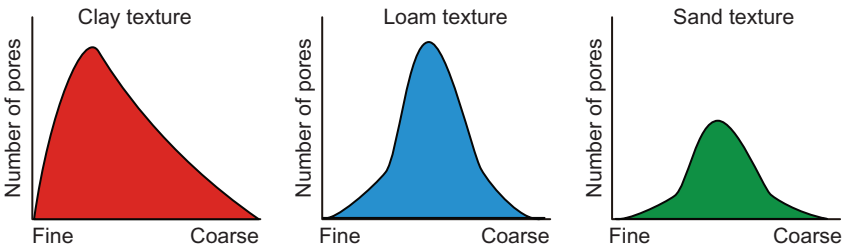

FIGURE 4.8 Typical pore size distributions for clay-, loam- and sand-textured horizons. Note that the clay-textured material has the smallest average pore size, but the greatest total volume of pore space.

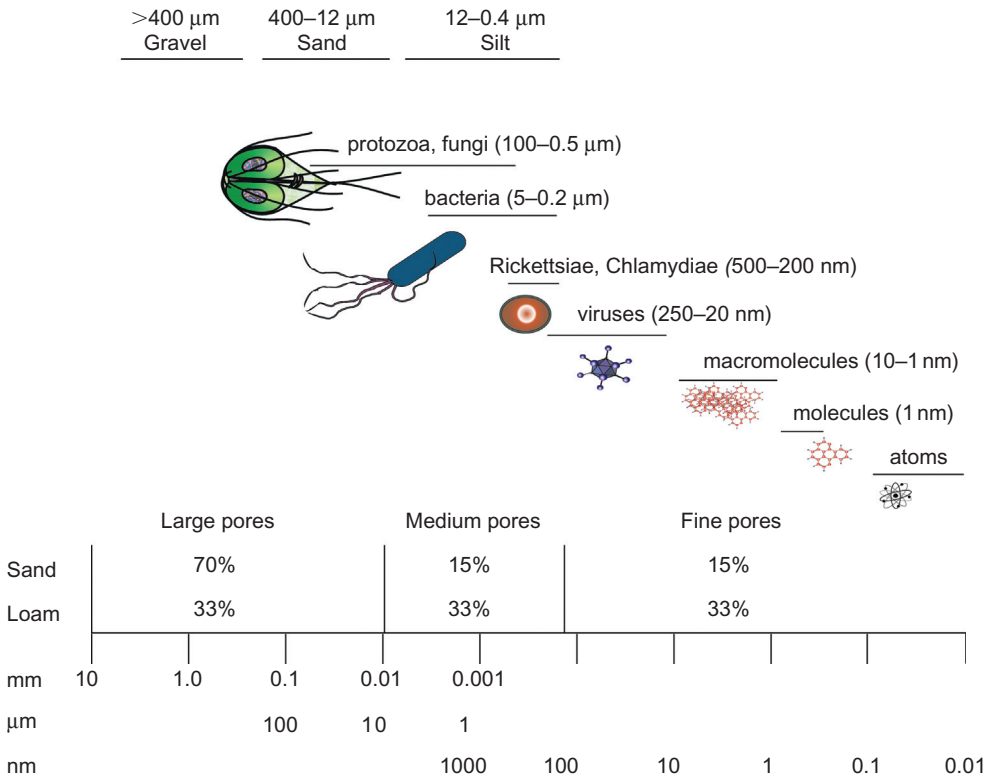

FIGURE 4.9 Comparison of sizes of bacteria, viruses and molecules with hydraulic equivalent diameters of pore canals. Adapted from Matthess *et al.* (1988).

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.

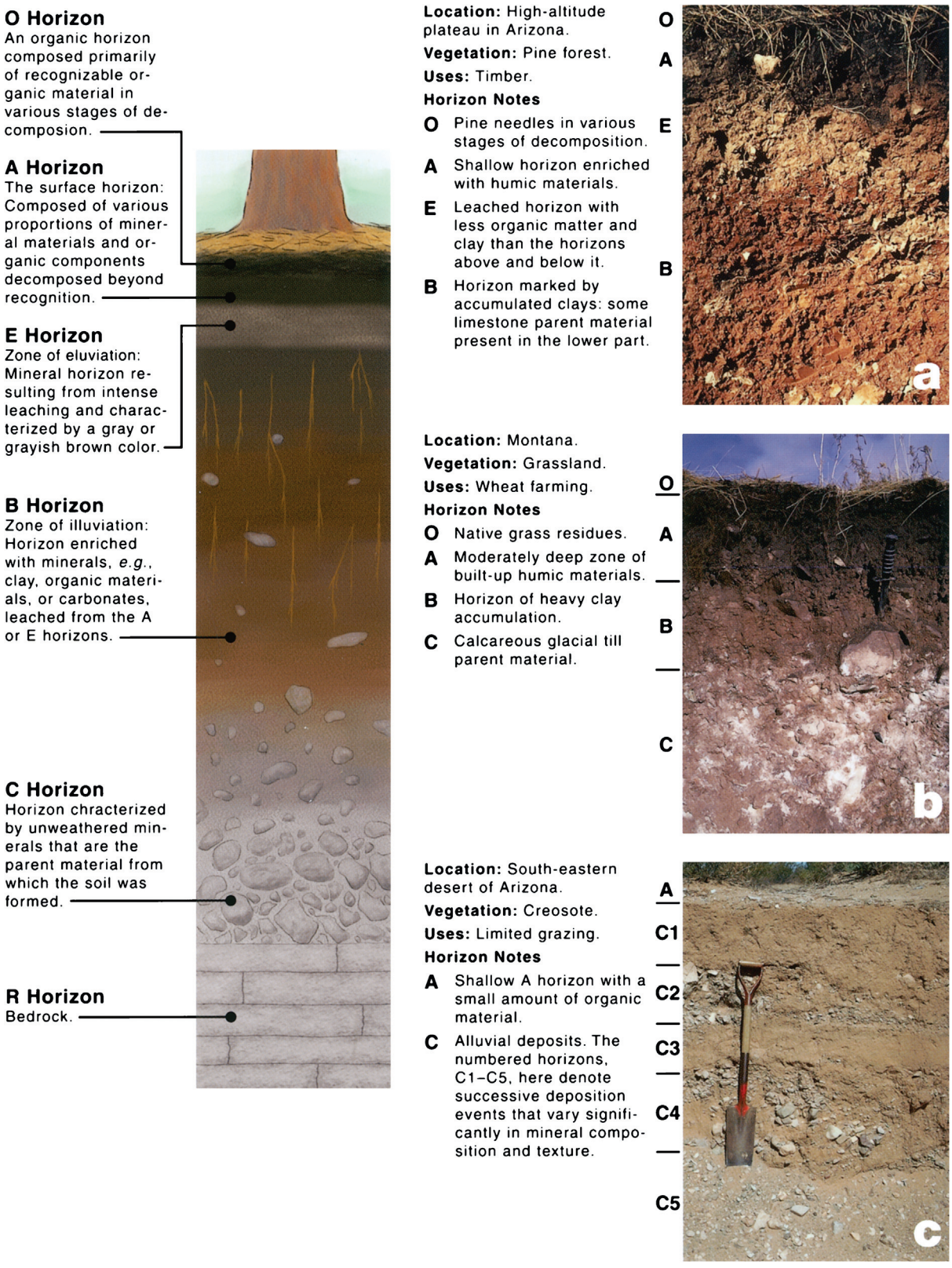

FIGURE 4.10 Typical soil profiles illustrating different soil horizons. These horizons develop under the influence of the five soil-forming factors and result in unique soils. From Pepper *et al.* (2006).

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.

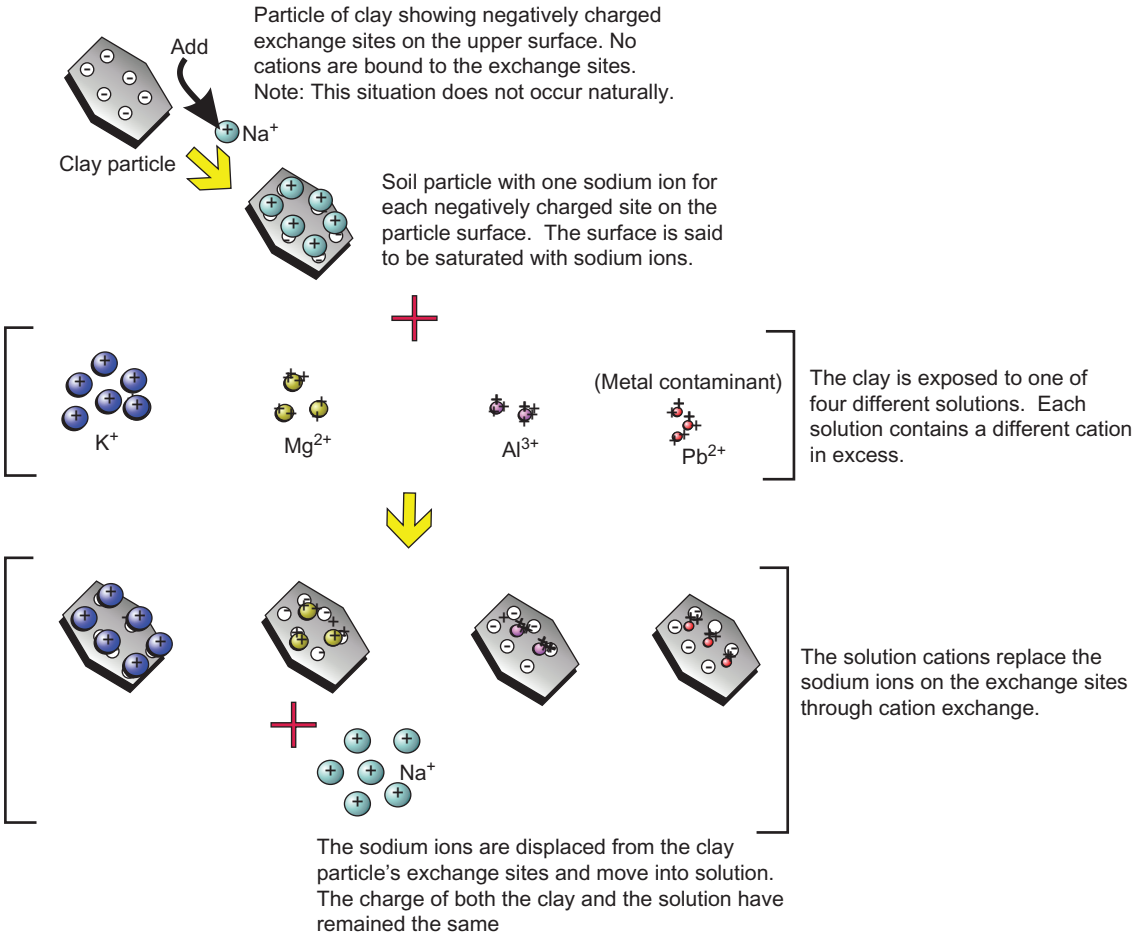

FIGURE 4.11 Cation exchange on clay particles. Adapted from Pepper *et al*. (2006).

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

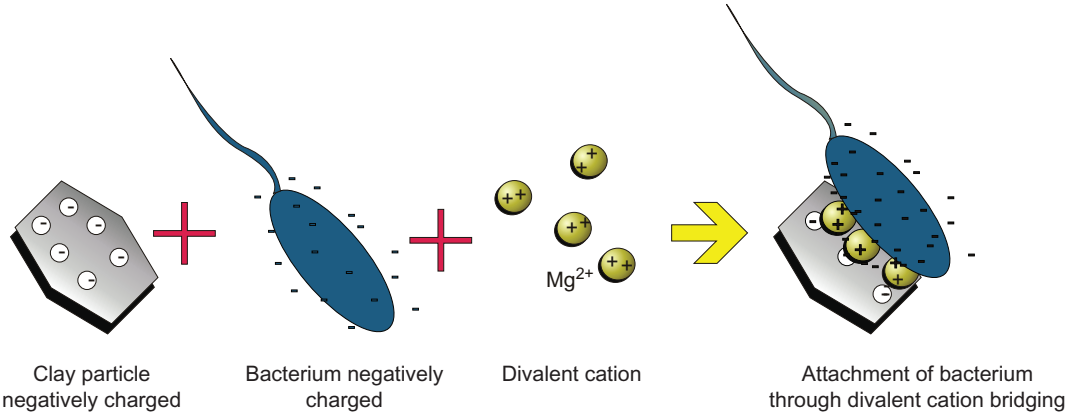

FIGURE 4.12 Attachment of a bacterial cell to a clay particle via cation bridging.

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,

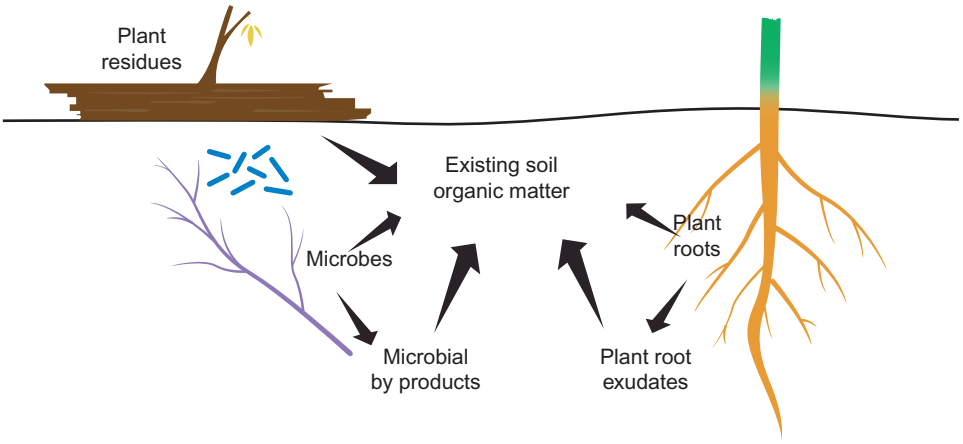

FIGURE 4.13 Schematic representation of the formation of soil organic matter.

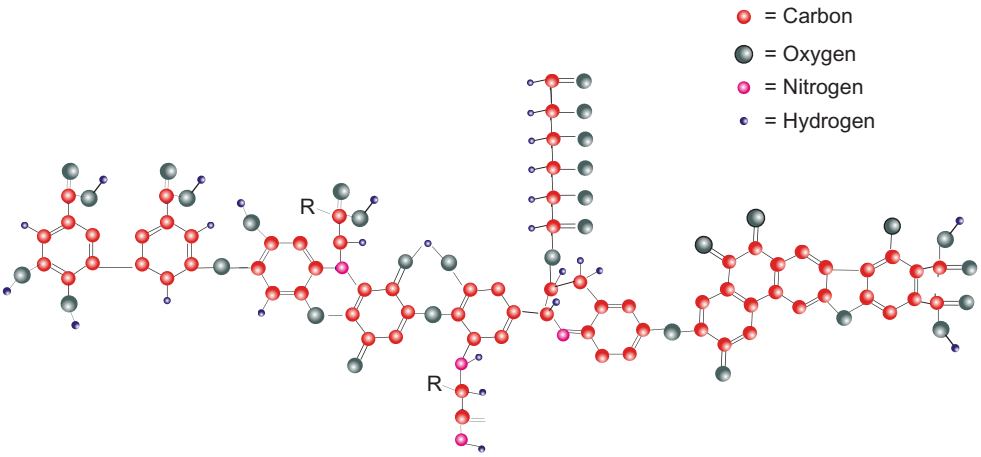

FIGURE 4.14 Humus polymer. R can represent various functional groups.

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

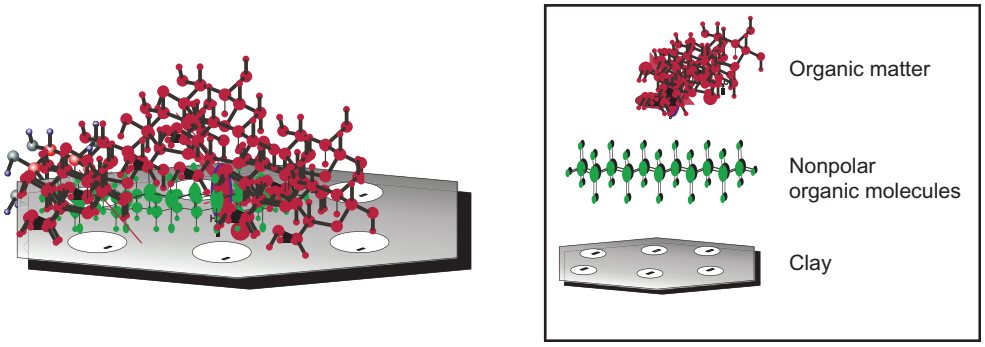

FIGURE 4.15 Hydrophobic sorption mechanisms. Nonpolar organic molecules tend to sorb to organic matter that is associated with solid mineral surfaces by diffusing into the sponge-like interior of organic matter molecules. Adapted from Schwarzenbach *et al.* (1993); reproduced by permission of John Wiley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

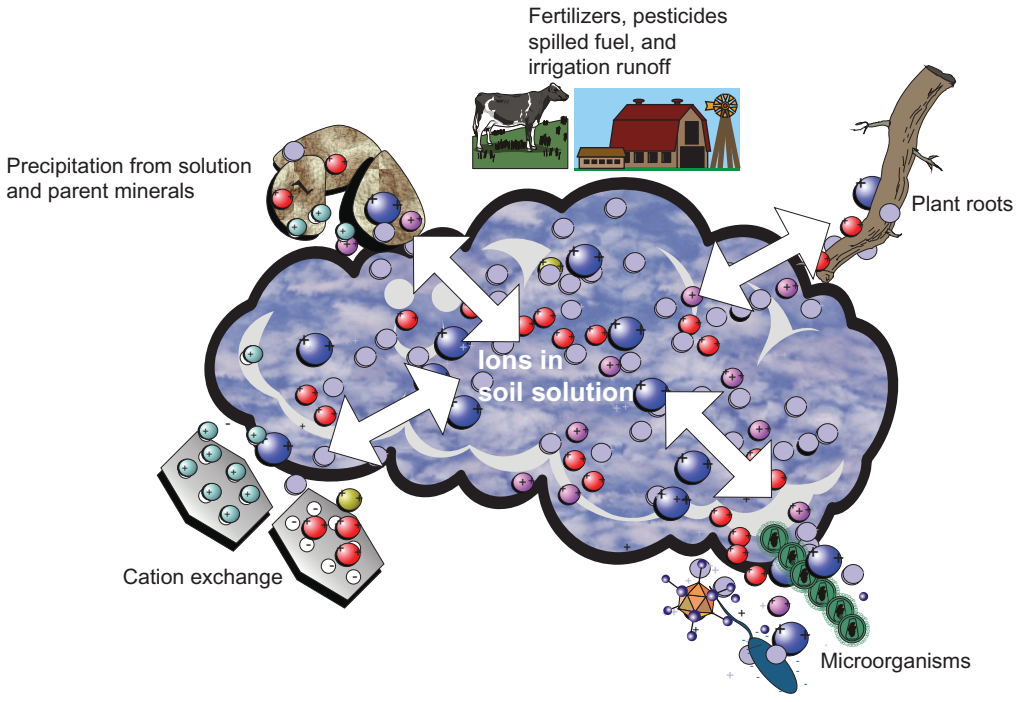

FIGURE 4.16 Paths of dissolution and uptake of minerals in the soil.

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

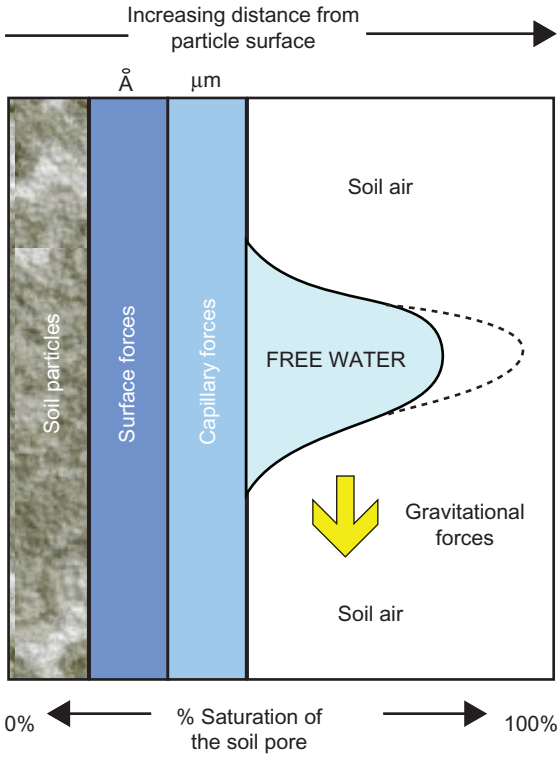

FIGURE 4.17 The continuum of soil water. Adapted from Dragun (1988). Reproduced with permission of ASP.

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.

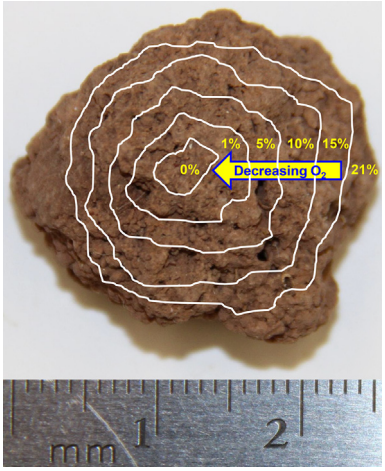

FIGURE 4.18 Gradient of oxygen concentrations in a typical soil aggregate. Adapted from Sextone *et al.* (1985).

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

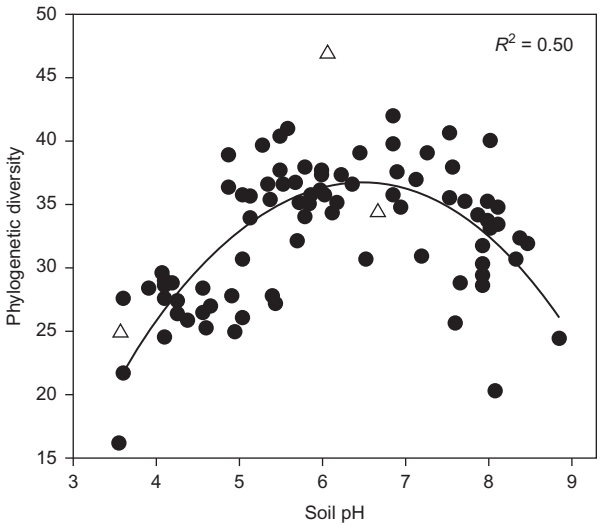

FIGURE 4.20 Soil bacterial diversity in soil samples from North and South America is strongly correlated with soil pH. From Lauber *et al.* (2009).

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.

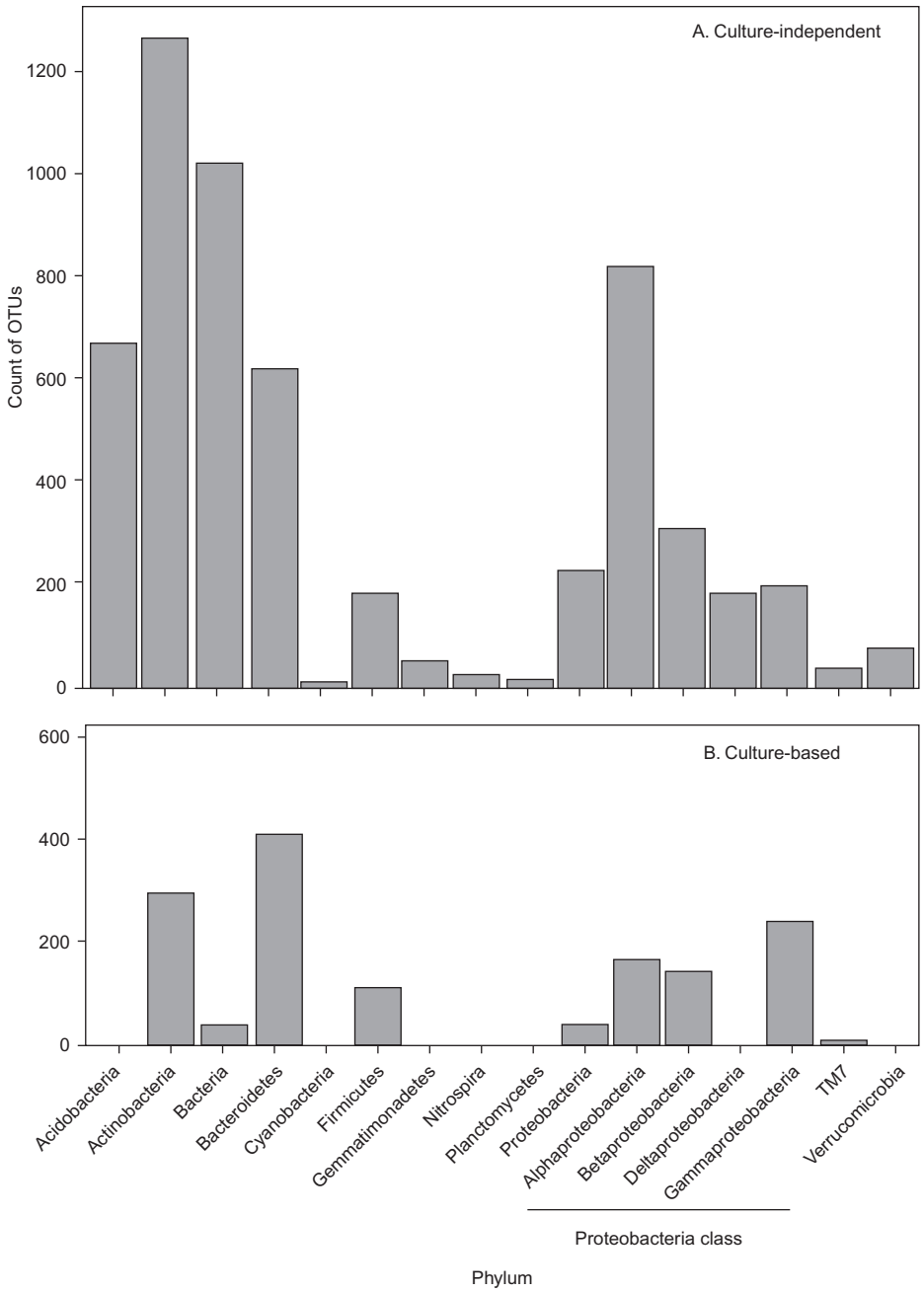

FIGURE 4.21 Impact of culture-based and -independent methods on characterization of a soil bacterial community. Note the greater number of phyla and operational taxonomic units (OTUs) detected using the culture-independent approach. From Shade *et al.* (2012).

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

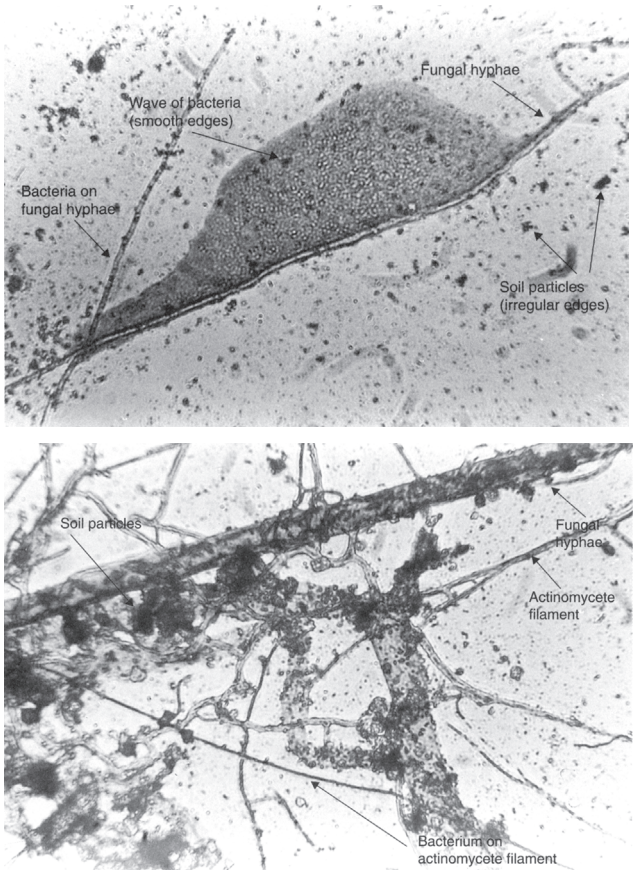

FIGURE 4.22 Comparison of soil bacteria, actinomycetes and fungi viewed under a light microscope. From Pepper *et al*. (2006).

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

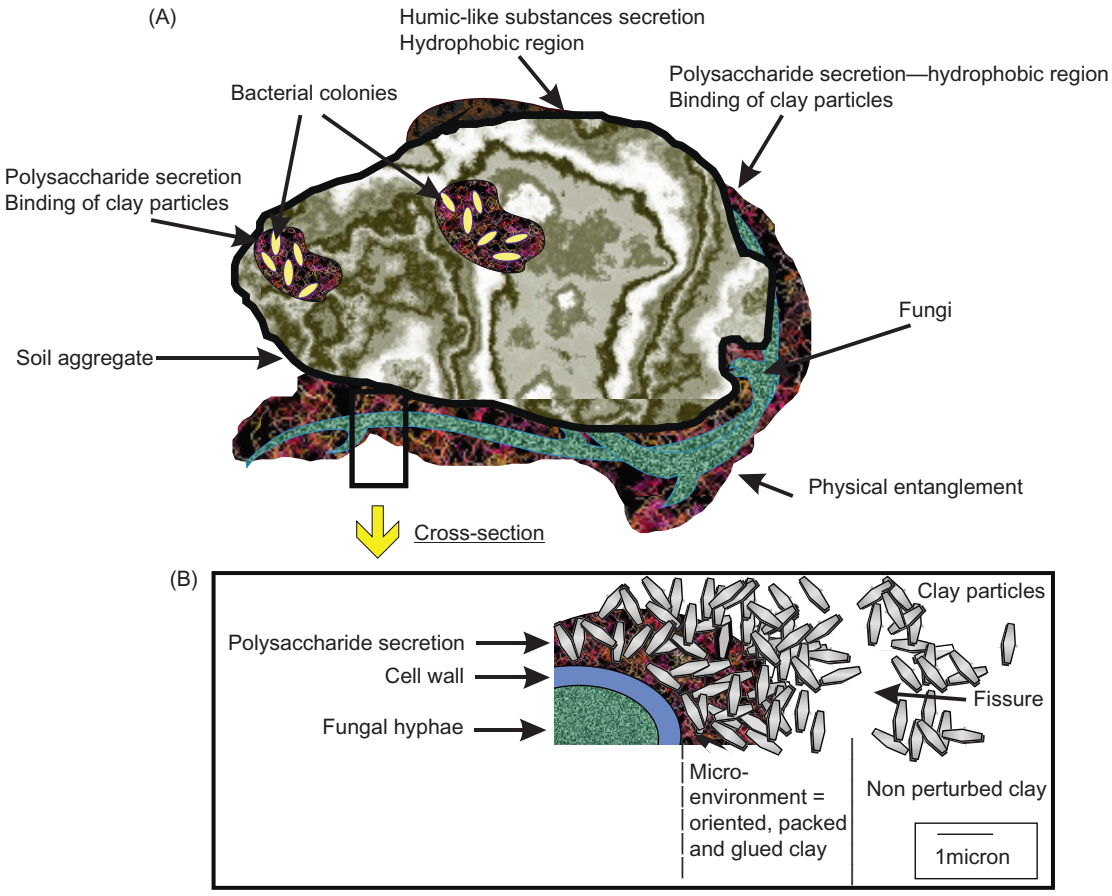

FIGURE 4.23 Microbially mediated aggregation: (A) schematic representation of the binding and stabilization of a soil aggregate by microorganisms; (B) detail of the microenvironment in the vicinity of a fungus. Adapted with permission from Robert and Chenu (1992).

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).

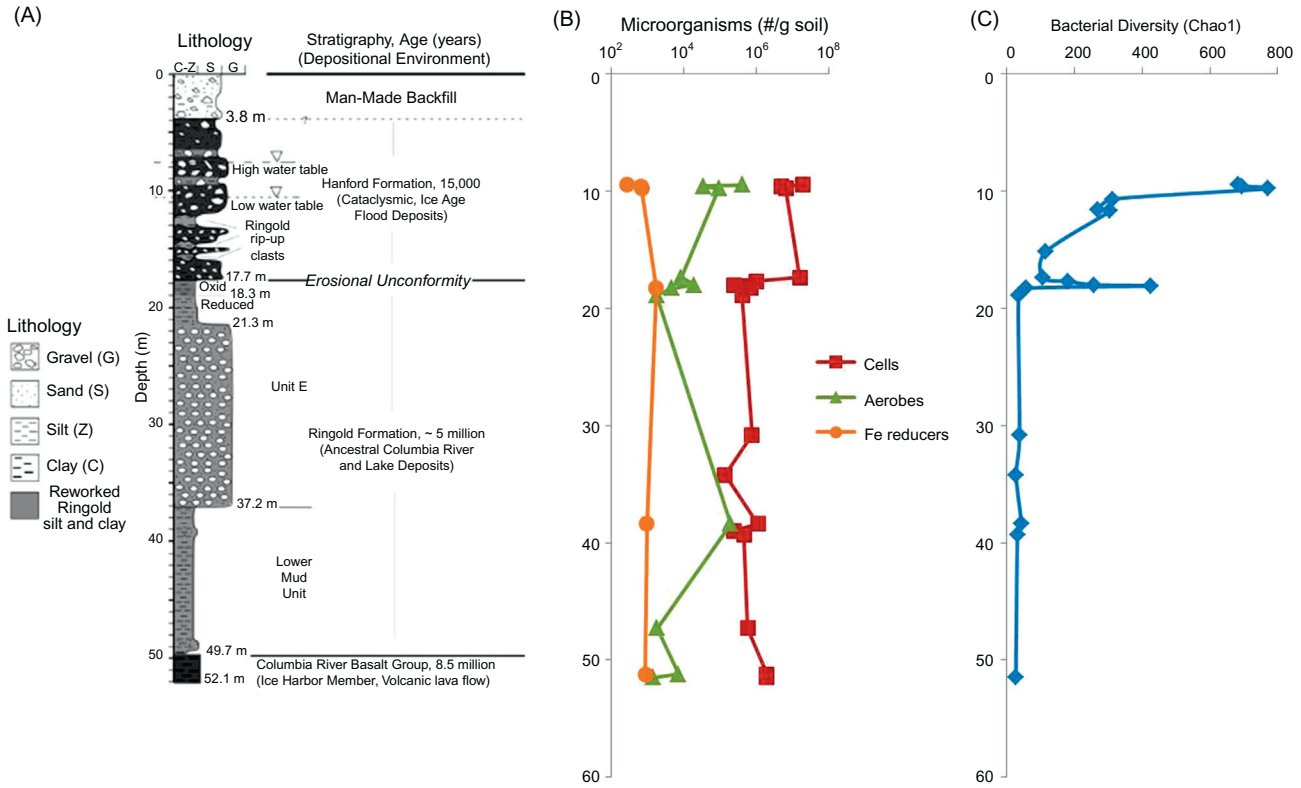

FIGURE 4.24 Distribution and diversity of microorganisms in a shallow subsurface environment. Adapted from Lin *et al*. (2012a,b).

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

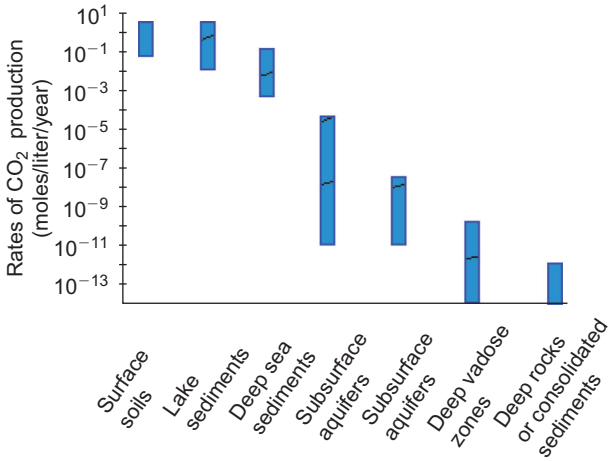

FIGURE 4.25 Ranges of rates of *in situ* CO_2 production for various surface and subsurface environments, as estimated by groundwater chemical analyses and geochemical modeling. Adapted from Kieft and Phelps (1997).

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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Chapter 5

Aeromicrobiology

Ian L. Pepper and Charles P. Gerba

5.1 INTRODUCTION

In the 1930s, F.C. Meier coined the term aerobiology to describe a project that involved the study of life in the air (Boehm and Leuschner, 1986). Since then, aerobiology has been defined by many as the study of the aerosolization, aerial transmission and deposition of biological materials. Others have defined it more specifically as the study of diseases that may be transmitted via the respiratory route (Dimmic and Akers, 1969). Despite the variations in definition, this evolving area is becoming increasingly important in many aspects of diverse fields including public health, environmental science, industrial and agricultural engineering, biological warfare and space exploration.

This chapter introduces the basics of aerobiology, including the nature of aerosols and the fundamentals of the aeromicrobiological (AMB) pathway. The remainder of the chapter focuses on a subset of the science that we shall term aeromicrobiology. Aeromicrobiology, as defined for the purpose of this text, involves various aspects of intramural (indoor) and extramural (outdoor) aerobiology, as they relate to the airborne transmission of environmentally relevant microorganisms, including viruses, bacteria, fungi, yeasts and protozoans.

5.2 AEROSOLS

Particles suspended in air are called aerosols. These pose a threat to human health mainly through respiratory intake and deposition in nasal and bronchial airways. In addition, soil or dust particles can act as a "raft" for biological entities known as bioaerosols (Brooks *et al.*, 2004). Smaller aerosols travel further into the respiratory system and generally cause more health problems than larger particles. For this reason, the United States Environmental Protection Agency (USEPA) has divided airborne particulates into two size categories: PM_{10}, which refers to particles with diameters less than or equal to 10 μm (10,000 nm), and $PM_{2.5}$, which are particles less than or equal to 2.5 μm

I.L. Pepper, C.P. Gerba, T.J. Gentry: Environmental Microbiology, Third edition. DOI: http://dx.doi.org/10.1016/B978-0-12-394626-3.00005-3

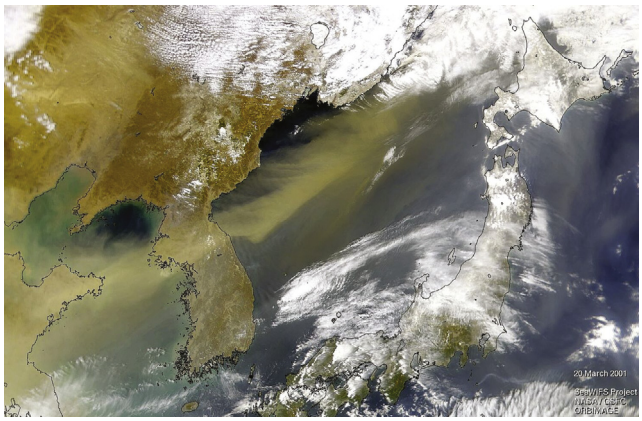

FIGURE 5.1 Mongolian dust over the Sea of Japan. Image provided by NASA.

(2500 nm) in diameter. For this classification, the diameter of aerosols is defined as the aerodynamic diameter:

$$d_{pa} = d_{ps}(\rho_p/\rho_w)^{1/2} \quad \text{(Eq. 5.1)}$$

where:

d_{pa} = aerodynamic particle diameter (μm)
d_{ps} = Stokes' diameter (μm)
ρ_p = particle density (g/cm^{-3})
ρ_w = density of water (g/cm^{-3})

Atmospheric particulate concentration is expressed in micrograms of particles per cubic meter of air (μg/m^3). The USEPA established a National Ambient Air Quality Standard (NAAQS) for PM_{10} of 150 μg/m^{-3} averaged over a 24-hour period, and 50 μg/m^{-3} averaged annually. More recently, separate standards for $PM_{2.5}$ of 65 μg/m^{-3} for 24 hours and 15 μg/m^{-3} annually have been introduced.

Symptoms of particulate matter inhalation include: decreased pulmonary function; chronic coughs; bronchitis; and asthmatic attacks. The specific causal mechanisms are poorly understood. One well-documented episode occurred in London in 1952, when levels of smoke and sulfur dioxide aerosols, largely associated with coal combustion, reached elevated levels due to local weather conditions. Over a 10-day period, approximately 4000 deaths were attributed to cardiovascular and lung disorders brought on or aggravated by these aerosols.

Airborne particles can travel great distances. Intense dust storms during 1998 and 2001 in the Gobi desert of western China and Mongolia (Figure 5.1) elevated aerosol levels to concentrations near the health standard in western North America several thousand miles away.

Smaller particles tend to travel greater distances than large particles. Stokes' law (Eq. 5.2) is used to describe the fall of particles through a dispersion medium, such as air or water:

$$V = [D^2 \times (\rho_p - \rho_1) \times g]/18\rho \quad \text{(Eq. 5.2)}$$

Information Box 5.1 Influence of Particle Size on Velocity of Deposition of Particles in Air, Calculated Using Stokes' Law

Particle Diameter (mm)	Particle Type	Rate of Fall in Air (cm s^{-1})
1	Sand	7880
0.1	Silt	79
0.001	Clay	7.9×10^{-5}
0.002	Clostridial spore	0.016

From Pepper et al., 2006.

where:

V = velocity of fall (cm/s^{-1})
g = acceleration of gravity (980 cm/s^{-2})
D = diameter of particle (cm)
ρ_p = density of particle (density of quartz particles is 2.65 g/cm^{-3})
ρ_1 = density of dispersion medium (air has a density of about 0.001213 g/cm^{-3}; water has a density of about 1 g/cm^{-3})
ρ = viscosity of the dispersion medium (about 1.83×10^{-4} poise or g cm^{-1}s^{-1} for air; 1.002×10^{-2} poise for water)

Using Stokes' law, we can calculate the rate of fall of particles in air (Information Box 5.1). Small particles are thus a greater concern than larger particles for several reasons. Small particles stay suspended longer and so they travel further and stay suspended longer. This results in an increased risk of exposure. Small particles also tend to move further into the respiratory system, exacerbating their effects on health. Stokes' law explains why we can expect viruses to persist as a bioaerosol longer than bacteria, which are much larger.

5.3 NATURE OF BIOAEROSOLS

Biological contaminants include whole entities such as bacterial and viral human pathogens. They also include airborne toxins, which can be parts or components of whole cells. In either case, biological airborne contaminants are known as bioaerosols, which can be ingested or inhaled by humans.

Bioaerosols vary considerably in size, and composition depends on a variety of factors including the type of microorganism or toxin, the types of particles they are associated with such as mist or dust, and the gases in which the bioaerosol is suspended. Bioaerosols in general range from 0.02 to 100 μm in diameter and are classified on the basis of their size. The smaller particles (<0.1 μm in diameter) are considered to be in the nuclei mode, those ranging from 0.1 to 2 μm are in the accumulation mode and larger

Information Box 5.2 Possible Modes of Respiratory Transmission of Influenza A

Direct Contact
Transmission occurs when the transfer of microorganism results from direct physical contact between an infected or colonized individual and a susceptible host.

Indirect Contact
Transmission occurs by the passive transfer of microorganisms to a susceptible host via inanimate contaminated object or fomite.

Droplet
Transmission occurs via large droplets (≥5 μm diameter) generated from the respiratory tract of the infected individual during coughing or sneezing, talking or during procedures such as suctioning or bronchoscopy. These droplets are propelled a distance of less than 1 m through the air, and are deposited on the nasal or oral mucosa of the new host or in their immediate environment. These large droplets do not remain suspended in the air and true aerosolization does not occur.

Airborne
Transmission occurs via the dissemination of microorganisms by aerosolization. Organisms are contained in droplet nuclei (airborne particles less than 5 μm that result from the evaporation of large droplets), or in dust particles containing skin cells and other debris that remain suspended in the air for long periods of time. Microorganisms are widely dispersed by air currents and inhaled by susceptible hosts.
See also Section 5.6.2 and Case Study 5.2.

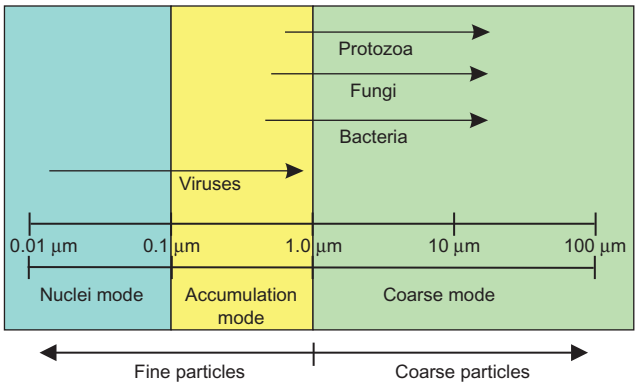

FIGURE 5.2 Diagrammatic representation of the relative sizes of bioaerosols. The depictions of the various kinds of organisms are indicative of their potential sizes when associated with airborne particles (rafts). The terminologies used to describe the various sizes of the bioaerosols are also indicated.

particles are considered to be in the coarse mode (Committee on Particulate Control Technology, 1980). As shown in Figure 5.2, particles in nuclei or accumulation mode are considered to be fine particles and those in coarse mode are considered coarse particles.

The composition of bioaerosols can be liquid or solid, or a mixture of the two, and should be thought of as microorganisms associated with airborne particles, or as airborne particles containing microorganisms. This is because it is rare to have microorganisms (or toxins) that are not associated with other airborne particles such as dust or water. This information is derived from particle size analysis experiments, which indicate that the average diameter of airborne bacterial particles is greater than 5 μm (Fengxiang *et al.*, 1992). By comparison, the average size of a soil-borne bacterium, 0.3 to 1 μm, is less than one-fifth this size. Similar particle size analysis experiments show the same to be true for aerosolized microorganisms other than bacteria, including viruses.

5.4 AEROMICROBIOLOGICAL PATHWAY

The aeromicrobiological pathway describes: (1) the launching of bioaerosols into the air; (2) the subsequent transport via diffusion and dispersion of these particles; and finally (3) their deposition. An example of this pathway is that of liquid aerosols containing the influenza virus launched into the air through a cough, sneeze or even through talking. These virus-associated aerosols are dispersed by a cough or sneeze, transported through the air, inhaled, and deposited in the lungs of a nearby person, where they can initiate a new infection (Figure 5.3). Traditionally, the deposition of viable microorganisms and the resultant infection are given the most attention, but all three processes (launching, transport and deposition) are of equal importance in understanding the aerobiological pathway.

5.4.1 Launching

The process whereby particles become suspended within Earth's atmosphere is termed launching. Because

FIGURE 5.3 A cough or sneeze launches infectious microbes into the air. Anyone in the vicinity may inhale the microbes, resulting in a potential infection.

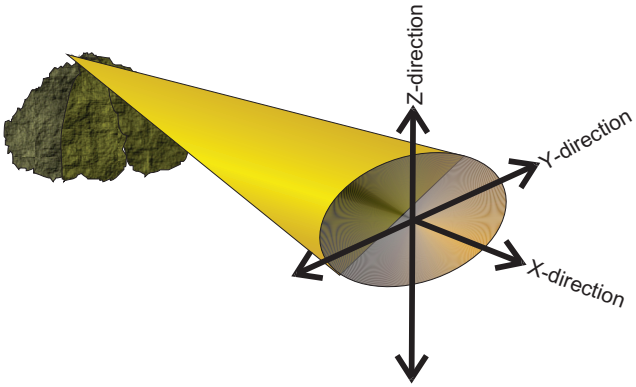

FIGURE 5.4 Schematic representation of the type of bioaerosol distribution expected from a point source, given three planes of diffusion: (1) the *X*-direction is the mean direction in which the wind is blowing; (2) the *Y*-direction is the lateral diffusion; and (3) the *Z*-direction is the vertical diffusion.

FIGURE 5.5 A linear bioaerosol source using the example of the release of biological warfare agents. This is an illustration of an instantaneous linear bioaerosol release.

bioaerosols must be launched into the atmosphere to be transported, it is important to understand this process. The launching of bioaerosols is mainly from terrestrial and aquatic sources, with greater airborne concentrations or atmospheric loading being associated with terrestrial sources than with aquatic sources. A recent model estimated that the total global emission of bacteria containing particles to the atmosphere to be 7.6×10^{23} to 3.5×10^{24} (Burrow *et al*., 2009). Some researchers speculate that there may even be atmospheric sources of bioaerosols in addition to terrestrial and aquatic ones. This phenomenon is related to the limited potential for microorganisms to reproduce while airborne. This, however, is an area of aeromicrobiology for which there is little available information.

Launching into the surface boundary layers can include, but is certainly not limited to, diverse mechanisms such as: air turbulence created by the movement of humans, animals and machines; the generation, storage, treatment and disposal of waste material; natural mechanical processes such as the action of water and wind on contaminated solid or liquid surfaces; and the release of fungal spores as a result of natural fungal life cycles.

Airborne particles can be launched from either point, linear or area sources. A point source is an isolated and well-defined site of launching such as a pile of biosolid material, before it is applied over a field. Point sources tend to display a general conical-type dispersion (Figure 5.4). Point sources can be further defined on the basis of the type of launching phenomenon: (1) instantaneous point sources, for example, a single event such as a sneeze; or (2) continuous point sources, from which launching occurs over extended periods of time, such as a biosolid pile.

In contrast to point sources, linear sources and area sources involve larger, less well-defined areas. When considered on the same size scale, linear and area sources display more particulate wave dispersion as opposed to the conical type of dispersion displayed by point sources. Linear and area sources can also be divided into instantaneous and continuous launching points of origin. For example, an instantaneous linear source might be a passing aircraft releasing a biological warfare agent (Figure 5.5). A continuous area source might be exemplified by release of bioaerosols from a large field that has received an application of biosolids or animal manures.

5.4.2 Transport

Transport or dispersion is the process by which kinetic energy provided by the movement of air is transferred to airborne particles, with resultant movement from one point to another. This "energy of motion" gained by airborne particles is considerable, and can result in dissemination of airborne microorganisms over long distances. Transport of bioaerosols can be defined in terms of time and distance. Submicroscale transport involves short periods of time, under 10 minutes, as well as relatively short distances, under 100 m. This type of transport is common within buildings or other confined spaces. Microscale transport ranges from 10 minutes to 1 hour, and from 100 m to 1 km, and is the most common type of transport phenomenon. Mesoscale transport refers to transport in terms of days and distances up to 100 km, and in macroscale transport, the time and distances are extended even further. Because most microorganisms have limited ability to survive when suspended in the atmosphere, the most common scales considered are the submicroscale and microscale. It should be noted, however, that some viruses, spores and spore-forming bacteria have been shown to enter into mesoscale and even macroscale transport.

As bioaerosols travel through time and space, different forces act upon them such as diffusion, inactivation and

ultimately deposition. Diffusion is the scattering and/or dissipation of bioaerosols in response to a concentration gradient as well as gravity, and is generally aided by airflow and atmospheric turbulence. The amount of turbulence associated with airflow, and thus the relative amount of diffusion that may occur in association with particulates such as bioaerosols, can be estimated using the method of Osbert Reynolds. Reynolds found that factors associated with mean wind velocity, the kinetic viscosity of the air and the relative dimension of the interfering structures could provide an indication of the amount of turbulence associated with linear airflow. Without turbulence, airborne particles from a point source would travel in a concentrated stream directly downwind. The Reynolds equation is written as follows:

$$\text{Reynolds number} = \frac{\text{velocity} \times \text{dimension}}{\text{viscosity}} \qquad \text{(Eq. 5.3)}$$

Consider, for instance, a situation in which there are relatively high winds (500 cm/sec) that are passing over a small bush (24 cm). Because the occurrence of frictional turbulence associated with an object depends on the wind velocity being high enough, and the object it is flowing over being large enough, we find that at normal air viscosity (0.14 cm^2/sec) the Reynolds number (Re) becomes:

$$\text{Re} = \frac{500\ \text{cm/sec} \times 24\ \text{cm}}{0.14\ \text{cm}^2\text{/sec}} = 85,700 \qquad \text{(Eq. 5.4)}$$

The limiting value for the Reynolds equation is usually considered to be 2000, with values above this number indicating turbulent conditions. The higher this value, the higher the relative turbulence of the airflow, and the greater the microorganism-associated particle diffusion that occurs per unit time. In the preceding example, one would expect a great deal of turbulence around items such as a bush, which would increase the diffusion rates of passing bioaerosols.

When dealing with particulate transport over time and distance, Tayler (1915) indicated that diffusion during horizontal transport could be viewed as an increase in the standard spatial deviation of particles from the source over time. What does this mean? For an instantaneous point source under the influence of a mean wind direction, spread would be a standard spatial deviation from a linear axis (x) extending from the source (origin) in the mean direction of wind flow, with diffusion caused by turbulence occurring in the lateral (y) and vertical (z) axes (Figure 5.4). The standard deviation of particulate diffusion cannot be considered constant over a particular spatial orientation, but is instead dependent on the time taken to reach the particular distance. Mathematical models that attempt to estimate the transport of airborne particles use this basic premise as a foundation for predictions. To picture this concept, imagine standing at the door of a room, where someone is holding a smoking candle. If there is no air current in the room the smoke will still eventually reach you at the door, but it will be very diffuse as it is also spreading in every other direction. However, if there is a fan behind the person holding the smoking candle and this fan is pointed at the door, then the smoke from the candle will be carried by this air current. It will travel the same distance as it did before, but it will travel faster, undergo less diffusion and as a result be more concentrated when it reaches you. This is the principle of time-dependent diffusion as indicated by Tayler's theory.

5.4.3 Deposition

The last step in the aeromicrobiology pathway is deposition. An airborne bioaerosol will eventually leave the turbulence of the suspending gas and will ultimately be deposited on a surface by one or a combination of interrelated mechanisms. These mechanisms are discussed in the following sections and include: gravitational settling; downward molecular diffusion; surface impaction; rain deposition; and electrostatic deposition. These processes are linked in many ways, and even though viewed separately, they all combine to create a constant, if not steady, deposition of particles.

5.4.3.1 Gravitational Settling

The main mechanism associated with deposition is the action of gravity on particles. The force of gravity acts upon all particles heavier than air, pulling them down and essentially providing spatial and temporal limitations to the spread of airborne particles. Steady-state gravitational deposition (Figure 5.6) in the absence of air movement can be described in very simplistic terms by Stokes' law, which takes into account gravitational pull, particle density, particle diameter and air viscosity (Section 5.2).

5.4.3.2 Downward Molecular Diffusion

Downward molecular diffusion, as indicated by the name, can be described as a randomly occurring process caused by natural air currents and eddies that promote and enhance the downward movement of airborne particulates (Figure 5.7). These random movements exist even in relatively still air and tend to be in the downward direction because of gravitational effects. As a result, measured rates of gravitational deposition tend to be greater than those predicted by the Stokes equation. The increase in the rate of deposition is due to the added effects of downward molecular diffusion. Molecular diffusion is also influenced by the force of the wind. Molecular diffusion-enhanced deposition rates tend to increase with increasing wind speed and turbulence.

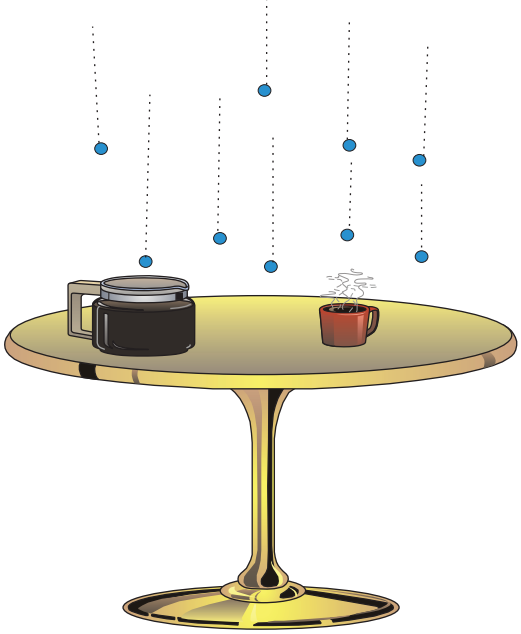

FIGURE 5.6 Schematic representation of gravitational settling, which is a function of Earth's gravitational pull, particle density, particle diameter and the viscosity of air. This figure does not take into account random air movement. Stokes' equation was developed to give an estimate of the terminal velocity achieved by particles as a function of gravitational settling.

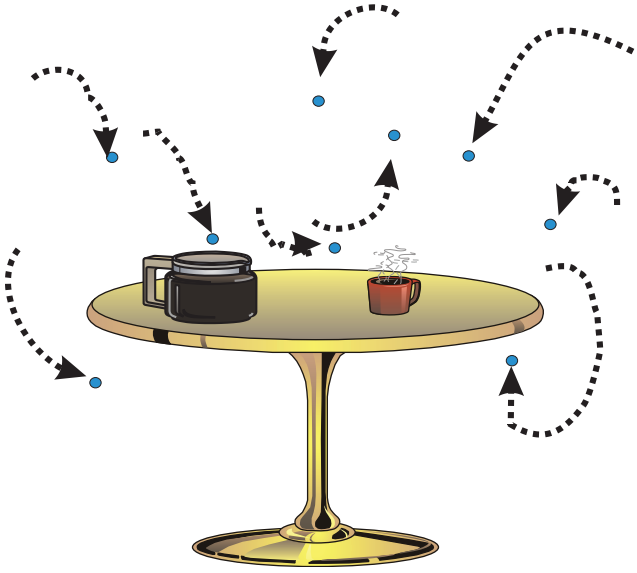

FIGURE 5.7 Schematic representation of downward molecular diffusion, a naturally occurring process caused by the air currents and eddies that promotes and enhance gravitational settling of airborne particles. Although molecular diffusion can occur in any direction, due to the effects of gravity the overall trend of the process results in net downward movement and deposition.

5.4.3.3 Surface Impaction

Surface impaction is the process by which particles make contact with surfaces, such as leaves, trees, walls and computers. With impaction there is an associated loss of kinetic energy. In nature, it is rare to find flat, smooth surfaces on which wind currents are unobstructed. Thus, surface impaction is a very critical factor influencing transport and deposition, especially for bioaerosols.

Impaction potential is the relative likelihood that an airborne object will collide with another object in its path. Impaction does not necessarily result in permanent deposition, however. Once a particle collides with an object, it has the potential to bounce. Bouncing off a surface causes the particle to reenter the air current at a lower rate, which can have one of two effects: (1) it can allow subsequent downward molecular diffusion and gravitational settling to occur, resulting in deposition on another nearby surface; or (2) it can allow the particle to escape the surface and once again reenter the air current. Studies have shown that impaction is influenced by the velocity and size of the particle, as well as the size and shape of the surface it is approaching.

5.4.3.4 Rain and Electrostatic Deposition

Rainfall and electrostatic charge also can affect deposition. Rainfall deposition occurs as a condensation reaction between two particles (raindrop and bioaerosol), which combine and create a bioaerosol with a greater mass, which settles faster. This can be described mathematically using the Stokes equation. In the example presented in Information Box 5.1, a clostridial spore alone has a calculated terminal velocity of 0.016 cm/sec. The same spore (bioaerosol), if it condensed with another particle such as a water droplet, has a greater mass and thus a greater terminal velocity. For instance, if the clostridial spore were to condense with a water droplet that doubled the bioaerosol density from 1.3 to 2.6 g/cm^3, the terminal velocity would be increased from 0.016 to 0.032 cm/sec. The overall efficiency of rain deposition also depends on the spread area of the particle plume. Larger, more diffuse plumes undergo stronger impaction than smaller, more concentrated plumes. Rain deposition is also affected by the intensity of the rainfall. The heavier the rainfall, the greater the overall rates and numbers of the condensation reactions, and the greater the subsequent increase in rain deposition.

Electrostatic deposition also condenses bioaerosols, but is based on electrovalent particle attraction. All particles tend to have some type of associated charge. Microorganisms typically have an overall negative charge associated with their surfaces at neutral pH. These negatively charged particles can associate with other positively charged airborne particles, resulting in electrostatic condensation. The major phenomenon occurring may be a

coagulation effect between particles (much like the condensation of the clostridial spore with the water droplet), which would increase the bioaerosol mass and enhance deposition. It might also be assumed that as an electromagnetically charged bioaerosol comes into close proximity with an electromagnetically charged surface, electroattractive or electrorepulsive influences may be present.

5.5 MICROBIAL SURVIVAL IN THE AIR

The atmosphere is an inhospitable climate for microorganisms mainly because of desiccation stress. This results in a limited time frame in which microbes can remain biologically active. Many microorganisms, however, have specific mechanisms that allow them to be somewhat resistant to the various environmental factors that promote loss of biological activity. Spore-forming bacteria, molds, fungi and cyst-forming protozoa all have specific mechanisms that protect them from harsh gaseous environments, increasing their ability to survive aerosolization. For organisms that have no such specific mechanisms, the survival in aerosols can often be measured in seconds. In contrast, organisms with these mechanisms can survive indefinitely.

As a result, viability is highly dependent on the environment, the amount of time the organism spends in the environment and the type of microorganism. In addition, microbes may be viable but nonculturable (Chapter 3), but for simplicity in this chapter we will use the term viable rather than the term culturable. Many environmental factors have been shown to influence the ability of microorganisms to survive. The most important of these are relative humidity and temperature. Oxygen content, specific ions, UV radiation, various pollutants and AOFs (air-associated factors) are also factors in the loss of biological activity. Each of these factors is discussed in the following sections.

The loss of biological activity can be termed inactivation and can generally be described using the following equation:

$$X_t = X_0 e^{-kt} \qquad \text{(Eq. 5.5)}$$

where:

- X_t represents the viable organisms at time t
- X_0 is the starting concentration
- k is the inactivation constant, which is dependent on the particular species of microorganisms as well as a variety of environmental conditions

5.5.1 Relative Humidity

The relative humidity or the relative water content of the air has been shown to be of major importance in the survival of airborne microorganisms. Wells and Riley (1937) were among the first to show this phenomenon, indicating that as the relative humidity approaches 100%, the death rate of *Escherichia coli* increases. In general, it has been reported that most Gram-negative bacteria associated with aerosols tend to survive for longer periods at low to mid levels of relative humidities, with enhanced decay at relative humidities above 80% (Brooks *et al.*, 2004). The opposite tends to be true for Gram-positive bacteria, which tend to remain viable longer in association with high relative humidities (Theunissen *et al.*, 1993). Thus, the ability of a microorganism to remain viable in a bioaerosol is related to the organism's surface biochemistry. One mechanism that explains loss of viability in association with very low relative humidity is a structural change in the lipid bilayers of the cell membrane. As water is lost from the cell, the cell membrane bilayer changes from the typical crystalline structure to a gel phase. This structural phase transition affects cell surface protein configurations and ultimately results in inactivation of the cell (Hurst *et al.*, 1997). In general, Gram-negative bacteria react unfavorably to desiccation, whereas Gram-positive cells are more tolerant of desiccation stress (Mohr, 2001).

Early studies by Loosli *et al.* (1943) showed that the influenza virus was also adversely affected by an increase in relative humidity. More recent work suggests that viruses possessing enveloped nucleocapsids (such as the influenza virus) have longer airborne survival when the relative humidity is below 50%, whereas viruses with naked nucleocapsids (such as the enteric viruses) are more stable at a relative humidity above 50% (Mohr, 2001). It should be noted that viruses with enveloped nucleocapsids tend to have better survival in aerosols than those without. Some viruses are also stable in the AMB pathway over large ranges of relative humidity, which makes them very successful airborne pathogens.

5.5.2 Temperature

Temperature is a major factor in the inactivation of microorganisms. In general, high temperatures promote inactivation, mainly associated with desiccation and protein denaturation, and lower temperatures promote longer survival times (Mohr, 2001). When temperatures approach freezing, however, some organisms lose viability because of the formation of ice crystals on their surfaces. The effects of temperature are closely linked with many other environmental factors, including relative humidity.

5.5.3 Radiation

The main sources of radiation damage to microorganisms including bacteria, viruses, fungi and protozoa are the shorter UV wavelengths and ionizing radiation such as

X-rays. The main target of UV irradiation damage is the nucleotides that make up DNA. Ionizing radiation or X-rays cause several types of DNA damage, including single strand breaks, double strand breaks and alterations in the structure of nucleic acid bases. UV radiation causes damage mainly in the form of intrastrand dimerization, with the DNA helix becoming distorted as thymidines are pulled toward one another (Freifelder, 1987). This in turn causes inhibition of biological activity such as replication of the genome, transcription and translation.

Several mechanisms have been shown to protect organisms from radiation damage. These include association of microbes with larger airborne particles, possession of pigments or carotenoids, high relative humidity and cloud cover, all of which tend to absorb or shield bioaerosols from radiation. Many types of organisms also have mechanisms for repair of the DNA damage caused by UV radiation. An example of an organism that has a radiation resistance mechanism is *Dienococcus radiodurans*. *D. radiodurans* is a soil bacterium that is considered the most highly radiation-resistant organism that has yet been isolated. An important component of its radiation resistance is the ability to enzymatically repair damage to chromosomal DNA. The repair mechanism used by these bacteria is so highly efficient that much of the metabolic energy of the cell is dedicated exclusively to this function.

5.5.4 Oxygen, OAF and Ions

Oxygen, open air factors (OAFs) and ions are environmental components of the atmosphere that are difficult to study at best. In general, it has been shown that these three factors combine to inactivate many species of airborne microbes. Oxygen toxicity is not related to the dimolecular form of oxygen (O_2), but is instead important in the inactivation of microorganisms when O_2 is converted to more reactive forms (Cox and Heckley, 1973). These include superoxide radicals, hydrogen peroxide and hydroxide radicals. These radicals arise naturally in the environment from the action of lightning, UV radiation, pollution, etc. Such reactive forms of oxygen cause damage to DNA by producing mutations, which can accumulate over time. The repair mechanisms described in the previous section are responsible for control of the damaging effects of reactive forms of oxygen.

Similarly, the open air factor (OAF) is a term coined to describe an environmental effect that cannot be replicated in laboratory experimental settings. It is closely linked to oxygen toxicity, and has come to be defined as a mixture of factors produced when ozone and hydrocarbons (generally related to ethylene) react. For example, high levels of hydrocarbons and ozone can cause increased inactivation rates for many organisms, probably because of damaging effects on enzymes and nucleic acids (Donaldson and Ferris, 1975). Therefore, OAFs have been strongly linked to microbial survival in the air.

The formation of other ions, such as those containing chlorine, nitrogen or sulfur, occurs naturally as the result of many processes. These include the action of lightning, shearing of water and the action of various forms of radiation that displace electrons from gas molecules, creating a wide variety of anions and cations not related to the oxygen radicals. These ions have a wide range of biological activity. Positive ions cause only physical decay of microorganisms, e.g., inactivation of cell surface proteins, whereas negative ions exhibit both physical and biological effects such as internal damage to DNA.

5.6 EXTRAMURAL AEROMICROBIOLOGY

Extramural aeromicrobiology is the study of microorganisms associated with outdoor environments. In the extramural environment, the expanse of space and the presence of air turbulence are two controlling factors in the movement of bioaerosols. Environmental factors such as UV radiation, temperature and relative humidity modify the effects of bioaerosols by limiting the amount of time that aerosolized microorganisms will remain viable. This section provides an overview of extramural aeromicrobiology that includes: aerosolization of indigenous soil pathogens; influenza pandemics; the spread of agricultural pathogens; the spread of airborne pathogens associated with waste environments; and important airborne toxins.

5.6.1 Aerosolization of Indigenous Soil Pathogens

Geo-indigenous pathogens are those found in soils that are capable of metabolism, growth and reproduction (Pepper *et al*., 2009). These are found in all soils and include both prokaryotic and eukaryotic organisms, many of which are spore formers. Such spores can potentially be aerosolized and cause human infections. *Bacillus anthracis* is a bacterial geo-indigenous pathogen that causes lethal disease in humans via pulmonary, gastrointestinal or cutaneous modes of infection (Gentry and Pepper, 2002). The organism is found worldwide and, because it is a spore former, can remain viable in soil for years.

Studies have shown the potential for anthrax to be disseminated by aerosols. Turnbull *et al.* (1998) found airborne concentrations of anthrax spores as high as 2.1×10^{-2} CFU L^{-1} of air, and airborne movement as far as 18 m from a contaminated carcass in Etosha National Park, Namibia. However, the majority of samples taken were negative, and the number of spores collected in positive samples was very low, making airborne contraction of disease at a distance from the carcass unlikely. A more

serious outbreak in humans resulting from a *B. anthracis* aerosol is described in Case Study 5.1.

Important fungal geo-indigenous pathogens include *Coccidioides immitis* and *Histoplasma capsulatum*. *Coccidiodes immitis* is a soil-borne fungi that causes a respiratory illness known as Valley Fever. It preferentially grows in the soils of semiarid regions of the Southwest United States, including California, Arizona, New Mexico and Texas (Baptista-Rosas *et al.*, 2007). Symptoms can be mild to fatal. *Histoplasma capsulatum*, another fungus causing respiratory infections, is found worldwide in soils, but, in the United States, it is endemic to southeastern and midwestern states (Deepe and Gibbons, 2008). Histoplasmosis can be asymptomatic or mild, but the infections can be very serious or even fatal for immunocompromised individuals.

Case Study 5.1 Anthrax

In 1979, an anthrax outbreak occurred in Sverdlovsk, in the then U.S.S.R., due to the accidental release of a bioaerosol from a military microbiological facility (Meselson *et al.*, 1994). At least 66 people died as a result of the release. Human anthrax cases extended 4 km along an axis to the south of the military facility and livestock cases extended up to 50 km in the same direction. The geographic distribution of human and animal cases was consistent with meteorological patterns existing when the accidental release was believed to have occurred. There has been no indication that human anthrax cases have occurred in Sverdlovsk since 1979.

Case Study 5.2 The Spanish Influenza Pandemic of 1918

This pandemic affected approximately one-third of the world population at that time, with 3–6% dying (Barry, 2004). The pandemic lasted from January 1918 to December 1920, the responsible virus being H1N1. This was the first outbreak resulting from H1N1, with the second epidemic occurring in 2009. Although the pandemic did not originate in Spain, the term "Spanish flu" was coined due to the severity of the infections in Spain. It is believed that the pandemic began in Haskell County, Kansas, before spreading rapidly to Europe. Estimates of the total number of deaths range from 50 to 100 million worldwide with 500,000 to 675,000 deaths in the U.S. A. (Barry, 2004).

5.6.2 Influenza Pandemics

Influenza pandemic is the term given to an epidemic of an influenza virus that occurs on a worldwide scale with a resultant infection of a large proportion of the human population. Known colloquially as the "flu," influenza is an infectious disease of birds and mammals caused by an RNA virus of the family *Orthomyxoviridae*. Influenza can cause the common flu symptoms of muscle ache, headache, coughing, weakness and fatigue, or pneumonia which can be fatal.

Avian influenza refers to a large group of influenza viruses that primarily affect birds, but have the potential to adapt and infect humans. An influenza pandemic occurs when an avian influenza virus adapts into a strain that is contagious among humans and that has not previously circulated within humans. Such adaptations can be devastating, as illustrated in Table 5.1.

Influenza virus transmission among humans can occur via four mechanisms: by direct contact with infected individuals; by indirect contact with contaminated objects of fomites; by inhalation of droplets that contain the virus; or by inhalation of aerosolized virus. Interestingly, despite 70 years of research since the influenza A virus was discovered, there is still debate about the modes of influenza transmission, specifically whether influenza is mainly transmitted via true bioaerosols, or by droplets, or by direct or indirect contact (Brankston *et al.*, 2007).

TABLE 5.1 History of Major Influenza Pandemics

Name of Pandemic	Period	Deaths	Influenza Subtype
Asiatic (Russian) flu	1889–1890	1 million	Unknown
Spanish flu	1918–1920	Up to 50 million	H1N1
Asian flu	1957–1958	2 million	H2N2
Hong Kong flu	1968–1969	1 million	H3N2
Swine flu	2009–2010	≅18,000	H1N1

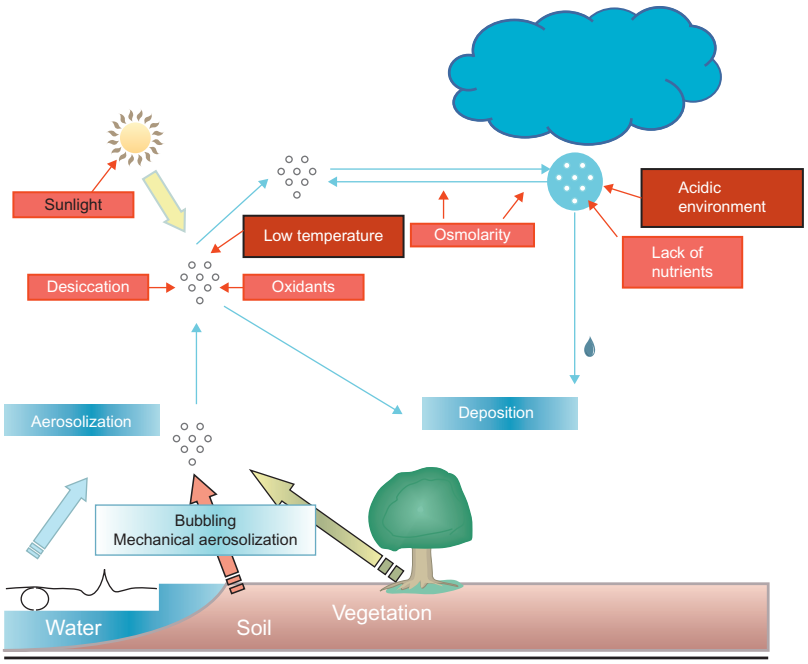

FIGURE 5.8 Cloud environmental factors that can adversely influence microbes.

5.6.3 Microbiology in the Clouds

Recent studies have suggested that microbes can potentially affect meteorological processes. In particular, some microorganisms, called ice nucleators, efficiently catalyze ice formation and may play a role in the formation and precipitation within clouds (Chistner, 2012). Based on recent studies, 95% of ice nuclei are biological particles and at least 40% originate from bacteria. Microorganisms are present in both clouds and fog. The abundance of culturable bacteria and fungi in clouds varies with the season, with greater numbers occurring in the summer and fall. While only 1% of the bacteria and 50% of the fungi in clouds are culturable, studies suggest that the majority are metabolically active (Delort *et al.*, 2010). Bacterial numbers range from 10^3 to 10^4/ml compared to fungal numbers of 10^2 to 10^4/ml. The cloud environment is a harsh environment with UV light irradiation, desiccation, low temperatures and other factors potentially adversely affecting microbes (Figure 5.8). Microorganisms may modify this environment by metabolizing organic compounds, and also by playing a role in cloud chemistry and physics, but much additional research is needed because the cloud environment is difficult to study.

5.6.4 Agriculture

Numerous plant pathogens are spread by the aeromicrobiological pathway (Information Box 5.3). Contamination of crops and animals via bioaerosols has a large worldwide economic impact. Rice and wheat are two of the major staple crops that are paramount to world food security. Major pathogens of such crops are the wheat rust fungi. These spore-forming fungi cause some of the most devastating diseases of wheat and other grains. In 1993, one type of wheat rust (leaf rust) was responsible for the loss of over 40 million bushels of wheat in Kansas and Nebraska alone. Even with selective breeding for resistance in wheat plants, leaf rust continues to have major economic impacts. The high concentration of wheat in areas ranging from northern Texas to Minnesota and up into the Dakotas makes this whole region highly susceptible to rust epidemics.

Spores of wheat rust are capable of spreading hundreds if not thousands of kilometers through the atmosphere (Ingold, 1971). The airborne spread of rust disease has been shown to follow a predictable trend, which starts during the fall with the planting of winter wheat in the southern plains. Any rust-infected plant produces thousands of spores, which are released into the air (Figure 5.9) by either natural atmospheric disturbance or mechanical disturbance during the harvesting process. Once airborne, these spores are capable of long-distance dispersal, which can cause downwind deposition onto other susceptible wheat plants. The generation time of new spores is measured in weeks, after which new spores are again released from vegetative fungi into the AMB pathway. For example, during the harvest of winter wheat in Texas, the prevailing wind currents are from south to north, which can allow rust epidemics to spread into the maturing crops farther north in Kansas and up into the young crops in the Dakotas (Figure 5.10). This epidemic spread of wheat rust and the resulting economic destruction produced are

Information Box 5.3 Examples of Airborne Plant Pathogens

Fungal Plant Disease	Pathogen
Dutch Elm disease	*Ceratocystis ulmi*
Potato late blight	*Phytophthora infestans*
Leaf rust	*Puccinia recondite*
Loose smut of wheat	*Ustilago tritici*
Downy mildew	*Pseudoperonospora humuli*
Maize rust	*Puccinia sorghi*
Powdery mildew of barley	*Erysiphe graminis*
Southern corn leaf blight	*Helminthosporium maydis*

The figure shows the airborne spread of late blight of potato that caused the 1845 epidemic known as the Irish potato famine. *Phytophthora infestans* spread from Belgium (mid-June) throughout Europe by mid-October. Famine related deaths are estimated from 750,000 to 1,000,000. Economic devastation from this famine caused the population of Ireland to decrease from approximately 8 million to 4 million from 1840 to 1911.

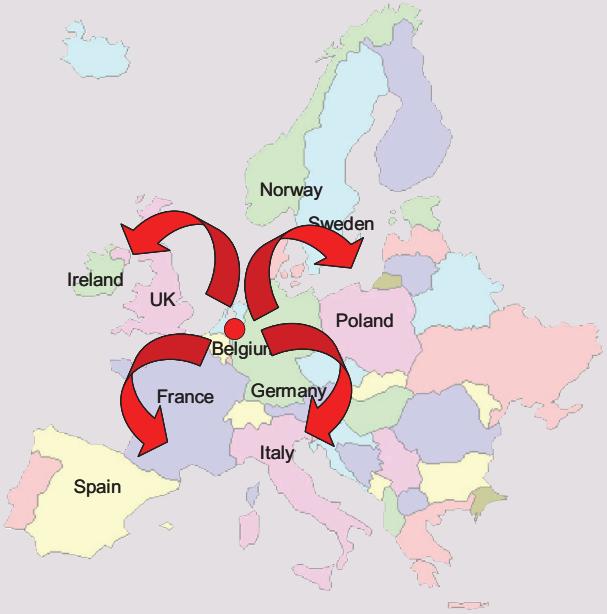

FIGURE 5.9 Field of wheat highly infected by phytopathogenic wheat rust. The field is being harvested by a hay machine, which is releasing a cloud of rust spores into the aeromicrobiological pathway. These spores can spread thousands of miles and infect other crops downwind, causing catastrophic losses to wheat crops.

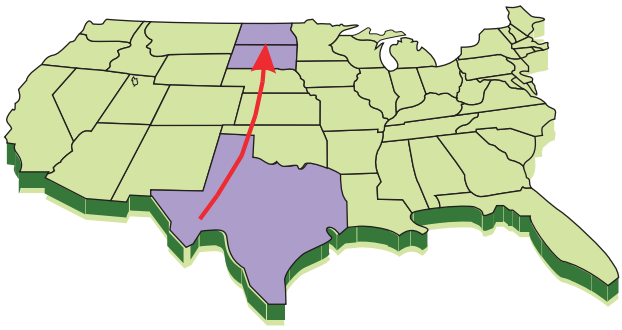

FIGURE 5.10 The arrow indicates the northern path of wheat rust infections as spread by the aeromicrobiological pathway. The wheat rust infection begins in the winter harvest in Texas and spreads northward with the prevailing wind currents. The epidemic spread of these phytopathogens infects maturing crops in Kansas and then moves up into the young crops in the Dakotas.

indicative of the impact that airborne microbial pathogens can have on agriculture.

A factor that complicates the control of such diseases is that chemical treatment for the control of pathogens is viewed as undesirable. This is because many pesticides have long half-lives and their residence in an ecosystem can be extremely harmful. Therefore, instead of using wheat rust fungicides, attempts are being made to breed strains of wheat that are more resistant to the fungi. Another method used for controlling phytopathogenic (plant pathogenic) fungi is spore monitoring as a disease control strategy. In this approach, the life cycle of the fungi, especially the release of spores, is monitored, and fungicide application is timed to coincide with spore release. This approach minimizes use of harmful chemicals. Thus, efficient aeromicrobiology pathway sampling, monitoring, detection and modeling have the ability to aid in the control of airborne pathogens.

The airborne spread of pathogenic microorganisms is also highly important in the animal husbandry industry (Information Box 5.4). The occurrence of foot-and-mouth disease is an example of the importance of bioaerosols in the spread of airborne disease (Case Study 5.3). It has long been thought that bioaerosol spread is linked primarily to respiratory pathogens, but there is growing evidence that gastrointestinal pathogens are also important in airborne transmission of disease among animals. One example of bioaerosol spread of a gastrointestinal pathogen is

Information Box 5.4 Examples of Airborne Animal Pathogens

Animal Disease	Pathogen
Bacterial diseases	
Tuberculosis	*Mycobacterium bovis*
Brucellosis	*Brucella* spp.
Fungal diseases	
Aspergillosis	*Aspergillus* spp.
Coccidioidomycosis	*Coccidioides immitis*
Viral diseases	
Influenza	Influenza virus
Rabies	Rhabdoviridae
Foot-and-mouth disease	Aphthovirus

Case Study 5.3 The United Kingdom Foot and Mouth Crisis 2001

Outbreaks of foot and mouth disease have occurred worldwide including multiple occurrences within the U.S.A. However, one of the more devastating outbreaks occurred in the U.K. in the spring and summer of 2001. Foot and mouth viruses are from the *Aphthovirus* genus of the family Picornaviridae, and are single-stranded RNA viruses. The 2001 U.K. outbreak was due to a type O pan-Asia strain that was thought to have arisen from infected meat illegally imported into the U.K. Transmission of the virus can occur via direct contact or via bioaerosols (Grubman and Baxt, 2004). Overall, 2000 cases were reported throughout Britain, resulting in the culling of 7 million sheep and cattle, costing the U.K. ≅ $16 million.

transmission of *Salmonella typhimurium* among calves that are housed individually in small pens (Hinton *et al.*, 1983). The potential for bioaerosol spread of this pathogen was recognized because the initial symptoms resembled those of pneumonia and appeared randomly within these animals, two factors that are not characteristic of oral transmission. Oral transmission generally occurs sequentially from one pen to the next, whereas aerial transmission can carry organisms past nearby pens, infecting calves randomly. Furthermore, Wathes *et al.* (1988) showed that *S. typhimurium* could survive for long periods in an airborne state, and calves and mice exposed to aerosolized *S. typhimurium* developed symptoms, proving that gastrointestinal pathogens could be spread by aerosolization. Finally, Baskerville *et al.* (1992) showed that aerosolized *Salmonella enteritidis* could infect laying hens. These hens showed clinical symptoms and were shedding the test strain of salmonellae in their feces

FIGURE 5.11 Application of secondary treated wastewater onto agricultural lands. This method is highly efficient at conserving water and has been shown to improve the fertility of soils. Due to the presence of pathogens in wastewater, and the nature of these land application systems, there are high concentrations of bioaerosols generated. Currently, however, there is little epidemiological and microbial risk assessment information available to determine if there may be health concerns for populations living in the vicinity of such operations, though there is a growing base of information on the concentration and types of pathogens found in these bioaerosols.

within a few days. Thus, the aeromicrobiology pathway can be important even in the spread of diseases for which pathogens are not normally considered airborne.

5.6.5 Waste Disposal

Waste disposal is a multibillion dollar industry in the United States. However, there are many hazards inherent in the treatment and disposal of wastewater (Figure 5.11), animal manures and biosolid material. Figures 5.12–5.14 illustrate the potential for bioaerosol production via various methods of land application of biosolids and also loading operations. Major hazards associated with waste effluents are pathogenic microorganisms including bacteria, viruses, protozoa and helminths. Wastewater treatment plants utilize activated sludge and trickling filter systems, and all of these treatment processes potentially create relatively large amounts of aerosols, which have been shown to include pathogenic microorganisms. Other aspects of the treatment process such as composting and land disposal are also associated with the generation of aerosols containing pathogenic microorganisms.

One of the primary methods for the disposal of biosolids and manure is agricultural land application. The major concern associated with the aerosolization process in relation to waste disposal operations is the exposure of waste disposal workers to pathogenic microorganisms (occupational risk). In addition, nearby population centers are also potential

FIGURE 5.12 Land application of liquid biosolids via a spray application, and collection of air samples via biosamplers.

FIGURE 5.13 Land application of liquid biosolids via a sprinkler system.

FIGURE 5.14 Land application of cake biosolids via a slinger in Solano County, California.

exposure risks (community risk). The potential for aerosolization of pathogens from land application of biosolids has become a nationally debated issue. A major national study on aerosolization from land application in the United States was conducted by Brooks *et al.* (2005a,b). This study showed that occupational risks of infection from bioaerosols was greater than for offsite communities, where risks were minimal (Brooks *et al.*, 2012) (Case Study 5.4). Baertsch *et al.* (2007) used DNA-based microbial source tracking to measure aerosolization during land application.

5.6.6 Important Airborne Toxins

Microbial toxins can also be airborne. For example, a toxin from *Clostridium botulinum* (botulinum A toxin) is a potential biological warfare agent (Amon *et al.*, 2001). Botulinum toxin is a neurotoxin that is normally associated with ingestion of contaminated food. However, the lethal dose is so small that aerosolization can also be a means of dissemination. The lethal dose for botulinum toxin by inhalation is 0.3 μg, with death occurring 12 hours after exposure. Death is due to asphyxiation caused by the paralysis of respiratory muscles. Another toxin produced by bacteria is staphylococcal enterotoxin. On occasion, this toxin can be fatal with the lethal dose estimated to be 25 μg by inhalation. The symptoms include cramping, vomiting and diarrhea, which occur within 1 hour of exposure by aerosolization.

An important airborne toxin is lipopolysaccharide (LPS) (Hurst *et al.*, 1997). Lipopolysaccharide is derived from the outer membrane of Gram-negative bacteria. It is also referred to as endotoxin and is a highly antigenic biological agent that, when associated with airborne particles such as dust, is often associated with acute respiratory symptoms such as chest tightness, coughing, shortness of breath, fever and wheezing. Due to the ubiquity of Gram-negative bacteria, especially in soil, LPS is considered by some to be the most important aerobiological allergen. LPS (Figure 5.15) has three major components: a lipid A moiety, which is a disaccharide of phosphorylated glucosamines with associated fatty acids; a core polysaccharide; and an O-side chain. The lipid A moiety and the core polysaccharide are similar among Gram-negative bacteria, but the O-side chain varies among species and even strains. It is the O-side chain that is responsible for the hyperallergenic reaction. There are many sources associated with the production of high levels of LPS, such as cotton mills, haystacks, sewage treatment plants, solid waste handling facilities, swine confinement buildings, poultry houses, and even homes and office buildings. LPS is liberated when Gram-negative bacteria in these environments are lysed but can also be released when they are actively growing.

In soils, bacterial concentrations routinely exceed 10^8 per gram and soil particles containing sorbed microbes can

Case Study 5.4 Occupational and Community Risks of Infection from Bioaerosols Generated During Land Application of Biosolids

In 2002, the National Research Council (NRC) issued a report titled, *Biosolids Applied to Land: Advancing Standards and Practices*. One of the recommendations of this report was the need to document and evaluate the risk of infections from bioaerosols generated during land application. In response to this the University of Arizona conducted a national study to evaluate occupational and community risks from such bioaerosols. Overall, more than 1000 aerosol samples were collected and analyzed for bacterial and viral pathogens (Tanner *et al.*, 2005; Brooks *et al.*, 2005b).

The study was undertaken in two parts. First, the emission rate of pathogens generated during loading of biosolids from trucks into spreaders, and also during land application of biosolids, was evaluated. This was assumed to be direct exposure to biosolid workers on-site, that is, occupational risk, since there was no pathogen transport required to for exposure. For community risk, fate and transport of pathogens was taken into account, since residents live off-site, allowing for natural attenuation of pathogens due to environmental factors such as desiccation and ultraviolet light.

Based on exposure data gathered on-site during land application, occupational risk of infection from Coxsackie virus A21 was determined using a one-hit exponential model: $P_i = 1 - \exp(-rN)$, where:

P_i = the probability of infection per work day,

r = parameter defining the probability of an organism initiating infection = 0.0253 for Coxsackie A21, and

N = number of pathogens inhaled per day

The annual risk of infection can be calculated from the daily risk using

$$P_{year} = 1 - (1 - P_i)^d$$

where d = number of days exposed per year.

Occupational Risk of Infection

Annual risk of infection for Coxsackie virus A21 during loading operations was 2.1×10^{-2}.

This risk suggests that approximately 1 worker per 50 is likely to be infected with Coxsackie virus A11 working on-site over the course of 1 year.

Community Risk of Infection

Risk was calculated for a distance of 30 m from a land application site assuming 6 days of land application annually, and 8 h exposure duration.

Annual risk of infection for Coxsackie virus A21 during loading was 3.8×10^{-5}.

Annual risk of infection for Coxsackie virus A21 during land application was 2.1×10^{-5}.

These data imply that community risks of infection are minimal. As a comparison, for drinking water a 1:10,000 risk of infection per hear is considered acceptable (Haas *et al.*, 2014).

be aerosolized, and hence act as a source of endotoxin. Farming operations such as driving a tractor across a field have been shown to result in endotoxin levels of 469 endotoxin units (EU) per cubic meter as measured by the *Limulus* amebocyte assay. These values are comparable to those found during land application of biosolids operations (Table 5.2) (Brooks *et al.*, 2006). Daily exposures of as little as 10 EU/m^{-3} from cotton dust can cause asthma and chronic bronchitis. However, dose response is dependent on the source of the material, the duration of exposure and the frequency of exposures (Brooks *et al.*, 2004). The data in Table 5.2 illustrate that endotoxin aerosolization can occur during both wastewater treatment and land application of biosolids. However, the data also show that endotoxin of soil origin resulting from dust generated during tractor operations results in similar amounts of aerosolized endotoxin (see also Section 26.3.2).

5.7 INTRAMURAL AEROMICROBIOLOGY

The home and workplace are environments in which airborne microorganisms create major public health concerns. In comparison with the extramural environment, intramural environments have limited circulation of external air and much less UV radiation exposure. Indoor environments also have controlled temperature and relative humidity, which are generally in the ranges that allow extended microbial survival. Thus, these conditions are suitable for the accumulation and survival of microorganisms within many enclosed environments, including office buildings, hospitals, laboratories and even spacecraft. In this section, we will consider these three diverse areas as examples of current topics related to intramural aeromicrobiology. Again, it should be noted that this section does not cover all aspects of intramural aeromicrobiology, but instead attempts to show the wide diversity of the science.

5.7.1 Buildings

Many factors can influence bioaerosols and therefore how "healthy" or how "sick" a building is. These include: the presence and/or efficiency of air filtering devices, the design and operation of the air circulation systems, the health and hygiene of the occupants, the amount of clean outdoor air

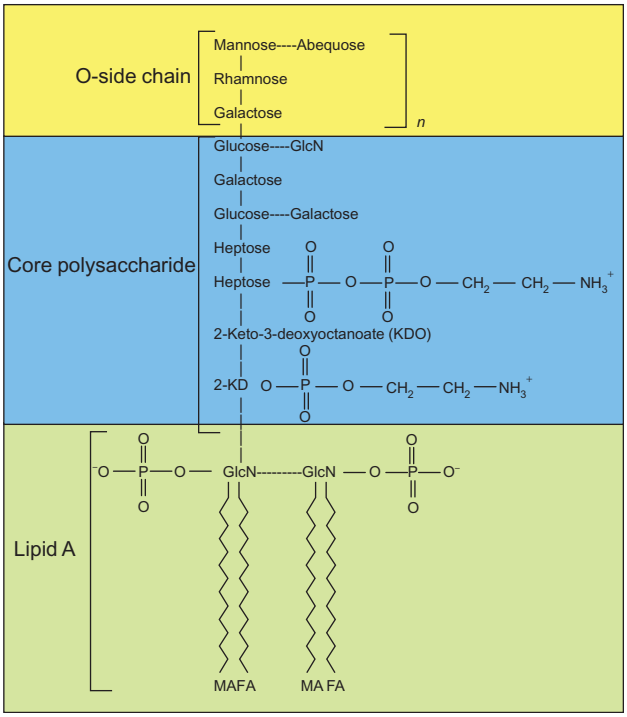

FIGURE 5.15 Schematic structural representation of the lipopolysaccharide from *Salmonella typhimurium*. The number of repeating units (*n*) in the side chain varies from 10 to 40. The sugars found in the side chain vary among bacterial species, whereas the composition of the core polysaccharide is usually the same. There is a molecule of beta-hydroxymyristic acid (MA), which is a14-carbon fatty acid attached to each N-acetylglucosamine (GlcN) residue. Other fatty acids (FA) are attached to these residues as well.

circulated through the building, the type of lighting used, the ambient temperature in the building, and the relative humidity (Information Box 5.5).

Some pathogens are uniquely adapted for survival and transmission in the intramural environment. One good example of such an organism is *Legionella pneumophilia*, the causative agent of both Legionnaires' disease and Pontiac fever. Legionnaires' disease or legionellosis is a pneumonia that causes disease in up to 5% of those exposed. Of those who contract the disease, up to 39% die from the infection. Pontiac fever is associated with flu-like symptoms and affects up to 100% of those exposed, although it is generally not associated with mortality. The causative agent of both diseases is a poorly staining, Gram-negative bacillus called *L. pneumophila*. This organism is named in association with the first highly characterized outbreak of the disease, which occurred in 1976 at an American Legion convention in Philadelphia.

Legionella spp. are ubiquitous in the environment. They are found in association with lakes, ponds, compost and streams, and have even been found in deep terrestrial subsurface environments. In addition to natural reservoirs, there are many human-made systems within which legionellae can find a niche. These include cooling towers, evaporative condensers, plumbing systems, whirlpools, shower heads and hot-water faucets (Bollin *et al.*, 1985). In the case of the American Legion convention, the reservoir for the organism that caused the outbreak was a poorly maintained cooling tower, which provided optimal conditions for *Legionella* proliferation. Because of the poor design of the air circulation system at the convention, this proliferation led to the subsequent aerosolization and spread of the organisms throughout the building.

What conditions promote the proliferation of *Legionella* spp.? Stagnant water and temperatures in the range of 35−46°C are factors that can lead to the rapid multiplication of background levels of *Legionella* spp. Another interesting aspect of the ecology of *Legionella* is that they can grow intracellularly within cyanobacteria and protozoa. How can growth and spread of *Legionella* spp. be avoided? Several strategies can be used. In the maintenance of hot-water plumbing systems, operating temperatures should be greater than 50°C. All potential places where water can stagnate in water pipes should be avoided. For cooling towers, the recommendations involve the installation of ozonization units, dry convective heat exchange designs and the avoidance of any design that could potentially mix the wet system with the supply air. Biocidal agents such as chlorine or copper can also be effective when used regularly at low levels.

5.7.2 Hospitals and Laboratories

Hospitals and microbiology laboratories are the two indoor environments with perhaps the greatest potential for the aerosolization of pathogenic microorganisms. Hospitals, because they are centers for the treatment of patients with diseases, have a high percentage of individuals, including patients and staff, who are active carriers of infectious, airborne pathogens. Of particular concern are neonatal wards, surgical transplant wards and surgical theaters, all critical areas where the control of nosocomial infection is imperative. Illustrating this point is a study by Portner *et al.* (1965) that evaluated airborne microbial concentrations in surgical theaters, industrial clean rooms, typical industrial manufacturing areas and a horizontal laminar flow clean room designed for the space industry. The surgical theater had by far the highest counts of pathogenic airborne microbial contaminants, followed by the industrial manufacturing area, the industrial clean room and finally the laminar flow room, which had the lowest counts of airborne microbes.

Because microbiology laboratories often handle pathogens, procedures have been developed and refined to protect laboratory workers. However, even under the strictest of conditions, aerosolization events may occur. In 1988, for

TABLE 5.2 Aerosolized Endotoxin Concentrations Detected Downwind of Biosolids Operations, a Wastewater Treatment Plant Aeration Basin, and a Tractor Operation

Sample Type	# of Samples Collected	Distance From Site (m)	Aerosolized Endotoxin (EU m[a])			
			Avg	Median	Minimum	Maximum
Controls						
Background	12	NA	2.6	2.49	2.33	3.84
Biosolids operations						
Loading	39	2–50	343.7	91.5	5.6	1807.6
Slinging	24	10–200	33.5	6.3	4.9	14.29
Biosolids pile	6	2	103	85.4	48.9	207.1
Total operation	33	10–200	133.9	55.6	5.6	623.6
Wastewater treatment plant						
Aeration basin	6	2	627.3	639	294.4	891.1
Nonbiosolids field						
Tractor	6	2	469.8	490.9	284.4	659.1

[a]*EU m = Endotoxin units per m*^{3}.

Information Box 5.5 Molds in Buildings

In moist environments within buildings mold and bacteria can proliferate rapidly within days and become established as colonies on solid surfaces, subsequently releasing toxins and/or allergens into the air. The most common indoor molds are *Cladosporium*, *Penicillium*, *Aspergillus* and *Alternaria*. Molds can cause both allergic reactions and chemical toxigenic responses from direct exposure to spores, cell wall components and mycotoxins. Molds and endotoxins can also be found within tobacco smoke (Pauly and Paszkiewicz, 2011).

instance, eight employees in a clinical microbiological laboratory developed acute brucellosis (Staszkiewicz *et al.*, 1991). A survey of the laboratory and the personnel showed that a cryogenically stored clinical isolate of *Brucella* sp. had been thawed and subcultured without the use of a biosafety cabinet. Other than this, the laboratory worker claimed to have used good technique. This example demonstrates the ease with which a bioaerosol can spread within areas where pathogens are handled for research and clinical purposes, and indicates the importance of bioaerosol control methodologies. The following sections describe how bioaerosol formation and spread is actually controlled in the laboratory.

5.8 BIOAEROSOL CONTROL

The control of airborne microorganisms can be handled in a variety of ways. Launching, transport and deposition are all points at which the airborne spread of pathogens can be controlled. The mechanisms used to control bioaerosols include ventilation, filtration, UV treatment, biocidal agents and physical isolation. These are discussed in the following sections.

5.8.1 Ventilation

Ventilation is the method most commonly used to prevent the accumulation of airborne particles. This mechanism involves creating a flow of air through areas where airborne contamination occurs. This can mean simply opening a window and allowing outside air to circulate inward, or use of air-conditioning and heating units that pump outside air into a room. Ventilation is considered one of the least effective methods for controlling airborne pathogens, but is still very important. Ventilation relies on mixing of intramural air with extramural air to reduce the concentration of airborne particles. However, in some cases the addition of extramural air can actually increase airborne particles. For example, one study showed that hospitals in Delhi, India, that relied on ventilation alone contained airborne fungal loads that were higher inside the hospital than those outside. This indicates that

ventilation alone may not be sufficient to significantly reduce circulating bioaerosols. Thus, for most public buildings, especially hospitals, other forms of bioaerosol control need to be implemented.

5.8.2 Filtration

Unidirectional airflow filtration is a relatively simple and yet effective method for control of airborne contamination. Some filters, for example, high-efficiency particulate air (HEPA) filters are reported to remove virtually all infectious particles. These types of filters are commonly used in biological safety hoods. However, because of their high cost, they are not often used in building filtration systems. Instead, other filtration systems that rely on baghouse filtration (a baghouse works on the same principle as a vacuum cleaner bag) are used. Typically, air filters (baghouse, HEPA, etc.) are rated using the dust-spot percentage, which is an index of the size of the particles efficiently removed by the filter, with higher percentages representing greater filtration efficiencies. The typical rating for the filters used in most buildings is 30 to 50%. Studies have shown that a 97% dust-spot rating is required to effectively remove virus particles from the air. Other factors that influence filtration efficiency are related to the type of circulation system and how well it mobilizes air within the building, the type of baghouse system used and the filter material chosen (nylon wound, spun fiberglass, etc.) as well as the filter's nominal porosity (1 μm−5 μm). All these factors combine to influence the efficiency of the air filtration and removal of particles including bioaerosols. In spite of the high level of efficiency that can be achieved with filtration, many systems still cannot stop the circulation of airborne microorganisms, especially viruses, and added treatments may be required to ensure that air is safe to breathe.

5.8.3 Biocidal Control

Biocidal control represents an added treatment that can be used to eradicate all airborne microorganisms, ensuring they are no longer viable and capable of causing infection. Many eradication methods are available, for example, superheating, superdehydration, ozonation and UV irradiation. The most commonly used of these methods is UVGI or ultraviolet germicidal radiation. UVGI has been shown to be able to control many types of pathogens, although some microbes show various levels of resistance. The control of contagion using UV irradiation was tested in a tuberculosis (TB) ward of a hospital. Contaminated air was removed from the TB ward through a split ventilation duct and channeled into two animal holding pens that contained guinea pigs. One pen received air that had been treated with UV irradiation; the other received untreated air. The guinea pigs in the untreated-air compartment developed TB, but none of the animals in the UV-treated compartment became infected. The American Hospital Association (1974) indicated that, properly utilized, UV radiation can kill nearly all infectious agents, although the effect is highly dependent on the UV intensity and exposure time. Thus, major factors that affect survival (temperature, relative humidity, UV radiation, ozone) in the extramural environment can be used to control the spread of contagion in the intramural environment.

5.8.4 Isolation

Isolation is the enclosure of an environment through the use of positive or negative pressurized air gradients and airtight seals. Negative pressure exists when cumulative airflow travels into the isolated region. Examples of this are the isolation chambers of the tuberculosis wards in hospitals used to protect others outside the TB wards from the infectious agent generated within these negative-pressure areas. This type of system is designed to protect other people in the hospital from the pathogens (*Mycobacterium tuberculosis*) present inside the isolation area. Air from these rooms is exhausted into the atmosphere after passing through a HEPA filter and biocidal control chamber.

Positive-pressure isolation chambers work on the opposite principle by forcing air out of the room, thus protecting the occupants of the room from outside contamination. One can reason that the TB ward is a negative-pressure isolation room, while the rest of the hospital, or at least the nearby anterooms, are under positive-pressure isolation. Other examples are the hospitals critical care wards for immunosuppressed patients such as organ transplant, human immunodeficiency virus (HIV)-infected and chemotherapy patients. These areas are protected from exposure to any type of pathogen or opportunistic pathogens. The air circulating into these critical care wards is filtered using HEPA filters, generating purified air essentially free of infectious agents.

5.9 BIOSAFETY IN THE LABORATORY

Many microbiological laboratories work specifically with pathogenic microorganisms, some of which are highly dangerous, especially in association with the aeromicrobiology pathway. Also, many types of equipment, such as centrifuges and vortexes (instruments for mixing of microbial suspensions) that are commonly used in microbiological laboratories can promote the aerosolization of microorganisms. Thus, laboratories and specialized equipment used in these laboratories (e.g., biosafety cabinets) are designed to control the spread of airborne microorganisms. There are essentially four levels of control designed

FIGURE 5.16 Biosafety cabinet Class II. From Telstar Life Science Solutions, photo courtesy J. Bliznick.

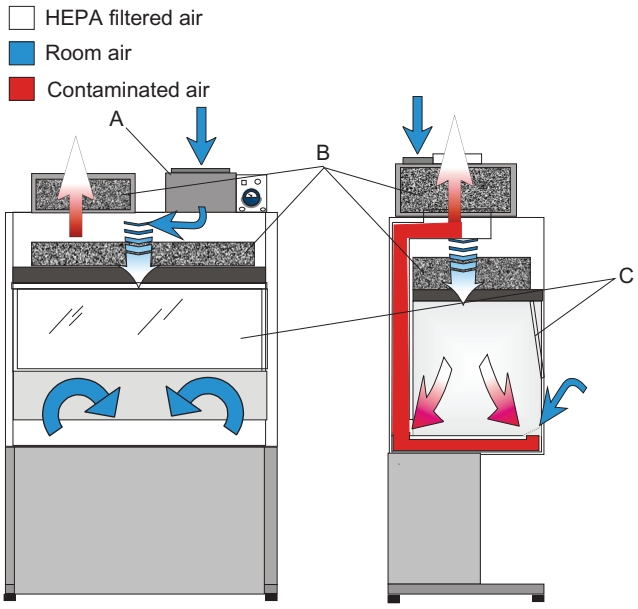

FIGURE 5.17 Schematic representation of the airflow paths within a typical Class II biosafety cabinet. Room air is dawn in from the top and from the front as indicated by the blue arrows. The nonpurified atmospheric air (blue) entering from the top of the cabinet is drawn in by an air pump (A) and then is purified by a HEPA filter (B) as it enters the workspace. Nonpurified air from the room (blue) entering from the front of the cabinet passes into the front grill and passes up through the top of the cabinet where it also passes through a HEPA filter before entering the workspace (C). This prevents the experiments in the workspace from being contaminated by airborne room contaminants. As the purified room air is exposed to the work environment and becomes contaminated (red) it is passed through yet another HEPA filter before being exhausted to the atmosphere. This pattern of airflow and purification ensures that the worker and the atmosphere are not exposed to the biohazards contained within the biosafety cabinet.

into laboratories, depending on the type of research being conducted. These levels of control are termed biosafety levels 1–4, with 1 being the lowest level of control and 4 the highest level of control. Within these laboratories, biosafety cabinets are essentially isolation chambers that provide safe environments for the manipulation of pathogenic microorganisms. In this section we will discuss biosafety cabinets and biosafety suits, followed by a short discussion of the actual biosafety levels imposed to achieve specific levels of control.

5.9.1 Biological Safety Cabinets

Biological safety cabinets (BSC) are among the most effective and commonly used biological containment devices in laboratories that work with infectious agents (US Department of Health and Human Services: CDC-NIH, 1993). There are two basic types of biosafety cabinets currently available (Class II and Class III), each of which has specific characteristics and applications that dictate the type of microorganism it is equipped to contain. Properly maintained biosafety cabinets provide safe environments for working with microorganisms. Class II biosafety cabinets are characterized by having considerable negative-pressure airflow that provides protection from infectious bioaerosols generated within the cabinet (Figures 5.16 and 5.17), and Class III biosafety cabinets are characterized by total containment (Figure 5.18). Class I cabinets are also in existence, but they are no longer produced and are being replaced by Class II cabinets for all applications.

Class II biosafety cabinets, of which there are several types, are suitable for most work with moderate-risk pathogens (Table 5.3). Class II biosafety cabinets operate by drawing airflow past the worker and down through the front grill. This air is then passed upward through conduits and downward to the work area after passing through a HEPA filter. Room air is also drawn into the cabinet through the top of the unit, where it joins the circulating air and passes through the HEPA filter and into the work area. About 70% of the air circulating in the work area is then removed by passing it through the rear grill of the cabinet, where it is discharged into the exhaust system. The remaining 30% is passed through the front grill, essentially recirculating in the cabinet (Figure 5.17).

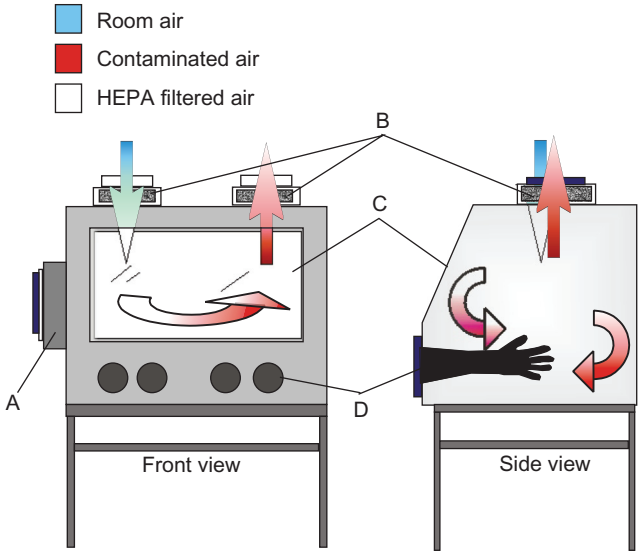

FIGURE 5.18 Schematic representation of a Class III biological safety cabinet. This cabinet is completely sealed from the environment. Any materials entering or leaving the cabinet are passed through a chemical dunk tank or autoclave (A) in order to sterilize them and prevent environmental contamination. Air entering or leaving these cabinets is passed through HEPA filters (B). Access to the workspace is by means of rubber gloves (D) and the workspace is visualized through a sealed window (C). These biosafety cabinets are utilized when working with highly pathogenic microorganisms to protect workers and the environment. Class III cabinets can be used to work with all biohazardous agents except those specifically designated for biosafety level 4 containment.

Laboratory personnel require special training in order to properly use Class II cabinets and to ensure proper containment of bioaerosols. One of the major hazards associated with Class II cabinets is the potential for the disruption of the negative airflow. Many mechanical actions can disrupt the protective airflow, such as repeated insertion and withdrawal of arms, opening or closing of doors in the laboratory, or even someone walking past the cabinet while it is in use. Any of these actions can potentially allow the escape of bioaerosols from the cabinet.

The Class III biosafety cabinet (Figure 5.18) is a completely enclosed environment that offers the highest degree of personnel and environmental protection from bioaerosols. Class III cabinets are used for high-risk pathogens (Table 5.3). All operations in the work area of the cabinet are performed through attached rubber gloves. Class III cabinets use complete isolation to protect workers. All air entering the cabinet is filtered using a HEPA filter, and the air leaving the cabinet is filtered by two HEPA filters in series. The exhaust may also include biocidal treatment such as incineration following the HEPA filtration to further ensure complete biological inactivation. In addition to these safeguards, Class III cabinets are connected with airtight seals to all other laboratory equipment (such as incubators, refrigerators and centrifuges)

TABLE 5.3 Examples of Classification of Biological Agents According to Risk

Class	Type of Agent	Agent
Class I	Bacterial	All those which have been assessed
	Fungal	for risk and do not belong in higher
	Protozoal	classes
	Viral	Influenza virus reference strains
		Newcastle virus
Class II	Bacterial	*Campylobacter* spp.
		Clostridium spp.
		E. coli spp.
		Klebsiella spp.
		Mycobacteria spp.
		Shigella spp.
		Vibrio spp.
		Salmonella spp.
	Fungal	*Penicillium* spp.
		Cryptococcus spp.
		Microsporum spp.
	Protozoal	*Cryptosporidium* spp.
		Giardia spp.
		Encephalitozoon spp.
		Enterocytozoon spp.
		Babesia spp.
		Echinococcus spp.
		Entamoeba spp.
		Fasciola spp.
		Leishmania spp.
		Plasmodium spp.
		Schistosoma spp.
		Trypanosoma spp.
	Viral	Adenoviruses
		Corona viruses
		Cowpox virus
		Coxsackie A and B viruses
		Echoviruses
		Hepatitis viruses A, B, C, D and E
		Epstein–Barr virus
		Influenza viruses
		Vaccinia virus
		Rhinoviruses
Class III	Bacterial	*Brucella* spp.
		Mycobacterium bovis
		Mycobacterium tuberculosis
		Rickettsia spp.
		Yersinia pestis
	Fungal	*Coccidioides immitis*
		Histoplasma capsulatum
	Protozoal	None
	Viral	Dengue virus
		Monkey pox virus
		Yellow fever virus
	Bacterial	None
Class IV	Fungal	None
	Protozoal	None
	Viral	Hemorrhagic fever agents
		Ebola fever virus
		Marburg virus

Adapted from University of Pennsylvania Biological Safety Manual.

that is needed for working with the pathogens while using the cabinet. The Class III cabinet must also be connected to autoclaves and chemical dunk tanks used to sterilize or disinfect all materials entering or exiting the cabinet.

Another type of containment that typically provides the same level of protection as a Class III biosafety hood is the biological safety suit (Figure 5.19). The biological suit, unlike biosafety cabinets, operates under positive pressure created by an external air supply, thus protecting the wearer. Like the biosafety cabinets, the biosafety suit isolates the laboratory worker wearing it from bioaerosols. Biosafety suits are typically used in airtight complete biocontainment areas, and are decontaminated by means of chemical showers upon exiting the biohazard area. Some biosafety suits are portable and can be used in environments outside the laboratory such as "hot zones" (epidemiological areas that are currently under the influence of epidemic cases of diseases caused by high-risk pathogens) so that microbiologists and physicians working in these areas can minimize their risk of exposure to pathogens. As in biosafety cabinets, the air entering and leaving the biosafety suit passes through two HEPA filters.

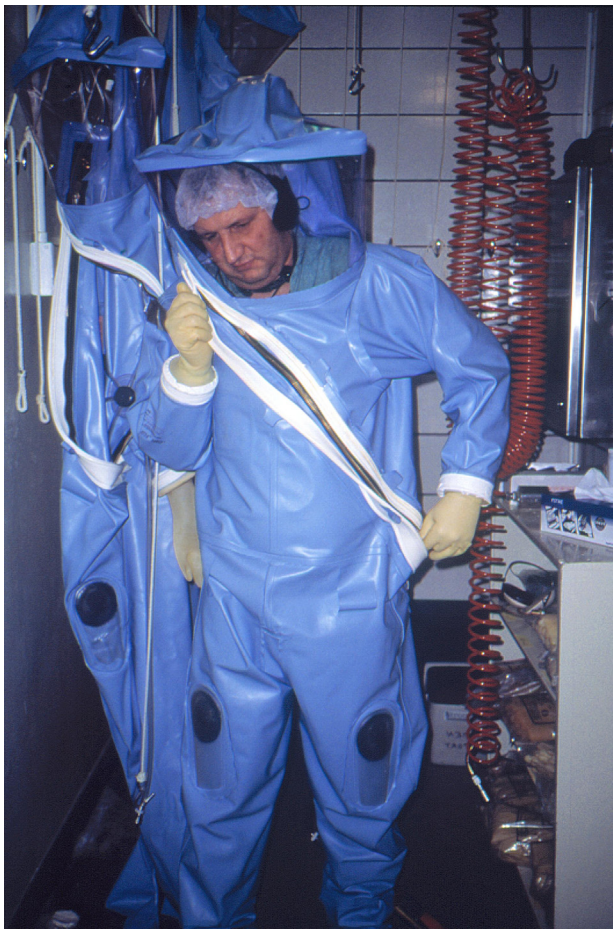

FIGURE 5.19 Biosafety suit. *Source*: Centers for Disease Control.

5.9.2 Biosafety Laboratories

Biosafety laboratories are carefully designed environments where infectious or potentially infectious agents are handled and/or contained for research or educational purposes. The purpose of a biosafety laboratory is to prevent the exposure of workers and the surrounding environment to biohazards. There are four levels of biohazard control, which are designated as biosafety levels 1 through 4.

Biosafety level 1, as defined by the Centers for Disease Control (US Department of Health and Human Services: CDC-NIH, 1993), indicates laboratories where well-characterized agents that are not associated with disease in healthy adult humans are handled. In general, no safety equipment is used other than sinks for hand washing, and only general restrictions are placed on public access to these laboratories. Work with the microorganisms can be done on bench tops using standard microbiological techniques. A good example of a biosafety 1 laboratory is a teaching laboratory used for undergraduate microbiology classes.

Biosafety 2 indicates an area where work is performed using agents that are of moderate hazard to humans and the environment. These laboratories differ from biosafety 1 laboratories in that the personnel have specialized training in the handling of pathogens, and access to the work areas is limited. Many procedures that may cause aerosolization of pathogenic microorganisms are conducted in biological safety level II cabinets or other physical containment equipment, to protect the laboratory workers.

Biosafety 3 indicates laboratories where agents that can cause serious or fatal disease as a result of AMB exposure are handled. As with biosafety 2, all personnel are specifically trained to handle pathogenic microorganisms. All procedures involving these infectious agents are conducted in biological safety level II cabinets or other physical containment devices. These facilities also have permanent locks to control access, negative airflow and filtered ventilation in order to protect the public and the surrounding environments. With certain pathogens used in biosafety 3 laboratories, Class III safety hoods may also be used, and clothes must be changed before leaving the premises.

Biosafety 4 is the highest level of control and is indicated for organisms that have high potential for life-threatening disease in association with aerosolization. To work in these facilities, personnel must have specialized training beyond that required for biosafety levels 2 and 3. Biosafety level 4 laboratories are 100% isolated from other areas of a building and may even be separated from other buildings altogether. Work in these areas is confined exclusively to Class III biological safety cabinets unless one-piece positive-pressure ventilation suits are worn, in which case Class II biosafety cabinets may be used. These laboratories are also specially designed to prevent microorganisms from being disseminated into the

environment. The laboratories have complete containment, and require personnel to wear specialized clothing, which is removed and sterilized before leaving the containment areas. Personnel are also required to shower before leaving the facility. In general, all air into and out of these laboratories is sterilized by filtration and germicidal treatment. These facilities represent the ultimate in our ability to control airborne pathogens.

5.9.3 Biological Agent Classification

For any microorganism, defined degrees of risk associated with its use indicate the type of containment needed to ensure the safety of laboratory workers, the public and the environment. There are five classes of organisms. Class I microorganisms are those that pose little or no hazard under ordinary conditions of handling and can be safely handled without special apparatus or equipment. In contrast, Class II are agents of low potential hazard that may cause disease if accidentally inoculated or injected but that can be contained by ordinary laboratory techniques. Class III agents are those that require special containment; they are associated with aerosol disease transmission and special permits are required to import them from outside the country. Class IV agents are those that require extreme containment and are extremely hazardous to laboratory personnel or may cause serious epidemic disease. Finally, Class V agents are restricted foreign pathogens whose importation, possession or use is prohibited by law.

QUESTIONS AND PROBLEMS

1. List the major factors important in the survival of microorganisms in aerosols.
2. What is the major component of biosafety cabinets that remove microorganisms?
3. What is the role of microorganisms in cloud formation?
4. Give an example of a continuous linear source and an example of an instantaneous area source of bioaerosols.
5. Considering a windspeed of 1.5 m/s, an object that is 12 cm tall and normal air viscosity, determine whether conditions around the object would be considered turbulent.
6. Consider an airborne virus and an airborne protozoan with a radius of 30 nm and 1 μm and particle densities of 2.0 and 1.1 g/cm^3, respectively. Under normal gravitational acceleration calculate the terminal velocity for each.
7. Calculate the annual community risk of infection given the following data:
   - aerosolized virus concentration = 7.16/m^3 of air
   - duration of exposure = 8 hours
   - infectivity constant $r = 0.0253$
   - breathing rate = 0.83 m^3 per hour

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Chapter 6

Aquatic Environments

Virginia I. Rich and Raina M. Maier

6.1 INTRODUCTION

The majority of the planet's habitat is aquatic: more than 80% of Earth's surface is aquatic, and the volume of habitat in aquatic systems is vast, spanning a range of environments (Table 6.1; Figure 6.1). These habitats are teeming with microbial life. Microorganisms are key drivers of the planet's biogeochemical cycles (Chapter 16), and this includes large roles for aquatic microbes. While the Amazon Forest has been called the lungs of the planet, roughly 50% of the oxygen that you breathe was actually produced by the photosynthesis of aquatic microbial primary producers. In addition, microbes are the base of aquatic food chains, which supply roughly 15% of the world's protein, and are projected to become an even larger share in the future. The water itself in aquatic environments is a vital resource, supplying water for drinking, agriculture, mining, power generation, semiconductor manufacturing and virtually every other industry. For some of these uses, aquatic microbes may be considered contaminants; as in the case of computer chip manufacturing. For potable water, contamination with pathogens results in approximately 11% of the world's population still lacking access to safe drinking water. In this chapter, we first define the main aquatic habitat types (planktonic, benthic and their interface), then examine how microbial lifestyles (primary and secondary production) are employed in them. Finally, we describe and provide general microbial characteristics of: (1) marine systems; (2) freshwater systems; and (3) select other aquatic environments.

6.2 MICROBIAL HABITATS IN THE AQUATIC ENVIRONMENT

6.2.1 Physical and Chemical Characteristics

There are a number of typical misconceptions about aquatic habitats, due to how we think about water, for example that it tends to be well mixed. First, aquatic habitats are not homogeneous. Stratification is an important physical structuring of aquatic environments, established due to temperature and salinity differences (see lake example in Figure 6.2). Surface waters are warmed by sunlight, and since warm water is less dense than cold

I.L. Pepper, C.P. Gerba, T.J. Gentry: Environmental Microbiology, Third edition. DOI: http://dx.doi.org/10.1016/B978-0-12-394626-3.00006-5

water (water is most dense at 4°C), this temperature-driven stratification tends to persist in the absence of mixing (which *does* occur, see below). The thermocline is the layer in aquatic systems where a rapid change in temperature occurs. Salinity differences can also establish stratification, when precipitation or other inputs bring fresher waters over saltier ones, which are denser. (The salinity of aquatic systems can range from freshwater at 0.5‰, to marine water between 33 and 37‰, to hypersaline systems such as the Dead Sea at 290‰; see Information Box 6.1.) This layering of aquatic environments can act as a barrier

TABLE 6.1 Distribution of Water on Earth

Habitat	Volume km^3	Percent of Total Water
Oceans, seas and bays	1,338,000,000	96.5
Ice caps, glaciers and permanent snow	24,064,000	1.74
Saline groundwater	12,870,000	0.94
Fresh groundwater	10,530	0.76
Ground ice and permafrost	300,000	0.22
Fresh lakes	91,000	0.007
Saline lakes	85,400	0.006
Atmosphere	12,900	0.001
Soil moisture	16,500	0.001
Swamp water	11,470	0.0008
Rivers	2120	0.0002
Biological water	1120	0.0001

From http://en.wikipedia.org/wiki/File:Earth_water_distribution.svg.

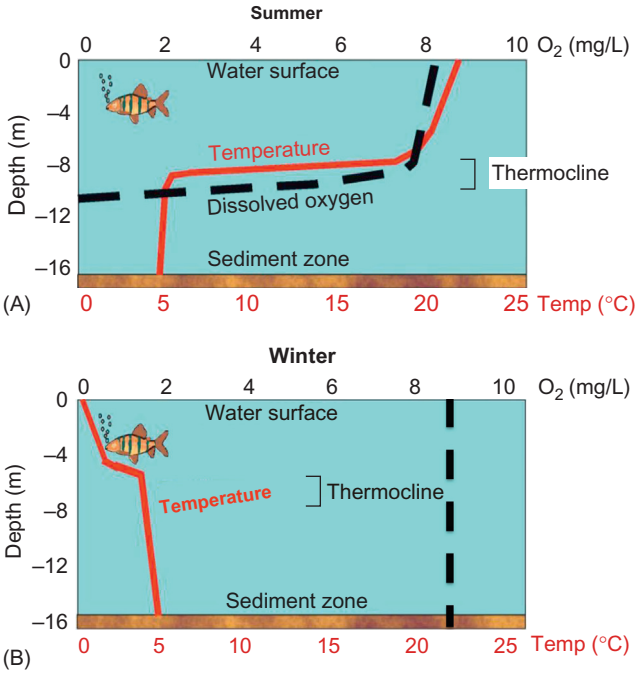


FIGURE 6.2 Stratification in a lake. This idealized view of a temperate-region, eutrophic (high-nutrient) lake shows temperature-driven stratification in the summer (A) due to warming of the surface waters. The thermocline, where the temperature drops sharply, acts as a barrier to mixing of deeper waters, thus preventing their oxygenation. In the fall and winter as the surface cools, the thermocline breaks down and mixing occurs, reoxygenating deeper waters (B).

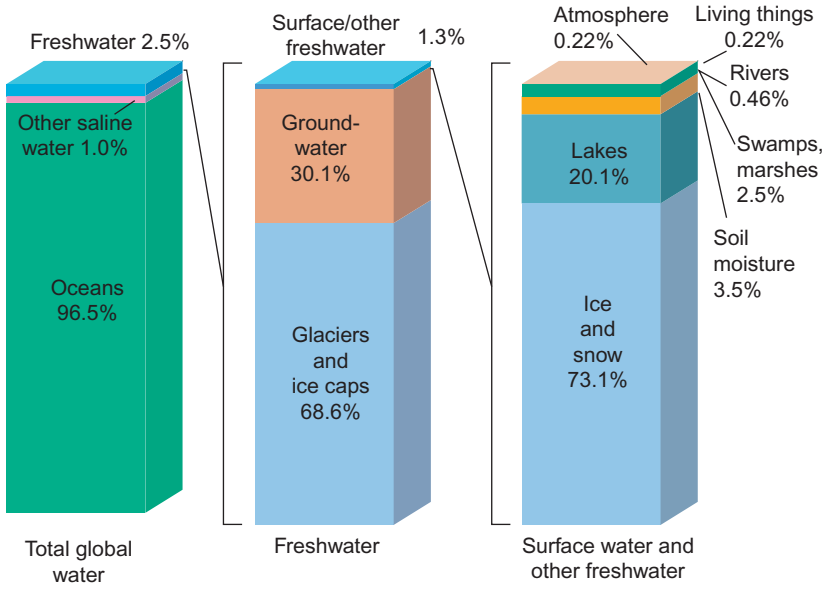


FIGURE 6.1 What are aquatic habitats? A view of the distribution of Earth's water. From http://ga.water.usgs.gov/edu/earthwherewater.html.

to vertical movement of organisms that move passively and/or are very small, including microbes. In addition, the layering can create markedly different chemical conditions in adjacent layers. Surface layers have higher oxygen concentrations due to diffusion and mixing from air, and they experience sunlight, but their typically high primary production can result in nutrient-limiting conditions. Microbes can be adapted for the conditions found in specific layers, and some have developed strategies for staying in certain layers. A hot topic in aquatic systems is thin layers (Sullivan *et al.*, 2010), which consist of layers of distinct chemistry and biology just centimeters to meters thick that can extend horizontally for kilometers. Interest in thin layers is due to the fascinating and very active biology within them, which until recently was simply missed by typically bulk-scale observations. The thermocline itself is often a transition layer of higher cell numbers and heterotrophic activity, because organic matter accumulates there. Likewise, the layer at the very bottom of the water column, directly above the sediment, often supports higher cell numbers, due to the resuspension of nutrients and carbon, as well as cells from the sediment.

A second misconception about aquatic environments is that they are static; that water's high thermal inertia and the vast size of many water bodies create extremely stable environments. In fact, aquatic systems are spatially and temporally highly dynamic. Mixing counteracts stratification, and is caused by the action of winds, currents, tides, upwelling, and temperature and salinity changes. Mixing is critical to bringing oxygen and nutrients to depleted waters. High evaporation rates such as occur in the Tropics can make surface waters saltier, which can help drive mixing as the denser saltier water then sinks. Seasonal temperature changes in surface waters are a major driver of mixing in aquatic systems. Surface waters are warmed by the summer sun and are cooled in the fall and winter. This results in a decrease in the thermocline strength, permitting deeper mixing (Figure 6.2). In addition, fall and winter often bring more storms, which further mix the water column. In some systems with extreme air temperature changes such as the Polar Regions or limited water volumes such as lakes, the thermocline breaks down altogether, allowing mixing throughout the water column. Where air temperatures drop below freezing, ice forms at the surface; in shallow lakes the ice may propagate all the way to the bottom. As an interesting aside, in marine systems, the formation of ice crystals pushes out the "impurities" of salts, creating extremely salty brine channels in sea ice, an extreme habitat where unique microbiology occurs. When ice thaws in springtime in temperate, alpine and polar aquatic systems, mixing through the water column occurs once more, before summer stratification is re-established.

Light is a critical driver of habitat differences in aquatic systems. Light is able to penetrate to a depth of 200 m or more, depending on the turbidity of the water (Figure 6.3). This depth defines the photic zone. In lakes and coastal areas, where the amount of suspended particulate matter in the water is high, light may penetrate less than 1 m. The aphotic zone is the dark water where light does not reach. The presence or absence of light results in very different microbial lifestyles, diversity and activity. It is essential to consider stratification and mixing in tandem with photic zone depth, when thinking about microbes that specialize in the sunlit surface waters. If an aquatic system is highly mixed (for example, in the fall in

Information Box 6.1 What Is Salinity?

The average salt concentration in the ocean is approximately 3.5‰. This is more precisely expressed in terms of salinity. Salinity (‰) is defined as the mass in grams of dissolved inorganic matter in 1 kg of seawater after all Br^- and I^- have been replaced by the equivalent quantity of Cl^-, and all HCO_3^- and CO_3^{2-} have been converted to oxide. In terms of salinity, marine waters range from 33 to 37‰, with an average of 35‰.

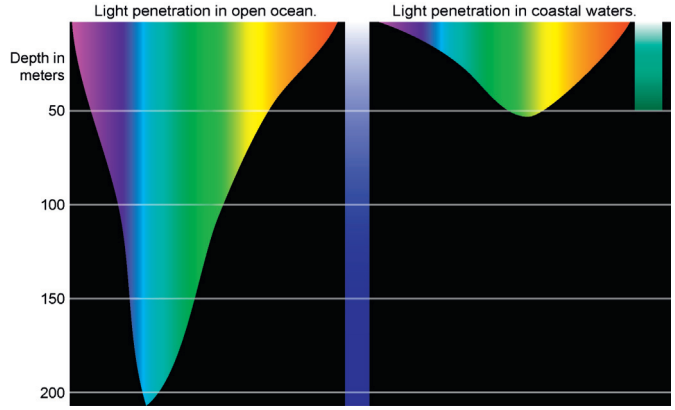


FIGURE 6.3 Light penetration through water, here shown for two ocean habitats, coastal vs. open ocean. Penetration is markedly different due to differences in turbidity resulting from dissolved and particulate matter and microbial cells. In the coastal waters, turbidity is higher, and light penetrates less deeply. Long wavelength light (red) is absorbed by water, while short wavelength light (purple) is scattered, such that the deepest penetrance is for mid-range green and blue wavelengths. Maximum light penetration may be several hundred meters in the open ocean but just tens of meters in coastal waters. From NOAA: Image courtesy Kyle Carothers, NOAA-OE.

many systems), photoautotrophic microbes may not stay in the photic zone long enough to accomplish much primary production. In contrast, stratification allows photoautotrophs to remain in the surface sunlit waters, but may result in nutrient depletion of those waters, which can also limit their primary production and growth.

Another way to consider the structuring of aquatic environments is based on habitats defined with respect to depth below surface. In particular, at the sea surface the air–water interface is referred to as the neuston (Figure 6.4), a habitat with high levels of harsh ultraviolet radiation, biochemicals and nutrients. Due to biomolecule interactions with the air–water interface that cause them to concentrate there, the neuston is comprised of a thin gel-like matrix of biomolecules (mainly lipids, proteins and polysaccharides) where microbes can attach or become trapped. The pelagic zone is a broad term used to describe the water column or planktonic habitat (see Section 6.2.2), and is subdivided on the basis of depth. In marine systems, depth from surface defines the epipelagic, mesopelagic, bathypelagic and abyssopelagic zones. Oceans range in depth up to 11,000 m in the deepest of ocean trenches. In lakes, which can be a few meters to more than 1000 m deep, depth is combined with light penetration to define the surface limnetic zone (where light intensity is at least 1% of sunlight), and deeper profundal zone. Within the pelagic zones, water can be comprised of many microhabitats and be highly structured. Floating or sinking particles create miniature islands of carbon, nutrients and substrates. Particle-associated microbes specialize in living on these islands, in contrast to the more intuitive free-living lifestyle we imagine in the pelagic habitat. Also, microbes and many macroorganisms produce exopolysaccharides, which cumulatively create an actual mesh structure to broad areas of water. Then, moving below the pelagic zone, the benthos is the sediment habitat underlying the water column (see Section 6.2.3).

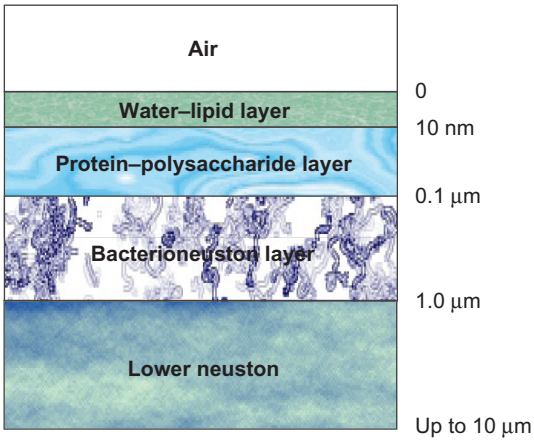


FIGURE 6.4 Schematic representation of the neuston, the upper layer of aquatic environments that can range from 1 to 10 μm in depth. The upper layer that interacts with the atmosphere consists of a water–lipid mixture that has increased surface tension. Below this is a layer of organic matter that accumulates from organic matter rising up the water column. Most scientists consider the neuston an extreme environment (see Chapter 7) because of many factors, including intense solar radiation, large temperature fluctuations, and the natural accumulation of toxic substances including chemicals, organic matter and heavy metals.

Another important and not immediately obvious set of aquatic habitats is defined by microbial associations with macroorganisms. These relationships define two additional habitats: epibiotic, which means attached to the surface of another organism, and endobiotic, which means living within another organism's tissues. Such microbe–macrobe relationships and communication increasingly appear to be the exception rather than the rule in nature (for example, in our own bodies, see Chapter 20), and can result in some particularly innovative and exciting biology. For example, many fish and squid employ bioluminescence generated through diverse microbial relationships. *Vibrio harveyii* is one microbe that uses luminescence in its fascinating endobiotic lifestyle (see Information Box 20.3).

6.2.2 Overview of Planktonic Microbes

Plankton, from Greek word meaning "wanderer" or "drifter," are organisms that live suspended in the water column and drift with the currents, with little or no ability to control their horizontal location. There are three functional groups of plankton, each with microbial members: phytoplankton, bacterioplankton and zooplankton. Pelagic microbial populations can be referred to as bacterioplankton (though notably, despite the name, these include archaeans as well as bacteria), and include photoautotrophs, chemoautotrophs (see Section 6.3.1.1) and heterotrophs. The phytoplankton (Figure 6.5) are the photoautotrophic plankton, which include microbes (cyanobacteria) and eukaryotes (algae, especially the single-celled dinoflagellates and diatoms). The zooplankton are larger heterotrophic plankton, including protozoans such as the intricate foraminiferans and radiolarians and larger organisms such as copepods. Figure 6.6 shows the relationship and interdependence of the various microbial components within a general planktonic food web.

Phytoplankton are the primary producers, which use photosynthesis to fix CO_2 into organic matter. This is a major source of organic carbon and energy, which is transferred to other trophic levels within the web (Figure 6.6). The organic compounds produced by phytoplankton can be divided into two classes, particulate or dissolved, depending on their size. Particulate organic matter (POM) compounds are large macromolecules such as polymers, which make up the structural components of the cells, including cell walls and membranes. Dissolved organic

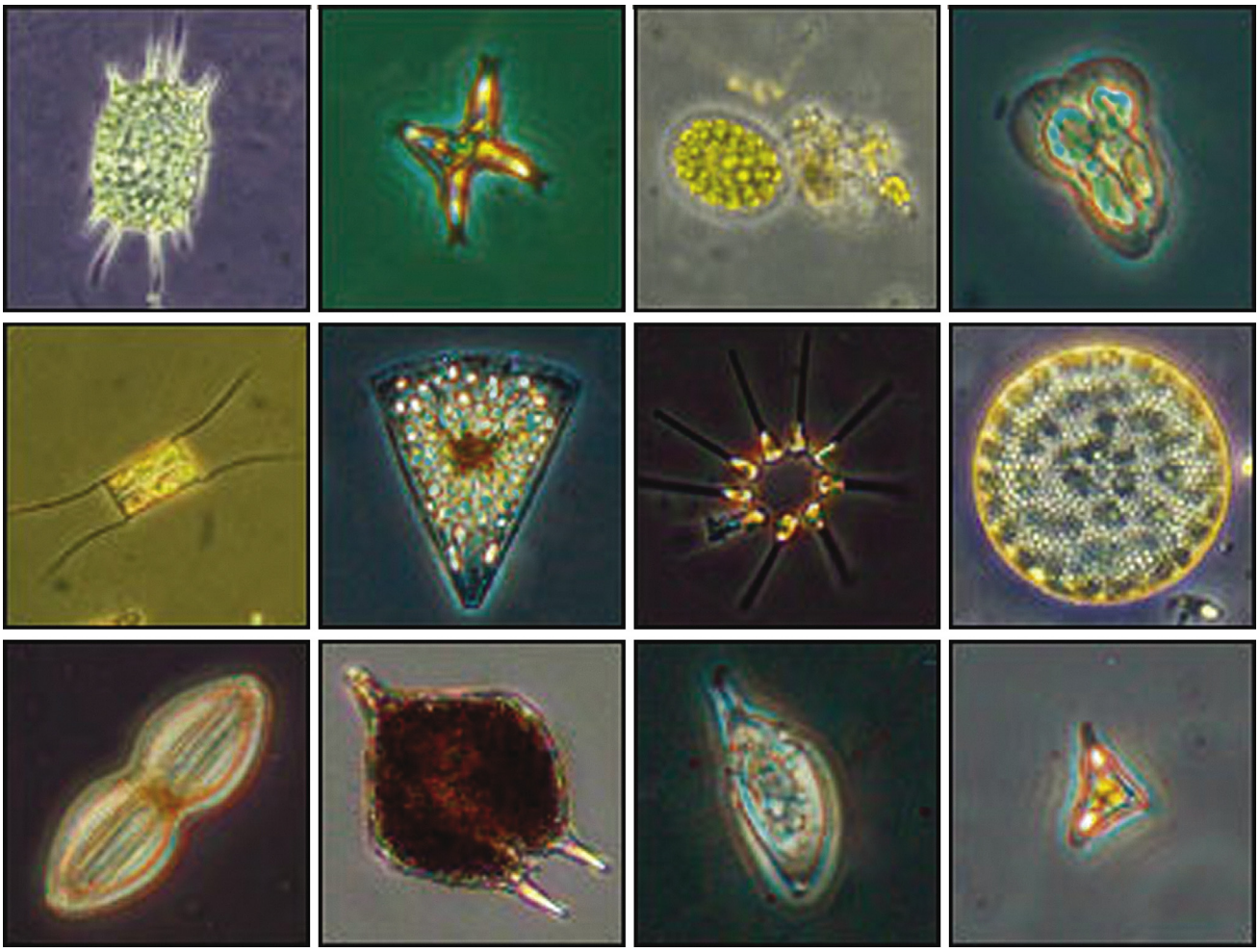


FIGURE 6.5 Examples of phytoplankton.

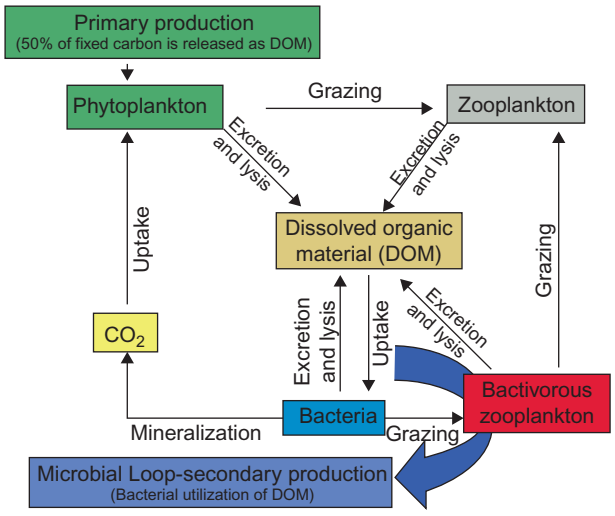


FIGURE 6.6 The planktonic food web. The microbial loop represents a pathway in which the dissolved organic products are efficiently utilized. The role of bacterioplankton is to mineralize important nutrients contained within organic compounds and to convert a portion of the dissolved carbon into biomass. Grazing by bactivorous protozoans provides a link to higher trophic levels. Modified from Fuhrman (1992).

matter (DOM) is composed of smaller soluble material that passes through a filter (pore size 0.7 μm) including amino acids, carbohydrates, organic acids and nucleic acids, which are rapidly taken up by microbes and metabolized (Kirchman, 2004). DOM is an extremely large carbon pool, equal in size to atmospheric CO_2 (Chapter 16).

6.2.3 Overview of Benthic Microbes

The benthos is the transition between the water column and the mineral subsurface. It collects organic material that settles from above, or that is deposited from nearby terrestrial environments (through river inputs, bulk overland flow or groundwater flow). At the surface of the benthos, nutrient and carbon levels are higher than in the directly overlying waters, which often causes dramatically higher microbial numbers (as much as five orders of magnitude) and activity than in the plankton. Since activity is high, oxygen is quickly used up, leading to a steep redox gradient in the sediment; that is, oxygen is replaced by

other terminal electron acceptors such as sulfate, nitrate or iron (see Chapter 3).

The benthos supports a physiologically diverse aquatic microbial community. This is because the redox and nutrient gradients described above create numerous microenvironmental niches, of which specific physiological groups of microorganisms are strategically positioned to take advantage. In shallow-water benthic communities (mud flats, river bottoms, etc.), the surface may be dominated by photoautotrophs. Below that, and in aphotic benthic habitats, heterotrophs and/or chemoautotrophs dominate, the latter fixing dissolved CO_2 into biomass using the energy of chemical bonds (see Chapter 3). The cycling of essential nutrients, such as carbon, nitrogen and sulfur, is dependent on a combination of aerobic and anaerobic microbial transformations (Figure 6.7), and can be viewed as biogeochemical cycling at small habitat scales (see Chapter 16).

In terms of carbon, the incoming organic matter (as POM or DOM) can be degraded aerobically to produce smaller compounds or CO_2, or anaerobically through fermentation into organic acids and CO_2. Organic acids can then act as electron donors for a group of strictly anaerobic bacteria that utilize CO_2 as the final electron acceptor in anaerobic respiration, thus generating methane (CH_4). The methanogenic activity in turn supports the activity of the methane-oxidizing bacteria (methanotrophs), which can use methane and other one-carbon compounds as an energy source, regenerating CO_2. Methanotrophic activity until recently was assumed restricted to the sediment–water interface zone, because it was believed to require oxygen. We now know that anaerobic methane oxidation

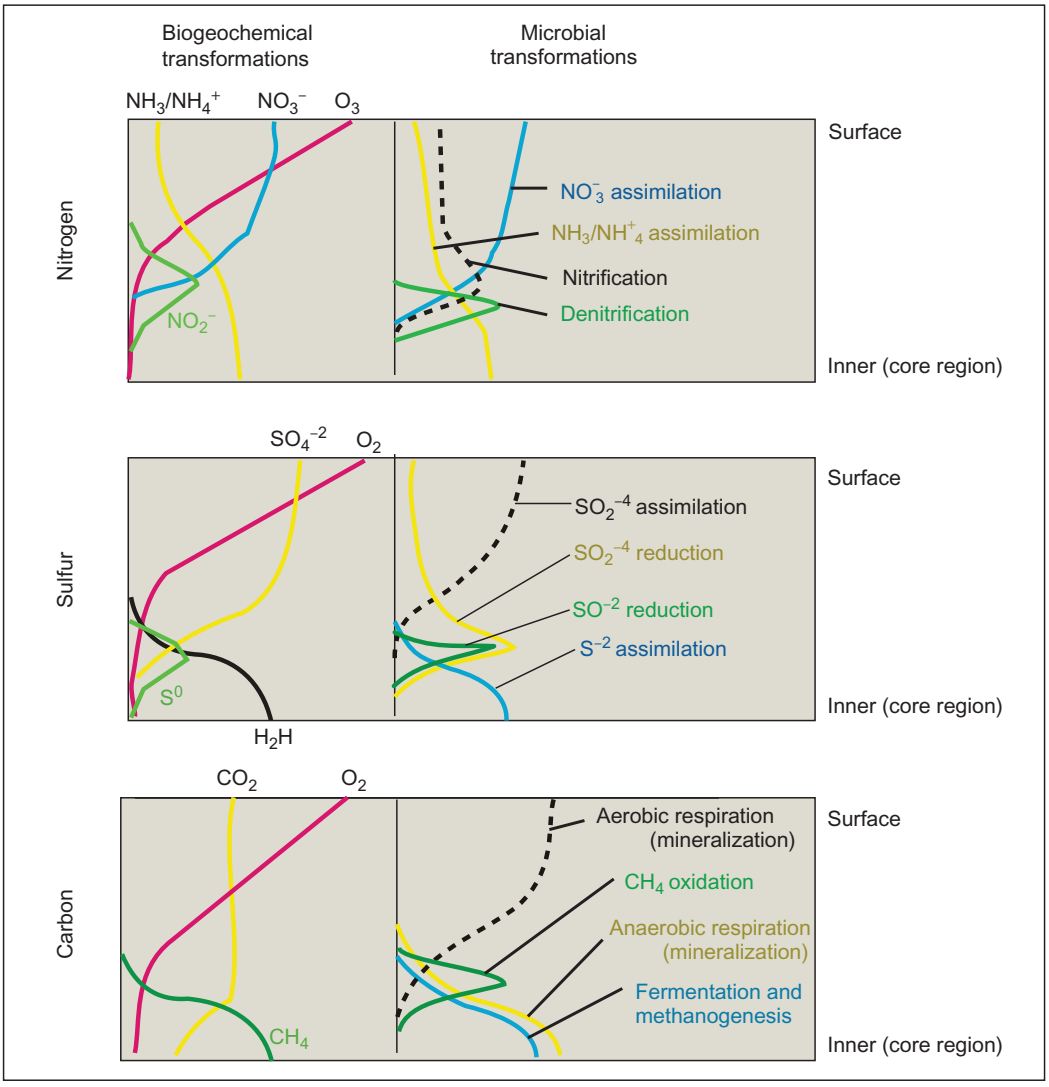


FIGURE 6.7 Biogeochemical profiles and major carbon, nitrogen and sulfur transformations that can be predicted for benthic environments in which oxygen levels are highest at the "surface" layer and are depleted by microbial activity to create anoxic conditions in the "inner" region. Adapted with permission from Pearl and Pinckney (1996).

occurs, performed by archaea and often in close syntrophic (cross-feeding) relationships with bacteria (Orphan *et al.*, 2009), although the details of this lifestyle and relationships continue to be unraveled.

In terms of nitrogen, the decomposition of organic material in the sediment layer generates ammonia from organic debris. Ammonia in the benthos may be used for two purposes: (1) biomass; that is, its assimilation as a source of essential nitrogen by planktonic and sediment microorganisms; and (2) energy; that is, its oxidation as an energy source by chemoautotrophic microorganisms. Ammonia oxidation is often localized at the sediment–water interface, where organisms utilize the release of ammonia by the decomposers and either oxygen (aerobic ammonia oxidation, by bacteria or archaea), or nitrite (anaerobic ammonia oxidation) as terminal electron acceptor (see also Section 16.3.4.2 and Case Study 19.3). The latter reaction, known as anammox, is thus far known only in the ubiquitous bacterial phylum *Planctomycetes* as its terminal electron acceptor (Kuenen, 2008). Nitrification is a two-step process of sequential oxidation of ammonia to nitrite, and then nitrite to nitrate, for example, performed in sequence by the bacterial genera *Nitrosomonas* and *Nitrobacter* (see also Section 16.3). The control of ammonia compounds can be important, especially in alkaline environments, where the undissociated NH_4OH form can be toxic to aquatic animals. The activity of the ammonia-oxidizing or nitrifying microbes can be highly sensitive to the presence of certain DOM, including naturally occurring and industrial chemicals. Therefore, the inhibition of nitrification (ammonia oxidation), which can be detected by an accumulation of ammonia or NO_2, provides a sensitive indicator of the environmental impact of certain toxic pollutants.

6.2.4 At the Interface: Biofilms and Microbial Mats

Interfacial habitats are special, as may have become clear above. They are defined by sharp environmental gradients (for example, of UV and oxygen in the neuston, and of redox conditions in the benthos), and these gradients can create distinct niches, which microbes take advantage of through diverse life history strategies. One strategy is to team up and specialize. While we think of microbial cells in isolation, in fact they are constantly interacting with one another (see Section 19.3.1). A common way is through quorum sensing, a process by which a single cell can "sense" whether a threshold number, or "quorum," of cells is nearby (see also Chapter 20). The coordination of microbial cells through physical, chemical and biological processes can result in the formation of complex, specialized and diversified structures. Here we discuss the two such structural types, common on surfaces in aquatic habitats.

6.2.4.1 Biofilms

A biofilm is a surface association of microorganisms that are strongly attached through the production of an extracellular polymer matrix. Biofilm-harboring surfaces are usually aquatic or at least moist, and include inert surfaces, such as rocks and the hulls of ships, and living ones, such as a copepod's exoskeleton, or an aquatic plant's submerged leaf. As elaborated below, biofilms have been extensively studied for their role in nutrient cycling and pollution control within the aquatic environment, as well as for their beneficial or detrimental effects on human health.

Biofilm development occurs through microbial attachment to a solid surface (Figure 6.8) in two stages: (1) reversible attachment, which is a transitory physicochemical attraction (including via hydrophobic, electrostatic and van der Waals forces; Marshall, 1985); and (2) irreversible attachment, which is a biologically mediated stabilization reaction (see Section 19.3.2.1). The attached bacteria excrete extracellular polymers, which create a matrix that surrounds the cells and forms a strong chemical bridge to the solid surface. The polymers then provide a matrix for the attachment of additional cells, form internal architecture in the biofilm structure and can create a visibly "slimy" layer on a solid surface (Marshall, 1992). The exopolymer matrix is also an integral component influencing the functioning and survival of biofilms in hostile environments.

Biofilms can be so highly organized that their architecture can rival that of simple macroorganisms. Examination of mature biofilms in their native states (using microscopic techniques, such as confocal laser scanning microscopy) has revealed a complex organization (Costerton *et al.*, 1995). Biofilms can be composed of cone-, mushroom- and column-shaped clusters of cells embedded within the extracellular polymer matrix and surrounded by large void spaces (Wolfaardt *et al.*, 1994; Korber *et al.*, 1995) (Figure 6.9A). The void spaces form channels (Figure 6.9B), which function as a primitive circulatory system by carrying limiting nutrients, such as oxygen, into the exopolymeric matrix. The presence of void spaces increases the biofilm surface area and the efficiency with which nutrients and gases are transferred between the biofilm and the surrounding water. The exact nature of the biofilm architecture depends on numerous factors, including the type of solid surface, the microbial composition of the biofilm and environmental conditions.

Microorganisms benefit from membership in a biofilm community. The extracellular matrix can have several

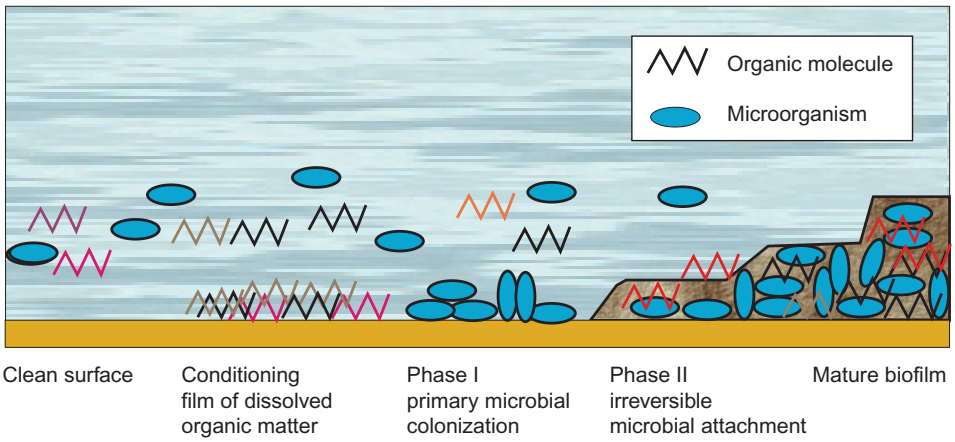


FIGURE 6.8 Representation of biofilm formation. Dissolved organic molecules of a hydrophobic nature accumulate at the solid surface−water interface and form a conditioning film. Bacteria approach the solid surface because of water flow and/or active motility. The initial adhesion (phase I) is controlled by various attractive or repulsive physicochemical forces leaking to passive, reversible attachment to the surface. An irreversible attachment is a biological, time-dependent process related to the proliferation of bacterial exopolymers forming a chemical bridge to the solid surface (phase II). By a combination of colonization and bacterial growth, the mature biofilm is formed. It is characterized by cell clusters surrounded by water-filled voids. Adapted from Marshall (1992).

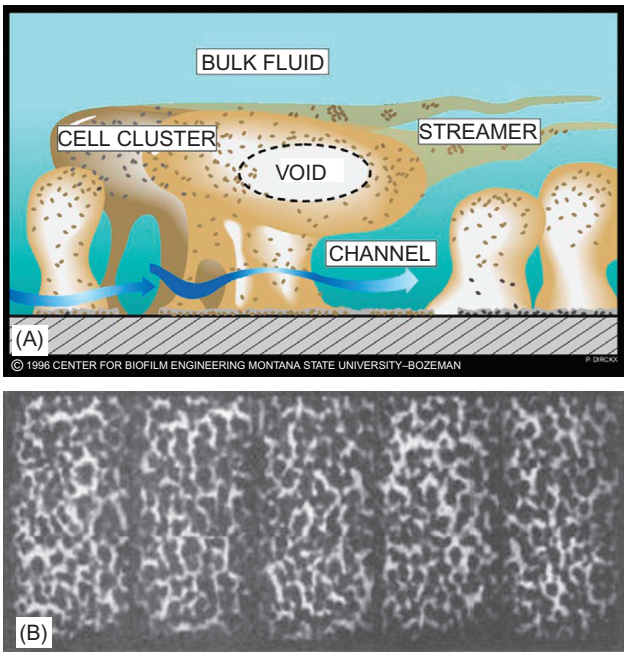


FIGURE 6.9 Biofilm communities are composed of cone-, column- and mushroom-shaped clusters of cells embedded in extracellular polysaccharide and other materials (A). Clusters of cells are surrounded by large void spaces (white networks of channels shown in biofilm in panel (B)) that allow convective flow of fluids through biofilm communities.

functions for the member cells: as an ion-exchange resin to filter and collect essential nutrients; as physicochemical protection against abiotic environmental stressors, such as desiccation or changes in pH and temperature; and against actual grazing by predators. Compared with planktonic cells, biofilm cells are far more resistant to antibacterial substances, such as antibiotics and disinfectants; this may be due to the barrier of the extracellular matrix, or to an altered physiological state of the attached bacterial cells.

Humans contain biofilms (the most obvious example being dental plaques) and have been using them intentionally for hundreds to thousands of years. Biofilms can act as water purification systems in natural environments, and remove DOM and other contaminants from flowing waters. This property has long been exploited for use in purifying water from municipal (sewage) or industrial sources (Case Study 6.1), with crude management in ancient times giving way to sophisticated "collaboration" with the biofilms in current water purification system designs, including trickling filters in wastewater treatment (Chapter 25) and point-of-use devices (Chapter 19). On the other hand, biofilms are responsible for tooth decay and compromised medical implants. Disease caused by biofilms on medical implants is estimated to account for nearly 60% of all hospital-borne infections, lengthen hospital visits by 2−3 days and increase healthcare costs by $1 billion per year (Davey and O'Toole, 2000). In industry, biofilm control is required for any type of pipeline (Chapter 28), in which biofilms lower the flow capacity, decrease heat-exchange efficiency and catalyze corrosion in the case of metal pipes.

6.2.4.2 Microbial Mats

Microbial mats can be considered a specialized type of biofilm. They are an extreme example of an interfacial aquatic habitat in which many microbial groups are

Case Study 6.1 Beneficial Biofilms Remove Cyanide from Gold Mine Effluent and Keep Mines in Business

The Homestake Mine in Lead, South Dakota, opened in 1877 during the Black Hills Gold Rush and operated profitably in the gold mining business for decades. As was the case with the operation of many other gold mines, cyanide was used to increase recovery of gold from ore obtained from the mine. As a result, an estimated 4 million gallons of cyanide-laden wastewater were released daily into nearby Whitewood Creek. These extraordinary levels of this toxic waste rendered aquatic life nearly nonexistent in the creek. By 1981, Whitewood Creek was listed as an Environmental Protection Agency (EPA) Superfund site. In 1977, the EPA required Homestake Mine to reduce its discharge of this toxic effluent. Traditional approaches to minimizing discharge of such toxic substances were expensive, and implementation of these approaches would result in closure of the mine.

The Homestake Mine needed an innovative, cost-effective strategy to deal with levels of cyanide in its wastestream. Jim Whitlock, a biochemist and South Dakota native, and Terry Mudder, an environmental engineer, were charged with addressing the problem (Whitlock, 1990). The solution the cross-disciplinary duo devised relied upon a bacterial biofilm, composed primarily of *Pseudomonas*, to remove cyanide and a host of other toxic substances, including ammonia and the metals nickel chromium, from the wastestream. Sets of large discs, called rotating biological contactors (RBCs), served as substrates upon which the pollutant-removing biofilm grew. Each RBC consisted of disks that harbored billions of bacteria across large surface areas (100,000 to 150,000 ft^2). Wastewater passed through a train of five of these RBCs. Each disk rotated at a rate of 1.5 revolutions per minute. Approximately 40% of each disk was submerged in the wastestream at all times. The rotation allowed the biofilm community to contact the wastestream and remove pollutants such as cyanide while meeting some microbial community members' requirements for oxygen. The first two RBCs contained primarily *Pseudomonas* for the removal of cyanide and the metal contaminants, while the remaining RBCs harbored nitrifying bacteria that allowed conversion of ammonia into a less toxic form, nitrate. End products resulting from this treatment were relatively innocuous and included sulfate, carbonate, nitrate and some solids, which were subsequently removed using a clarifier. The treatment facility began operation in 1984 and became more efficient and economical over time. Cyanide removal rates of 99% (from influent levels of 4.1 mg/L to effluent levels of 0.06 mg/L) were obtained. Copper and iron were removed quite efficiently—removal rates of 95–98% were common. Removal of other metals, particularly nickel, chromium and zinc, was less remarkable. Nonetheless, the effluent was free enough of pollutants to allow rainbow trout to reinhabit Whitewood Creek. Thus, this innovative use of biofilms dramatically reduced pollution introduced into the environment by the Homestake Mine, and allowed the mine to continue operations until its closing in 2002. To date, thousands of similar RBCs have been employed worldwide to reduce cyanide levels from industrial wastestreams.

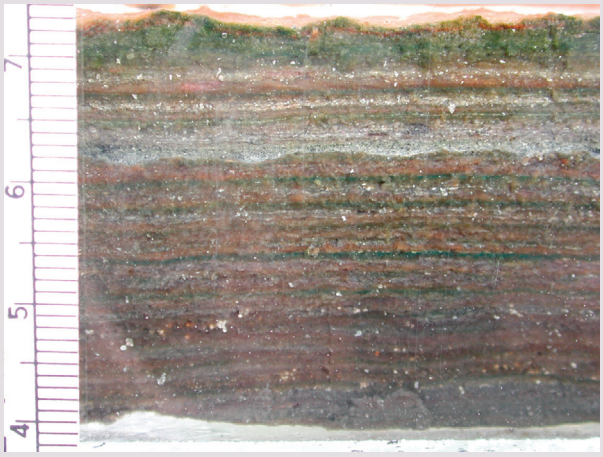
laterally tightly compressed into a thin mat of biological activity. While biofilms are typically one to several cell layers thick, microbial mats range from several millimeters to a centimeter thick, and are vertically stratified into distinct layers (Information Box 6.2). Another distinguishing characteristic of microbial mats is that they are based on autotrophy, the fixation of inorganic carbon into biomass, which occurs either photosynthetically or chemosynthetically. Similarly to biofilms, mat microbial groups interact with each other in close spatial and temporal physiological couplings. Microbial mats have been found associated with environments such as the benthic–planktonic interface of hot springs, deep-sea vents, hypersaline lakes and marine estuaries. By supporting most of the major biogeochemical cycles, these mats are largely self-sufficient.

In a photosynthetic microbial mat, the photosynthetic activity of the cyanobacteria creates an oxygenic environment in the upper layer of the mat. Oxygen can become supersaturated during the day, but at night, in the absence of sunlight, microbial respiration rapidly depletes all the available oxygen. Respiration by sulfate-reducing bacteria, considered a strictly anaerobic process, helps decompose the DOM from the cyanobacteria in the upper generally aerobic layers. This apparent contradiction may be resolved temporally, with oxygenic photosynthesis occurring during the day and anaerobic sulfate reduction occurring at night, or spatially, due to the formation of anaerobic microenvironments even in the upper layers, due to the high demand for oxygen by heterotrophic activity.

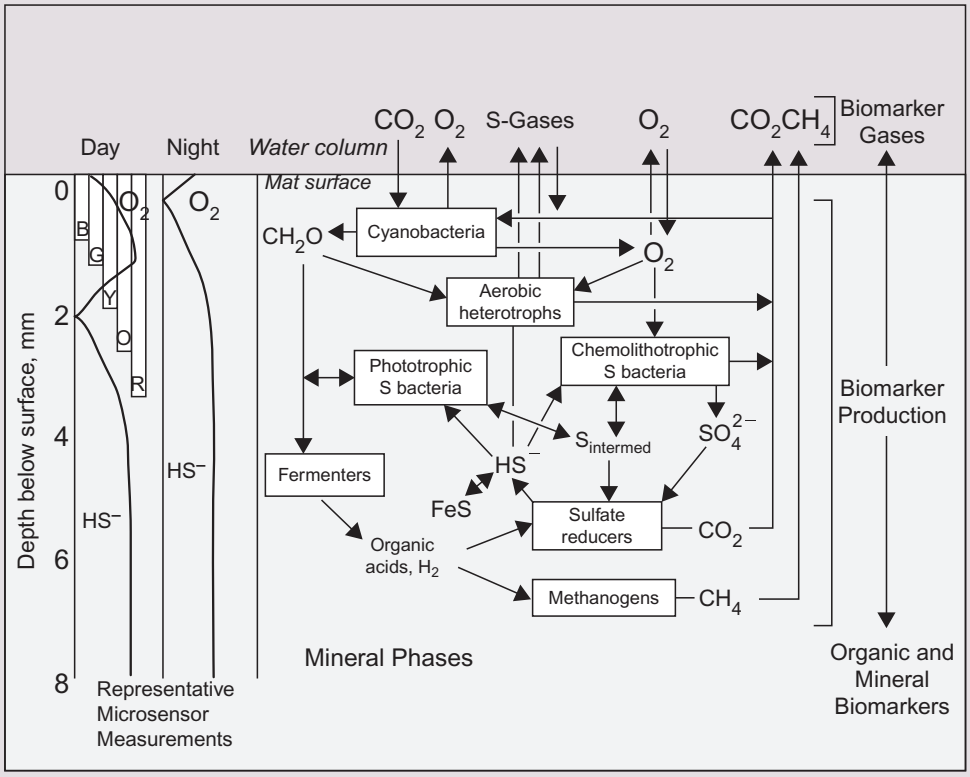
Microbial mats are unique communities because the interdependent microbial components form clearly stratified and often distinctively colored zones. Mats are often found in extreme environments or in environments where conditions fluctuate rapidly. The cyanobacteria are known to be tolerant of extreme conditions, such as high temperatures or highly saline waters, and thrive in locations where competition from other microbial groups and predation by grazing organisms are limited by the inhospitable environment. Fossilized microbial mats, known as stromatolites, dating back 3.5 billion years were among the first indications of life on Earth (see Information Box 6.2, and Section 16.1.2). At that time, Earth's atmosphere lacked oxygen, and the stromatolites from that era were probably formed with anoxygenic phototrophic bacteria (purple and green sulfur bacteria—see also Section 16.4.3.2).

Information Box 6.2 The Importance of Microbial Mats on Early Earth

The figure (top) shows a cross section of a microbial mat collected by NASA scientists from a hypersaline pond at one of the world's largest salt production facilities in Guerrero Negro, Baja California Sur, Mexico. The smallest gradations in the ruler are in millimeters.



It has been suggested that such mats forced the close proximity of the first aerobic photosynthetic microbes (cyanobacteria) with anaerobic heterotrophs. This proximity was in turn responsible for the adaptation of anaerobic heterotrophic microbes on early Earth to the presence of oxygen, which was extremely toxic to these first heterotrophic forms of life (Hoehler *et al.*, 2001). Today, as shown in the figure below, these mats provide another example of the complexity of biogeochemical cycling in aquatic environments (Des Marais, 2003). In these mats, cyanobacteria photosynthetically generate organic matter (required by heterotrophs) and oxygen (toxic for strict anaerobes). On the other hand, anaerobic heterotrophic activity recycles required nutrients back to the phototrophic community while generating toxic sulfide. Mat microbes have developed strategies to cope with the conundrum posed by these different populations. Note that in this case, the community is driven directly by photosynthesis while activity in the benthic environment is driven indirectly by photosynthesis in the form of DOM.



From (top) NASA, 2005 and (bottom) from Fig. 1 from Des Marais, D. 2003. *Biol. Bull.* **204**, 160–167. Reprinted with permission from the Marine Biological Laboratory, Woods Hole, MA.

6.3 MICROBIAL LIFESTYLES IN AQUATIC ENVIRONMENTS

6.3.1 Primary Production

Primary production in the ocean is estimated to be 50–60 petagrams (Pg = 10^{15} g) of carbon per year (De la Rocha, 2006), with freshwater likely accounting for an additional one-to-several Pg (Tranvik *et al.*, 2009). This represents 50% of the total primary production globally. The amount of primary production within a given water column depends on a variety of environmental factors. These factors include: the availability of essential inorganic nutrients, particularly nitrogen and phosphorus; water temperature; the turbidity of the water, which affects the amount of light transmitted through the water column; and the degree of vertical mixing, as described above. The concentration of primary producers in aquatic environments ranges from 10^0 organisms/ml in some benthic habitats, to 10^8 organisms/ml in surface zones.

Open oceans have relatively low primary productivity because of low levels of the essential nutrients nitrogen and phosphorus. The exceptions are areas where currents cause upwelling of deeper waters bringing nutrients from the deep sea. Coastal areas are productive because of the introduction of dissolved and particulate organic material from river outflows and surface runoff from the terrestrial environment. Upwelling can also increase productivity due to wind driven nutrient rich waters, such as off the coast of California, where upwelling-driven productivity supported the large sardine fishery made famous in John Steinbeck's *Cannery Row*. For freshwater environments, smaller and shallower freshwater bodies tend to be nutrient rich or eutrophic, supporting high productivity. Large, deep lakes can be nutrient poor or oligotrophic like the open ocean, with low productivity. However, human activities can significantly increase nutrient loading. Sources of natural nutrient loading include terrestrial runoff, rivers that feed into the lake and plant debris such as leaves. Nutrient loading from human activities includes runoff from animal manures and agricultural runoff, both of which contain high levels of nitrogen and phosphorus, the nutrients most often limiting in aquatic environments.

6.3.1.1 Photoautotrophy vs. Chemoautotrophy

Aquatic primary production is considered, and quantified, almost exclusively as photoautotrophy occurring in sunlit waters. Because photosynthesis is mediated by photopigments with characteristic absorption spectra such as

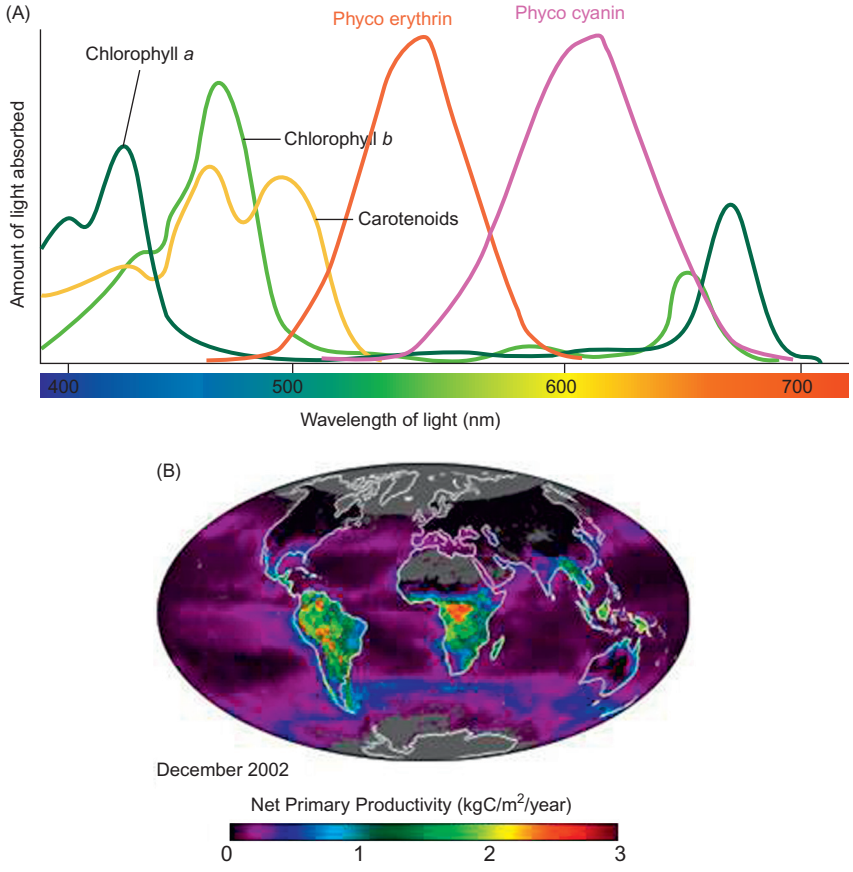


FIGURE 6.10 The absorption spectra of photosynthetic pigments (A). Modeled global primary production (B), from satellite data on chlorophyll abundance, measured by its characteristic spectral signature. *Source*: NASA.

chlorophyll (Figure 6.10A), it can be measured from space through satellite imagery (Figure 6.10B). Chemoautotrophy, the ability to fix carbon using chemical bond energy (i.e., the oxidation of reduced compounds) (see Chapter 3), does not have a similar such marker that is visible from space! Initially, chemoautotropy was believed to occur in extreme aquatic environments such as geothermal hot springs and oceanic hydrothermal vents, but gradually it has been increasingly recognized to occur in many more aquatic habitats. The question has now evolved from "where does chemoautotrophy occur?" to "where does chemoautotrophy not occur?"

Chemoautotrophs are seen throughout the water column, especially in oxygen minimum areas, which occur when high microbial decomposition (usually linked to high overlying primary productivity) strips oxygen from the water. The Gulf of Mexico's "Dead Zone" (Figure 6.11) is an example of a particularly large oxygen minimum area, and received its name because the extreme lack of oxygen in mid-depth waters causes fish die-offs. These areas are expected to increase with global change, due to increased water stratification from warmer temperatures, combined with more extreme precipitation events flushing agricultural fertilizers into aquatic systems. Chemoautotrophs have been discovered to be abundant in oxygen minimum waters, as well as in benthic systems (see Section 6.4.2.2), and are present at varying levels throughout aquatic habitats (Reinthaler *et al.*, 2010; Swan *et al.*, 2011). When inorganic carbon fixation is measured throughout the water column, consistent though low amounts can be seen through the dark subphotic waters (Figure 6.12), cumulatively (due to the large volume involved) representing a large and important though still poorly defined contribution to marine primary production.

Ammonia oxidizing archaeans are one form of ubiquitous pelagic chemoautrophs, virtually unknown until the mid-1990s (Schleper and Nicol, 2010). These marine pelagic Crenarchaota are a minor component of photic communities, but reach 35–40% of open-ocean microbes below 1000 m (Figure 6.13) (Karner *et al.*, 2001). A variety of molecular methods, including gene and transcript surveys, genomics and metagenomics (see Chapter 21), show that chemoautotrophy via aerobic ammonia oxidation is likely the dominant lifestyle for these microbes (Church *et al.*, 2010).

6.3.2 Secondary Production

Although we may think of the general planktonic food web in the aquatic systems as simply involving

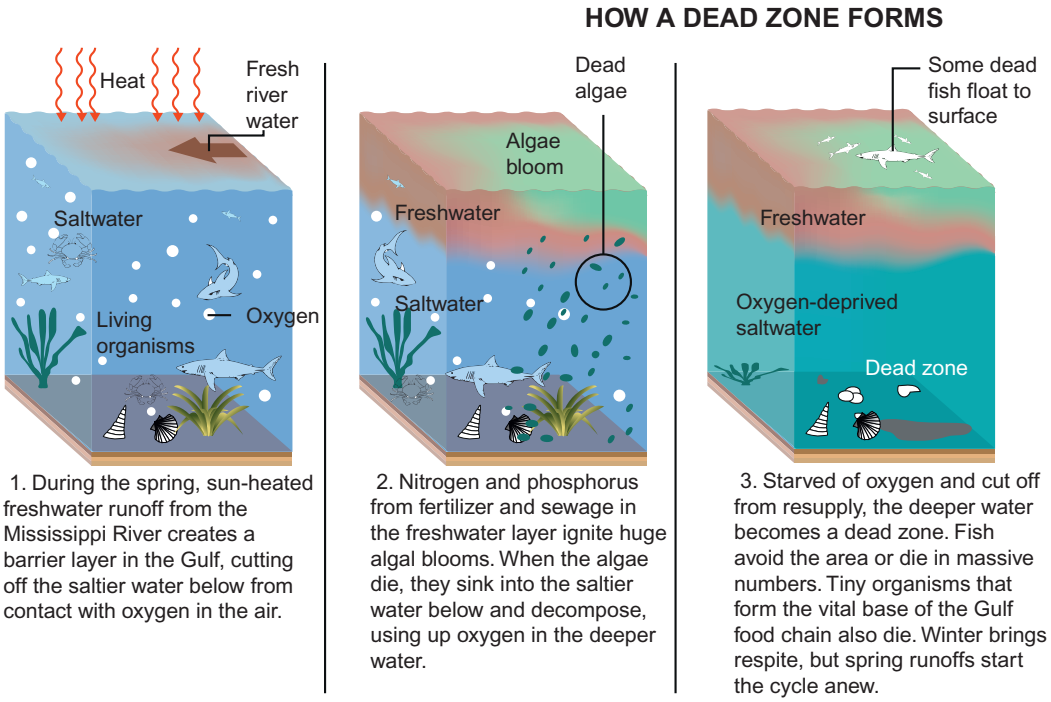


FIGURE 6.11 A so-called Dead Zone, where heterotrophic decomposition has stripped the water of oxygen, leading to large regions of anoxia and sometimes resulting in massive fish die-offs.

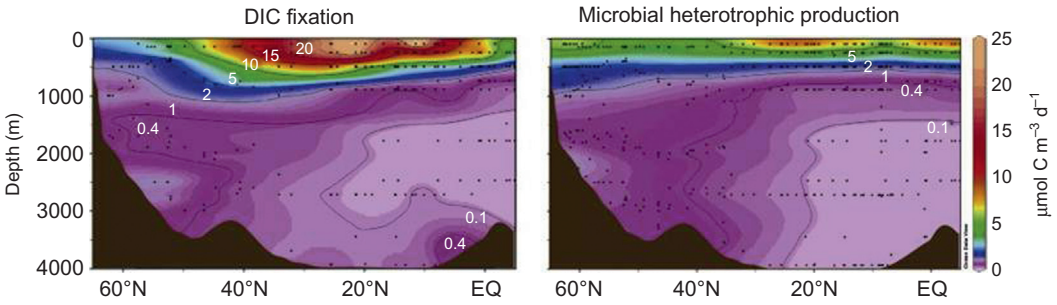


FIGURE 6.12 Chemoautotrophy in the eastern North Atlantic. On the left, dissolved inorganic carbon (DIC) fixation through the water column, measured by ^{14}C-bicarbonate fixation. The strong signal of photosynthesis in surface waters is clear, but low amounts of fixation continue throughout the vast dark waters below. On the right, microbial heterotrophic production as measured by ^{3}H-leucine incorporation showing that the subphotic pelagic chemoautotrophy is on the same order of magnitude as the heterotrophic production at those depths. Image from Reinthaler *et al.* (2010).

bacterioplankton and zooplankton consuming phytoplankton, the primary producers, which in turn are consumed by progressively larger organisms, the actual transfer of carbon and energy between trophic levels is much more complex (Figure 6.6; see also Figure 2.19 for an alternate image of a pelagic food chain). DOM represents a very large pool of carbon, roughly equivalent to the CO_2 in the atmosphere. This is because >50% of the carbon fixed by photosynthesis is released into the water column as DOM, which is rapidly utilized by heterotrophic microbes in a pathway in the aquatic food web referred to as the microbial loop. In this loop, bacterioplankton remineralize a portion of the DOM into CO_2 and nutrients, which in turn fuel new primary production—in fact, microbially recycled nutrients in the ocean's surface waters fuel roughly 80% of marine primary production (Duce *et al.*, 2008). Bacterioplankton also assimilate DOM to produce new biomass of their own, which is referred to as microbial secondary production. Thus, the microbial loop serves to efficiently utilize the DOM released into the water column. Because there is so much DOM, and the microbial loop allows its nutrients, carbon and energy to be retained in the sunlit surface waters to support more growth, the microbial loop is a key concept in aquatic systems.

Why is there so much DOM, and where does it come from? The DOM pool comes primarily from phytoplankton, with contributions from zooplankton and bacterioplankton, as well as from larger organisms through excretion and the lysis of dead cells. Among the phytoplankton, it is known that both "healthy" cells and "stressed" cells (those under some form of environmental stress) release DOM into the water column. In addition, "sloppy" feeding habits of zooplankton and larger organisms that eat phytoplankton may release a portion of their biomass as DOM into the water column. Finally, evidence indicates that as much as 6 to 26% of DOM is released during the lysis of phytoplankton and bacterioplankton by viruses (Ashelford *et al.*, 2003) (see Figure 6.6).

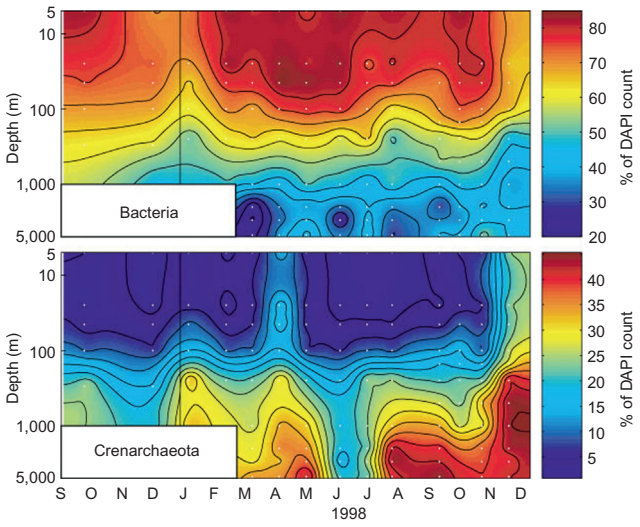


FIGURE 6.13 Marine Crenarchaea are abundant over space and time. This example shows the relative abundance of Bacteria (top) and Crenarchaeota (bottom) at Station ALOHA in the north Pacific off Hawai'i, through the water column over 15 months. Total cell counts were made by epifluorescence using the DAPI nucleic acid stain (see Chapter 13), and the relative proportion of each group was obtained using targeted probes with fluorescence *in situ* hybridization (FISH; see Chapter 13).

Thus, in a real aquatic food web, the heterotrophs (the bacterioplankton and zooplankton) consume each other, DOM, POM and autotrophs (phytoplankton, the main primary producers). The zooplankton in turn are consumed by larger organisms such as fish and other filter feeders. In the open ocean it takes approximately five steps or trophic levels to produce exploitable fish. In coastal zones it takes 1.5 to 3.5 steps to produce fish because primary production levels are higher. There is often a temporal lag between primary and secondary production; Figure 6.14 shows that an increase in phototrophs (as measured via chlorophyll *a*, a photosynthetic pigment) is followed by an increase in heterotrophs in a marine system.

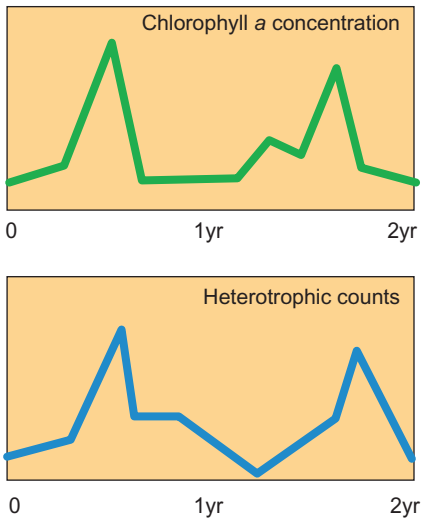


FIGURE 6.14 Diagram of the interrelationship between the concentration of chlorophyll *a*, a photosynthetic pigment and heterotroph density. The concentration of chlorophyll in water is related to the amount of primary production. This in turn influences the amount of secondary production by heterotrophic populations. In this figure, it can be seen that as the chlorophyll *a* concentration increases, it is closely followed by an increase in heterotrophic populations. Thus, secondary production is intimately tied to primary production. Adapted from Rheinheimer (1985).

6.3.2.1 Photoheterotrophy: A Newly Appreciated Microbial Lifestyle

Until recently, microbial life in aquatic systems was imagined to occupy the two main categories of (1) photosynthesizers, living in the surface water and forming the base of the food chain, and (2) the heterotrophs, living solely off that sunlight-fueled primary production. Increasingly, we know this was too simplistic a view. As described above, chemoautotrophy is likely an appreciable additional though poorly understood source of primary production in many aquatic systems. In addition, heterotrophs may not be as exclusively reliant on primary production as we have understood. They may be able to supplement their energy, but not carbon, requirements through the use of sunlight. This photoheterotrophy is not photosynthesis because it does not involve carbon fixation, and, since CO_2 is not being fixed, these organisms are still fundamentally "heterotrophs." There are several different ways that heterotrophs can directly, or indirectly, use sunlight for energy (reviewed in Moran and Miller, 2007), and two of these ways are highlighted briefly here.

First, aerobic anoxygenic phototrophy (AAnP), which uses bacteriochlorophyll to capture sunlight for energy without producing oxygen, appears to be widespread among diverse marine microbial lineages (Moran and Miller, 2007). In the freshwater and marine sites examined so far, the percentage of surface microbial cells carrying the genes for AAnP ranges from $<1\%$ to 10% of the community (Figure 6.15A) (Yutin *et al.*, 2007). Second, a simple type of light-harvesting molecule related to the rhodopsins in our own retinas was discovered in marine microbes (reviewed in DeLong and Béjà, 2010). Since it was initially described in a Proteobacteria, it was termed proteorhodopsin, and was demonstrated in the lab to be a light-activated proton pump that generates proton gradients available for ATP production (Figure 6.15B). In addition, it is expressed in the environment, tuned to different wavelengths of light at different depths, and confers growth benefits in some cultured microbes. The gene encoding proteorhodopsin is present in a remarkable 13–80% of marine surface bacteria and archaeans (DeLong and Béjà, 2010). The current understanding for how both of these strategies works is as a way for heterotrophs to survive lean times, and be faster to respond when new food is again present. This has significant impacts on marine carbon cycling, energy budgets and ideas about the ecology and evolution of these communities. Yet, the prevalence of photoheterotrophy was unknown until very recently, and there is much about it that remains to be understood.

6.4 MARINE ENVIRONMENTS

Marine environments are one of the dominant groups of habitats on the planet. Oceans cover $\approx 70\%$ of Earth's surface, such that a more accurate name for the planet might be Ocean. Beyond surface area, the ocean's vast volume makes its importance as a habitat even greater. The ocean's average depth is ≈ 4000 m, and its deepest spots are $\approx 11{,}000$ m; this ocean habitat is mostly dark (except for bioluminescence) and under high pressure (Figure 6.16 and Table 6.2). It encompasses a remarkable diversity of conditions and life forms, and we are only at the early stages of mapping these.

Marine microbiology has had a major role in propelling environmental microbiology forward (see Information Box 6.3), through the discovery of new physiologies (e.g., photoheterotrophy, Section 6.3.2.1), and the use of cutting-edge methods (e.g., metagenomics; see Chapter 21). These advances were led over the last several decades by a small number of individuals, and have resulted in a now large, robust community of marine microbiologists and microbial oceanographers. The number of global exploration efforts centered on marine microbiology are one indication of the success of this field; these include the International Census of Marine Microorganisms (ICoMM), which has profiled microbial communities from more than 500 global sites using the 16S rRNA gene (see Chapter 21 for how such profiling works); the Global Ocean Survey, which profiled roughly 150 communities using shotgun sequencing (thus capturing a random sampling of microbial genes rather than just the 16S rRNA marker gene; see Chapter 21 for details);

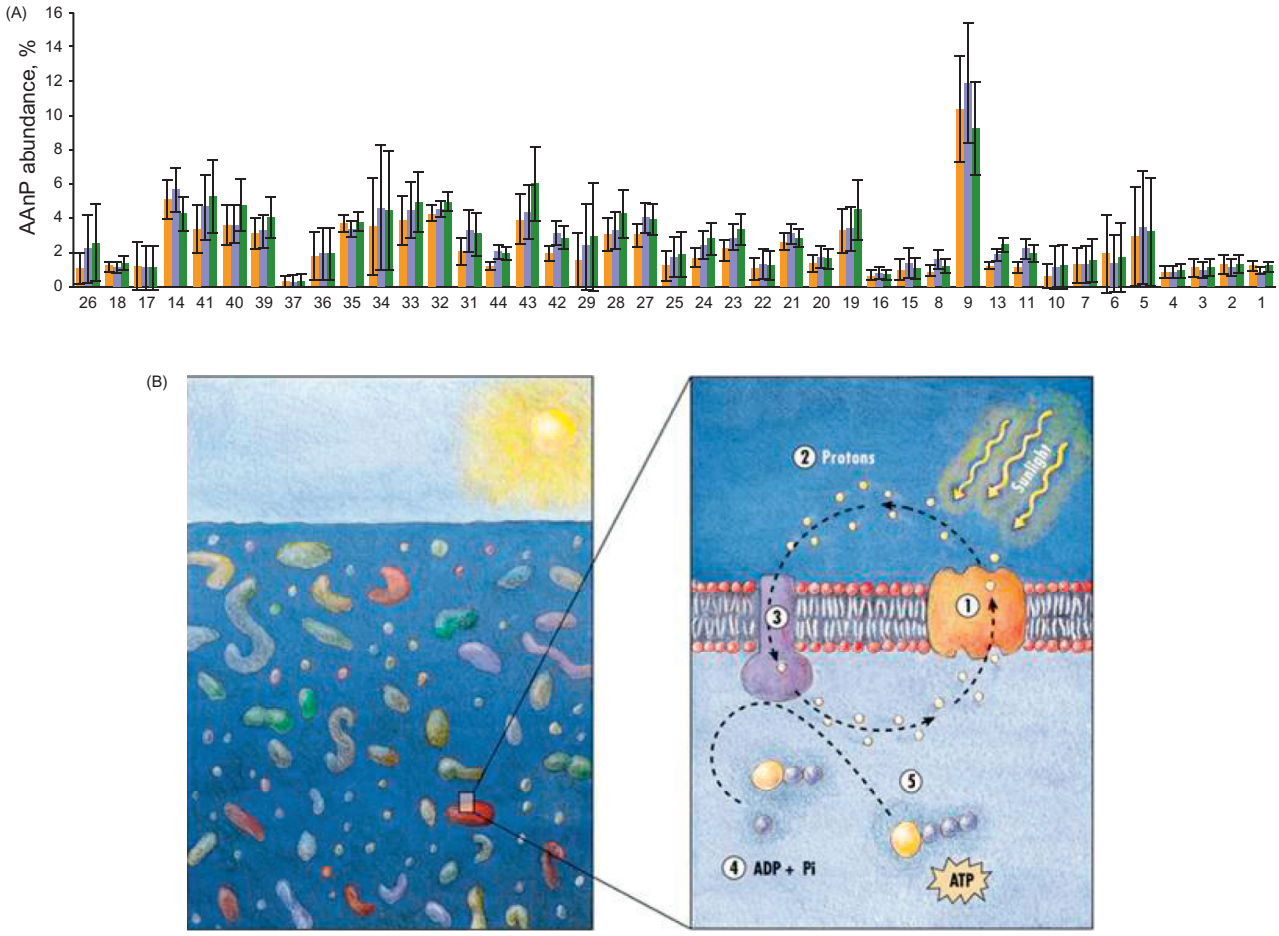


FIGURE 6.15 Photoheterotrophy. (A) The percentage of bacterial cells calculated to contain genes involved in anaerobic anoxygenic phototrophy, in Atlantic and Pacific surface waters, using the metagenomic data from the Global Ocean Survey (numbers on the X-axis refer to sampling station numbers). From Yutin *et al.* (2007). (B) An artist's rendition of microbial cells in the surface ocean, and how proteorhodopsin works in the cell membrane to capture light energy to pump protons, producing the proton gradient that ATPase can then harness to make ATP. From DeLong and Béjà (2010).

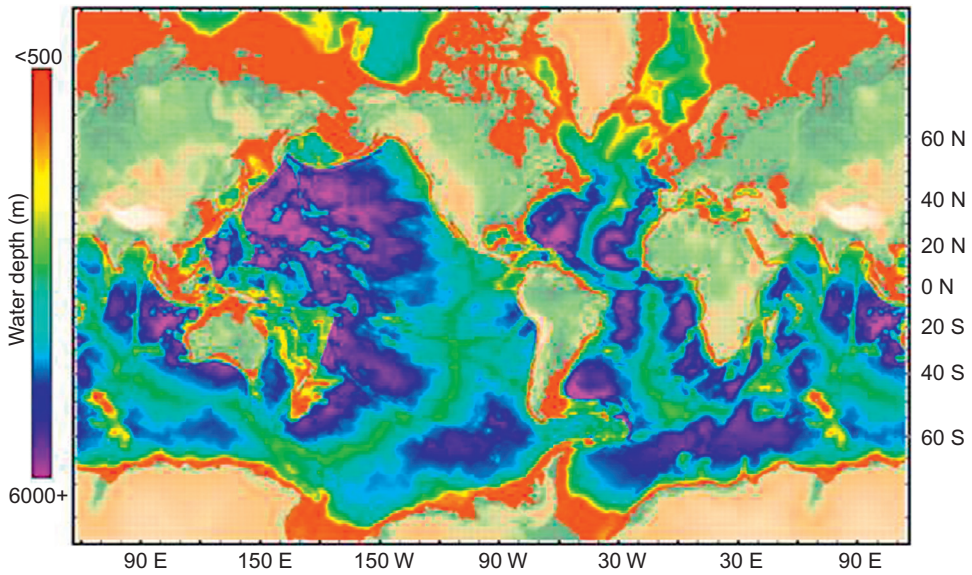


FIGURE 6.16 Global map of ocean water depth. The water depth scale is from less than 500 m (red) to 6,000+ m (purple). From Orcutt *et al.* (2011).

and the Tara Oceans survey, which is undertaking a trophically integrated virus-through-zooplankton survey across more than 150 global sites, using a variety of molecular and microscopic techniques. The extremely large volumes of data coming from such efforts continue to fuel discoveries and novel analyses of marine microorganisms' ecology and evolution that are not yet possible for microbes in most other habitats.

TABLE 6.2 Estimated Volumes of Ocean Habitats

Habitat	Vol. (m^3)
Water column (<200 m below sea level)	3.0×10^{16}
Water column (200+ m below sea level)	1.3×10^{18}
Hydrothermal plumes[a]	7.2×10^{13} (/yr)
Subsurface ocean	$!10^{16}$
Sediment, all	4.5×10^{17}
Shelf sediment	7.5×10^{16}
Slope sediment	2×10^{17}
Rise sediment	1.5×10^{17}
Abyssal sediment	2.5×10^{16}
0- to 10-cm layer	3.6×10^{13}
Ocean crust[b]	2.3×10^{18}

Adapted from Orcutt et al. (2011).
[a]*The volume of hydrothermal plumes is given as the volume of plume fluid produced per year.*
[b]*The volume of oceanic crust was assumed by multiplying the average thickness of the oceanic crust (7 km by the assumed area of seafloor underlain by crust (65% of Earth's surface, or 3.3×10^{14} m^2)).*

6.4.1 Marine Planktonic Communities

The ocean contains diverse microbial habitats, both vertically (neuston to abyssopelagic depths) and horizontally (coastal upwelling regions versus open ocean gyres, the Mediterranean versus the Antarctic Ocean). As a general rule, microbial concentrations are highest within the neuston and drop markedly below this region (Figure 6.17); surface waters contain up to $\approx 10^8$ microorganisms/ml and decrease by more than 10-fold at a depth of 100 m. Coastal oceans support on average 10-fold higher microbial numbers than open oceans, due to terrestrial nutrient and carbon inputs; this is especially true in populated coastal areas (Rheinheimer, 1985). Oceans have profiles similar to those

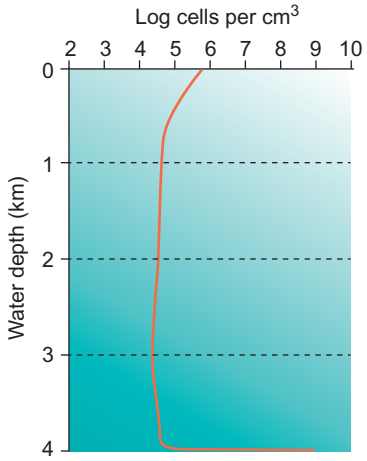


FIGURE 6.17 Cell density through the water column and surface sediments at station BIOTRANS in the north-east Atlantic. Note log scale. From Jørgensen and Boetius (2007).

Information box 6.3 Marine Microbes as Drivers of Discovery: Doubling the World's Known Proteins by Sequencing the Sargasso Sea

The oceans harbor a staggering number of microorganisms that mediate biogeochemical cycles which affect the entire planet. In 2003, a team of scientists led by J. Craig Venter (a leader of sequencing the human genome) embarked on round-the-world yacht trip to sample surface marine microorganisms and sequence their genomes. This monumental effort, termed the Global Ocean Survey, undertook the use of metagenomic analysis (sequence analysis of all the DNA in a sample, see Chapter 21) to increase knowledge of ocean microorganisms (Falkowski and de Vargas, 2004; Venter *et al.*, 1994). The nutrient-limited Sargasso Sea, a two-million square mile portion of the North Atlantic Ocean, was chosen for the journey's starting place on the assumption that its oligotrophic status would support a simpler community, more amenable to sequencing.

Sargasso Sea water samples were passed through filters (from 0.8 μm down to 0.1 μm) to capture microbial members of this marine environment. Once microorganisms were harvested, the scientists extracted DNA from the filters. Shotgun sequencing, a high-throughput technique commonly used in the field of genomics, was then used to characterize the community DNA. Using this approach, the team generated truly staggering amounts of data over the next years of their journey. Yooseph *et al.* (2007) organized these data into protein clusters of similar sequences, as a means of analyzing it without relying on reference databases for matching and identification (an approach which remains more common as it is easier, but is highly biased by, and limited to, the composition of the reference database(s) used and typically only permits analysis of a subset of the data). Using this approach, Yooseph *et al.* discovered that the marine microbial protein diversity discovered by their team to date—which was just a portion of the final total—doubled the world's known diversity of protein types.

of lakes presented later in the chapter, depending on whether the marine environment is oligotrophic like the open ocean, or eutrophic like coastal waters, especially coastal waters where sewage outpours may be present. Due to numerous efforts such as the International Census of Marine Microorganisms (ICoMM), we now know fairly definitively that pelagic bacterial communities are dominated by surface Cyanobacteria (particularly *Prochlorococcus*, described below), Alphaproteobacteria (driven by SAR11, described below) and Gammaproteobacteria (Zinger *et al.*, 2011) (Figure 6.18A and B).

6.4.1.1 Making Half the Oxygen You Breathe: Marine Phytoplankton

Overall, marine phytoplankton (Figure 6.5) make half of the oxygen you breathe, and this contribution is in turn divided about 50:50 between the cyanobacteria (Figure 6.19) and the eukaryotic algae. Cyanobacteria, dominated by the genera *Prochlorococcus* and *Synechococcus*, account for a quarter of global primary production. As the "coccus" of their names implies, they are small, round cells that are morphologically relatively

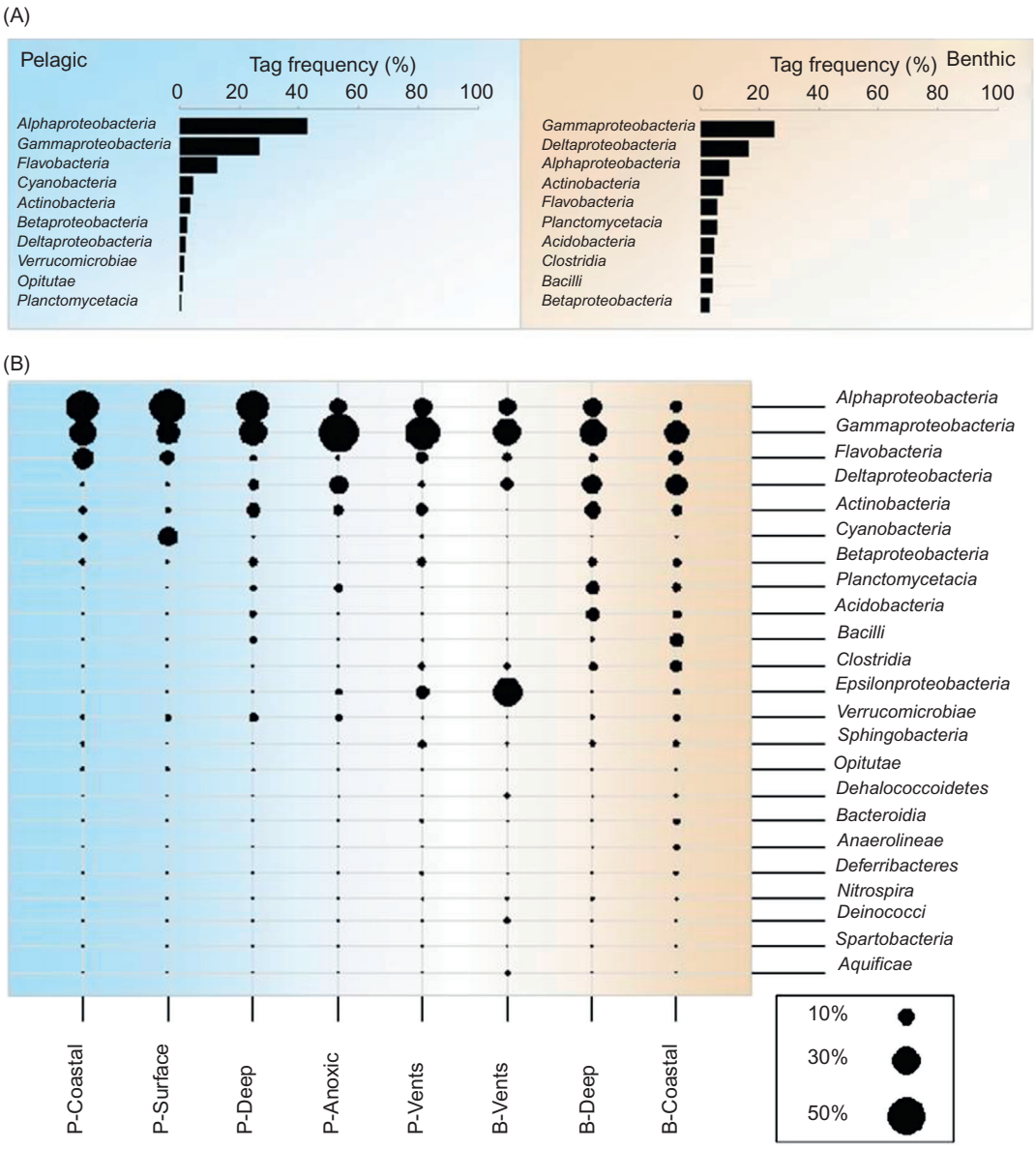


FIGURE 6.18 Bacterial community composition across 509 samples spanning the ocean's pelagic and benthic habitats, from the International Census of Marine Microorganisms. This represents 9.6 million sequences of amplicons of the 16S rRNA gene V6 hypervariable region, sequenced by 454 pyrosequencing (see Chapter 21). (A) The top 10 bacterial classes in each habitat (not including hydrothermal vents and anoxic habitats in the benthic averages). (B) Average abundances of bacterial taxa in various pelagic (P) and benthic (B) ecosystem types. From Zinger *et al.* (2011).

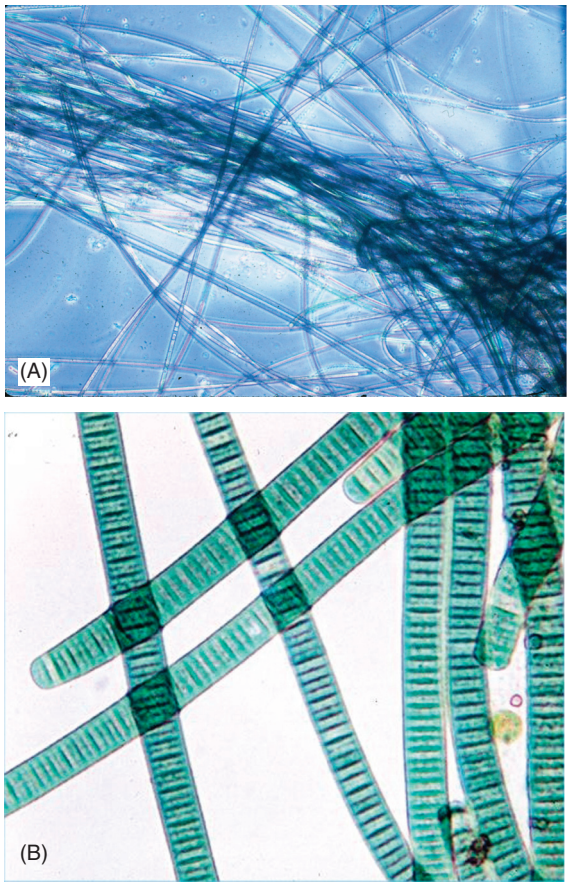


FIGURE 6.19 Example of cyanobacterial phytoplankton. (A) *Lyngbya* and (B) *Oscillatoria*.

nondescript. *Synechococcus* tend to dominate in coastal regions, while *Prochlorococcus* rule the vast oligotrophic surface waters in the center of oceans. In fact, *Prochlorococcus* are the numerically dominant photosynthetic cells on the planet—and yet were not discovered until 1988! These two genera have also been cultured in the lab, and been the focus of extensive physiological studies including the distributions and diversity. These cyanobacterial genera have played an important role in linking microbial diversity to physiology and ecology in both cultures and wild populations. Their studies leant great support to the microbial "ecotype" concept: microbial "species" are extremely difficult to define (see Section 19.2.3), but the rigorous diversity and ecological studies of *Prochlorococcus* clearly revealed related phylogenetic clusters that share defining ecological characteristics (for example, high-light adapted vs. low-light adapted) (Moore *et al.*, 1998). Other important cyanobacteria include lineages that fix nitrogen, including *Trichodesmium*, *Crocosphaera* and *Anabaena*, with *Trichodesmium* believed to account for about half of global marine nitrogen fixation (Bergman *et al.*, 2013). As in all habitats, in the oceans nitrogen gas must be fixed into ammonia to be biologically available (see Chapter 16 for details of this process and the nitrogen cycle). Although heterotrophic marine nitrogen fixers exist, cyanobacterial nitrogen fixers are currently considered to be the group performing the vast majority of marine nitrogen fixation.

Eukaryotic phytoplankton are responsible for roughly the other half of marine primary production, thus about a quarter of the primary production on the planet. They are also some of the singularly most beautiful microorganisms known (Figure 6.5). The remarkable morphologies many possess are created by hard outer coverings with intricate designs, the shapes of which can help keep them from sinking out of the photic zone. Eukaryotic phytoplankton are also responsible for much of the periodic bioluminescence seen in surface waters at night. Important groups include the diatoms, coccolithophores and dinoflagellates. Diatoms are responsible for about 20% of global photosynthetic primary production, and thus the majority of eukaryotic phytoplankton's contribution. They are also involved in the cycling of silica in the oceans: 4−50% of the dry weight of their cells is made of silica, which is used in a two-valved extracellular skeleton called a frustule. Their cells may be unicellular or form long chains, depending on the species and conditions. Coccolithophores are a second major group of eukaryotic phytoplankton, whose name comes from the Greek word for "round stone-bearers" due to the calcium carbonate ($CaCO_3$, i.e., chalk) plates that cover their surfaces. They are small but significant primary producers (on the order of $\approx$5% of the global signal), and like many phytoplankton grow to high abundance known as "blooms" in places like the Sargasso Sea and the Gulf of Alaska. The settling of such blooms to old ocean floors millennia ago during the Cretaceous formed the famed White Cliffs of Dover, England. The final important group of eukaryotic phytoplankton is the dinoflagellates. This diverse group actually includes members unable to photosynthesize (and thus living heterotrophic lifestyles), as well as groups that live symbiotically with marine organisms such as corals. Much of the planktonic bioluminescence in the sea is due to dinoflagellates.

Phytoplankton abundance and community composition vary depending on the season and conditions. Algal blooms occur when waters are eutrophic, warm and calm, and certain algae or cyanobacteria proliferate rapidly resulting in blooms. Such blooms are a natural part of the yearly cycling of many lake and ocean ecosystems. However, extreme eutrophication and bloom events can adversely affect the water quality in several ways. As described above, high amounts of primary production settling through the water column can strip oxygen from the water during its decomposition. In addition, water experiencing algal blooms tends to be unpleasant for recreation (fishing, boating and swimming) because of odors and slime. In the worst cases, harmful algal blooms are composed of algae that

produce potent toxins, sometimes resulting in coastal red tides. These get their name from the most notorious sources, red-pigmented dinoflagellates. The red tides of Florida, known for massive fish-kills washing onto beaches and human respiratory irritation, are caused by the dinoflagellate *Karenia brevis* blooming throughout the Gulf of Mexico, and then accumulating along the Southeast U.S. coast. *K. brevis* is a highly toxic alga that causes human illness, shellfish toxicity, animal and bird mortalities, and reddish water discoloration. As global change conditions exacerbate eutrophication and bloom-supporting conditions (warmer, more stratified waters), harmful algal blooms and red tides are likely to become more frequent and intense.

6.4.1.2 Heterotrophic Marine Microbes

There are several ubiquitous, abundant marine heterotrophic microbes, analogs to the major photoautotrophs *Prochlorococcus* and *Synechococcus*, described above. By far the most important is the alphaproteobacterial SAR11 clade typified by *Pelagibacter ubique*, whose name literally means ubiquitous pelagic bacteria. This clade was discovered in 1990 in 16S rRNA gene surveys and is seen throughout the world's oceans, from the tropics to the poles. Since it could not be grown, it bore the name SAR11, based on its first description as a sequence clade, from the 11th clone sequenced from a clone library made from Sargasso ("SAR") seawater. Finally, in 2002, this clade's "birth announcement" came after *Pelagibacter ubique* was successfully grown in the lab and its physiology began to be studied (Rappé *et al.*, 2002). Remarkably, about a third of all bacterial cells in the ocean are from the SAR11 lineage, which may be its own family in the Alphaproteobacteria. They are equally abundant in shallow and deep waters (Zinger *et al.*, 2011). Why is SAR11 so successful? It has a highly streamlined genome, which allows it to survive and reproduce even in very low-nutrient conditions. It also has diversified into a number of "ecotypes" specialized to different subhabitats, like *Prochlorococcus*. Lastly, its success may be partly due to its ability to supplement its energy needs (though not its carbon needs) by phototrophy, since at least some of the clade's members have the ability to perform photoheterotrophy (see Section 6.3.2.1) (Giovannoni *et al.*, 2005), which may allow them to get through lean times and channel more scarce resources into biomass rather than energy.

There are a number of other important heterotrophic groups in the oceans. They include microbes, such as the Deltaproteobacteria that often dominate deeper water, and Euryarchaeota. Euryarchaeota are less abundant overall than pelagic Crenarchaeota (which are highlighted in Section 6.3.1.1), but are ubiquitous, and appear to be seasonally important members of surface communities. A recent population genome assembly of one (from a metagenome, see Chapter 21) suggests a photoheterotrophic lifestyle via proteorhodopsin (see Section 6.3.2.1) with their carbon substrates of choice being proteins and lipids (Iverson *et al.*, 2012). Other important marine pelagic heterotrophs include protozoa such as flagellates and viruses (see next section), as well as fungi. Heterotrophs not only consume DOM and POM, but can be active predators; it is estimated that protozoa and viruses are responsible for similar amounts of bacterial mortality (Wommack and Colwell, 2000).

Fungi in the marine habitat occur in endolithic associations with limestone, the shells of sea creatures, sponges and corals (Golubic *et al.*, 2005). They have also been isolated from carbon-rich areas of the water column and benthic habitat. The distribution of fungi in aquatic environments is not well studied; however, there is increasing interest in useful secondary metabolites that marine fungi may produce. Another surprising fact is that fungi have been isolated from sediment samples taken at depths of 5000 m in the Central Indian Basin (Damarem *et al.*, 2006).

6.4.1.3 Marine Viruses

Viruses are important to almost any ecosystem so far studied (see Section 2.4 for a general overview of viruses), and are the most abundant biological entities on the planet (10^{31} on Earth), commonly outnumbering bacteria about 10 to 1. If laid end to end, 10^{31} viruses would stretch 10^{8} light years away from Earth, and comprise a biomass equivalent to $\approx$75 million blue whales. Such astronomical numbers have led to speculation that viruses represent the largest unexplored genetic reservoir on Earth.

Ocean viruses were thought nearly nonexistent until 1989, when seawater was concentrated onto electron microscopy grids, allowing their direct observation. Previous culture-based studies had used nonmarine microbial hosts, and thus had unsurprisingly failed to recover marine viruses. Now marine viruses are perhaps the best-studied environmental viruses, and are known to play diverse roles in marine ecosystems (reviewed in Breitbart, 2011). They impact microbes through mortality (cell lysis), horizontal gene transfer and the modulation of host metabolisms. Additionally, they alter global biogeochemistry. In fact, through lysis and the production of POM and DOM, they are responsible for the largest ocean carbon flux (150 Gt/yr) dwarfing all others by >five-fold). Viruses that infect phytoplankton are also important to nutrient cycling, because their lysis of phytoplankton's primary production promotes secondary production, as described in Section 6.3.2. Marine viruses also infect larger marine animals such as fish and crabs, and can

thereby cause considerable economic losses for the fishing industry, particularly in aquaculture settings with high host densities.

With new genomics-based windows (Chapter 21) into wild viral communities, the extent of viral impacts on large-scale biogeochemistry, beyond simple lysis, has been increasingly revealed. For example, viruses can contain "host" genes, which are expressed during infection to metabolically reprogram infected cells, in order to maximize viral production. The mechanisms by which this occurs can have profound biogeochemical impacts. The most surprising discovery of this is that cyanobacterial viruses carry "host" photosynthesis genes (reviewed in Breitbart, 2011). These genes are expressed during infection, allowing the infected cells to maintain photosynthesis (and thus energy production) longer, and are hypothesized to power the production of more progeny viruses. A significant portion of marine cyanobacterial photosynthesis in fact appears to be performed by phage-encoded photosynthesis proteins (Sharon *et al.*, 2007). Evolutionarily, these virally encoded photosynthesis genes are a dynamic gene pool, with the diversity generated among phage copies able to recombine back into the host gene pool (Sullivan *et al.*, 2006). This means that viruses appear to help shape global photosystem evolution! Such discoveries demonstrate the central ecological and evolutionary roles of viruses. However, in spite of advances in marine virology, environmental virology is bottlenecked by "unknowns." Fundamental questions such as "who infects whom?" remain open; 90% of each viral genome and metagenome is typically new to science, and cultured model systems insufficiently represent wild diversity. This is an area open for many more discoveries.

6.4.2 Marine Benthic Communities

Benthic environments are habitats of steep redox gradients and abundant microniches, as described in Section 6.2.3 along with the general characteristics of benthic aquatic microbes. The seafloor provides diverse habitats that can be grossly divided into soft-bottom sediments, hard-bottom rocky ocean crusts and hydrothermal vents. The latter is an extreme environment covered in detail in Section 7.4.1. There are a number of other notable but less abundant benthic habitats whose microbial communities have been studied in some detail; these include cold seeps and, though the concept may be surprising, "whale-falls," where the carcasses of whales fuel thriving successional ecosystems (see *Osedax* the "bone-eating" worm, Figure 6.20). Symbioses between microbes and macrobes are a hallmark of many of these systems.

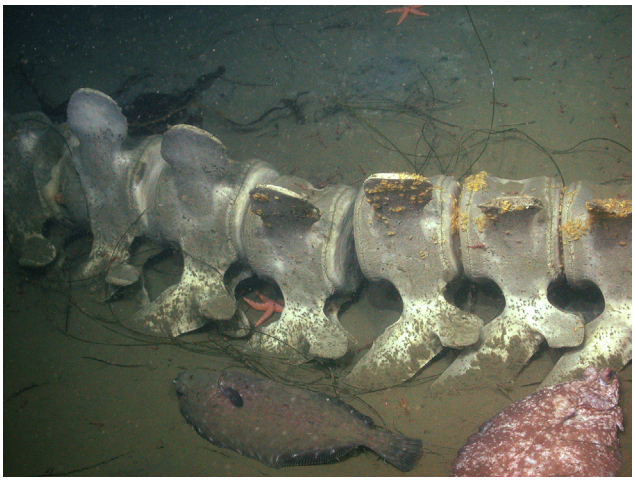


FIGURE 6.20 Two flatfish lurk near the vertebrae of a dead whale about 600 meters below the ocean surface in Monterey Canyon. Researchers at the Monterey Bay Aquarium Institute (MBARI) towed this whale carcass off a beach in Monterey Bay and placed it on the seafloor so that they could study the animals that fed on and colonized the carcass over time. The orange specks on the bones are amphipods, which consume the flesh of the recently sunk whale. MBARI researchers also discovered a new genus of worms (*Osedax*) that burrow into the whale bones after the flesh has been consumed. Instead of legs, these worms have "roots" which infiltrate the whale bone and contain unique endosymbiotic bacteria that can digest collagen within the whale bones and thus provide nutrition for the worms. © 2007 MBARI.

6.4.2.1 Marine Sediments

The seafloor is the final resting place for "marine snow" (sinking organic particles) and larger dead organisms, and this rain of organic matter from above can be an important food source for heterotrophic marine microorganisms. It can also add seasonality to the seafloor, where temperatures and other physicochemical conditions may remain more constant through the year. Rates of decomposition typically decline with depth, as does the amount of organic matter reaching the ocean floor. Concomitantly, the number of cells in the surface sediments typically declines with the seafloor depth.

From the global perspective enabled by ICoMM (one of the few broad efforts to survey diverse benthic habitats), Gammaproteobacteria comprise an average of $\approx$25% of global benthic bacterial communities surveyed (their analyses so far have focused on bacteria), while Deltaproteobacteria are the next most abundant phylum at $\approx$16% (Zinger *et al.*, 2011) (Figure 6.18). Overall, these groups, along with the next three most abundant phyla, the Planctomycetales, Actinobacteria and Acidobacteria, contain chemoautotrophs and anaerobic or microaerophilic heterotrophs, making them suited for benthic living. Coastal sediments are distinguished by lineages typically considered terrestrial or indicative of human contamination, the Clostridia and Bacilli (Zinger *et al.*, 2011). In deep-sea sediments, it appears that Acidobacteria in the

upper sediments give way to Chloroflexi and the candidate division JS1 in lower sediments (Zinger *et al*., 2011).

Other research reveals that Archaea and Eukarya are important components of the soft benthos. Crenarchaeota and Euryarchaeota are abundant in sediments, and benthic diatoms, forams and radioladiarans contribute to the benthic food web.

6.4.2.2 Rock-eaters Under the Sea

When we consider seafloor communities, we typically think of soft-bottomed habitats. However, much of the ocean's floor is made of basaltic crust, which extends several kilometers below the seafloor. Due to the resulting massive volume, basaltic crust is actually the largest potential habitat on Earth. But is it actually a habitat—can things live on rocks at, and below, the bottom of the sea? Discoveries just within the last decade suggest that chemolithoautotrophs—"autotrophs" meaning they fix their own carbon, "chemo" meaning they use chemical energy to do it and "lithos" meaning that the chemical energy comes from rock—are abundant, diverse and active in basaltic crusts. In fact, microbial cell numbers on seafloor basalt are typically 3−4 *orders of magnitude* higher than in the overlying waters (Santelli *et al.*, 2008), then decrease in the subseafloor. The physiologies of these abundant cells are just beginning to be investigated, and will likely be diverse since their communities appear more diverse than those in either deep or surface ocean waters (Santelli *et al.*, 2008). However, chemolithoautotrophy appears to be a major lifestyle, through various proposed mechanisms including iron, sulfur and manganese oxidation (Santelli *et al.*, 2008), and as supported by the presence of diagnostic gene sequences, isotopic investigations and incubation experiments (Lever *et al.*, 2013). Even before the last decade's discoveries in this habitat, subseafloor microbes had been estimated to comprise 10−30% of the total living biomass of Earth (Whitman *et al*., 1998). Understanding planetary carbon and energy cycles is simply not possible without understanding these systems, and their continued exploration is certain to yield new discoveries for years to come.

6.5 FRESHWATER ENVIRONMENTS

Freshwater environments, such as springs, rivers and streams, and lakes, are those not directly influenced by marine waters. The science that focuses on the study of freshwater habitats is called limnology, and the study of freshwater microorganisms is microlimnology. There are two types of freshwater environments: running water, including springs, streams and rivers; and standing water, including lakes, ponds and bogs. These freshwater environments have very different physical and chemical characteristics, and correspondingly different microbial communities and activities. For instance, the microbial community in a lake in Egypt is not the same as the microbial community in one of the Great Lakes in the northeastern United States. In this section we define various freshwater environments and outline the types of microorganisms that inhabit them.

6.5.1 Springs, Streams and Rivers

Springs form wherever subterranean water reaches Earth's surface. Microorganisms, especially bacteria and algae, are often the only inhabitants of springs. In general, photosynthetic bacteria and algae dominate spring environments, with communities ranging from 10^2 to 10^8 organisms/ml. These primary producers are present in the highest concentrations (10^6 to 10^9 organisms/ml) along the shallower edges of the spring and in association with rock surfaces, where light is available and inorganic nutrients are in highest concentrations (Rheinheimer, 1985; Kaplan and Newbold, 1993). Although heterotrophs are also present, numbers are usually low (10^1 to 10^6 organisms/ml) because DOM is low. As they mature and die, photosynthetic populations provide the initial source of organic matter for downstream heterotrophic populations. However, the largest portion of DOM found in surface freshwater originates from surrounding terrestrial sources. This organic input, which originates from sources such as plant exudates, dead plants, animals and microbial biomass, is transported into standing water habitats by mechanisms such as terrestrial runoff, seepage and wind deposition. Thus, we have the image of spring water starting at its source with very low concentrations of DOM and heterotrophs. The DOM and the heterotrophic populations steadily increase as the spring moves away from the source and as inputs of terrestrial organic matter and microbial biomass continue to accumulate (Kaplan and Newbold, 1993).

Springs, as they flow away from their subsurface source, merge with other water sources to form streams and rivers that eventually flow into other bodies of water such as lakes or seas. Streams contain primary producer communities, especially when light can penetrate to the bottom of the stream. Photosynthetic populations range from 10^0 to 10^8 organisms/ml and tend to be present as attached communities associated with biofilms because of the flowing nature of the water column. Phytoplankton (free-living) communities also exist in streams, but because of the constant water movement, they are not spatially stable populations (Rheinheimer, 1985).

As a stream progresses and becomes larger, it tends to accumulate DOM from surface runoff and sediments. The increase in DOM limits the penetration of light and consequently begins to limit photoautotrophic populations.

In turn, heterotrophic populations begin to increase in response to increased DOM. In general, the concentration of heterotrophs in streams and rivers ranges from 10^4 to 10^9 organisms/ml, with microbial numbers increasing as DOM increases. Because of their flow patterns, stream and river waters are for the most part well aerated, meaning that their microbial inhabitants are predominantly aerobic or facultatively aerobic. Although isolated pools that form in rivers act as DOM and POM sinks and support fairly stable heterotrophic planktonic communities, the only truly stable populations in the flowing habitats of streams and rivers are the biofilm and sediment (benthic) communities (Rheinheimer, 1985).

6.5.2 Lakes

Lakes are among the most complex of the freshwater environments. They may range from small ponds to vast lakes that generate their own weather patterns, such as Russia's Lake Baikal, which holds roughly one-fifth of the world's unfrozen surface freshwater, and is the deepest lake on the planet, and the U.S.A.'s Great Lakes. Although often regarded as nonflowing environments, lakes have inflows and outflows. Lakes may have unique chemical composition, and can form extreme environments (see Chapter 7); examples include salt lakes (see Section 6.6.2), bitter lakes that are rich in $MgSO_4$, borax lakes that are high in $Na_2B_4O_7$ and soda lakes that are high in $NaHCO_3$.

Lake microbial communities and their interactions are complex and diverse, reflecting the complexity of the habitat. Lakes contain extensive primary and secondary productive populations that interact dynamically. The primary productivity in the shallow near-shore waters is high, driven predominantly by algae and secondarily by cyanobacteria. The attached communities here are dominated by the presence of filamentous and epiphytic algae. Central lake waters are dominated by phytoplankton, which form distinct community gradients based upon the wavelength and the amount of light that penetrates to a given depth (Figure 6.21). One example of a lake-dwelling microbial phototroph with a specialized niche is *Chlorobium*, a green sulfur bacterium. *Chlorobium* can use longer wavelengths of light than many other phototrophs, meaning they can live deeper. They are also anaerobic organisms, using H_2S rather than H_2O for photosynthesis (see Section 16.4.3.2). Thus, they have a competitive advantage in establishing a niche at depths lower in the water column or even in the surfaces of sediments, where only small amounts of light penetrate, little or no oxygen is present, but hydrogen sulfide is available.

In addition to their phototrophic populations, lakes have extensive heterotrophic communities. Heterotrophic concentrations vary with depth, but there are three areas

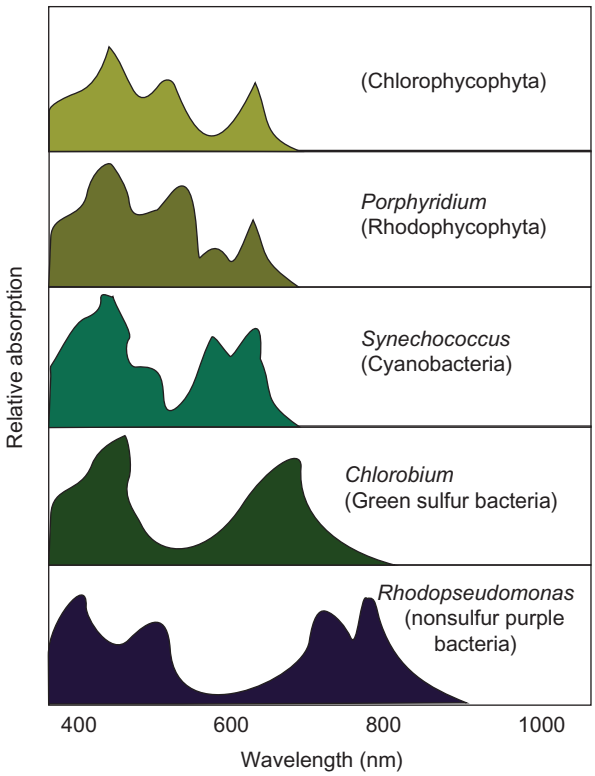


FIGURE 6.21 Graphs showing the light absorbance spectrum of common phytoplanktonic algae and photosynthetic bacteria. It can be seen that each of these groups has a different profile. This enables groups to take advantage of their niche. In general, organisms that are capable of utilizing longer wavelengths are found deeper in the water column. Thus, they do not have to compete with organisms higher in the water column that absorb the shorter wavelengths. Adapted from Atlas and Bartha (1993).

that generally have elevated numbers of heterotrophs (with some parallels to marine systems). These are areas with higher nutrients and organic matter: the neuston layer; the thermocline, where organic debris tends to settle and accumulate; and the upper layer of the benthos, where the heterotrophic populations are mainly anaerobic.

There are some striking differences between oligotrophic and eutrophic lakes; Figure 6.22A and B compare the major bacterial populations typically found in each (Konopka, 1993). Oligotrophic lakes have higher rates (four- to 20-fold) of primary production than eutrophic lakes, due to their deeper light penetration. In oligotrophic lakes, as might be expected, the amount of secondary production is directly coupled to primary production, and secondary production in the photic zone is generally 20 to 30% of primary production. Eutrophic lakes have much higher ($\approx$three to 80 times) rates of secondary production than oligotrophic lakes, and of a decoupling from primary production than oligotrophic lakes (Atlas and Bartha, 1993).

Apart from their microbial and algal populations, streams, rivers and lakes also contain fungi, protozoa and

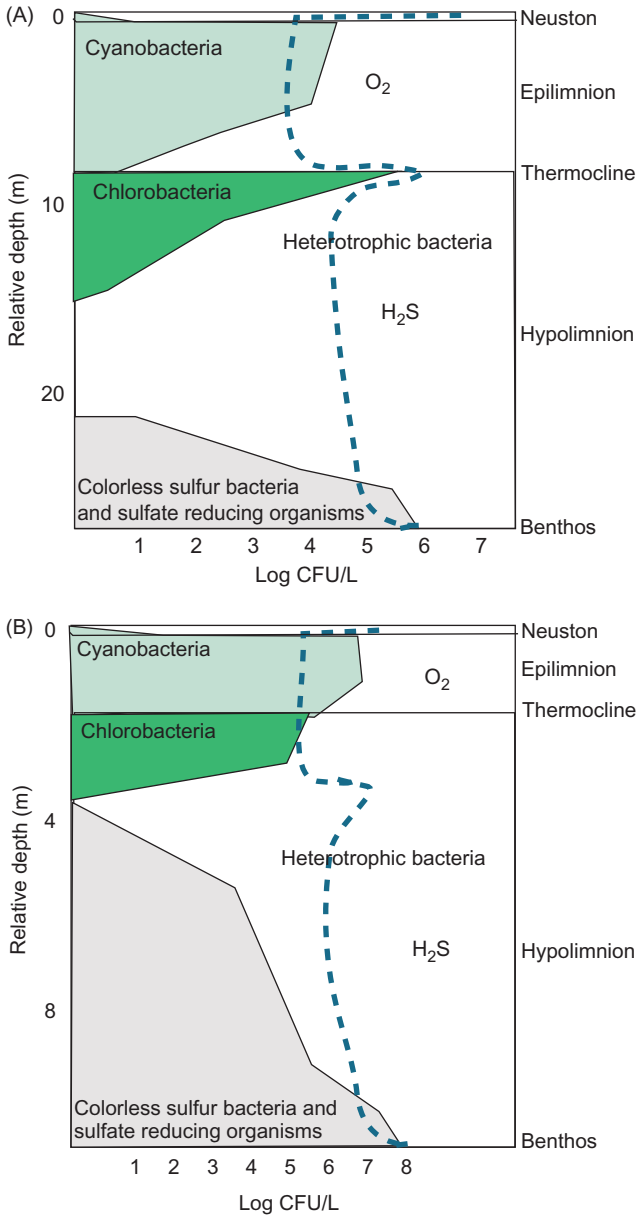


FIGURE 6.22 (A) Schematic representation of bacterial distribution in a typical oligotrophic lake. Notice especially the distribution and concentrations of the photosynthetic populations. Also note the lower concentration of heterotrophs in the upper zone, where cyanobacteria predominate. The large increase in the heterotrophic population between the epilimnion and the hypolimnion is related to the presence of a zone where organic matter accumulates. This area is known as a thermocline and is a zone where the sunlight-warmed surface water (less dense) and the deeper colder water (more dense) meet, forming a density gradient where organic matter accumulates. (B) Schematic representation of a typical eutrophic lake. This figure shows the same groups of organisms as in (A) indicating the localization and relative concentrations throughout the water column. Notice that both the photosynthetic and the heterotrophic populations are considerably higher in a eutrophic lake. Adapted from Rheinheimer (1985).

viruses, which interact and contribute to the functioning of the food web. Fungi serve as parasites of planktonic algae, preventing overpopulation and allowing light to penetrate farther into the water column. Other fungi have simple lifestyles, colonizing surfaces and often forming fungal lawns, whereas still others can have complex predatory lifestyles. A fascinating example is *Zoophagus insidians*, which live attached to filamentous green algae in rivers and lakes. The fungi have long hyphae which trail down into the water column forming fishing lines: when touched by prey such as rotifers, the hyphae rapidly secrete a sticky substance, ensnaring the microscopic animal. The hyphae then grow quickly into the mouth of the rotifer and form a fungal mycelium that absorbs the contents of the animal's body from the inside.

Protozoa and viruses are important predators of aquatic microorganisms. Protozoan populations are typically several orders of magnitude lower than bacterial numbers. They are able to affect the numbers of bacteria and algae because each protozoan is able to consume hundreds of bacteria and algae per day. Protozoa and their food species often exhibit the cyclic, temporally offset population dynamics typical of predator–prey relationships, where the prey is abundant first, then the predators' numbers rise and cull the prey, until food is limiting and predators decrease again. Viral and host populations fluctuate in a similar fashion (Wommack and Colwell, 2000). Viruses in freshwater environments can be very abundant, with viral numbers exceeding bacterial by two orders of magnitude (versus one in most marine habitats). Like marine viruses, freshwater viruses have diverse hosts, including microbes, microalgae, protozoa and larger organisms. As in marine systems, viruses can cause an appreciable amount of bacterial mortality (20 to 60%) (Suttle, 1994; Hennes and Simon, 1995), and the associated release of DOM fuels secondary production (Middledoe *et al.*, 1996), as discussed in Section 6.3.2. Together, protozoan and viral populations help to control the concentrations and biomass of the bacterial and algal communities (Wommack and Colwell, 2000).

6.6 OTHER NOTABLE AQUATIC ENVIRONMENTS

6.6.1 Brackish Waters

Brackish water is a broad term used to describe water whose salinity is between that of fresh and marine water, and these are often transitional areas where such waters mix. An estuary, which is the part of a river that meets the sea, is the best-known example of brackish water.

Estuaries are highly variable environments because the salinity can change drastically over a relatively short distance, ranging from 10‰ to 32‰ (Information Box 6.1), and over time of day due to tidal cycles (for example, high tide bringing saltier marine waters farther up into the estuary). Seasonal increases in freshwater due to rainfall or snowmelt will decrease the salinity at a given point in the estuary. In order to survive here, resident microbes must be adapted to these large fluctuations in salinity. Despite this challenge, estuaries are very productive environments. In general, estuarine primary production is low, due to poor sunlight penetration as a result of high turbidity, which occurs from suspended organic matter brought by river inflow and tidal mixing (Ducklow and Shiah, 1993). However, heterotrophic activity and secondary production are high. Primary and secondary production are decoupled in these systems, because of the large amounts of organic carbon brought by terrestrial runoff and river inflow. In fact, the supply of carbon and nutrients can be so great that in many cases estuaries can actually become anoxic for whole seasons during the year (Ducklow and Shiah, 1993).

6.6.2 Hypersaline Waters

Hypersaline environments include coastal lagoons, salt and soda lakes, salterns (human-made hypersaline ponds for producing salt; Figure 6.23A), deep-sea brine pools (formed from the dissolution of salt during seafloor tectonic activity), brine channels in sea ice, and fermented foods and pickling brines. Hypersaline environments have higher salinities than seawater ($\approx$35‰) and may even be salt saturated. At room temperature, saturation of freshwater with sodium chloride results in $\approx$270‰. The saltiest aquatic habitat on Earth may be the hypersaline lakes of McMurdo Dry Valleys in Antarctica, where the salinity can reach $\approx$440‰—see Section 7.1.1 for a detailed discussion of these lakes. Hypersaline environments are considered extreme because normal cell physiologies cannot withstand the strong salt concentrations: the salinity gradient from inside to outside the cell causes it to rapidly desiccate, losing its cellular water.

Halophiles, a type of extremophile (see Chapter 7 for other examples) adapted to salty habitats, have mechanisms for accumulating or producing nontoxic solutes inside their cells to be isosmotic with the external environment. Their proteins are also specially modified to prevent denaturation at high salt concentrations. For surface-sunlit hypersaline habitats such as salterns, high UV levels are also a challenge, and many of the microbes there have pigments to protect them, as well as efficient DNA protection and repair systems. The microbial pigments, often carotenoids and bacteriorhodopsins, typically lend shallow salt habitats a pink or orange color. While halophiles occur in all three domains of life, there are comparatively few eukaryotic halophiles and an abundance of archaeal ones. One of the most notable halophiles is the ubiquitous genus *Haloquadratum* (Figure 6.23B), a euryarchaean within the class Halobacteria (note the counterintuitive "*-bacteria*" ending despite being within the Archaea), which is shaped just like a flat, square salt crystal! Like other extreme environments, the harsh conditions of hypersaline waters result in lower microbial community diversity, since fewer lineages are able to survive in them (Figure 6.23C). Halophiles are of particular interest to astrobiologists (biologists who study life on Earth that may be similar to life on other planets, and search for that extraterrestrial life), since remnant water on Mars is likely to be highly salty, and also UV levels at the Martian surface are high.

6.6.3 Subterranean Waters

The groundwater environment is in the subsurface and includes shallow and deep aquifers. The characteristics and microbial communities of the groundwater environment have been discussed in Sections 4.2.1.3 and 4.6. Briefly, microorganisms are the sole inhabitants of these environments and bacteria and archaeans are the dominant types of microbe present. In general, levels of microbial activity are low, especially in intermediate and deep aquifers. As shown in Figure 4.18, activity is orders of magnitude lower in these aquifers than in other aquatic habitats, due to low nutrient levels. Many subsurface environments may even be considered extreme from a nutrient perspective (Chapter 7).

6.6.4 Wetlands

Wetlands are a habitat type where soils are seasonally or permanently saturated with water, which can be freshwater, brackish or marine. They contain distinct plant and microbial communities, and represent a diverse and important aquatic habitat. They were discussed in Section 4.2.1.4, and are simply highlighted here. Marine wetlands include mangrove swamps that provide "nursery" habitat for the larvae of many fish species. Brackish wetlands include estuarine marshes that can act as massive filtration systems to decrease total carbon and nutrient loading on coastal waters. Related to this, human-made wetlands are often created as part of wastewater treatment (described in Section 25.6). Freshwater wetlands are the largest natural source of the potent greenhouse gas methane (CH_4) to the atmosphere (Denman *et al.*, IPCC report, 2007). Freshwater wetlands also include peat bogs, which contain a vast amount ($>$30%) of the planet's stored soil carbon pool. This stored carbon may be in danger of release to the atmosphere under continued climate change (Denman *et al.*, 2007).

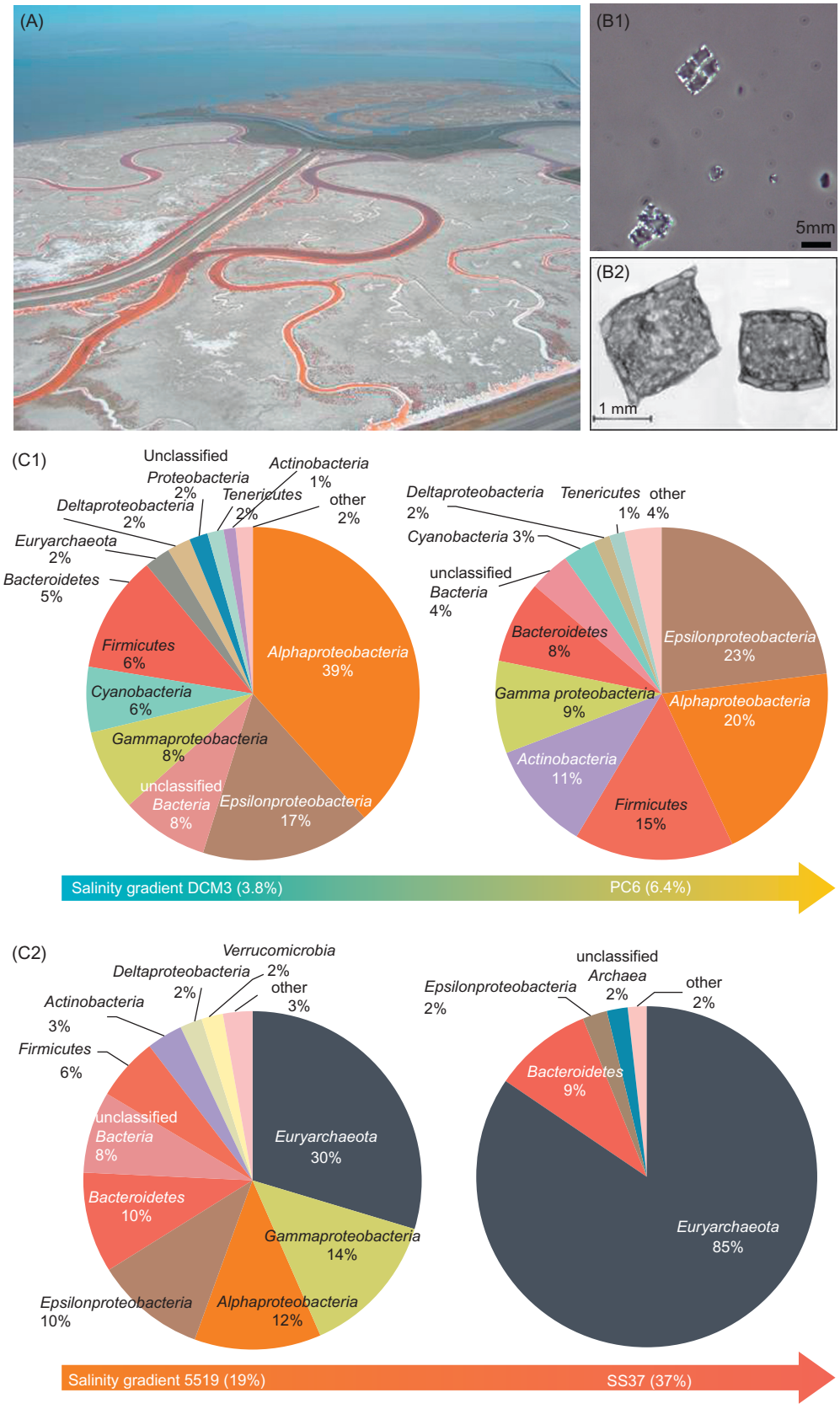


FIGURE 6.23 Hypersaline communities. Diverse hypersaline habitats exist including salt ponds, like those on the shores of California's San Francisco Bay (A). (B) *Haloquadratum* is one of the most memorable halophiles for its box-like shape, reminiscent of a salt crystal. (C) Microbial community composition shifts and becomes less diverse as salinity increases. A study compared Mediterranean warm waters at 35‰ salinity, a hypersaline coastal lagoon in the Galapagos Islands at 6‰, and two Spanish salterns (salt ponds) at 190‰ and 370‰. The research team profiled the communities using metagenomic sequencing (Chapter 21), and these pie charts show the taxonomic breakdown of 16S rRNA gene sequences pulled from those metagenomic datasets (using sequences longer than 100 bp, identity >95% to the taxonomy indicated, total number of 16S rRNA reads per dataset ranging from 1408 to 3333). *Haloquadratum walsbyi* (shown in the inset of (B)), within the Euryarchaeota, was by far the dominant microbe in the two most saline sites, at 15% and 64% of the total reads, respectively. (B) © Mike Dyall-Smith. http://www.nature.com/news/2004/041011/full/news041011-3.html and http://web.aanet.com.au/~aanet/DGBHome/Research/PC_square4_7_opt_L.jpg. (C) From Ghai *et al.* (2011).

QUESTIONS AND PROBLEMS

1. What are the most "important" aquatic habitats by volume and/or by relevance to humans?
2. Describe large-scale and small-scale ways in which aquatic environments are not homogeneous.
3. What is meant by the term microbial loop? Roughly how much primary production is fueled by recycled nutrients?
4. What are biofilms and microbial mats? Where would you expect to find each? Why are they of particular interest to humans?
5. A newly trained environmental engineer is hired to investigate solutions to clogging of water distribution lines by a persistent, thick and gelatinous material. The astute engineer quickly recognizes that this recurring problem may be caused by microorganisms and rushes to isolate and characterize the microorganisms clogging the pipelines. The engineer is successful in culturing several microorganisms in broth cultures (i.e., flasks containing liquid microbiological media) from the material found in the pipelines. In these broth cultures, the engineer determines the amount of an antimicrobial compound necessary to kill these microorganisms. To be certain an adequate amount of this antimicrobial compound is delivered, the engineer adds twice as much as the broth culture-based tests suggested would be necessary. Much to the engineer's surprise and dissatisfaction, the treatment is ineffective in killing the microorganisms found within the pipelines. Why did this treatment fail? What additional measures might the engineer need to take to solve the company's problem with the clogged pipes? Can you devise any novel strategies based on material presented in this chapter?
6. What is photoheterotrophy? Give two specific examples of microbes that live this lifestyle.
7. What is aquatic chemoautotrophy, and why may it be important?
8. What is a thermocline?
9. Describe how marine environments differ from freshwater environments physically, chemically and microbially.
10. What roles do aquatic viruses play in ecosystems?
11. Your first job is as an environmental microbiologist is at a wastewater treatment plant, where you are in charge of the sludge bioreactors. Even before you read the later chapter on wastewater treatment, why do you care about aquatic viruses in your system?
12. You have been hired fresh out of college by a geoengineering consulting firm, Geoengineering Real Solutions. You are put on a team evaluating ocean fertilization with iron as a way to sequester carbon from the atmosphere. The idea is that iron is a limiting micronutrient for a number of phytoplankton in the oligotrophic open oceans. Fertilizing large areas of ocean by dumping iron filings off of tanker ships should cause phytoplankton to bloom, fixing more CO_2 out of the atmosphere, resulting in more carbon getting buried in the deep sea due to sinking particles. How would you use your logic to evaluate whether this solution is likely to be successful, and what additional pieces of information might you need to help your team decide?

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